STUDIES ON PERINATAL LAMB MORTALITY WITH PARTICULAR REFERENCE TO IMMUNOLOGICAL AND NUTRITIONAL FACTORS

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This thesis is presented for the Degree of Doctor of Philosophy of the University of Edinburgh

1977

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ACKNOWLEDGEMENTS

The author wishes to record his gratitude to the Iraqi Government, Ministry of Higher Education and Scientific Research for the financial support they offered throughout the period of the study.

I am very grateful to Professor J. T. Baxter (Professor and Head of the Department of Veterinary Medicine, University of Edinburgh) for the opportunity to undertake this work, for his continuous encouragement and also for the excellent provision of facilities in his Department without which, this work would not have been completed.

My special and sincere thanks to Dr. D. L. Doxey for his efficient guidance and advice through all the stages of the study. To him I will remain greatly indebted.

I am most grateful to Dr. W. J. M. Black for making it possible for me to have access to the flocks of sheep used in the study, for the very useful remarks he made during preparation for the work, and also for the provision of facilities on farms of The Edinburgh School of Agriculture.

I accord my sincere thanks to Mr. R. Brown (Chief Technician of the Department of Veterinary Medicine) and other members of his staff, notably Mr. G. Keay (Senior Technician), for their skilful technical assistance.

I am most grateful to Mr. J. FitzSimons (Animal Recorder of the Edinburgh School of Agriculture), Mr. A. Cowe (the then low-ground Shepherd of the same School) and Mr. I. A. Walton (Veterinary student) for the assistance they offered on the farm, particularly during the lambing periods.

For the various kinds of help received from other staff of the Royal (Dick) School of Veterinary Studies, namely: Drs. G. R. Scott and A. G. Luckins (Centre for Tropical Veterinary Medicine), Mrs. G. McConnell (Data Programmer), Mrs. H. London (Librarian), Mrs. M. Brand (Secretary of the Department of Veterinary Medicine) and Mr. Munro (Photographic Department), I am duly grateful.

I am indebted to Mrs. M. Allison for the meticulous way in which she typed this thesis.

Finally, I accord my thanks to all the friends I met in Edinburgh, whose encouragement and support during my long stay away from home are greatly appreciated.

SUMMARY

This study was concerned with perinatal lamb mortality (PLM). Over a three year period, 263 ewes and 576 lambs were studied. The work was divided into three major sections.

The first section was a preliminary study divided into two parts. In the first part, basic data on PLM were collected from a variety of sheep to ascertain which aspects of the topic were worthy of further investigation. In the second part, the effects of ewe nutrition during the last eight weeks of pregnancy on lamb performance were investigated. Particular attention was paid in both studies to mortality rates, causes of death and birth weight and growth rate of lambs. The effect of litter size and the passive immune status of the lambs were also observed.

The preliminary results lead on to the second major section in which two topics were investigated. Firstly, the effect on PLM, subsequent lamb performance and the lamb's immunoglobulin status was investigated when twin lambs were deprived of colostrum. The deprivation appeared to have no demonstrable detrimental effect. Secondly, the effect of ewe nutrition during the last eight weeks of pregnancy on PLM, the immune status of lambs and the subsequent performance of both ewes and

lambs was studied. Nutritional levels affected both ewe and lamb performance.

In the last major part of the work the combined effect of inadequate ewe nutrition during late pregnancy and colostrum deprivation of twin lambs was studied. A detrimental effect on lamb performance was observed when the two factors were imposed together.

Throughout the various stages of this work, blood levels of 3-hydroxybutyrate, urea and albumin were used to monitor the ewe's nutritional status. The major laboratory investigations were concerned with levels of immunoglobulins (IgG_1, IgG_2, IgM and IgA) in lamb's serum and also in ewe's serum and colostrum. In addition, blood glucose, PCV and serum alkaline phosphatase levels were measured in lambs. The value of these parameters was assessed in relation to PLM.

C H A P T E R O N E

INTRODUCTION

DEFINITION OF PERINATAL LAMB MORTALITY

Sheep play a very important role in farming systems and whether it is a major or a complementary enterprise, it has to be a profitable one.

One of the factors that can affect profitability in sheep is losses due to perinatal lamb mortality (PLM) (Meat and Livestock Commission Report, 1972; Barton and Blyth, 1977; Dickson, 1977; Maxwell, 1977). This can be a major factor in lowering sheep productivity, causing the loss of millions of pounds to the sheep-producing industry.

The efficiency of sheep production can be improved, for example, by the production of larger numbers of strong lambs per lambing or by achieving more than one lambing per year and more lambings in a ewe's lifetime. The whole object of this increased efficiency is lost if lamb mortality rises to keep pace with increased production.

There are many scattered reports about lamb losses in relation to various factors, but only a few of these describe precisely the term "perinatal period" in relation to parturition and there seems to be no international definition for this term.

McFarlane (1961) mentions only the period covering the seven days after parturition. Stamp (1967) gives

figures for a ten-day period after parturition and Dennis (1972) extends the time to 28 days after birth. Some authors talk about lamb mortality until marking time (Moule, 1954; Gunn and Robinson, 1963) and others cover a period as late as three months after birth (Vetter, Norton and Garrigus, 1960; Seth, Pandey and Roy, 1972; Harker, 1977). Many workers have reported figures for mortality up to weaning time (Purser and Young, 1959; Davies, 1964; Watson, 1972; Wiener, Deeble, Broadbent and Talbot, 1973; Whitelaw, 1976). In some reports, data is presented which has been collected over a two-year period and lambs of almost any age are included. However, despite these differences, most authors agree that the highest percentage of PLM occurs within 10 days of birth and the majority of these losses take place during the first few days of life (Venkatachalam, Nelson, Thorp, Luecke and Gray, 1949; Thomson and Thomson, 1949; Alexander and Peterson, 1961; McFarlane, 1961; Watson and Elder, 1961; Davies, 1964; Purser and Young, 1964; Booth, 1972; Dennis, 1972; Pout, 1973; Whitelaw, 1976; Harker, 1977; Saunders, 1977). I have, therefore, decided to define PLM as "the death of fetuses or newborn lambs occurring before, during or within 10 days of parturition".

Theoretically, it may not be valid to compare figures reported for lamb mortality unless these figures represent losses during a fixed period of time after lambing.

However, despite this, the reports on lamb mortality agree that losses from this complicated problem are too numerous and costly for the sheep industry to continue to accept and that a reduction in the level of losses would be of great economic importance.

EXTENT AND SIGNIFICANCE OF PLM

PLM is a difficult problem to study because of the factors involved, the absence of fixed criteria and agreed definitions, plus the fact that most farmers tend to believe that PLM is not a problem and, consequently, fail to investigate it or allow others to investigate for them.

In many cases, the surveys that have been undertaken have been conducted on individual flocks and deal with one or just a few of the many factors involved. These surveys show contrasting results about losses in lambs.

Perinatal mortality is a problem not only in sheep but also in other farm animals and even in humans, particularly in some under-developed countries and Alexander (1971 - 1972a) in his general appraisal of the problem gave an average loss of 20 per cent for all lambs and piglets born, 10 per cent for calves and 1·8 to 8·2 per cent for humans.

Twenty per cent of all lambs which are born die before weaning in most sheep-rearing countries (Stamp, 1967) and this is an average figure which can vary from

country to country and, in a particular area, it even varies from flock to flock. According to Watson (1972) lamb mortality in Victoria, Australia, varied from five per cent to 77 per cent of all lambs born and, in Scotland, Barton (personal communication), in his three years' survey of commercial, low ground flocks, found that PLM varied from six per cent to 48 per cent. Surveys done in different countries of the world revealed the levels of lamb losses shown in Table 1.

For sheep farming in Great Britain, these percentages mean losses of 1·5 to four million lambs annually (Wiener et al., 1973). In Scotland, a 15 per cent lamb mortality in hill flocks represents a loss of 350,000 lambs per year. In New Zealand, two million lambs are lost annually when the lamb mortality is represented by a level of only 10 per cent (Thomson and Aitken, 1959). In Australia, where high levels of losses in lambs have been reported, a 20 to 25 per cent PLM means a loss of 13 to 17 million lambs a year (Watson, 1972).

With such a high level of losses, it is essential for all workers involved in the sheep industry to be aware of the different causes of this problem, so that once the role of each cause in a particular area is known, then suitable preventive measures can be taken.

TABLE 1.

Level of lamb losses in different countries

Country	Level of Losses as percentage of lambs born	Reference
Australia	36	Moule (1954)
"	15	McFarlane (1961)
"	21·1	Smith (1962)
"	22·8	Dennis (1970)
"	17·7	Dennis and Nairn (1970)
"	25 - 35	Booth (1972)
"	20 - 25	Watson (1972)
Great Britain	12 - 15	Stamp (1967)
"	17·6	Saunders (1977)
India	23	Singh and Singh (1970)
"	14	Malik and Acharya (1972)
New Zealand	12 - 15	Hartley and Boyes (1964)
"	17·8	Hight and Jury (1969)
Scotland	12 - 19	Purser and Young (1959)
"	15 - 20	Houston (1972 - 1973)
"	13·4	Edin. School of Agric., Annual Report (1973)
"	6 - 48	Barton (1974)
South Africa	9·4 - 13·5	Reyneke and Colyn (1966)
Sweden	13·5	Gunnarsson et al. (1972)
U.S.A.	28·6	Venkatachalam et al. (1949)
"	10·1	Mathews and Ogden (1957)
"	18	Vetter et al. (1960)

CLASSIFICATION AND CAUSES OF PLM

McFarlane (1961, 1965) classified PLM according to the time of death into three main classes -

1. Ante-partum deaths: this represents all deaths before the start of the birth process.
2. Partum deaths: this represents all deaths during the birth process.
3. Post-partum deaths: this represents all lambs that died after surviving the birth process.

Within these three major classes he included a total of 21 sub-classes. By applying this method during post-mortem examination on 3,543 dead lambs, he found that the first class was represented by seven per cent, the second by 36 per cent and the third and largest represented 58 per cent of the losses.

Dennis (1966), using McFarlane's criteria during a three-year investigation of causes of PLM in Western Australia, found that ante-partum, partum and post-partum classes were represented by five, 20·5 and over 74·0 per cent respectively and this seems to be the generally accepted picture for the different classes of PLM.

Stamp (1967) classified PLM into the following:

1. Ante-natal or ante-partum stillbirth (often termed "abortion").

2. Intra-natal or intra-partum stillbirths and early post-natal deaths.
3. Neonatal mortality.

Dennis (1972), although he agreed with McFarlane's classification of PLM, proposed a perinatal period based on the perinatal period for man, and he classified lamb deaths into the following two major classes:

1. Fetal deaths, to include deaths before or during parturition.
2. Neonatal deaths, to include deaths of newborn lambs within the first 28 days of life.

In their classifications of PLM, both McFarlane and Dennis tried to avoid the use of vague terms such as stillbirths. However, Stamp (1967) eliminated any confusion resulting from the use of terms like abortion or stillbirth by referring also to the time of death in relation to birth.

In the present study of PLM the following categories will be used:

1. <u>Ante-partum deaths</u>:

In all cases the lambs have died in utero before the birth process commenced. Abortion means that the lamb was produced at least 10 days prior to the expected date of birth. Stillbirth is the term applied to lambs dying in utero which were born within 10 days of the expected parturition

date and includes lambs that were born dead during a normal parturition process.

2. Partum deaths (dystocia):

All lamb deaths directly attributable to any part of the birth process.

3. Post-partum deaths:

This category is divisible into two sub-divisions.

Neonatal: Deaths occurring between birth and 10 days of age.

Late post-natal: Deaths occurring between 10 - 28 days of age. This subdivision is outside but related to PLM and will be included when applicable.

There are various causes of death operating in these classes and subclasses of PLM. Stamp (1967) gave a detailed explanation of the role of different infectious and non-infectious factors within each of these classes. From what has been shown during the investigations into the causes of PLM by different workers (Thomson and Fraser, 1939; Thomas, 1945; Wallace, 1948; Thomson and Thomson, 1949; Venkatachalam et al., 1949; Moule, 1954; Alexander and Peterson, 1961; McFarlane, 1961; McDonald, 1962; Dennis, 1964, 1965a,b, 1966, 1970, 1972; Stamp, 1967; McMeniman and Young, 1968; Dennis and Nairn, 1970; Alexander, 1971-72b; Plant, Beh and Acland, 1972;

Houston and Maddox, 1974; Owen, 1976; Whitelaw, 1976) it can be said that, in general terms, PLM is usually a result of various kinds of nutritional, managemental, infectious, environmental, maternal and other factors but, in order to be more specific, causes of PLM can be placed into seven categories.

1. Starvation - mismothering - exposure - *Escherichia coli* complex.
2. Undernourishment of the ewe during late pregnancy.
3. Dystocia.
4. Infections.
5. Congenital malformations.
6. Predators.
7. Accidents.

I will now consider the importance of these categories in more detail.

C H A P T E R T W O

REVIEW OF LITERATURE

CAUSES OF PERINATAL LAMB MORTALITY

Perinatal lamb mortality is a complicated problem and most of the workers who investigated it covered one or more of the many factors that have been implicated. PLM has been studied in relation to the following factors.

Breed, (Mathews and Ogden, 1957; Gunn and Robinson, 1963; Wiener, 1969; Fogarty, 1972; Watson, 1972; Wiener et al., 1973; Barker, 1975; Doney and Gunn, 1976; Saunders, 1977).

Age and parity of the ewe, (Mathews and Ogden, 1957; Purser and Young, 1959, 1964; Gunn and Robinson, 1963; McDonald, 1966; Hight and Jury, 1969; Mullaney, 1969; Turner, 1969; Watson, 1972; Marais, 1974; Doney and Gunn, 1976; Harker, 1977; Saunders, 1977).

Gestation length, (Moule, 1954; Alexander, 1956; Alexander and Peterson, 1961; Dawes and Parry, 1965; Mullaney and Lear, 1969; Shevah, 1974).

Litter size, (Thomson and Thomson, 1949; Moule, 1954; Mathews and Ogden, 1957; Watson and Elder, 1961; Gunn and Robinson, 1963; Davies, 1964; Purser and Young, 1964; Shelton, 1964; Smith, 1964; Hight and Jury, 1969; Mullaney, 1969; Singh and

Singh, 1970; Seth *et al.*, 1972; Watson, 1972; Harker, 1973, 1977; Barker, 1975; Slee, 1976; Saunders, 1977).

Lamb birth weight, (Wallace, 1948a; Thomson and Thomson, 1949; Moule, 1954; Purser and Young, 1959, 1964; Watson and Elder, 1961; Gunn and Robinson, 1963; Davies, 1964; Shelton, 1964; Smith, 1964; Dennis, 1965a; Hight and Jury, 1969; Mullaney, 1969; Singh and Singh, 1970; Alexander, 1971 - 1972a; Fogarty, 1972; Malik and Acharya, 1972; Seth *et al.*, 1972; Watson, 1972; Harker, 1973, 1977; McFarlane, 1973; Emady, Noakes, Hadley and Arthur, 1974; Juma *et al.*, 1974; Holmes, 1975; Slee, 1976; Maxwell, 1977; Saunders, 1977).

Sex of newborn lamb, (Moule, 1954; Vetter *et al.*, 1960; Gunn and Robinson, 1963; Hight and Jury, 1969; Mullaney, 1969; Mullaney and Lear, 1969; Singh and Singh, 1970; Juma *et al.*, 1974; Marais, 1974; Pout, 1973).

Type of birth coat, (Alexander, 1964; Davies, 1964; Obst and Evans, 1970; Watson, 1972).

Behaviour of ewe and lamb, (Moule, 1954; Alexander and Peterson, 1961; Alexander, 1964, 1971 - 1972b; Smith, 1964; Holmes, 1975; Bareham, 1976; Slee, 1976).

The authors named above approached the problem of perinatal

lamb mortality by concentrating on a few topics which were of particular importance or interest to them. Other workers used a general approach, trying to classify all the causes of mortality which they encountered.

Moule (1954) made eleven observations on 2,467 Australian Merino lambs during which he attributed lamb deaths to the following: dystocia, accidents, starvation, predators, exposure, infections and unknown causes.

In classifying and defining factors associated with perinatal lamb losses, McFarlane (1961) listed the following: stillbirth, dystocia, starvation and mismothering, exposure, miscellaneous accidents, predators and drowning.

Dennis (1966), in estimating the overall incidence of PLM in Western Australia during 1963 - 1965, and also during his assessment of the incidence of lamb losses in other sheep flocks in Australia and in the U.S.A., mentioned the following major causes: starvation - mismothering - exposure - complex, dystocia, flock pathogens or intra-uterine infections, neonatal infections, predators and congenital abnormalities.

Stamp (1967), in diagnosing PLM, stated that the different causes in Great Britain are: infections of the uterus and placenta as a cause of abortion and also infections of the newborn lamb, nutritional deficiency of the ewe as a direct or indirect cause of the different classes of PLM, starvation - mismothering - exposure

complex of newborn lambs, dystocia, toxic factors, genetic defects and predation.

Halliday (1968a), while studying the levels of serum gamma-globulin in dead lambs from hill flocks in Scotland, used the following terms for indicating causes of lamb death: stillbirth, starvation - exposure, infections, accidents (including damage during birth) and deaths from unknown causes.

In reviewing the incidence of lamb losses in Australia in relation to various factors, Watson (1972) concluded that the causes of PLM were: inadequate nutrition of ewe during late pregnancy, failure of lambs to survive the birth process (dystocia), starvation and exposure of the newborn, infections and predators.

Houston and Maddox (1974) surveyed the causes of mortality among young Scottish Blackface lambs and mentioned starvation, stillbirth, diseases and accidents as important factors.

Whitelaw (1976) stated that the components of perinatal losses in intensive hill sheep farming are: stillbirth and late abortion, dystocia, starvation - exposure - stress, predation and congenital defects.

It can be concluded that the seven causes of PLM listed at the end of the introduction agree with the views of most of the authors quoted above and can be used as a basis for a more detailed study of the literature.

The general opinion is that five of the causes are of minor, sporadic or localised importance. They can be listed as follows:

1 Predators.

2 Congenital malformation.

3 Accidents.

4 Infections.

5 Dystocia.

The other two categories can be considered as widespread major causes of PLM and they are

6 Undernourishment of the ewe.

7 Starvation - mismothering - exposure - *E. coli* complex.

1. PREDATORS

Losses in newborn lambs as a result of attack by predators have been reported several times but the extent of these losses varies from property to property and from flock to flock. Most of the reports come from areas where predators like crows (raven), foxes, feral pig, eagles or dogs exist in large numbers. Predation can be primary, that is the predator kills a healthy lamb which would otherwise have survived, or it can be secondary in which case the lamb is dead, dying or severely ill before the attack takes place.

Only a few observations on certain flocks have shown predation as an important cause of PLM. Moule (1954)

reported that during 11 observations in Queensland, 155 out of 453 lamb deaths were attributed to predation, mainly by crows and foxes, but 111 of these were lost during one of the observations and only 44 lost in the remaining ten. He did not specify whether the predation was a primary cause of death or a mere mutilation. In Western Australia, Smith (1964) claimed that the raven was responsible for 36·2 per cent of the lamb mortality in a Border Leicester flock. From post mortem examination of 981 lambs in a Merino flock, he found that 27 per cent of them had been attacked by predators, mainly crows and eagles, but only 14 per cent of lambs killed by predators were not starving at the time of death. Davies (1964), in his observations on the effect of stocking rate and lambing time upon PLM in a Merino flock in South-Western Australia, reported losses due to predation that varied between 3·4 and 9·4 per cent but he stated that it was impossible to assess how many of these losses were associated with primary predation.

In another Merino flock in Australia, 12·1 per cent of total lamb losses were due to primary predation by foxes, representing just over two per cent of the total lambs born to that flock (Moore, Donald and Messenger, 1966). However, the extensive work on PLM conducted by Dennis (1965c, 1966, 1969, 1974a) and Dennis and Nairn (1970) in that part of the world (Australia) showed that

predators did not actually play any important role in primary lamb losses but simply acted as scavengers. They reported lamb losses varying between 0·6 and 2·7 per cent of all lambs born as a result of predation.

In Great Britain, Stamp (1967) stated that foxes and dogs, but not eagles, do cause considerable losses on occasion, and Houston (1972 - 1973), reporting on hoodie crows in Argyll in Scotland, found that predation represents only about one per cent of all lamb deaths. Eighty one per cent of the attacked lambs were dead before the attack. Of the remaining 19 per cent which were alive when attacked, 14 per cent were starved, two per cent had diseases and three per cent showed normal fat levels.

During our investigation of PLM in low ground sheep in Scotland, there have been no lambs lost as a result of primary predation although mutilations did occur.

In the main, predators do not play a significant primary role in PLM in Britain although they might constitute a problem on a particular farm. Usually it is the very weak, starving or dying lambs that are attacked, in which case the first task in reducing this particular kind of loss will not be to prevent predation but to produce strong healthy lambs from ewes with good mothering abilities.

2. CONGENITAL MALFORMATIONS

Lethal and non-lethal deformities acquired in the uterus and existing at birth have been observed in man and all classes of domestic animals including sheep. They can be caused by genetic or environmental factors or their interaction.

Dennis (1965d) reported 60 different forms of congenital abnormality among dead lambs in Western Australia. It has been shown from systematic post mortem examinations of large numbers of lamb carcases that these abnormalities mainly involved the musculo-skeletal, cardiovascular, central nervous and digestive systems and, to a lesser extent, the urogenital and respiratory systems. The common defects include agnathia, atresia ani, heart defects, limb deformities and embryonic duplication (Dennis, 1965d, 1974a, 1975a, b, c, d; Dennis and Leipold, 1968; Hughes, 1971 - 1972a; Haughey, 1973a).

Congenital malformation usually forms a very small percentage of total lamb losses. In New Zealand, Hartley and Kater (1964) found that only one per cent of the lambs autopsied had died as a result of lethal congenital defects. In Australia, Hughes, Hartley, Haughey and McFarlane (1964) reported defects in 1·5 to 2·1 per cent of the 3,503 lambs they autopsied on three farms. Dennis (1966) reported that the overall losses due to congenital abnormalities were 0·5 to one per cent of

total lambs born. Among lamb losses in a pure Southdown flock and in a Merino flock, congenital deformities represented one per cent and 0·6 per cent of all lambs born, respectively (Dennis, 1970; Dennis and Nairn, 1970). Gunnarsson, Jacobsson, Lindberg and Möllerberg (1970) found that developmental defects played only a minor role as a cause of PLM in eight flocks of sheep near Stockholm. Haughey (1973b), in reporting on birth injury to the central nervous system as a component of PLM, stated that lethal congenital malformations comprise on average about one per cent of losses.

Although there are a wide variety of malformations described in the literature it is clearly recognised that they account for only a small percentage of lamb deaths and are of minor economic significance on the majority of farms.

3. ACCIDENTS

Many authors have related lamb losses to accidents (Moule, 1954; McFarlane, 1961; Halliday, 1968a; Daly, 1973; Houston and Maddox, 1974; Whitelaw, 1976). The main types of accident reported are drowning in water troughs and ditches, strangling in fences and fractures and other injuries due to the lamb being crushed by the ewe. These accidents may be secondary as, for example, when a careless or sick ewe is confined to a small pen

along with its very weak lamb or lambs and the lambs are lain on and crushed. In many cases, these weak lambs would have succumbed to some other factor, like starvation, if they had not been killed accidentally.
In other cases, unavoidable accidents happen to strong lambs but it is a rare occurrence in a well run flock.

Losses due to accidents are always low and range from nil to one per cent in very well managed and closely supervised flocks, to as high as three per cent in flocks that have only minimal shepherding.

4. INFECTIONS

As a cause of PLM, infections can be divided into the following two groups:

i) Intra-uterine infections.

ii) Neonatal infections.

i) Intra-uterine infections

Intra-uterine infection of the fetus is caused by an infectious agent that has been contracted by the pregnant ewe. This will usually result in the expulsion of a dead fetus (infectious abortion) or the birth of a weak lamb with a poor chance of survival. The most common pathogenes incriminated in this type of infection are Vibrio fetus, Brucella abortus ovis, Listeria monocytogenes, Toxoplasma gondii, enzootic abortion agents (Chlamydia spp.), Salmonella spp., and to a lesser extent

Pasteurella spp., Corynebacterium pyogenes, Escherichia coli, Bacillus spp., streptococci and staphylococci (Hughes et al., 1964; Hartley and Boyes, 1964; Watt, 1965, 1977; Haughey, 1967; Broadbent, 1971, 1975; Hughes, 1971 - 1972b, 1975; Gunnarsson et al., 1972; Plant et al., 1972; Smith, 1972; Dennis, 1974b, 1975e).

Ovine abortion can be the result of some other infectious diseases caused by agents that do not involve the uterus directly. Among these diseases are the following:- tick-borne fever, louping-ill, Foot-and-Mouth disease, blue tongue, Rift Valley fever and Nairobi sheep disease (Stamp, 1967).

ii) Neonatal infections

Infection of lambs through the umbilicus and other common routes, during and shortly after birth, can result in the death of some lambs, especially those which are small, weak or lacking in immunity. Escherichia coli, Clostridium spp., Pasteurella spp., Corynebacterium spp. and Staphylococcus aureus are among these infectious agents (Moule, 1954; Hughes, 1971 - 1972b; Hughes, Haughey and Hartley, 1971; Shaw, 1971; Daly, 1973; Dennis, 1974c).

Comparatively small numbers of lamb deaths can be attributed to infections. Moule (1954) found that only eight out of 453 neonatal deaths were caused by infections.

In a flock of 105 maiden Merino ewes, infectious agents were not implicated in any of the 22 lamb deaths that occurred during and within three days of birth (Alexander and Peterson, 1961).

Hughes et al. (1964) autopsied 3,503 lambs that had been collected from three farms in New South Wales, Australia. Infections were recognised in only 1·1 to 7·1 per cent of the lambs autopsied. Smith (1964), while studying lamb mortality in Western Queensland, found that pneumonia, the only infectious condition reported in his study, was responsible for only 0·84 and 3·8 per cent of mortalities in the Merino and Border Leicester flocks respectively.

In Western Australia, surveys into causes of abortion in sheep and perinatal lamb losses, on different properties, showed that only 6·5 per cent of the properties submitted lambs which were infected with a known abortion-producing disease. Only eight per cent of the lambs showed evidence of individual infections (Dennis, 1965a). Dennis (1966) found also that intra-uterine and neonatal infections were responsible for the death of two to four per cent of the total number of lambs born in a situation where PLM amounted to 15 to 25 per cent of total lambs born. He (Dennis, 1970; Dennis and Nairn, 1970) further reported that infections were responsible for losses of 0·6 per cent and 2·2 per cent of the total lambs born to a Merino flock and a pure Southdown flock respectively.

In Scotland, neonatal infections were found in only 11 out of 117 dead lambs in a hill flock (Halliday, 1968a). Among 282 dead Scottish Blackface lambs, Houston and Maddox (1974) found diseases to be a possible cause of 10 per cent of the deaths.

Hughes et al. (1971) reported that in 2,158 carcases autopsied, infections, presumably acquired after birth, were responsible for the deaths of only 0·2 to 0·3 per cent of all lambs born.

Alexander (1971 - 1972b) stated that infections probably contribute to the death of less than two per cent of lambs born and Watson (1972) considered the percentage to be less than one.

Haughey (1967, 1973b) concluded that infections are of widespread occurrence but at a low level of incidence and this seems to be the true picture upon which most of the investigators tend to agree. Nevertheless, it must be emphasised that occasionally on specific farms, in an intensively used lambing area, for example, losses may reach catastrophic levels as a result of an "abortion storm" caused by a single infectious agent or of a neonatal infection with coliform organisms. These occasional large losses caused by infections are often associated with poor management and other stress factors acting on ewes and lambs. By the use of proper hygienic measures and preventive medicine, e.g. vaccination, most of these

losses can be avoided.

It is important to mention that E. coli infections of lambs in the neonatal period are of considerable frequency under intensive sheep production systems (Stamp, 1967; Daly, 1973; Dennis, 1974a; Whitelaw, 1976; Donald, 1977; Harker, 1977). However, such infections are usually common only among weak lambs that have been starved due to some behavioural or other reason, exposed to a spell of inclement weather or other predisposing factors. For this reason, the risk of E. coli in PLM should not be separated from these factors and will be discussed under the "starvation - mismothering - exposure - E. coli complex".

5. DYSTOCIA

Dystocia is a term used to describe a prolonged or difficult birth which in the absence of skilled assistance may lead to the death of the lamb or lambs. Deaths due to dystocia are usually a result of asphyxiation, inhalation of fetal fluid and trauma. In this connection, Haughey (1973a) observed an almost complete association (93·5 per cent) between the presence of vascular abnormalities in the CNS caused by trauma and anoxia during birth, and the parturient death.

Causes of dystocia are many, among them are uterine inertia, rupture or torsion of the uterus, discrepancy in

size between the fetus or fetuses presented and that of the maternal pelvis, the degree of dilation of the birth canal and malpresentation of fetus or fetuses. The most common forms of dystocia in ewes are postural abnormalities of all kinds, multiple births and disproportion between the fetal size and the diameter of the maternal pelvis.

Losses due to dystocia are more common in heavier lambs and the incidence is higher among primiparous ewes due to maternal - fetal size discrepancy (Purser and Young, 1959; McFarlane, 1961; Watson and Elder, 1961; Gunn and Robinson, 1963; McDonald, 1966; Stamp, 1967; Singh and Singh, 1970). However, the adverse effect of large sized lambs is probably more important in the smaller, hill breeds where the additional hazard of being on the hill and thus being seen less often, may allow dystocia to go uncorrected for too long a period (Watt, 1965).

Wide variations in the incidence of dystocia have been reported among breeds (Gunn and Robinson, 1963; Fogarty, 1972; George, 1973), flocks (Purser and Young, 1959; Owen, 1976) and properties (Hughes et al., 1964).

Mathews and Ogden (1957) studied mortality among 13,086 lambs in an experimental flock in Utah, U.S.A., and reported that 15·27 per cent of the dead lambs were lost either before birth or due to parturition

difficulties. McFarlane (1961), while classifying perinatal deaths, found, however, that 36 per cent of dead lambs are parturient deaths. At the same time he noticed that 39 per cent of 1,474 lambs showing parturient lesions had ante-parturient lesions as well, and indicated that the incidence of dystocia could be increased by ante-parturient diseases.

Hartley and Kater (1964) reported that about four per cent of the total lambs born to Romney ewes were lost in association with dystocia. Moore et al. (1966) investigated lamb mortality in a flock of 545 Merino ewes and found that only 8·9 per cent of all dead lambs were lost due to dystocia.

Dennis (1966) estimated lamb losses in West Australia caused by dystocia to vary from one to five per cent of total lambs born. Dennis and Nairn (1970) studied the incidence of various causes of losses in a Merino flock and found that with a PLM of 17·7 per cent, only 1·8 per cent of total lambs born were lost due to dystocia. However, lamb losses due to dystocia can occasionally be very high. In one case concerning a pure Southdown flock in Australia, Dennis (1970) found dystocia to be responsible for the death of 63·9 per cent of lambs submitted for necropsy.

In the life of the lamb, the intra-partum phase is very short. It can be very precipitous too. Because

of that, good supervision is very vital at lambing. Even after a successful but difficult birth, both the ewe and the lamb may show marked behavioural changes. The lamb is usually weak and unable to stand, walk or suck. At the same time, the dam is exhausted and has disturbed mothering ability. All these changes will definitely reduce the lamb's chance of survival.

The literature reviewed indicates that dystocia is responsible for only a small percentage of PLM losses.

6. FEEDING OF THE EWE DURING PREGNANCY AND LACTATION

It is well established that nutrition is an important factor which influences the reproductive performance of the ewe.

Reproductive performance is usually expressed as the number of lambs produced per ewe (litter size), the lamb birth weight and also by the quantity and quality of the ewe's milk supply which is essential for the survival and future performance of the newborn lamb.

In order to achieve a successful breeding season, a ewe must be supplied efficiently with the food that will leave her in a balanced nutritional status during all the stages of her breeding cycle.

In studying the relationship between nutrition and ewe reproductive efficiency, different workers have either designated the levels of nutrition arbitrarily as

low, medium and high without even referring to the level of feed intake, or have used energy intake calculated after offering different levels of feed with known energy contents to different groups of ewes. The indicators used to assess the nutritional status of the ewe were initially confined to ewe body weight changes. In later work body score was introduced together with studies on the levels of plasma free fatty acids (FFA), ketones and blood glucose. Russel, Doney and Reid (1967a, b) successfully detected different levels of undernourishment by measuring plasma FFA and ketones. They recommended the use of the first parameter in circumstances where a moderate degree of undernourishment had been imposed on the ewe but suggested the use of plasma ketone levels for assessing and adjusting feed requirements in experimental situations. Bowden (1971) also considered that blood parameters such as non-esterfied fatty acids and ketones can be useful in defining the ewes' nutritional status during pregnancy and lactation.

In relation to PLM, late pregnancy is the most critical stage during which the ewe must be fed adequately not only to meet her requirement but also to meet the energy needs of a fast growing fetus or fetuses. During the last six to eight weeks of pregnancy, approximately 70 per cent of the fetal growth takes place (Curson and Malan, 1935; Wallace, 1948b, c; Reid, 1958;

Alexander, 1971 - 1972b; Lodge and Heaney, 1973; Meat and Livestock Commission, 1973; Robinson, 1973), and at this time the fetal energy requirement is high. The necessary energy is supplied either from food or, if nutrition is inadequate, by the ewe drawing on her own body reserves. If body reserves are badly depleted then the lives of both the ewe and her lambs can be endangered.

Although most of the investigators stressed the importance of nutrition during late pregnancy in relation to the ewes' reproductive efficiency, others reported on the general effects of nutrition throughout the breeding cycle, while some concentrated on the levels of nutrition during the premating, mating, early pregnancy, mid-pregnancy and lactation periods.

Among those authors who took an overall view of ewe nutrition throughout the breeding cycle was Bell (1944) who considered that half the lamb deaths in a Merino and Shropshire flock could be attributed to faulty ewe nutrition.

In New Zealand, Coop (1950) carried out several experiments involving 1,750 grazing Corriedale ewes to investigate the effect of level of nutrition during pregnancy and during lactation. The high-level nutrition group was represented by ewes that gained 25 to 40 lb from tupping to lambing while the low-level nutrition group

had a live-weight gain of ± 5 lb. The high level of nutrition during pregnancy resulted in an increase of 0·5 lb in lamb birth weight but did not reduce ewe or lamb mortality. The level of nutrition during lactation significantly affected lamb losses and weaning weight of lambs.

Van Horn, Burkitt, Willson, Flower, Drummond and Payne (1951) separated over 1,000 ewes into three groups immediately after breeding. The first group of ewes was grazed on range during the winter without any pellet supplementation. The second group was grazed also but received a daily supplementation of one-third lb per head of concentrate pellets ranging from 10 to 40 per cent protein. The third group was wintered on a full feed of hay plus one-third lb of concentrate pellets and one-third lb of dehydrated alfalfa pellets per head. Both kinds of pellets contained about 20 per cent protein. The results from these three groups were compared to that of a fourth (control) group of about 1,000 ewes that had been kept on high plane of nutrition before and during breeding as well as through the winter. The percentages of lambs born to the first, second, third and fourth groups were 80, 105, 117 and 126 respectively.

Robinson and Forbes (1968) conducted two experiments on 64 Border Leicester cross Scottish Blackface ewes to

assess the effect of protein intake throughout pregnancy. Lambs born to ewes with low protein intakes had lower birth weights and grew less quickly than lambs born to ewes with high protein intakes.

Severe undernourishment of the pregnant ewe has various adverse effects including shortened gestation lengths (Alexander, 1956), reduced lamb birth weights, poor lamb vigour, reduction in birth coat density and inhibition of maternal behaviour (Alexander, 1971 - 1972b). In addition, the onset of lactation is delayed and production of milk reduced (McCance and Alexander, 1959).

Adequate planes of nutrition have the opposite effects and consequently improve both the number of lambs born and the number surviving (Mullaney and Lear, 1969; Cloete, 1972).

In intensive hill sheep systems, improving the level of nutrition according to the ewes' requirements ensures a high conception rate and the milk supplies necessary for lamb survival and growth (Whitelaw, 1976). Hill sheep production cannot be increased, even by the introduction of new breeds, when nutrition is a limiting factor (Russel, 1971). In fact, nutrition rather than genotype can be the limiting factor in determining a particular breed's reproductive performance (Doney and Gunn, 1976).

Having looked at the general effects of ewe nutrition, it seems appropriate to consider now the effects of nutrition during the pre-mating period.

Underwood and Shier (1941) studied the effect of nutritional "flushing", which is the practice of keeping lean ewes on a high level of nutrition for two to three weeks before mating, upon the fertility of 700 Border Leicester cross Merino ewes in Western Australia. In this experiment, flushing of the ewes for a fortnight on oats and clover resulted in a higher fertility rate due entirely to an increase in the proportion of twins. Lambing percentage was 114·6 and 94·6 for the flushed and non-flushed groups respectively.

Darroch, Nordskog and Van Horn (1950) who investigated the effects of feeding supplementary concentrates to 462 Columbia - Rambouillet ewes during various parts of the breeding cycle found that pre-breeding supplementation increased flock fertility by 10 per cent. Flushing immediately before mating increased the lambing percentage of 320 Border Leicester cross Merino sheep by nine to 13 (Tribe and Seebeck, 1962). Killeen (1967) found that the level of nutrition during the three weeks before mating and ewe body weight at mating independently affected the multiple birth and ovulation rate but had only minor effects on early reproductive failure. Gunn, Doney and Russel (1969) studied the effect on fertility

in 273 Scottish Blackface ewes of nutrition and body condition. They used a body condition score ranging from one (very thin) to five (very fat) and decided that:-

a) Ewe body condition at mating had a significant positive effect on both ovulation and lambing rates but had no effect on infertility.

b) The level of food intake before and at mating did not affect ovulation or lambing rates of moderately fat ewes (body condition score of three) but significantly affected the ovulation and lambing rates of the very thin ewes (body condition score of 1·5).

In the period immediately following mating maintenance of adequate nutrition continues to be important.

Edey (1969) reviewed the subject of prenatal mortality in sheep and gave it an estimated percentage of 20 - 30. He pointed out that these early losses are a common occurrence in the first three to four weeks of pregnancy but rare after day 30, and emphasised the importance of post-mating levels of nutrition as a measure to reduce embryonic mortality. Cumming (1972a) showed that among a flock of Border Leicester cross Merino ewes, rising and falling planes of nutrition did not differ significantly in their effect on ovulation rate but the rising plane caused significant improvement in the number of ewes lambing and

the number of lambs born per ewe mated or per ewe lambing. Ewes that lost weight had lower embryonic survival. The same author (1972b) also demonstrated that by restricting nutrition in the first three weeks of pregnancy, an increase in the percentage of unsuccessful embryonic implantations occurred. There is a reduction in lambing rate, particularly among lean ewes, if nutrition is inadequate directly after mating (Gunn and Doney, 1973). The ewe's nutritional status around the time of mating also affects lamb birth weight (Russel and Foot, 1973).

Once pregnancy is established consideration can be given to restriction of the quantity of feed supplied to ewes. Parry (1956) studied the occurrence of pregnancy toxaemia among 50 lowland sheep flocks in Britain and found that ewes which achieved high weight gain in early pregnancy (fat ewes) were more likely to suffer from pregnancy toxaemia. He suggested that ewe body weight must be maintained by restricting food intake until six to eight weeks before lambing when good body weight gain should be ensured by improving the grazing or by feeding concentrate supplements.

Watt (1965), while commenting on the prevention of pregnancy toxaemia, considered that it appeared satisfactory to allow the ewe to lose weight in the first six to eight weeks after mating, then to maintain that weight until eight weeks before lambing when she should be fed

adequately to produce a gradual increase in her weight until lambing.

Stamp (1967) stressed the role of proper levels of nutrition in the ewe during pregnancy to prevent pregnancy toxaemia which may result in some lamb losses.

The National Research Council (1968) reported that the energy requirement for the ewe during the first 15 weeks of pregnancy was only slightly more than that needed for maintenance.

In New Zealand, Coop and Clark (1969) undertook three trials involving 3,500 Border Leicester cross Corriedale ewes to study early pregnancy nutrition and concluded that to restrict nutrition for five to six weeks starting at two to three or five to seven weeks of pregnancy, had no effect on the ewes' reproductive performance. They recommended such restriction as a way of saving feed for the critical late pregnancy period.

The effect of nutrition during mid and late pregnancy on various aspects of ewe and lamb performance has been studied extensively.

The authors who, as part of their work, looked into these problems include Barnicoat, Logan and Grant (1949a,b); Thomson and Thomson (1953); Guyer and Dyer (1954); Thomson and Aitken (1959); Alexander and Peterson (1961); Alexander (1964); The National Research Council (1968); Watson (1972); Robinson (1973); O'Hara, Wolff and Oliver

(1975); Bareham (1976). Detailed work on the topic started many years ago.

Thomson and Fraser (1939) carried out an in-door feeding trial on three groups of 14 Greyface ewes, using a diet of turnips, hay and concentrates. The first group of ewes was fed sufficient to maintain weight and strength. The second group was fed as much as they could eat. The third group of ewes was offered the same amount of food as group one but they were allowed to eat as much as they could during the last month of pregnancy. While in lamb, the average increase in body weight per ewe was eight, 50 and 20 lb for groups 1, 2 and 3 respectively. The experiment showed that in the first group the lambs were born weak and of low birth weight, and the ewes themselves were short of milk. Heavy feeding throughout pregnancy (group 2) was no more effective in producing lambs of normal strength and size to mothers with sufficient milk supply than was heavy feeding during the last month of pregnancy. The mean lamb birth weight of twins in group 1, 2 and 3 was 7·8, 10·1 and 10·3 lb respectively.

Underwood and Shier (1942), in a trial on 108 ewes during the last four to six weeks of pregnancy, found that feed supplements had no effect on lamb birth weight or growth rate. Ewes supplemented with 0·5 lb of wheat per ewe per day lost only five lambs compared to 22 lambs

lost in the unsupplemented group. However, the following year Underwood, Shier and Cariss (1943) reported that supplementation with concentrates during the last third of pregnancy not only reduced lamb losses but increased the birth weight and growth rate of lambs.

Wallace (1948a, b, c) worked with 20 Suffolk or Halfbred ewes, over three years at Cambridge, and showed that the level of nutrition of the ewe during the last six weeks of pregnancy had a considerable influence on both the lamb birth weight and the ewe milk yield. Low levels of feeding during late pregnancy markedly reduced lamb vigour at birth. With lamb birth weight, lamb vigour is of vital importance not only to avoid lamb losses but also to help the lamb make the best use of the dam's milk supply. Wallace also reported that, after birth, the growth rate of lambs was highly influenced by the level of milk intake.

In Scotland, Thomson and Thomson (1949) conducted somewhat similar experiments to those of Wallace in Cambridge, but they used a larger number of ewes. At lambing there were 30 high-plane ewes that had received a liberal high-protein diet and 44 low-plane ewes that had been kept on a restricted protein diet in the second half of pregnancy. These feeding treatments resulted in an increase in body weight of about 30 per cent for the first treatment and a weight loss of five per cent for

the second group. The main results of dietary restriction during the second half of pregnancy were a reduction in lamb birth weight and ewe milk supply, an impairment of the maternal instinct and seriously reduced vitality of twin lambs at birth. They stated that these findings have obvious importance for sheep farming.

Blaxter (1957) combined results from several nutritional experiments conducted in Great Britain. He reported the effect of low levels of feeding during late pregnancy on lamb losses as follows

	No. of Lambs	Lambs stillborn (%)	Lambs that died shortly after birth (%)	Lambs that survived (%)
High plane of nutrition	58	5·2	3·4	91·4
Low plane of nutrition	86	21·0	33·7	45·3

Perrin (1958) reported that ewe nutrition during late pregnancy affected the chemical composition of the colostrum. The colostrum of the low-plane group of ewes had a lower level of fat and protein but higher lactose and total mineral contents than that of the high-plane group of ewes.

McDonald (1961), in reviewing the physiological limitations on reproduction in sheep stated that "the later stages of pregnancy are vitally influenced by

maternal nutrition and that the ewe becomes progressively more vulnerable to nutritional stress as pregnancy proceeds". The same worker (McDonald, 1962) quoted data which indicated that ewes fed ad libitum and which became fat by day 100 of pregnancy reduced their food intake near term.

Smith (1962) investigated lamb losses in a Merino flock in Central Western Queensland and found that because of the decline in the plane of nutrition, the group of ewes which lambed in November lost weight during the last three weeks of pregnancy and had a PLM of 72·3 per cent whereas those which lambed in September slightly increased their weight and lost 21·1 per cent of their lambs.

Russel, Doney and Reid (1967a, b) also studied the effects of undernourishment in late pregnancy. Undernourishment reduced birth weight of singles and twins by 10 per cent when the undernourishment was moderate and by 25 per cent when it was severe. They reported a strong relation between the level of undernourishment in late pregnancy and the lamb birth weight. They also estimated an additional nutritional requirement of 100 g digestible organic matter per kg fetus per day for ewes during late pregnancy.

Treacher (1970) observed significant differences in the birth weight of twins when, during the last six weeks of pregnancy, their mothers were on nutritional levels

that increased body weight by 20, 10 and nil per cent of their 14 week pregnancy weight. The greater the ewe body weight increase the heavier were the lambs born.

The Ministry of Agriculture, Fisheries and Food (1971) recommended that, to avoid any health risk to the ewe and her lambs on intensive sheep production systems, the level of nutrition in late pregnancy should be 60 per cent above the basic maintenance requirement.

About this period attempts were made to quantify the levels of nutrition required, especially in late pregnancy.

Robinson, Fraser and Bennett (1971) used 57 North country Cheviot ewes to study the energy requirements during late pregnancy, relying on the relationship between plasma FFA levels, energy intake, lamb birth weight and ewe body weight. For an 80 kg ewe producing twin lambs with a total weight of 8·4 kg, they estimated a daily energy requirement of approximately 15·47, 17·14 and 22·2 MJ at prepartum days 45, 25 and five respectively. Robinson, Brown and Lucas (1973) reported that for Welsh Mountain ewes the level of feeding needed to maintain body weight at one week before lambing is approximately 50 per cent more than that required at four to five weeks of pregnancy. They estimated that by supplying these pregnant ewes with only 80 per cent of their optimal energy requirement, a 10 per cent ewe body weight loss and a reduction in lamb birth weight should be expected.

Robinson (1974) observed the performance of 96 Finnish Landrace cross Dorset Horn ewes accommodated in a very sophisticated experimental intensive ewe unit. He was able to adjust the nutrition, lactation and day light length during a 205-day reproductive cycle to levels that might be uneconomic and impractical under existing farming systems. The late pregnancy feeding intake of these ewes varied from about 12 MJ per day for ewes carrying singles to about 17 MJ per day for those carrying quadruplets. Under these circumstances, lamb mortality was 7·6 per cent of all lambs born.

Shevah, Black and Land (1975) studied the effect of energy intake during the last six weeks of pregnancy on the performance of 84 Finn cross Dorset ewes using different levels of energy, ranging from approximately 9·6 to 18·4 MJ of metabolisable energy per ewe per day and they found no significant effect on lamb birth weight, litter size or neonatal lamb mortality. They also stressed the importance of ewe body weight and weight gain during early pregnancy.

Lactation can be affected by the level of feeding during late pregnancy and also by the amount of food given during the lactation period.

Guyer and Dyer (1954) using 63 ewes over a two-year period studied the factors affecting sheep production under farm conditions. The ewes were divided into two

groups. Both groups were left to graze during pregnancy and had liberal allowances of concentrates during lactation but, to one of them, a daily supplementation of two lb of concentrates per ewe had been given during the last 60 days of pregnancy. This resulted in greater milk yield and heavier lambs at 16 weeks of age. They indicated that lamb growth rate was influenced by the milk intake, number of lambs nursed and lamb birth weight but not by sex of lamb. They found also that ewes suckling multiples produced more milk than those suckling singles. However, during the whole of their work, they reported that the ewes offered concentrates during late pregnancy lost 13 lambs at or near birth in comparison to only six lambs lost to the other group. They offered no explanation for this unexpected result concerning lamb mortality.

Robinson and Forbes (1968) found that low protein intake during pregnancy resulted in decreased ewe milk production three weeks after parturition and Treacher (1970) reported that ewe undernourishment during the last six weeks of pregnancy caused both the quality and quantity of milk to fall.

Louca, Mavrogenis and Lawlor (1974) observed that the levels of nutrition in late pregnancy were closely related to the birth weight of twins and triplets and also to the quality and quantity of milk produced by the ewe in early lactation.

A summary of the various kinds of practical information available on the feeding of the ewe at different stages of her breeding cycle has been issued by the Meat and Livestock Commission (1973). They recommended the following procedures:

a) A good standard of feeding and management before mating. At mating the ewes should be in optimum body condition and weight to ensure successful embryonic implantation.

b) Pregnant ewes should be kept on a high plane of nutrition during the first month of pregnancy, in order to achieve successful embryonic implantation.

c) During the second and third month of pregnancy the report stated that even a five per cent body weight loss caused by mild undernourishment of ewes in good body condition is not detrimental to the growth and development of the fetus. For fat ewes, this level of undernourishment could be considered necessary to avoid any risk that can result from sudden sharp drops in feed intake later in pregnancy.

d) During the late pregnancy period, more nutrients are needed to meet the need of the rapid fetal and mammary tissue development. During this stage, a twin-bearing ewe of 70 kg body weight

will need approximately 13·5 to 17·0 MJ of metabolisable energy (ME) per day, compared to only 8·4 MJ ME per day for the same ewe during early pregnancy.

e) For the lactating ewe, severe undernutrition during late pregnancy can reduce ewe milk production by 10 to 35 per cent. Other factors that can affect milk yield, which is very important for the survival and growth rate of the newborn, include:

i) level of feeding of the lactating ewe,

ii) breed, body weight and age of the ewe, and

iii) number of lambs suckled.

The conclusions drawn in the MLC report are based on their own work and on the work done by many of the authors whose articles have just been reviewed. It is fair to say that these conclusions are an accurate assessment of the general consensus of opinion reached by most of the authors I have cited and reflect the present opinions concerning variations in ewe energy requirement throughout the breeding cycle.

7. STARVATION - MISMOTHERING - EXPOSURE - *ESCHERICHIA COLI* COMPLEX

The four interrelated factors, i.e. starvation, mismothering, exposure and *E. coli* infection are an

important cause of PLM. When a factor operates independently of the others it is referred to as an "uncomplicated factor". More usually two or more factors operate together to cause lamb deaths when they are referred to as "complicated factors".

Starvation means that the lamb has been deprived of colostrum or milk for a variety of causes. The ewe may have a normal udder yet fail to secrete milk or let the milk down properly. The udder or teats may be diseased or, the ewe, because of her poor mothering ability, may prevent lambs, especially small weak ones, from sucking.

As a result of starvation, the lamb will have to rely on its fat reserve to stay alive. In suitable circumstances it may survive for two to three days but if subjected to environmental stress the survival period will be reduced to a few hours.

Mismothering is a break in the natural bond that attaches the ewe to her lamb or lambs. This may come about for behavioural, managemental or health reasons and results in the neglect and starvation of the newborn lamb leaving it very vulnerable to other stressing factors and infections.

Exposure in the case of British farms means the exposure of the lamb to adverse weather conditions including low ambient temperature, wind, rain and snow fall. In some tropical countries, the exposure to high climatic

temperature could inflict a considerable degree of stress on the newborn.

During the neonatal period and in circumstances where lambs, usually the weak and small ones, fail to get enough colostrum or are exposed to chilling weather or left in unsanitary situations, enteric or septicaemic E. coli infections can account for further lamb losses.

The four factors are closely interrelated and the effect of any of them on lamb survival can be aggravated by the others.

Because of this close relationship, I decided to consider them together as a single causal entity for PLM. However, some workers dealt mainly with the non-infectious aspects of the complex and I will review this first.

Starvation and exposure were considered to be the most important causes of early lamb death by Thomas (1945). Other authors, from a variety of countries, have similar opinions about the effects of starvation and exposure on lamb survival. They include Reid (1958), Alexander and Peterson (1961), McDonald (1961, 1966), McFarlane (1961), Alexander (1964), Dennis (1964, 1972), Hartley and Kater (1964), Stamp (1967), Booth (1972), Glimp (1972), Owen (1976) and Slee (1976, 1977).

Among the causes of starvation are mismothering or desertion of lambs by the ewe (Moule, 1954; Alexander and Peterson, 1961; McFarlane, 1961; Dennis, 1964, 1966,

1972; Smith, 1964; Alexander, 1971 - 1972a; Edinburgh School of Agriculture, Annual Report, 1973; Daly, 1973; Haughey, 1973a, b).

Lambs may be deprived of colostrum or milk as a result of their inherent weakness associated with immaturity (Hight and Jury, 1969) or low birth weight (Booth, 1972).

Lambs which have starved can be detected by the absence of colostrum or milk in the intestinal tract and by the fact that they have either completely or in part catabolised their body fat reserves (McDonald, 1961; McFarlane, 1961; Smith, 1964; Halliday, 1968a). This depletion of body fat reserves makes the newborn lamb vulnerable to poor environmental conditions, especially those involving cold, rain or wind (Alexander, 1964, 1971 - 1972a). Slee (1976, 1977) regarded starvation and cold exposure of the newborn as strongly related factors in connection with PLM and suggested that lambs born to well fed ewes can survive on their own glycogen and fat reserves for the first four days of life provided the weather is warm. In cold, wet and windy conditions the lambs' energy reserves will last for only six to 16 hours.

These views are supported by the high numbers of lamb deaths reported in the literature as a result of starvation, mismothering and exposure. In 11 observations

made in Australia by Moule (1954), starvation was responsible for 135 and exposure for 50 out of 434 lamb deaths. In New Zealand, Hartley and Boyes (1964) found that out of 880 lambs born dead or dying up to four weeks of age 27·5 per cent were lost as a result of starvation. Ten to 15 per cent of lambs born in Western Australia die for the same reason (Dennis, 1966) and Hight and Jury (1969) reported that physiological starvation was responsible for 15·1 per cent of deaths in single lambs and 41·7 per cent of deaths in multiple litters. Dennis and Nairn (1970) reported a lamb mortality of 17·7 per cent in a Merino flock, 12·4 per cent being due to starvation, and the remaining 5·3 per cent covering all other causes of death. Houston (1972 - 1973) examined 248 dead lambs from Scottish hill farms and of these 120 had completely exhausted fat reserves and a further 15 partially depleted reserves. Houston and Maddox (1974) reported starvation to be responsible for 60 per cent of the deaths in young Scottish Blackface lambs, while Johnston (1977) considered starvation/exposure to be the cause of 35 per cent of all lamb deaths on 10 Scottish lowland farms. Saunders (1977) studied the Meat and Livestock Commission records for 306 lowland sheep flocks in different parts of Great Britain and found that 56·5 per cent of the lambs dying during the first thirty days of life had no milk in their stomachs at autopsy.

As I mentioned earlier, the starvation/exposure complex is incomplete without consideration of the part infection, especially that due to E. coli, may play in the problem.

Watt (1965), reviewing some important causes of PLM, referred to colibacillosis as an important disease of the lambing field and lambing shed which could result in large lamb losses if managemental precautions were ignored. He suggested that this condition should be differentiated from 'watery mouth' caused by retention of meconium. Stamp (1967) also referred to E. coli infection as a common occurrence, especially among intensively kept sheep. Shaw (1971) studied the pathogenicity of E. coli strains in newborn lambs and found that oral dosing of lambs with E. coli before taking colostrum resulted in diarrhoea and the death of more lambs than if the dosing was done after feeding colostrum. He also noticed that exposure of lambs to an environment contaminated with E. coli only caused illness after chilling the lambs with cold water. Hughes (1971 - 1972b), in New Zealand, referred to infection with E. coli as a common flock problem although it was often only associated with sporadic deaths. Gunnarsson, Jacobsson and Möllerberg (1972) investigated lamb mortality and its causes on 13 Swedish sheep farms and found that weakness, gastro-enteritis and septicaemia are frequent causes of lamb loss.

E. coli was one of the commonest isolates from dead lambs.

Dennis (1972) indicated that starvation, mismothering and exposure complicated by concurrent infections are the major causes of lamb loss in most sheep rearing countries of the world. The same author (1974c) found that E. coli was the main bacterium incriminated in neonatal infections and deaths in lambs but in many instances the infection was superimposed on cases of starvation. Daly (1973) considered that acute E. coli infections were a major problem among newborn lambs. Although Broadbent (1975) isolated E. coli from most of the dead lambs he examined, their pathogenicity was not assessed. In the North of Scotland, septicaemia and entero-toxaemia caused by E. coli infection were not uncommon during the neonatal period (Owen, 1976).

It is noticeable that most of the authors cited consider that there is an important relationship between the four factors but that starvation is regarded as the most serious single factor.

As ewe nutrition and milk production appear to be connected, and lamb starvation is universally agreed to be an important factor in PLM, it seemed desirable to consider the link between these factors and at the same time study the role of colostrum, and the immunoglobulins it contains, in relation to ewe nutrition, ewe milk production and the subsequent performance of the lamb.

C H A P T E R T H R E E

REVIEW OF LITERATURE

NUTRITION AND IMMUNOGLOBULINS

The literature already reviewed in Chapter Two, concerning the causes of PLM, illustrated the ideas and observations of the different workers who have shown an interest in this problem. The two most important causes of PLM, i.e. ewe undernourishment during late pregnancy, and the starvation - mismothering - exposure - E. coli complex, received most of the investigators' attention.

In this chapter I will deal with both of these causes and with other related factors in more detail as follows:-

1. The levels of late pregnancy feeding and their effect on ewe reproduction with special reference to PLM.
2. Immunoglobulins.
3. Immunoglobulins in sheep.
4. The role of colostrum in lamb survival.

LEVELS OF FEEDING DURING LATE PREGNANCY AND THEIR EFFECT ON EWE REPRODUCTIVE PERFORMANCE WITH SPECIAL REFERENCE TO PLM

Literature review

The references previously dealt with concerning ewe nutrition during different stages of the breeding cycle highlighted the importance of late pregnancy feeding

(the last eight weeks of pregnancy) in ewe reproduction efficiency. This conclusion was reached as a result of surveys and investigations carried out on different breeds and flocks of sheep.

The following relevant literature, briefly described previously, will be considered now in detail:

Underwood and Shier (1942) compared the performance of two groups of Border Leicester cross Merino ewes. A "control group" received oat silage during the last five to six weeks of pregnancy, and a "fed group" received 0·5 lb per head per day of wheat grain in addition to the silage, during the same period. The difference in lamb losses between the two groups was highly significant ($P < 0{\cdot}01$). Their results concerning lamb mortality are given below.

After Underwood and Shier (1942)

Group of ewes	No. of lambs ear-tagged		No. lost at or shortly before birth		No. died shortly after ear-tagging		Total lamb deaths
	Twins	Singles	Twins	Singles	Twins	Singles	
Fed-group	22	93	-	4	-	1	5
Control-group	15	91	*9	*5	4	4	22

*Including lambs lost with ewes dying of pregnancy toxaemia.

In another experiment, Underwood et al. (1943) aimed for further information on lamb production in relation to the level of ewe feeding during the last third of pregnancy using the same feeding treatments as that of Underwood and Shier (1942). They found a significant effect due to feeding treatments, on ewe and lamb losses, lamb birth weight and lamb growth rate. Some of their results are summarised in the following tables.

After Underwood et al. (1943)

Group of ewes	Total No. of ewes	Ewe Losses			Lamb Losses			Total No. lambs tagged
		Pregnancy toxaemia	Other causes	Total	At or before birth	Shortly after birth	Total	
Control-group	350	26	2	28	*60	11	71	328
Fed-group	358	3	1	4	*14	6	20	395

*Including lambs lost with ewes dying of pregnancy toxaemia.

After Underwood et al. (1943)

		1942		1943	
		Mean birth weight (lb)	Age (days) at 65 lb live weight	Mean birth weight (lb)	Age (days) at 65 lb live weight
SINGLES	Control-group	9.5	93	8.9	95
	Fed-group	10.5	89	10.5	90
TWINS	Control-group	6.9	109	7.8	124
	Fed-group	9.5	101	9.3	112

Wallace (1948a, c) studied the effect of feeding levels in the last six weeks of pregnancy on ewe performance. He put three groups of ewes (five ewes in each) on super-maintenance, maintenance and sub-maintenance rations using different amounts of hay or straw with or without concentrate supplementation. During this period, the first group gained an average of 40·0 lb, the second had a constant live weight, while the third lost an average of 10·4 lb of live weight. He reported a significant effect of the feeding treatments on fetus weight at 144 days of gestation, lamb birth weight and ewe milk production as shown in the following table.

After Wallace (1948a, c)

Feeding treatment	Mean fetus weight at 144 days of gestation (g)	Average lamb birth weight (lb)		Average milk yield (16 weeks) of ewes with singles only (lb)
		Singles	Twins	
Super-maintenance (High)	10,107	13·7	10·9	301·2
Maintenance (Medium)	-	8·8	7·7	254·8
Sub-maintenance (Low)	5,548	8·9	5·7	131·5

All but one of the lambs born to the sub-maintenance group died at or shortly after birth. For the maintenance and super-maintenance groups, all the lambs survived although those from the maintenance group, especially the twins, looked less vigorous than those from the super-

maintenance group. Ewes suckling twins produced more milk than those suckling singles and that was probably due to the suckling capacity of lambs.

In another experiment, Wallace (1948a) showed that lambs from ewes on high and low levels of nutrition during the last six weeks of pregnancy, showed mean weaning weights of 84 and 68 lb respectively. He concluded that lamb's growth rate can be affected by levels of feeding during late pregnancy.

Barnicoat *et al.* (1949a, b) studied the various factors affecting the milk yield of 59 New Zealand Romney ewes. Among the factors studied was level of feeding during the last six weeks of pregnancy. Both ewe milk production and lamb birth weight were affected by the feeding treatments as shown in the following table made from pooling some of their results.

After Barnicoat *et al.* (1949a, b)

Level of feeding during late pregnancy	Av. ewe wt. gain during last 6 weeks of pregnancy (lb)	Av. milk production during first 12 weeks of lactation (Gal)	Av. lamb birth weight (lb)		Av. lamb weight at 12 weeks of age (lb)	
			Singles	Twins	Singles	Twins
HIGH	16·5	22	10·2	8·4	42·2	31·4
LOW	6·0	18	9·6	7·5	37·7	27·8

Lamb losses during and after lambing were very low (only three lambs lost, presumably to the low nutrition group). The small difference in PLM was due to both nutritional regimes being adequate as reflected by the small difference

in weight gain between the two groups during pregnancy.

These workers also found that low levels of feeding resulted in milk of lower protein content. They also reported that feeding during lactation had profound effects on ewe milk production and lamb growth rate during the first 12 weeks after lambing.

In Scotland, the work of Thomson and Thomson (1949) showed that the level of feeding during the second half of pregnancy had a profound effect on lamb birth weight and perinatal lamb losses. The two feeding treatments resulted in an increase in body weight of about 30 per cent in the case of "high groups" (30 ewes) and a fall in body weight of about five per cent in the case of "low groups" (44 ewes). Ewes on low levels of feeding showed weakness after parturition and also produced little or negligible amounts of milk. Other effects of the feeding treatments on ewe reproduction efficiency are given in the following table.

After Thomson and Thomson (1949)

Feeding treatment	Lamb birth weight (kg)		*Perinatal lamb losses		Live but initially inactive lambs	Ewes with less than 146 days gestation period	Ewes with poor mothering ability
	Singles	Twins	Singles	Twins			
High group	4·8	3·5	-	5	-	2	-
Low group	3·7	2·3	8	38	20	17	11

*Including "presumptive neonatal deaths", i.e. lambs starving progressively until hand fed or fostered on another ewe.

Coop (1950) reported on his three-year trials concerning the effects of levels of feeding during pregnancy on ewe and lamb performance in New Zealand. He used poor and good quality pasture with or without concentrate supplementation and concluded that this had no effect on ewe weight, ewe and lamb mortality, growth rate and weaning weight of lambs.

Lamb losses at and after lambing were not affected by the level of nutrition during late pregnancy. In one of his trials, for example, he supplemented group 2 ewes with 0·5 lb of concentrate per ewe per day during the last 30 days of pregnancy, while group 1 (the control group) did not receive any supplementation. The results of this trial can be summarised as follows.

After Coop (1950)

Groups	Average ewe weight after lambing (lb)	Lambs weaned per 100 ewes	Lambs born dead or died before weaning %	Singles weaning weight (lb)	Twins weaning weight (lb)
1	112·5	100	20	62·3	46·6
2	112·6	108	20	61·9	50·0

From the figures he showed concerning ewe body weight change and a difference in lamb birth weight of about 0·5 lb, it can be suggested that what he called a "low plane" of nutrition was actually high enough to leave the ewes in a satisfactory condition for good production performance.

He also reported that levels of feeding during lactation were the most necessary and important factors. In two different groups of ewes that were left on low levels of feeding (poor pasture) during pregnancy, one of them had been changed to a high-plane of feeding (thus called Low - High) using oats and new pasture. The other group was kept on a low-plane of feeding during the first four weeks of lactation (thus called Low - Low). From birth to weaning, the low - high group lost only 10 per cent of its lambs while the low - low group lost 23 per cent.

Darroch et al. (1950) compared the effect of supplementing a group of 237 ewes ("fed ewes") with 0·5 lb concentrates per ewe per day during late pregnancy to another group of 223 ewes ("not fed") which received no concentrates. Both groups were of the same breed (Columbia - Rambouillet crossbreds). They found a difference in the level of lamb survival between the two groups which was statistically not significant. The following table shows some of their findings.

After Darroch et al. (1950)

Feeding treatment	No. of ewes	Lamb birth weight (lb)	Lamb weaning weight (lb)	No. of lambs per 100 ewes			
				Born	Born dead	Dead or missing to weaning	Weaned
"Fed"	237	11·1	80·5	127	10	18	99
"Not fed"	223	10·9	79·0	123	13	20	90
Difference		+0·2	+1·5	+4	-3	-2	+9

Thomson and Thomson (1953) reported on the effect of level of feeding (High or Low) during late pregnancy on the ewes' lactation performance and the lambs' growth rate, as a second part of the work already cited (Thomson and Thomson, 1949). After lambing, the high-plane ewes either suckled their own lambs (H H group), or lambs from low-plane ewes (L H group). The low-plane ewes suckled their own lambs (L L group) or lambs from high-plane ewes (H L group). During lactation, all the ewes in these four groups were kept on the same diet consisting of concentrates, hay and swedes or grass. All lambs had access to concentrates, hay and grass as soon as they would eat them.

The average milk yield of the Cheviot ewes that had been on the high-plane of nutrition during late pregnancy was approximately 20 gal. in 13 weeks of lactation compared to only 11 gal. for those maintained on slightly higher than half the quantity of nutrient supplied to the high-plane ewes. Improving nutrition of the low-plane ewes immediately after lambing did not increase the milk supply quickly enough to be of full benefit to the lambs. In the milk-yield trial, all ewes were left to suckle one lamb only. Some of their findings concerning ewe milk yield and lamb performance can be briefly summarised as follows.

After Thomson and Thomson (1953)

Feeding treatment	No. of ewes in the milk-yield trial	**Total milk yield, 5-91 days (Gal)	Lamb weight at 5 days of age (kg)	Lamb weight increase at 91 days of age (kg)	Total No. of lambs born to all trials	*No. of lambs lost during lactn.
H-H	4	19·7±1·30	6·0±1·73	19·8±1·52	21	None
L-H	4	19·0±1·30	3·8±0·40	21·9±1·52	9	None
L-L	8	10·9±0·92	3·8±0·28	14·5±1·08	17	1
H-L	3	13·8±1·50	4·7±0·46	14·9±1·76	9	3

*Presumably as a result of milk shortage.

**Milk yield was evaluated by weighing lambs before and after suckling.

Increase in lamb weight was highly correlated with ewe milk yield in both high and low plane of nutrition groups during five to 28 days post-partum, but not with their milk production at 28 to 91 days post-partum. These workers concluded that undernourishment in late pregnancy greatly affects vitality of the newborn lambs and has a severe effect on ewe's milk supply, particularly during early lactation.

Guyer and Dyer (1954) in their two-year investigation of ewes' concentrate feeding during late pregnancy and its effect on birth weight and survival of lambs, used two groups of ewes that were left to graze during pregnancy. Group A had a liberal allowance of concentrates during late pregnancy while group B received no concentrates at any stage of pregnancy. Their findings can be summarised

as follows.

After Guyer and Dyer (1954)

Year		1952		1953	
Treatment		A	B	A	B
No. of ewes lambing		31	29	28	28
Average No. of lambs born per ewe		1·55	1·41	1·82	1·68
Average No. of lambs raised per ewe		1·46	1·36	1·68	1·50
Average birth weight of lambs born alive (lb)	Singles	11·1	10·3	12·1	11·9
	Twins	9·0	7·4	9·3	9·0
	Triplets	7·5	6·8	8·2	8·9
No. of lambs died at or near birth		7	1	6	5

Lambs born to group A were in most cases heavier than those born to group B. The authors did not explain their unexpected finding of higher lamb losses in group A.

Russel et al. (1967a) studied the effect of level of nutrition during the last six weeks of pregnancy on the performance of 51 Scottish Blackface ewes. The ewes were divided into three groups and fed hay and concentrates, adequately in group 1, with a moderate degree of undernourishment in group 2, and to produce a severe degree of undernourishment in group 3. At 10 days before lambing, the ewes in group 1, 2 and 3 showed mean plasma ketone levels of about two, four and nine mg per 100 ml respectively. Average lamb birth weight for

the three feeding treatments was as follows.

After Russel et al. (1967a)

Feeding treatments	1	2	3
Average singles birth weight (kg)	4·6	4·3	3·5
Average of the total twins birth weight (kg)	8·2	7·1	5·4

The reduction in birth weight of both singles and twins, as a result of undernourishment (group 3), was highly significant in relation to the birth weights reported for lambs in group 1 ($P < 0{\cdot}01$). Even a moderate degree of undernourishment (group 2) resulted in a noticeable lamb birth weight reduction.

Treacher (1970) used ground, pelleted dried grass in feeding three groups of Scottish Half-bred ewes during the last six weeks of pregnancy to achieve live-weight gains of 20, 10 and 0 per cent of their live weight at 14 weeks of pregnancy. Thirty-two ewes were used, all carrying twin fetuses. The effect of the feeding level on lamb birth weight and ewe milk production was as follows.

After Treacher (1970)

Feeding treatment	1. (20% gain)	2. (10% gain)	3. (0% gain)
Total ewe feed intake in the last six weeks of pregnancy (kg)	67·1	46·5	36·1
Average total birth weight of twins (kg)	10·10	9·44	8·18
Total milk yield, 0-6 weeks (kg)	58·8	43·5	26·9

He also reported on the quality of milk produced by different groups. In the first and third day of lactation, milk from treatment 3 showed higher fat and protein content as an indication of a slower onset of lactation due to undernourishment.

Ferguson (personal communication) studied the ewe hay intake during the last eight weeks of pregnancy. During this period, four groups of Scottish Halfbred ewes were fed the same quality of hay ad libitum, plus 30, 20, 10 or 0 kg of concentrates to the first, second, third and fourth group respectively. He found that the level of lamb losses from birth to weaning in the first, second, third and fourth groups were 6·7, 5·9, 11·6 and 16·3 per cent respectively.

Louca et al. (1974) conducted two trials to study the effect of plane of nutrition in the last six weeks of pregnancy on some Cyprus breeds of sheep. In the first of their trials, they kept two groups of ewes either on medium (straw ad libitum plus 0·5 kg concentrates per ewe per day), or high (straw ad libitum plus 1·0 kg concentrates per ewe per day) levels of nutrition. Both groups of ewes received the same level of feeding until 14 days after lambing. In a second trial, using the same breed, two groups on the same late pregnancy feeding treatments as those of the first trial were placed on high level of feeding (ad libitum straw and

concentrates) for 28 days after lambing. They found that these feeding treatments had significant effects on twin and triplet birth weight. Early ewe milk production was also affected, mainly due to plane of nutrition during lactation and, to a certain extent, during late pregnancy.

Their important findings can be summarised below.

After Louca et al. (1974)

Trial No.		1		2	
Feeding treatments		Medium	High	Medium	High
No. of ewes		14	14	27	26
Ewes' average weight at ten weeks of pregnancy (kg)		58·4	59·5	61·9	60·5
Ewe daily ME intake (MJ)		11·66	16·63	10·03	14·74
Ewes' weight loss during the second half of pregnancy (kg)		9·7	6·2	7·9	2·9
First 14-days of lactation average ewe milk yield (kg)		15·2	25·6	21·9	21·8
Mean lamb birth weight (kg)	Singles	4·6	4·8	5·0	5·0
	Twins	3·3	3·8	3·1	3·7
	Triplets	2·4	2·9	omitted because of very small No.	

The workers did not refer to level of lamb losses in the 2 trials.

Shevah et al. (1975) studied the performance of 84 Finn cross Dorset ewes in two experiments carried out during 1971 and 1972. The ewes were allocated to groups receiving different levels of feeding during the

last six weeks of pregnancy. The different feeding treatments did not affect lamb birth weight or neonatal lamb mortality.

The description and results of their work can be briefly summarised as follows.

After Shevah et al. (1975)

	Exp. 1 (1971)			Exp. 2 (1972)	
Feeds	250 g Hay + concentrate			Complete ruminant diet	
No. of ewes per treatment	12	12	12	24	24
Energy levels (% of requirement)	ad.lib.	100	80	100	50
Daily ME intake per ewe (MJ)	18·4	17·3	14·2	17·2	9·45
Plasma ketones (mg/100 ml) during last six weeks of pregnancy	2·9	2·4	1·8	2·7	4·2
Average ewe live wt. gain (kg) during last six weeks of gest^{n}.	7·0	7·0	6·0	8·0	0
Total No. of lambs born	24	25	22	52	53
Mean lamb birth wt. of twins (kg)	3·2	3·5	3·3	3·8	3·4
Lamb mortality in first week of life (no.)	5	1	3	5	2

Conclusion

It can be concluded that the work conducted so far, concerning levels of feeding during late pregnancy and its effect on PLM, is far from being complete.

Most of the workers in the field of ewe nutrition and its relation with the ewe reproductive performance, referred to parameters like ewe body weight, lamb birth weight, ewe milk production and the like. Unfortunately, in many cases, these reports were contradictory. More than that, the same workers have failed to give enough attention to the subject of PLM and its role in affecting sheep production and the national economy in general. The data in this connection is obviously lacking and further investigation is needed.

IMMUNOGLOBULINS

Introduction

The following account is based on publications by Franklin (1971), Hobbs (1971), Weir (1973), Cline (1975) and Topley and Wilson (1975).

As defined by the World Health Organization, 1964, immunoglobulins are proteins of animal origin endowed with antibody activity and also certain proteins, like myeloma and Bence-Jones' proteins, related to them by chemical structure and hence antigenic specificity.

Immunoglobulins are heterogeneous with regard to structure, size and biological activity and this is a reflection of the heterogeneity of the cells that produce them. This heterogenicity has made immunoglobulin studies difficult, nevertheless, a vast amount of

information has accumulated during this century concerning immunoglobulins, mainly as a result of studies conducted on man and to a lesser extent on other mammalian species. In the human, five major classes of immunoglobulin are now recognised, according to their physico-chemical characteristics, and immuno-chemical, metabolic and functional features. They are designated: IgG, IgM, IgA, IgD and IgE (World Health Organization, 1964; Rowe and Fahey, 1965; Ishizaka, Ishizaka and Hornbeck, 1966) and have average serum levels of 12·4, 1·2, 2·5, 0·03 and 0·0003 mg per ml respectively. Within most of these classes of immunoglobulins, sub-classes which are closely related to each other have been defined. In the human, for example, there are four sub-classes of IgG (IgG_1, G_2, G_3 and G_4) and two sub-classes of IgA (IgA_1 and A_2). Immunoglobulins are produced by plasma cells and lymphocytes. It seems likely that a single antibody producing cell, when stimulated, undergoes division and produces many progeny (a clone) and that these cells produce only antibody molecules of unique specificity (Burnet, 1967), i.e. they produce only a single immunoglobulin class and sub-class such as IgG_1.

Antigen recognition

There are in the simplest terms two types of lymphocyte, T cells and B cells. Both of them are derived from

a common precursor stem cell principally originating from the bone marrow. The first type develops in the thymus and it is responsible for "cell mediated immunity". The second type of lymphoid cells develops (independently of the thymus gland) in the avian bursa of Fabricius or mammalian equivalent. In mammals the B cells are found in the spleen, lymph node and the blood and are the precursors of immunoglobulin producing plasma cells which are capable of secreting immunoglobulins in the blood (humoral antibody), or even producing it locally (IgA in particular) in different parts of the body such as the mammary and exocrine glands, and respiratory, digestive and urogenital tracts. It seems that cellular interaction between T cells, B cells and macrophages is necessary for antibody synthesis. When an antigen such as a bacterium enters the body it is detected, recognised and bound by T cells which as a result undergo proliferation. The cells resulting from this proliferation react with the antigen in the presence of macrophages and this process informs and stimulates B cells to proliferate. The progeny of the stimulated B cell change into plasma cells which have great specific immunoglobulin producing capability.

In short, T cells detect and bind antigen and then stimulate or induce B cells to produce specific antibody.

Structure of immunoglobulins

The basic human immunoglobulin is IgG (Fig. 3·1). It has four polypeptide chains and attached carbohydrate, the chains being linked by disulphide bonds and hydrogen binding. Two of the chains are called heavy chains, which are each composed of 420 amino-acids and have a molecular weight of 52,000. They are class specific and indicated by the Greek letter corresponding to their class of immunoglobulin (Gamma, Mū, Alpha, Delta and Epsilon for IgG, IgM, IgA, IgD and IgE respectively). The other two chains are called light chains, each composed of 214 amino-acids and with a molecular weight of 22,000. The light chains can be further subdivided on the basis of their structure into two major types, Kappa (k) and Lambda (λ). They are common to all classes but in a given immunoglobulin molecule, only one type is found (both types never occur together).

Each polypeptide chain has regions of 110 amino-acids and a linking disulphide bridge. There are two such regions in each light chain and four in each heavy chain. Some of these regions are of constant amino-acid composition (common regions) and others are of variable composition (variable regions). A variable region is represented by the N-terminal half of the light chain and the N-terminal quarter of the heavy chain and is involved in antigen binding. The remainder

of each chain represents the common region (Fig. 3·1). The region between amino-acids 220 and 240 of the heavy chain is called the hinge region and the immunoglobulin can be split at this point by substances such as papain or pepsin. Cleavage caused by papain for example, will produce three fragments (Fig. 3·1): two identical Fab (antigen-binding fragments) and one Fc (crystalizable fragment). The hinge region is a very flexible one and this presumably will allow the Fab piece to move over great distances when reacting with an antigen.

Functions and other features of immunoglobulins

The most significant character of immunoglobulins is their capacity to specifically bind antigens and this is the role of the Fab piece, whereas the biological properties such as complement fixation and skin sensitization are associated with the Fc piece.

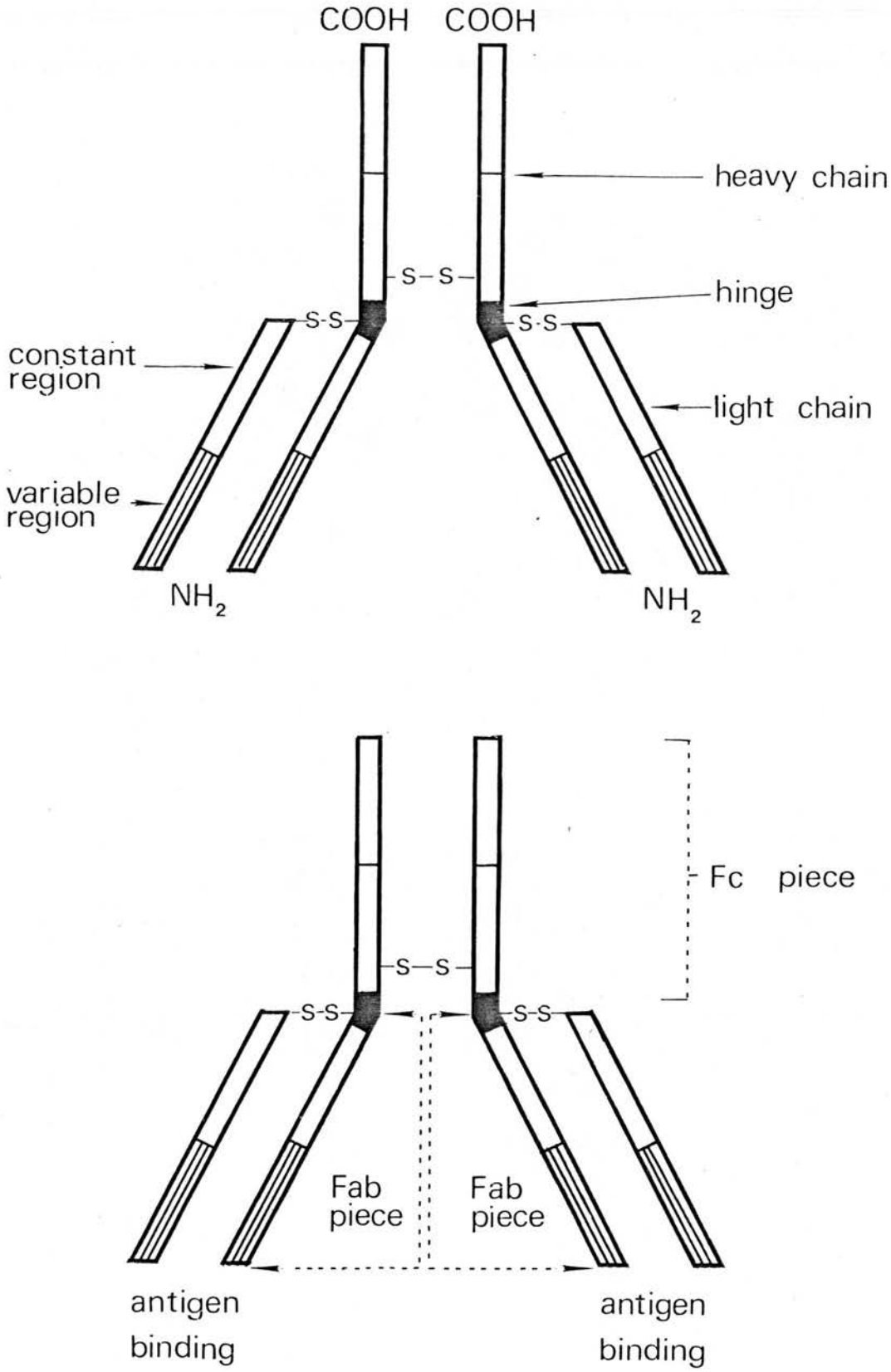
Immunoglobulins can react specifically to antigens and render them harmless by one or more of the following ways: neutralization, lysis, agglutination, precipitation and opsonization.

IgG

Among the different classes of immunoglobulins, IgG is the most abundant in human serum and accounts for 73 per cent of the normal serum immunoglobulins. It has a sedimentation coefficient of 7_S, a half-life of 24 days and a molecular weight varying from

FIG. 3·1

Structure of the IgG molecule.



150,000 to 180,000.

Functions of the different sub-classes of IgG include efficient precipitation and complement fixation, neutralization of viruses and soluble antigens (example, bacterial toxins), agglutination and opsonization (IgG is not as efficient as IgM in the last two reactions).

IgM

IgM accounts for only seven per cent of the immunoglobulin in normal human serum. It is of a large molecular weight with a sedimentation coefficient of 19_S, a half-life of five days and a molecular weight of 900,000. The molecule consists of five 7_S sub-units (pentameric structure, Fig. 3·2). A J - (junction) chain of approximately 25,000 molecular weight has been described. It is thought that this chain plays an important role in joining and perhaps holding together the 7_S sub-units with the consequent formation of polymeric IgM and IgA (Halpern and Koshland, 1970).

IgM is the first immunoglobulin to be detected after an antigen injection (first line of defence). It is a very potent agglutinating, complement fixing, opsonizing and lysing antibody. It also possesses some virus neutralizing activity, but in this connection, it is much less efficient than IgG (Svehag, 1964).

IgA

This immunoglobulin exists in the serum of man and other mammals as a 7_S monomer and also as polymeric 9_S, 11_S and 13_S forms (Fig. 3·2 a - c). Certain parts of the body seem to have special immune systems capable of producing a secretory 11_S IgA locally (at the lamina propria underlying the mucous membrane) and secreting it to the associated surface in, e.g. colostrum, tears, saliva, intestinal and respiratory secretions.

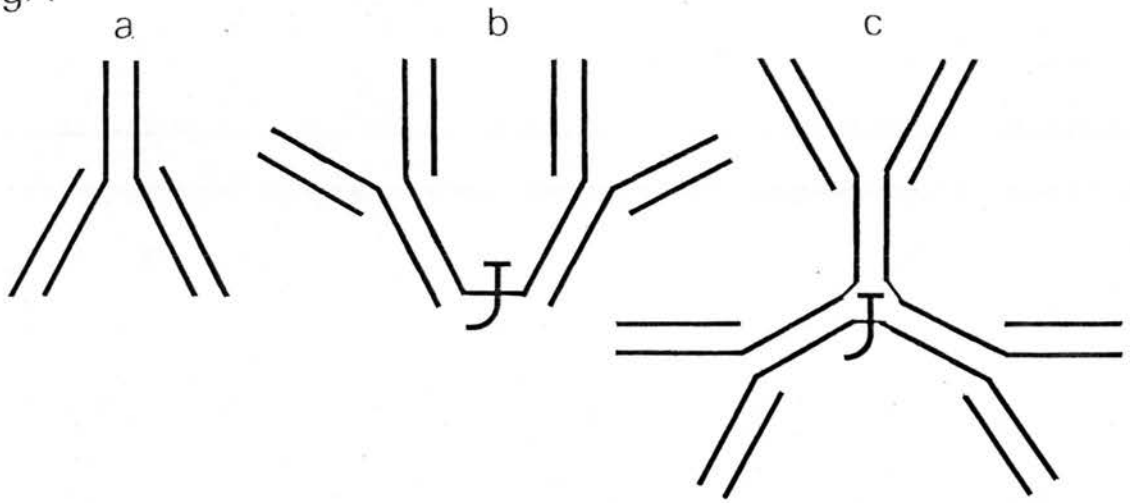
The secretory IgA exists as a dimer linked by a J - chain and also associated with a "secretory" (S) piece (Fig. 3·2). The S - piece is a glycoprotein of 58,000 molecular weight and is believed to facilitate the secretion of the IgA in the external secretions. Also by being resistant to digestive enzymes, it protects the colostral IgA while it passes through the stomach to the intestine where it will perform its antibody protective role against micro-organisms. The 7_S human serum IgA forms about nine per cent of normal serum immunoglobulins. It has a five day half-life and a molecular weight of 150,000 to 170,000.

The secretory IgA is of 390,000 molecular weight (7_S dimer produced by plasma cells, plus

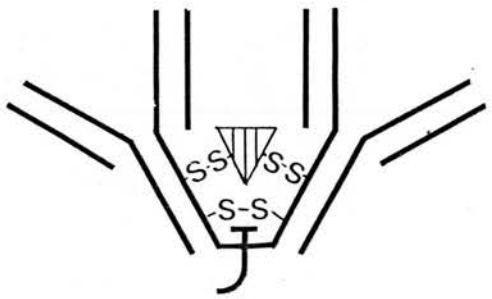
FIG. 3·2

Structure of the IgA and IgM molecules. Notice the position of the secretory piece and the J-chain.

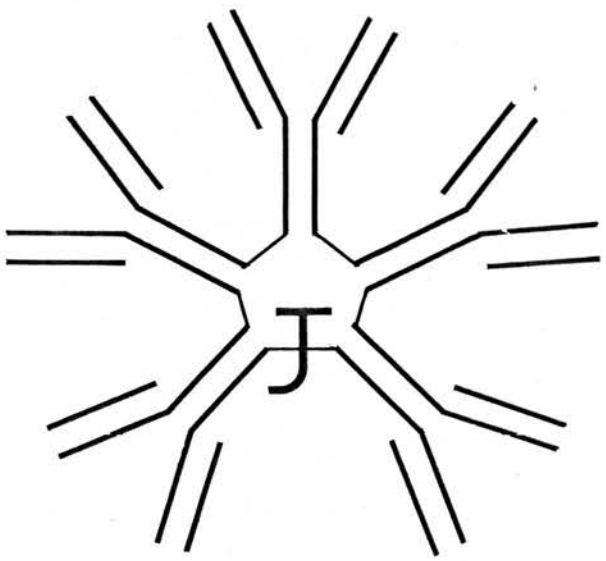
IgA



IgA with secretory piece



IgM



S - piece synthesized in epithelial cells). It is able to protect internal body surfaces. It has haemagglutinating activity and can neutralize some viruses. It can fix complement while 7_S IgA cannot (Hobbs, 1971). Secretory IgA is also shown to be efficient in opsonizing bacteria (Wernet, Breu, Knop and Rowley, 1971).

IgD

A monomeric immunoglobulin, occurring in low levels in human serum and forming about one per cent of total normal serum immunoglobulins. It has a half-life of 2·8 days and a molecular weight of 150,000 to 184,000. Very little is known about IgD, particularly in relation to its function, however, antibody activity has been reported by Gleich, Bieger and Stankievic (1969).

IgE

A very minute amount of this immunoglobulin occurs in human serum and this makes its study very difficult. It appears as a monomer with a molecular weight of 185,000 to 200,000. It has a sedimentation rate of $7·9_S$ and a very short half-life (2·2 days). It is a "reaginic antibody", i.e. it has the ability to fix to skin and other tissues, and sensitize mast cells which by contact

with antigen (allergen) will release biologically active substances (amines) such as histamine, resulting in immediate hypersensitivity. IgE may have some role against helminth infestations.

OVINE SERUM AND COLOSTRAL IMMUNOGLOBULIN: TYPES AND IMPORTANCE

Introduction

Immunoglobulins have been comprehensively studied in the human species and considerable work has been done in cattle, sheep and pigs.

Of these species, cattle have been the most extensively investigated and work concerning the role of serum and colostrum gamma-globulins in the newborn was undertaken more than fifty years ago (Orcutt and Howe, 1922; Smith and Little, 1922). The different classes and sub-classes of bovine immunoglobulins have been identified as IgG_1, IgG_2, IgM and IgA (Butler, 1971; Butler and Maxwell, 1972) and possibly IgE (Wells and Eyre, 1972). These immunoglobulins were studied and reviewed in relation to the following factors:-

Colostrum and serum levels (Porter, 1972; Wilson, Duncan, Heistand and Brown, 1972; Williams, Maxwell and Spooner, 1975); colostrum feeding of calves (Kruse, 1970; Penhale, Logan, Selman, Fisher and McEwan, 1973; Logan, 1974); infections and mortality

among newborn calves (Logan and Penhale, 1971, 1972; McEwan, Fisher and Selman, 1970; Fisher and Martinez, 1975); neonatal management (Selman, McEwan and Fisher, 1971; McBeath and Logan, 1974); age of the cow (Williams et al., 1975) and litter size (Kay, Little and Kitchenham, 1976).

In the pig, on the other hand, immunoglobulins have been studied only in the last few years and observations concerning the importance of the four pig immunoglobulins, IgG_1, IgG_2, IgM and IgA have been reported by Bourne (1969, 1971a, 1971b, 1973), Curtis and Bourne (1971) and Hill and Porter (1974).

As the sheep is the only species to be included in my investigation of PLM, and because serum and colostrum immunoglobulins are an important parameter in the investigations, the whole of the next section will be directed to sheep immunoglobulins particularly those of the serum and colostrum.

Literature review

As early as 1888, Chauveau investigated the vaccination of ewes against anthrax and reported on the importance of acquired immunity to protect young lambs against this disease. Since that date, and increasingly in the last few years, immunological studies have been conducted on sheep and there is now a wide range of data concerning

the type and significance of different classes and sub-classes of ovine immunoglobulins defined by their cross reaction with the corresponding human and bovine specific immunoglobulins, and also by their physico-chemical and biological properties.

McCarthy and McDougall (1953) reported that feeding colostrum to newborn lambs within 29 hours after birth resulted in an increase in their serum globulin mainly due to one electrophoretic component that seemed to be the gamma-globulin.

Silverstein, Uhr, Kraner and Lukes (1963) used immunoelectrophoresis to demonstrate the presence, in sheep serum, of four types of immunoglobulins designated as: fast gamma-globulin, slow gamma-globulin, IgM and IgA. The first and second gamma-globulins are now called IgG_1 and IgG_2 (after the WHO Committee for immunoglobulin nomenclature, 1964).

Aalund, Osebold and Murphy (1965) isolated IgG_1, IgG_2 and IgM from ovine serum using immunoelectrophoresis, analytical ultracentrifugation and also by comparing these immunoglobulins to their counterparts in human serum. Using the last phenomenon, they also reported the existence of a fourth immunoglobulin homologous to human serum IgA.

Halliday (1965a) studied the transfer of antibodies against Salmonella pullorum and Brucella abortus from

ewes' colostrum to their lambs. He found that these lambs absorbed both types of antibodies non-selectively, and with equal degree of efficiency.

Harrison and Mage (1967) reported the isolation of two distinct 7_S ($gamma_1$ and $gamma_2$) immunoglobulins from sheep serum. They also separated each of these immunoglobulins into its heavy and light polypeptide chains. The criteria they used included antibody activity, electrophoretic behaviour and amino-acid composition.

Jonas (1968) used immunoelectrophoresis to study the distribution of immunoglobulins in some ovine body fluids. For ewe serum and presuckling colostrum, he reported the presence of slow and fast gamma-globulins and IgM which were all present in the sera of lambs after 24 hours of suckling and throughout the first four weeks of the lambs' life. IgM was absent from many other body fluids examined, including those collected from small intestine, trachea and lung.

Mackenzie and Lascelles (1968) injected 19 lactating ewes intravenously with fast and slow IgG which had been labelled with iodine [^{131}I]. They found that the fast one transferred more efficiently into the ewes' milk than the slow one. As the two sub-classes of IgG have the same molecular size, they suggested a selective transfer mechanism (during lactation) for the fast IgG similar to the one which exists during colostrum formation.

Pan, Kaplan, Morter and Freeman (1968) examined normal ovine serum for the presence of different classes and sub-classes of immunoglobulins and confirmed the presence of IgG_1, IgG_2, IgM and IgA. They also reported the presence of delta and epsilon minor immunoglobulin classes and suggested that at least the last one seems to be analogous to human IgE in that it possess a homocytotropic antibody activity that was not related to any of the major immunoglobulin classes reported in this study.

Curtain (1969) claimed that an immunoglobulin sub-class exists in the serum of some sheep. He considered it as a new IgG sub-class antigenically different from IgG_1 and IgG_2 and named it IgG_{1A}. He reported that IgG_{1A} was absent from all colostral and some serum samples.

Feinstein and Hobart (1969) reported that ovine serum IgG is of two sub-classes that were analogous to ox serum IgG_1 and IgG_2. They reported that both sub-classes had complement fixing activity but with different degrees of efficiency as shown by the capability of only IgG_1 to fix low doses of guinea pig and rabbit complement.

Georgiev (1969) examined sera from fetuses and also sera from newborn lambs before colostrum sucking. Using electrophoresis, he found no gamma-globulin in these sera.

The work of Heimer, Clark and Maurer (1969) showed that ovine serum immunoglobulins include at least four antigenically different types: slow IgG_2, fast IgG_1, IgM and IgA. They also reported the presence of a fifth one which might be either a third IgG sub-class or an analogue of human IgD.

Heimer, Jones and Maurer (1969) reported three classes of immunoglobulins in ovine colostrum: a $6{\cdot}5_s$ IgG_1 which constituted the major immunoglobulin of colostrum, a 15_s IgA_2 that was associated with a secretory piece and always higher in amount than the third one which was a $10{\cdot}8_s$ IgA_1 that was lacking the secretory piece. They also reported minute traces of other IgG sub-classes.

Jonas (1969a) reported that the immunoelectrophoresis (IE) pattern of ewes' serum and presuckling colostrum treated with heat ($62{\cdot}5^{\circ}C$ for 30 minutes) or 2-Mercaptoethanol, lost the IgM arc and showed new arcs. Both slow and fast IgG were not affected by any of the treatments.

Jonas (1969b) prepared specific antisera to IgM, fast IgG (IgG_1) and slow IgG (IgG_2) and used them to show that ewes vaccinated subcutaneously with killed S. typhimurium produced high titres of IgM antibody, in one week, but a much lower titre of IgG_1 and IgG_2 antibodies even after a booster injection. Ewes which were

challenged orally with living S. typhimurium produced in nine to 15 days a high titre of IgM antibody and also lower titres of IgG_1 and IgG_2 antibodies. He stressed the importance of route of administration, the dose of the antigen and the use of adjuvant for better antibody production.

Smeaton (1969) studied the metabolism of gamma-globulins in newborn lambs and reported that the intestinal absorption of colostral IgM, IgG_1 and IgG_2 is non-selective. He also reported the occurrence of rapid morphological changes in the gut epithelium of the newborn lamb, immediately after birth, and ascribed to these changes the cessation of colostrum gamma-globulin absorption as early as 36 hours of age.

Sullivan, Prendergast, Antunes, Silverstein and Tomasi (1969) reported a similarity between the cow and the sheep secretory immune system. In the saliva and colostrum of both species, they reported the presence of a fast 7_s IgG_1 as the main protein which is immunologically identical to that of the serum. Their work also showed evidence of a secretory 10_s immunoglobulin, presumably a secretory IgA.

Hudson, Bandy and Kitts (1970) used an immunoelectrophoresis method to quantify different classes of ovine serum immunoglobulins. They referred to the main classes as 7_s - IgG_1, 7_s - IgG_2, IgM and IgA.

Lascelles and McDowell (1970) reported the isolation of a locally produced IgA in the whey of ewe following an intra-mammary infusion with killed Br. abortus antigen three to four weeks before lambing. They suggested that this antigen had actually awakened a dormant local immune system. The IgA, they reported, had no secretory piece.

Lee and Lascelles (1970) reported on the local production of immunoglobulin in sheep and demonstrated the existence of numerous fluorescing pyroninophilic cells in the mammary glands that were stimulated by killed Brucella antigen. Most of these cells were IgA-specific and only a few of them were IgG_1- and IgM-specific. The gastro-intestinal tract and the lymph nodes also showed an abundance of these fluorescing cells which were mainly IgA-specific in the first and IgG_1- and IgG_2-specific in the second location.

Pahud and Mach (1970) identified IgA in the serum, colostrum, milk, saliva and urine of sheep by its cross reaction with anti-human and anti-bovine IgA. These workers also purified secretory IgA, serum IgA and free secretory component (the secretory piece). By applying the single radial immuno-diffusion (SRID) technique of Mancini, Carbonara and Heremans (1965) using cross-reacting anti-bovine serum or specific anti-ovine serum, They reported the following normal values for ovine-specific immunoglobulins in the different body fluids:-

After Pahud and Mach (1970)

Type of fluid	IgG (mg/100 ml)	IgM (mg/100 ml)	IgA (mg/100 ml)
Serum	2100 (1800 - 2400)	120 (80 - 180)	25 (5 - 100)
Colostrum	6000 (5200 - 6400)	410 (180 - 560)	200 (90 - 300)
Milk	30 (10 - 50)	3 (1 - 4)	6 (3 - 9)
Saliva	10 (1 - 25)	Trace	20 (4 - 50)

By reporting high levels of IgA in the saliva of the cow, the ewe and the goat, they concluded that this immunoglobulin is the major secretory one in these species.

Curtain and Anderson (1971) investigated the local production of IgG_1, IgG_2, IgA and what they called IgG_{1A} in the alimentary tract and associated lymph nodes of Merino sheep infested with Ostertagia and Trichostrongylus spp. and also in parasite-free sheep, using immunofluorescence. They found a large number of IgG_1 and IgG_{1A} containing cells in the lamina propria and the base of the abomasal villi in parasitised sheep but none in the parasite-free sheep. The mucosa of the jejunum and ileum of both groups of sheep showed IgG_1, IgG_{1A} and IgA containing cells, but the parasitised intestines had significantly higher IgG_{1A} containing cells than the non-parasitised ones. This was possibly due to the involvement of this particular immunoglobulin in intestinal

parasitic infestation.

Vaerman (1971) identified IgA from the sera of eight mammalian species including the sheep, by cross-reacting this immunoglobulin with an antiserum specific for human IgA. He reported that IgA is a minor immunoglobulin in the colostrum and milk of the sheep as well as of the goat and cow.

Watson and Lascelles (1971) reported the presence of IgA in the sera and also in most of the mucous surface secretions of sheep including colostral whey.

Varela-Diaz and Soulsby (1972) conducted an immunoelectrophoretic study on sera of 14 colostrum-fed and six colostrum-deprived lambs and reported the existence of a hypogammaglobulinaemic state in the sera of the second group for a few weeks after birth. They claimed that only IgG_1 and not IgG_2 can pass from the colostrum of the ewes to their lambs' sera. In the sera of colostrum-deprived lambs, IgG_1 appeared within a week of birth but IgG_2 failed to appear until three to four weeks after birth, or even until five to six weeks after birth in the case of colostrum fed lambs. These workers also reported only traces of IgG_2 in the ewes' colostrum.

Janusz, Godzńska and Lisowski (1973) reported the preparation of immunoelectrophoretically pure IgG_1 and IgG_2 fractions and also of IgA and IgM rich, but not pure, fractions from the colostrum of sheep using chromatography

on DEAE-cellulose with a multibuffer system. They reported that IgG_1 is the predominant immunoglobulin in ovine colostrum. During their study, they gave values of 77, 12, 0·4 and 10 per cent of total ovine colostrum immunoglobulins, for IgG_1, IgG_2, IgM and IgA respectively.

Watson and Lascelles (1973a) studied the capacity of the glandular epithelium of the ovine mammary gland for selectively transferring IgA and IgM into secretions. They found that preferential transfer exists but it is related to the activity of the local immune system. They proved this by infusing the left mammary glands of eight ewes with killed Br. abortus organisms three weeks before parturition and then calculating the milk whey: lymph ratio of IgA and IgM four weeks after lambing. They found that this ratio was three to four times higher for infused than non-infused glands.

A similar conclusion was drawn by Watson and Lascelles (1973b) on the selective transfer of IgA in salivary glands when they reported high levels of IgA in non-parotid salivary gland secretion (15·7 mg per 100 ml) which are on constant antigenic stimulation. The parotid salivary glands, which experience very low antigenic stimulation because of the continuous flow of large amounts of saliva, showed very low levels of IgA (0·3 mg per 100 ml). During both of their studies, these workers reported values for IgG_1, IgG_2, IgM and IgA in

the serum of lactating ewes ranging from about 1700 to 2000, 500 to 660, 170 to 210 and 16 to 110 mg per 100 ml respectively.

Beh and Lascelles (1974) carried out investigations that involved canulation of the lymphatic ducts of intestinal lymph nodes and antigenically stimulated popliteal lymph node in 10 Merino ewes. The aim was to determine the importance of immunoglobulin containing cells as potential IgA producing cells in these two locations. By using fluorescein-conjugated specific antisera, they found that almost all the immunoglobulin-containing cells in the intestinal lymph nodes were IgA-specific, originating from the lamina propria or Peyer's patches. These workers found that most of the cells which appeared in the popliteal lymph node following antigenic stimulation were IgM and IgG_1 specific. They suggested that these cells might serve as precursors of IgG_1 and IgM specific plasma cells in the gut.

By comparing specific radio-activities of the different ovine immunoglobulins in serum and intestinal secretions, Cripps, Husband and Lascelles (1974) found that all IgG_2, most of IgG_1 (both appear to have equal secretion facility), most of IgM and only negligible amounts of IgA in these secretions were blood plasma derived. They stated that 97 per cent of IgA was locally produced.

Cripps and Lascelles (1974) studied the biological half-lives of IgG_1 and IgG_2 in adult wethers, non-pregnant ewes, ewes in their last week of pregnancy and three to six day old lambs using radio-immunoassay. Their findings can be summarised as follows:-

After Cripps and Lascelles (1974)

		Adult wethers	Non-pregnant ewes	Ewes during colostrum formation	Lambs
Biological "half-lives" (days) of:	IgG_1	7.85	7.83	3.73	6.60
	IgG_2	8.08	8.15	6.95	6.93

All figures are similar except for IgG_1 in ewes at late pregnancy, the period of colostrum formation and transfer of IgG_1 from serum to colostrum, where the half-life was much shorter than that of IgG_2 and very much less than that of non-pregnant ewes.

These workers also demonstrated the capability of both adult sheep and lambs as young as three weeks to produce antibodies as a result of local antigenic stimulation of the jejunum by ferritin. These antibodies were associated with IgA in the intestinal secretions and mostly with IgG in the serum.

Curtain (1975) reviewed briefly but comprehensively the subject of ovine immunoglobulin. He collected different data which showed that ovine serum levels of

IgG_1, IgG_2, IgM and IgA range from 1300 to 1800, 100 to 1500, 50 to 200 and 25 to 80 mg per 100 ml respectively and that the levels of secretory immunoglobulins IgG_1 and IgA in ovine colostrum are 1400 to 3200 and 150 to 280 mg per 100 ml respectively. He reported also on what he had previously described as IgG_{1A} sub-class (Curtain and Anderson, 1971). Because of its association with homocytotropic antibody activity to sheep intestinal nematodes and also the cross-reactivity between it and the human anti-IgE, he concluded that this IgG_{1A} fraction is identical with ovine IgE.

Lisowski, Janusz, Tyran, Morawiecki, Gołab and Białkowska (1975) conducted a comparative physico-chemical study on ovine colostral IgG_1 and IgG_2. They suggested that colostral IgG_1 might be of both local and serum origin, while colostral IgG_2 is locally produced.

Smith, Dawson, Wells and Burrells (1975) reported on the preparation of specific antisera to ovine IgG, IgM and IgA immunoglobulins purified from colostrum, serum and lung fluid respectively. By applying the single radial immuno-diffusion (SRID) technique using specific antisera, they were able to measure the levels of the three classes of immunoglobulins in different ovine body fluids. They reported IgG as a major class in serum and colostrum while IgA predominated in others such as lung fluid and saliva. IgM was detected and

measured in different body fluids. Lung fluid, however, was completely lacking in this immunoglobulin. They measured IgG as a class without differentiating between IgG_1 and IgG_2 sub-classes. The values which they reported for IgG, IgM and IgA in sheep serum, colostrum and lung fluid were as follows:

After Smith, Dawson, Wells and Burrells (1975)

Type of fluid	Mean (& range) immunoglobulin concentration, mg/100 ml		
	IgG	IgM	IgA
Serum	1,882 (1100 - 3000)	201·7 (95 -470)	30·9 (8 - 84)
Colostrum	10,121 (5000 - 16400)	291 (82-445)	624 (153 -1728)
Lung fluid (Pulmonary adenomatosis)	95 (25 - 200)	0	213.8 (55 - 420)

Wells, Dawson, Smith and Smith (1975) studied the transfer of IgG_1 and IgG_2 from plasma to nasal secretions of four newborn colostrum-deprived lambs using radio-immunoassay. Their work showed that both sub-classes are transferred with equal facility to the mucosal secretion of upper respiratory tract.

Ciupercescu (1976) reported the production of mono-specific antisera to sheep IgG_1 and IgG_2 in the goat. Activity of the anti-sheep IgG_2 was very high, while that of anti-sheep IgG_1 was low, and because of this

only the former was recommended for extensive SRID testing.

Halliday and Williams (1976) while studying colostral conferred passive immunity in lambs reported only on the IgG class of immunoglobulin, as a parameter for assessing the lambs' immune status. They demonstrated considerable individual variations in the levels of IgG in sera of lambs at 24 hours of age. These levels ranged from 600 to 1770 mg per 100 ml.

Pearson and Brandon (1976) investigated the effect of thymectomy, in utero, at 55 to 66 days of gestation, on the subsequent concentrations of IgG_1, IgG_2, IgM and IgA in lambs. By comparing the levels of these immunoglobulins in six thymectomised and five control non-thymectomised lambs, they found that, at 64 and 128 days after birth, the first group had significantly lower levels of IgG_1 and IgG_2 while levels of IgM and IgA were similar in both groups. They suggested that although the thymus seems to be non-essential for ovine immunoglobulin production, it might regulate the production of IgG. They also reported increased serum levels of IgG_1, IgM and IgA but not IgG_2 in lambs as a result of colostrum absorption. They reported the following levels for the different classes and sub-classes of immunoglobulins:

After Pearson and Brandon (1976)

Age of lambs (days)	Average serum immunoglobulin levels (mg per 100 ml) of normal (N) and thymectomised (T) lambs							
	IgG_1		IgG_2		IgM		IgA	
	N	T	N	T	N	T	N	T
0	4	4	Neg.	1	26	25	13	Neg.
2	1226	1479	5	3	218	190	26	31
16	830	1020	7	4	50	40	Neg.	Neg.
32	713	355	10	2	70	65	3	Neg.
64	506	334	46	15	114	74	6	5
128	942	357	182	60	175	209	11	13

Ciupercescu (1977) prepared pure immunoglobulins IgG_1, IgG_2 and IgM from pooled sheep serum using ion-exchange chromatography. To each of them he prepared specific antiserum which he used in the SRID technique for measuring levels of these immunoglobulins in 16 pregnant and 12 non-pregnant ewes, eight rams and 30 newborn lambs, all Finn cross Dorset.

His main findings were:-

i) A sharp decrease in ewe serum levels of IgG_1 and IgM but not IgG_2 during the last two weeks of pregnancy.

ii) No seasonal variation in serum levels of the three different immunoglobulins using non-pregnant ewes.

iii) Lamb serum at three days of age contained all the three immunoglobulins. These sera and also sera collected from lambs at different times during the first 14 weeks after birth showed no sex or litter size effect on immunoglobulin levels.

iv) By comparing levels of IgG_1, IgG_2 and IgM in lambs at three days, six weeks and 14 weeks of age to their growth rate between 0 to six and six to 12 weeks, the only significant correlation was between lamb growth rate from six to 12 weeks, and their serum IgG_1 and IgG_2 concentrations at 14 weeks of age [$r = -0{\cdot}630$ ($P < 0{\cdot}01$) and $-0{\cdot}368$ ($P < 0{\cdot}05$) respectively].

Some of his findings concerning mean serum immunoglobulin levels (mg per 100 ml) can be briefly summarised in the following table.

After Ciupercescu (1977)

Ig class or sub-class	Pregnant ewes			Lambs		Non-pregnant ewes		
	Time before lambing			Age in days		June	Sept.	Dec.
	8 weeks	2 weeks	Lambing day	3	42			
IgG_1	2031	1900	1365	2448	777	2158	1945	2172
IgG_2	494	458	509	23	47	360	370	458
IgM	253	277	183	173	110	332	364	336

Conclusion

Reports considered in this section concerning ovine serum and colostrum immunoglobulins have shown that there are four major classes and sub-classes of immunoglobulins in the ovine serum and lacteal secretions. These are IgG_1, IgG_2, IgM and IgA. The existence of other, but very minor, classes which might be analogous to human IgD (Heimer, Clark and Maurer, 1969) and IgE (Pan et al., 1968; Curtain, 1975) have been reported but very little is known about them in sheep.

Lambs at birth have none or very negligible amounts of circulating gamma-globulins but once they consume colostrum, all the above-mentioned four immunoglobulins can be detected in their sera in quantities that might be related to the colostral content of each immunoglobulin.

Of the four immunoglobulins, IgG_1 is the predominant one, both in serum and colostrum. It passes in large quantities to the newborn lamb in the first two days of life as a result of intestinal absorption from the ingested colostrum in which IgG_1 might account for over 80 per cent of the total immunoglobulins.

The SRID technique of Mancini et al. (1965) was used for quantitating levels of the different specific ovine immunoglobulins in serum and colostrum. However,

reports of these quantitations are very few. Very wide variations in the concentration of these immunoglobulins seem to exist in ovine serum and colostrum.

THE ROLE OF COLOSTRUM IN LAMB SURVIVAL

Literature review

The importance of colostrum to the newborn lamb has been acknowledged for a long time. In lambs, as in calves, piglets and foals, maternal antibody, a very necessary means for protection against neonatal diseases, comes from the mother's colostrum. This is because there is little, if any, placental transfer of antibody in utero.

A search of the literature has shown that ovine colostrum and its role in the survival of the newborn lamb is far from being well documented (unlike the case for cattle and, to a certain extent, for swine).

Starvation of the newborn lamb, for any reason, has been shown to be a major cause of neonatal deaths. When I started my investigation into the subject of PLM in relation to late pregnancy feeding of the ewe, availability of colostrum at lambing was one of the parameters included. As a continuation of that, I carried out some observations on the effect of colostrum deprivation for certain lengths of time, on the survival of the newly born lamb and its subsequent performance. In this connection, and also in relation to the role of

colostrum as a source of antibody and energy, relatively few references could be located.

McCarthy and McDougall (1953) used 17 Cheviot, Blackface and Half-bred lambs to study the effect of delaying colostrum ingestion on the changes in lamb's serum proteins. Seven lambs were allowed to take colostrum immediately after birth, six after delays of 12, 24, 29 and 48 hours, and three and nine days respectively (only one lamb for each time of delay), and four were deprived of colostrum.

Their work indicates the following -

1 Lambs absorb their immunoglobulin from colostrum.

2 There was an increase in serum globulin mainly due to what is now called gamma-globulin, when colostrum was given up to 29 hours but not at 48 hours or more after birth.

However, the above mentioned workers did not refer to the later performance of the small number of lambs they used.

Lecce and Morgan (1962), in one of their experiments, tested the ability of newborn lambs to absorb bovine colostrum, egg protein and polyvinylpyrrolidone (PVP), which is a non-protein, non-toxic polymer, with high molecular weight suitable for testing intestinal absorption as it has similar molecular weight and osmotic properties to those of serum proteins. Although they

used only five newly born lambs, their investigation showed that lambs starved for 24 to 48 hours after birth were still able to absorb the three substances named at these times or even six hours later (i.e. at 30 or 54 hours of age). Lambs which were fed as much bovine colostrum as they could take during the first 20 hours after birth, failed to absorb PVP or egg proteins when 24 hours old. They suggested that the ability of lambs to absorb large molecules may not depend upon the age of the animal but on whether the animal was fed and also the amount of food (colostrum) it consumed. They also stated that colostrum was not only necessary as an antibody carrier but also to ensure rapid gut "closure", i.e. to ensure that it became non-permeable to particles of high molecular weight as part of its defence mechanism against invasion by micro-organisms like E. coli.

Halliday (1968a) investigated causes of death and also levels of serum gamma-globulin in 472 dead lambs, mainly of the Scottish Blackface breed. At autopsy, the majority of these lambs showed no colostrum or milk in the stomach or intestine. By using electrophoresis to fractionate serum proteins (heart blood), he detected no gamma-globulin in 84 per cent of the lambs dying on the day of birth, or in 43 per cent of all other lambs dying aged nine days or less.

Starvation, as indicated by the absence of visceral fat, was the major cause of death in lambs aged up to nine days (excluding those which died on the first day and did not walk). Lambs in this category had significantly lower levels of gamma-globulin than lambs dying of other causes.

Lambs dying of all causes before the age of six days had significantly lower gamma-globulin than surviving lambs of the same age. The gamma-globulin levels in dying lambs, as represented by percentage of total protein, was 15·6 per cent compared to 32·1 per cent in surviving lambs. The levels in dying and surviving lambs of older ages (seven to 34 days old) were not significantly different.

Ignatēva (1971) studied the chemical composition of ewe's colostrum and milk. He reported that during the first day after lambing, immunoglobulin formed more than 60 per cent of colostral total protein and this ratio decreased markedly by the third day of lactation. He stressed the importance of feeding colostrum to lambs, particularly during the first 36 to 48 hours of life, as that is the period when the permeability of the lamb gut for gamma-globulins is still preserved.

Shaw (1971) investigated the protective importance of colostrum in two groups of 27 newly born lambs against a very pathogenic strain of E. coli. The first group

were dosed orally with *E. coli* and then deprived of colostrum for 12 hours after birth. The second group were allowed to suck for the first two hours of their life and then dosed orally with the same amount and strain of *E. coli*. The treatments resulted in fewer deaths in the second group than the first. Fatal diarrhoea in the first two days of life occurred in 13 and nine lambs of the first and second group respectively.

Reid (1972), Findlay (1973) and Harker (1973, 1974), all stressed the importance of colostrum for the survival of the newly born lamb. During their investigations, they all used the zinc sulphate turbidity (ZST) test of McEwan, Fisher, Selman and Penhale (1970) to measure levels of immunoglobulins in the sera of dead and live lambs during the neonatal period. The dead lambs (mostly dying due to infection with *E. coli* or Pasteurella spp.) showed very low readings. Their work indicated that colostrum deprivation could be an important cause of neonatal lamb losses.

Bem and Popescu (1973) reported preventive and curative effects for whole ovine serum gamma-globulin given orally or intramuscularly to newborn lambs. The ovine serum gamma-globulin was precipitated from blood of healthy sheep using saturated ammonium sulphate. The total protein content of this bio-preparation was 11 g per 100 ml, of which 90 per cent was gamma-globulins.

As assessed by immunoelectrophoresis, all the major immunoglobulins, i.e. IgG_1, IgG_2, IgM and IgA were found in this preparation. In one of their experiments, for example, they found that in a group of lambs treated with this preparation at birth, the losses in the first nine days of life were about six per cent compared to 20 per cent losses in the untreated control group.

They also investigated in lambs the intestinal absorption of the same preparation and also the effects of colostrum deprivation on levels of lamb serum gamma-globulin (measured by electrophoresis). They used two groups of lambs which were both deprived of colostrum for six hours after birth. Lambs in the first (treated) group received 10 ml (each) of whole ovine serum gamma-globulin immediately after birth, while the control group received none. Both groups were allowed to suck their mothers normally, after six hours of deprivation. Levels of serum gamma-globulins in the two groups were as follows.

After Bem and Popescu (1973)

Group	Serum gamma-globulin values (%) at		
	Birth	6 hours	24 hours
Treated	0	15	37
Control	0	0	9

They concluded that the first six hours in the neonatal lamb's life is very critical for normal intestinal absorption of gamma-globulins. According to Ermekov, Ten, Sadykov and Nurmagambetov (1973), even a delay of about two to three hours in the colostrum intake of lambs can result in the deaths of some lambs, or in poor performance of the survivors. Among 100 lambs observed, they recorded five deaths in lambs which received their first colostrum about 2·5 hours after birth. The surviving lambs that experienced the same kind of delay had an average daily weight gain of 245 g compared to 255 g weight gain achieved by lambs that took colostrum within the first hour of life.

Knight and Leek (1973) studied the effects of three management systems on lamb serum protein levels. They used three groups of pure Dorset lambs. Group 1 consisted of 11 bottle-raised colostrum-deprived lambs (lambs in this group received milk by bottle immediately after birth). Group 2 consisted of nine lambs that were allowed to suck their mothers for 10 to 22 hours after birth then were raised on milk by bottle. Group 3 consisted of 20 lambs that were left with their mothers throughout the study. Using electrophoresis, these workers showed that of the different serum protein fractions studied (i.e. albumin, alpha-, beta- and gamma-globulin) only gamma-globulins were affected by the

treatments during the first seven weeks of the lamb's life. Gamma-globulin values for the second and third (colostrum-fed) groups did not differ significantly from each other, but were significantly higher than those of the first group. At two weeks of age, for example, the lambs in the first, second and third groups showed mean total gamma-globulin values of 0·33, 1·36 and 1·25 g per 100 ml respectively. During their investigations, the above mentioned authors did not refer to the effects of the treatments on lamb performance.

Campbell (1974) investigated the role of colostrum in preventing lamb losses from E. coli infections. He used five groups of 10 newly born lambs. The first group was allowed to suck their dams four times a day. The second group was allowed to suck their mothers and four hours later they were orally dosed with saline suspension of a very pathogenic E. coli, then allowed to suck their mother four times a day. The third group was dosed at birth with the same saline suspension of E. coli and, four hours later, they were allowed to suck their own mothers four times a day. The fourth group of lambs were taken from their mothers at birth and allowed to suck milk from foster ewes four times a day. The foster ewes were chosen from ewes that had lambed two weeks previously. In the fifth group, lambs were

taken from their mother before suckling and left to suck milk from foster ewes as in group 4. They were dosed with the same E. coli suspension at this time.
The effects of these treatments on lamb serum total protein and on lamb losses in the first week of life can be summarised as follows.

After Campbell (1974)

Treatment groups	Total serum protein at 48 hrs (g/100 ml)	Lamb losses in the first week of life (no.)
1	5·42	1
2	5·08	2
3	4·58	3
4	4·32	5
5	4·05	8

He concluded that colostrum feeding is very important in reducing levels of neonatal lamb losses caused by infection.

Larson, Ward, Frederiksen, Ardrey and Frank (1974) conducted an experiment to study the lamb's capability to absorb immunoglobulins from freeze dried bovine colostrum. They allocated 25 newly born lambs to three groups. Lambs in the first group (six lambs) were left to suck their mothers naturally. The second group (nine lambs) were fed about 0·7 litre of freeze dried bovine colostrum during the first 24 hours after

birth and then kept on liquid milk replacer. The third group (10 lambs) were reared from birth on liquid milk replacer and received no colostrum at all. Serum total gamma-globulin levels and serum antibody activity against a pathogenic strain of E. coli were affected by the treatments, as shown in the following table.

After Larson et al. (1974)

Group No.	Gamma-globulin concentration (g/100 ml) at:		Mean indirect haem-agglutination titre at:	
	24 hours	7 days	24 hours	7 days
1	2·74	1·51	353	136
2	1·21	0·65	178	54
3	0·03	0·05	Negative	Negative

Ducker and Fraser (1976) reported on the effect of the uptake of colostrum on blood gamma-globulin levels, mortality and growth rate of housed lambs. They used all lambs (singles and twins) born to 76 Grey-face ewes. The lambs were either allowed to suck their dams immediately, or were deprived of colostrum for six or 18 hours after birth. All the lambs received an intensive degree of care from the time of first suckling. Lamb serum gamma-globulin levels were estimated by the ZST test at different occasions until

six weeks of age. The three treatments showed no difference in the levels of serum gamma-globulin or lamb losses. However, lambs which were allowed to suck immediately after birth were heavier at six weeks of age than those in the other two treatments. Some of their findings can be briefly summarised as follows.

After Ducker and Fraser (1976)

	Duration of restriction from suckling (hours)	No. of lambs	Gamma-globulin levels at 72 hours of age (ZST units)	Live weight gain to 6 weeks of age (kg)	No. of lamb deaths
Singles	0	10	23.5	12.3	0
	6	10	29.7	10.8	1
	18	11	27.5	10.5	1
Twins	0	29	24.6	8.5	1
	6	29	26.5	7.8	1
	18	28	20.1	7.7	2

Of the six lambs which died, five showed low levels of gamma-globulin (less than 15 ZST units) and four died in the first week of life.

Halliday and Williams (1976) studied the importance of the number of colostrum feeds given to newly born lambs and its effect on their passive immune status. Two groups of 15 lambs of different breeds were used in the study. All lambs received pooled colostrum (at the rate of 30 g per kg body weight) with added anti-egg

albumin antibodies one hour after birth. One group received a second feed of colostrum without added antibodies six hours after the first feed. A third group of lambs received untreated colostrum only and were used as a control group. From 24 hours of age, all lambs were kept on milk substitute.

By measuring serum antibody concentration (using the haemagglutination test) and also the levels of immunoglobulin-G using the micro-Kjeldahl technique and electrophoresis, they found that more than one feed of colostrum is necessary to improve a lamb's immune status and that a single feed of colostrum is not sufficient. Their work indicated that the second colostrum feed improved antibody absorption from the first feed by ensuring the rapid arrival of sufficient amounts of colostrum in the jejunum.

Conclusion

The reports already considered concerning colostrum availability and its usefulness as an antibody and energy supplier for the newborn lamb are few. However, most of these reports suggested that colostrum is very necessary for lambs and, to make the best use of it, lambs need to ingest sufficient in the first few hours of their lives.

Information about the different factors that might

affect colostrum quantity and quality, and consequently the survival and subsequent performance on lambs is sparse.

No work has been reported on the effect of the level of feeding of ewes in late pregnancy, on levels of different classes of immunoglobulins in their sera and colostrum, and consequently in their lambs' sera.

A search of the literature also failed to reveal any information concerning the effects of delay in colostrum intake on the levels of the different classes of immunoglobulins in the sera of the lambs and on their survival and subsequent performance.

The present study, or part of it, was therefore conducted with the aim of clarifying some of these points.

C H A P T E R F O U R

MATERIALS AND METHODS

Five sections will be included in this chapter. The first one will describe the collection methods of all samples needed for the study. The second will deal with the preparation of pure ovine IgG_1, IgG_2, IgM and IgA immunoglobulins. In the third section, the raising of different antisera will be discussed. All other laboratory estimation methods will be referred to in the fourth section. In the fifth section a brief description of experimental sheep management will be presented.

COLLECTION OF SAMPLES

Ovine blood

Blood required for laboratory estimations was collected from the jugular vein of ewes or lambs using either disposable plastic syringes in conjunction with 20 G by 1·5 inch hypodermic needles or by vacutainer tubes (Becton Dickinson Ltd.) using similar gauge needles. Blood collected in this manner was treated in one of the following ways, depending on the type of estimation to be conducted:

Serum samples were obtained by allowing blood to clot, retract and then centrifuging at 3,000 rpm for 10 minutes. Plasma samples for total ketone estimation were obtained from heparinised blood samples (one mg heparin per one ml blood), which were centrifuged at

2,400 rpm for 20 minutes. Blood for packed cell volume (PCV) was collected in bottles containing EDTA (five mg potassium ethylene diamine tetra-acetic acid per 2·5 ml blood), for blood urea in bottles containing potassium oxalate (1·2 mg per one ml blood) and for blood glucose, in bottles containing a mixture of potassium oxalate and sodium fluoride in the proportions of three parts to one (two mg of the mixture per one ml blood).

When large amounts of ovine blood were needed for the isolation of serum IgG_1, IgG_2 and IgM immunoglobulins, five litres of blood were collected from the slaughter house in conical flasks containing glass beads. After defibrinization by immediate and vigorous shaking for 15 minutes, the serum was separated by centrifugation at 2,000 rpm for two hours, using 0·5 litre centrifuge bottles (M.S.E. Centrifuge, England).

All samples were stored at $+4^{\circ}C$ immediately after collection but when it was not possible to analyse sera or plasma for some parameters within two days of collection, they were stored at $-20^{\circ}C$.

Ovine colostral whey

Pre-suckling colostrum was collected from the ewe's udder as soon as possible after lambing (usually within one hour). The colostrum samples were then diluted with an equal volume of normal saline (0·85 per cent NaCl), loaded in eight ml cellulose nitrate tubes

(Beckman) and spun for two hours at 50,000 rpm at 4°C using an L2 65B ultracentrifuge (Beckman).
The resulting clear whey separated from the fat and casein was then withdrawn by a plastic syringe and needle. For each ewe, two ml of colostral whey was collected for measuring levels of total protein, gamma-globulins and specific immunoglobulins. The extra whey was pooled and used as a source of ovine IgG_1 and IgG_2 immunoglobulins. Colostral whey was stored at -20°C until all required estimations and preparations were performed.

Ovine lung fluid

Ovine lung fluid is a good source of IgA (Smith et al., 1975). This material was collected from some sheep that were suffering from pulmonary adenomatosis (Jaagsiekte). By lifting the hind part of affected sheep, lung fluid could be induced to run from the nostrils and this was collected in a suitable glass container. The fluid was then strained through gauze and centrifuged to remove particles. 260 ml of this fluid was collected and stored at -20°C and used for the isolation of IgA.

PREPARATION OF THE DIFFERENT CLASSES OF OVINE IMMUNOGLOBULINS

This involved:

Precipitation of whole gamma-globulin from sera, colostral whey or lung fluid.

Preparation of columns used for ion-exchange and gel-filtration chromatography.

Isolation of pure IgG_1, IgG_2, IgM and IgA immunoglobulins.

Precipitation of whole gamma-globulin from sera, colostral whey and lung fluid

This was performed by salt precipitation or by dialysing against running tap-water.

Salt precipitation

Gamma-globulin fractions were precipitated from two litres of ovine serum, 150 ml of ovine colostral whey and 260 ml of lung fluid, using 41 per cent saturated ammonium sulphate (SAS) at the rate of one volume SAS to two volumes serum, whey or lung fluid. The addition of SAS was gradual and involved continuous stirring. The mixture was left to stand for two to three hours, centrifuged at 3,000 rpm for 15 minutes and the supernatant discarded. The precipitate was washed twice with 41 per cent SAS and then dissolved in a minimum amount of 0·01 M phosphate

buffer (PO_4), pH 7·6, in the case of serum and whey gamma-globulins. This was used later as a starting material to prepare pure IgG_1 and IgG_2 immunoglobulins. Precipitate from the lung fluid, on the other hand, was dissolved in distilled water and used for preparation of IgA immunoglobulin. All of these preparations were dialysed for 48 hours in 24/32 visking tubings, against 0·01 M phosphate buffer, pH 7·6.

Precipitation by dialysis against running tap-water

About two litres of ovine serum were loaded in bags made of two inch visking tubings and dialysed against running tap-water. Dialysis which lasted for 72 hours resulted in the precipitation of a reasonable amount of gamma-globulin in the bottom of the bags. After centrifugation of the serum at 2,000 rpm for 15 minutes at 4°C (M.S.E. MISTRAL-4L Centrifuge, England), the supernatant was discarded and the precipitate was then collected and dissolved in 48 ml of 0·1 M Tris-NaCl buffer, pH 8·0. This preparation was then stored at -20°C, in four ml aliquots and was later used as starting material for isolating IgM immunoglobulin by gel-filtration.

Preparation of chromatography columns

During my work, both ion-exchange and gel-filtration chromatography has been employed. The preparation of chromatography columns can be briefly described as follows.

Ion-exchange chromatography

This was conducted on a pre-swollen diethyl-aminoethyl (DEAE) cellulose (Whatman, DE52), using a 40 by 2·5 cm column (Pharmacia, London). The DE52 cellulose can be used without the initial precycling treatment. As suggested by the manufacturer and according to the dimensions of the ion-exchanger bed, the required amount of DE52 cellulose was suspended in the required amount of the acid component (KH_2PO_4) of the 0·5 M phosphate buffer, pH 7·6. The slurry was then degassed using vacuum pressure, along with continuous stirring using a magnetic stirrer. The slurry was filtered in a Buchner funnel using a 15 cm filter paper (Whatman 54) under reduced pressure provided by a Venturi water pump. The filtrate was discarded and the ion-exchanger suspended in the basic component (Na_2HPO_4) of the same buffer. The ion-exchanger was then washed once with a 0·1 M phosphate buffer, pH 7·6. Equilibration of the ion-exchanger was achieved by repeated

buffer washing (using the starting buffer which was always a 0·01 M phosphate buffer, pH 7·6) until the filtrate had exactly the same pH and conductivity as that of the starting buffer. Fines were then removed by leaving the slurry to stand for about 30 minutes. After this, some of the supernatant buffer containing fines was removed to leave a final volume of 'wet settled volume' plus 20 per cent. A fresh starting buffer was then added to make the final volume of the slurry 150 per cent of the 'wet settled volume' of the ion-exchanger. This final preparation was then packed into columns.

For re-use, the DE52 column was dismantled and the ion-exchanger was washed with a strong salt solution (0·5 M NaCl), and then with a 0·1 M phosphate buffer, pH 7·6. Equilibration, removal of fines and packing of the column were performed as described before.

Gel-filtration chromatography

This involved the use of either Bio-gel A 1·5 m (BIO-RAD Laboratories Ltd., England) or Sephadex G 200 (Pharmacia Ltd., London). Both of these preparations were built in a 100 by 25 cm column (Pharmacia Ltd., London) with a resulting gel bed

length of about 80 to 90 cm.

For the Bio-gel column, a 500 ml settled bed volume of the gel slurry was poured into the column. After packing, the gel bed was then washed with about two litres of 0·1 M Tris-NaCl buffer, pH 8·0, as recommended by Smith et al. (1975) for the isolation of ovine IgM immunoglobulin. The buffer and the samples were applied in an ascending flow, at a rate of 25 ml per hour.

To prepare a Sephadex column, the required amount of sephadex G 200 (20 g, is recommended by the manufacterers, Pharmacia Ltd., London) was left to swell in about 750 ml of 0·1 M Tris-HCl 1 M NaCl buffer, pH 8·0 (Smith et al., 1975). The gel slurry was then degassed and poured into the column. The gel bed was left to settle and proper packing was achieved by gently running an ample amount of buffer (about two litres) through the column in a descending way, at a rate of 16 ml per hour. Buffer then was run in an ascending flow for about four hours.

The packed sephadex G 200 column was checked for homogeneity of the bed by running two ml of 0·2 per cent blue dextran 2000 (Pharmacia Ltd., London). After proper packing, this column was used for further purification of ovine IgA.

As an anti-microbial agent, 0·02 per cent sodium azide (NaN_3) was always added to all buffers used in Bio-gel and Sephadex chromatography. Before re-using a Bio-gel or a Sephadex G 200 column, the bed was eluted with at least two litres of the buffer in use for each of the columns. An upward running of buffer and samples through the DE52, Bio-gel and Sephadex G 200 columns was always accomplished by the use of a P-3 peristaltic pump (Pharmacia Ltd., London).

Both the ion-exchange and the gel-filtration chromatography were conducted in an LKB unit at 4°C. The unit consisted of an Ultra Rac fraction collector, a Uvicord II ultraviolet absorptiometer at 280 nm wavelength, and a chopper bar recorder. Concentration of all eluates was always performed by dialysis in 18/32 visking tubings, against 40 per cent polyethylene glycol 6000 (BDH Chemicals Ltd., England).

Isolation of pure ovine IgG_1, IgG_2, IgM and IgA immunoglobulins

IgG_1 and IgG_2

See Figs. 4·1 and 4·2. Pure IgG_1 and IgG_2 immunoglobulins were isolated using stepwise elution from the material previously obtained by

FIG. 4·1: IgG_1 and IgG_2 chromatogram.

Fractionation of ovine serum or colostral whey gamma-globulins on DE52 column; flow rate, 45 ml per hour.

Buffers:

B1, 0·01 M phosphate buffer, pH 7·6;

B2, 0·035 M phosphate buffer, pH 7·6.

FIG. 4·2: Immunoelectrophoresis of IgG_1 and IgG_2 preparations.

Wells:

Upper well, pure IgG_1;

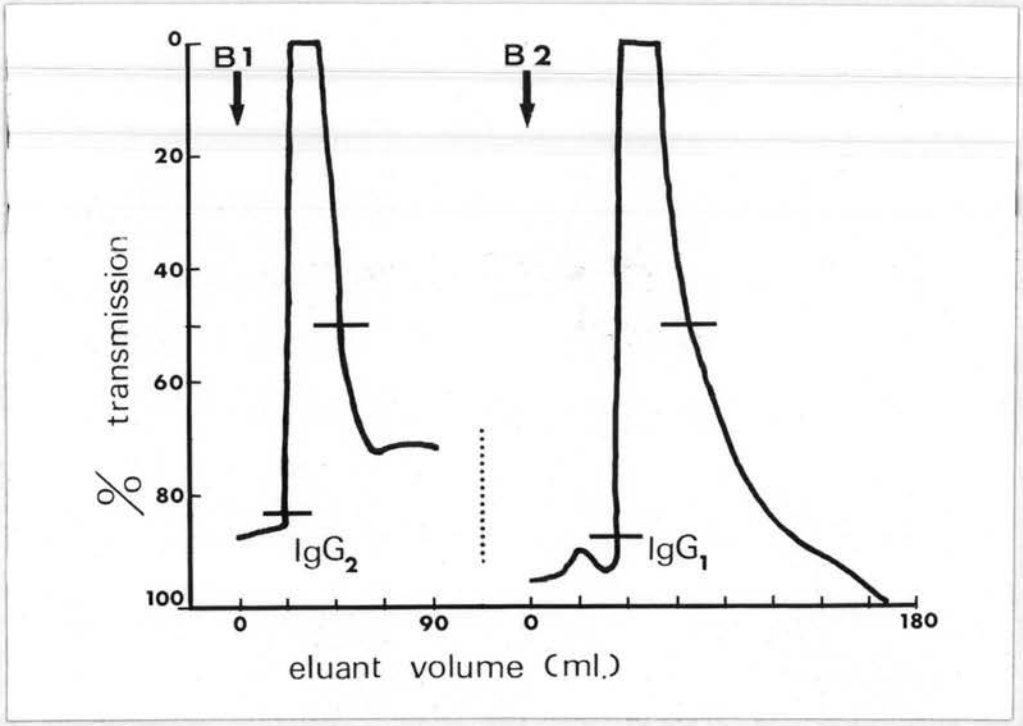
second upper well, contaminated IgG_1;

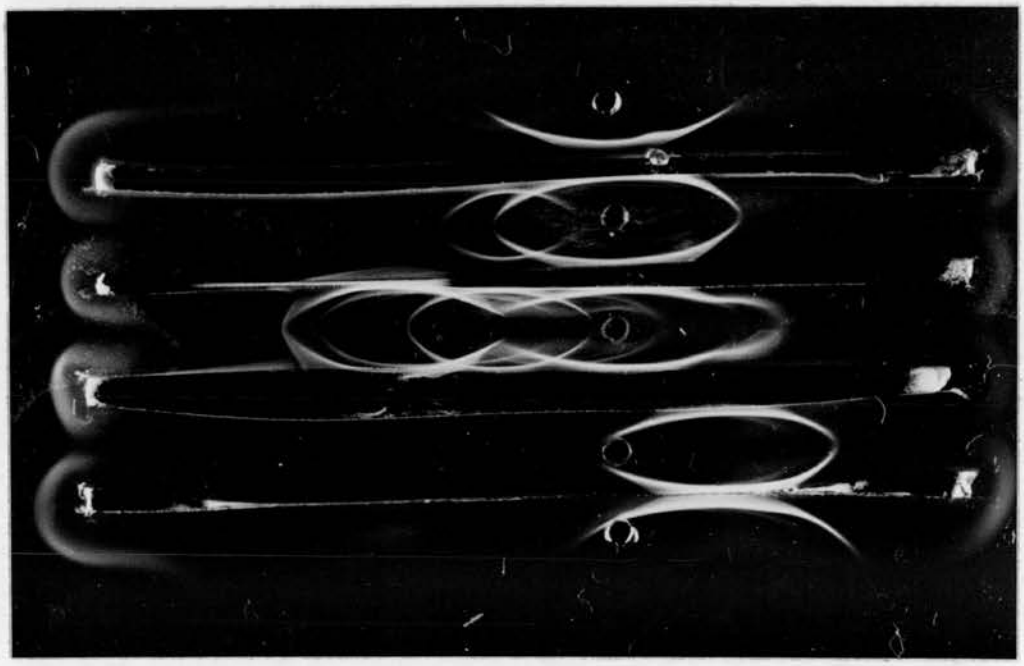
central well, sheep serum;

two lower wells, pure IgG_2.

Troughs:

Rabbit anti-sheep sera.


B1
B2
0
20
40
60
80
100
% transmission
IgG2
IgG1
0
90
0
180
eluant volume (ml.)



salt precipitation from ovine serum and colostral whey. The method used is basically similar to that described by Brandon, Watson and Lascelles (1971) or Ciupercescu (personal communication and 1977) and adopted with some modifications, which included the use of DE52 ion-exchanger and 0·035 M phosphate buffer, pH 7·6.

About 80 ml of serum gamma-globulin and 40 ml of colostral whey gamma-globulin preparations, which were already dialysed against the starting buffer, were applied in five ml aliquots to DE52 columns (with bed dimensions of 30 by 2·5 cm, flow rate 45 ml per hour). This was followed by the starting buffer (0·01 M phosphate buffer, pH 7·6) which resulted in eluting IgG_2 in the first peak. IgG_1 sub-class was separated by applying a second stronger buffer (0·035 M phosphate buffer, pH 7·6) (Fig. 4·1). Eluates from the first three-quarters of each peak representing IgG_1 or IgG_2 sub-classes were collected. The two sub-classes were pooled separately and concentrated by dialysis against polyethylene glycol 6000.

The two preparations, which appeared on immuno-electrophoresis to be slightly cross-contaminated, were used mostly for preparing anti-sheep IgG_1 and

anti-sheep IgG_2 antisera.

For preparing standard IgG_1 and IgG_2 for estimation purposes, these slightly contaminated preparations were further purified (Fig. 4·2) by re-running them once and some times twice more through newly prepared and equilibrated DE52 columns.

IgM

See Figs. 4·3 and 4·4. This was isolated from ovine serum gamma-globulin that had been precipitated by dialysis against running tap-water and then dissolved in 48 ml of 0·1 M Tris-NaCl buffer, pH 8·0.

Four ml aliquots of this preparation were then filtrated through a column of Bio-gel, using the above mentioned buffer, at a flow rate of 25 ml per hour. Two protein peaks were produced (Fig. 4·3). Only the ascending part of all first peaks was collected, pooled and concentrated to about 15 ml. On immunoelectrophoresis, this protein fraction gave one precipitation arc (Fig. 4·4) against rabbit anti-sheep sera and also against rabbit anti-bovine IgM*, and the two lines were identical. This preparation was used as standard IgM for the Single Radial Immunodiffusion

*Anti-bovine IgM was kindly supplied by Dr. A. G. Luckins, C.T.V.M., Edinburgh University.

FIG. 4·3: IgM chromatogram.

Filtration of ovine serum IgM through a Bio-gel A 1·5 m column.

Buffer:

0·1 M Tris-NaCl; pH 8·0;

flow rate, 25 ml per hour.

FIG. 4·4: Immunoelectrophoresis of IgM preparation.

Wells:

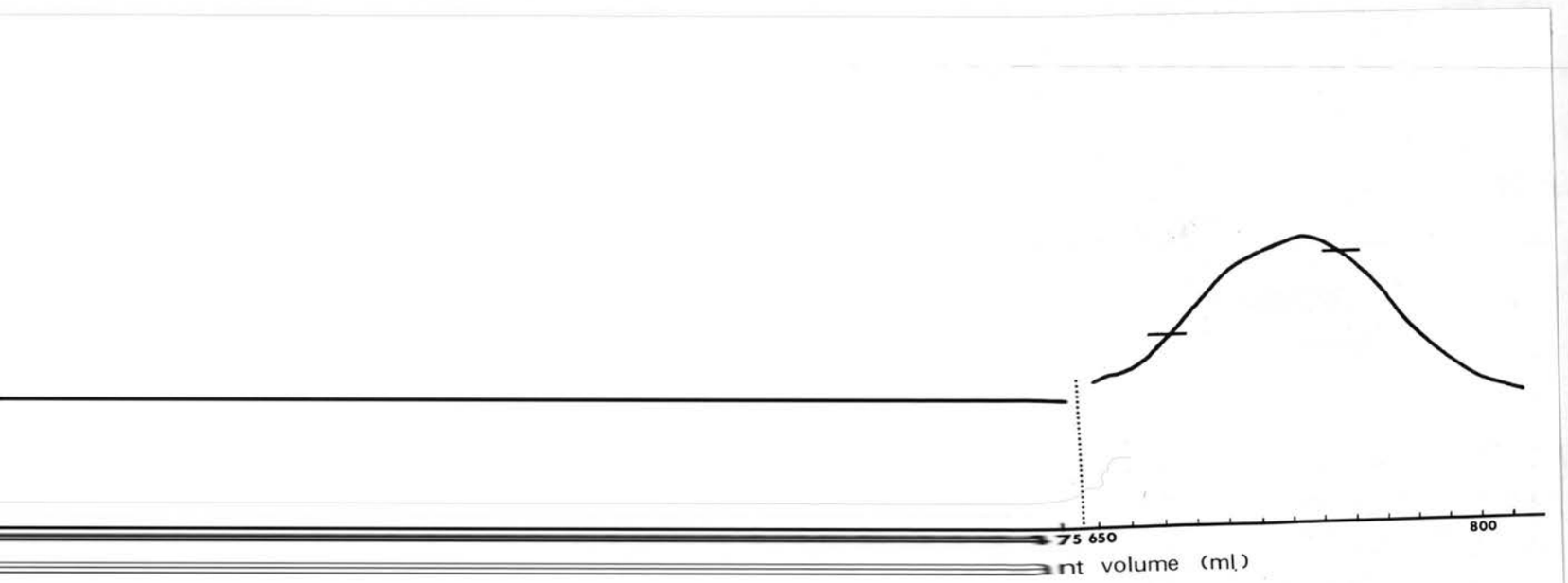
Upper well, sheep serum;

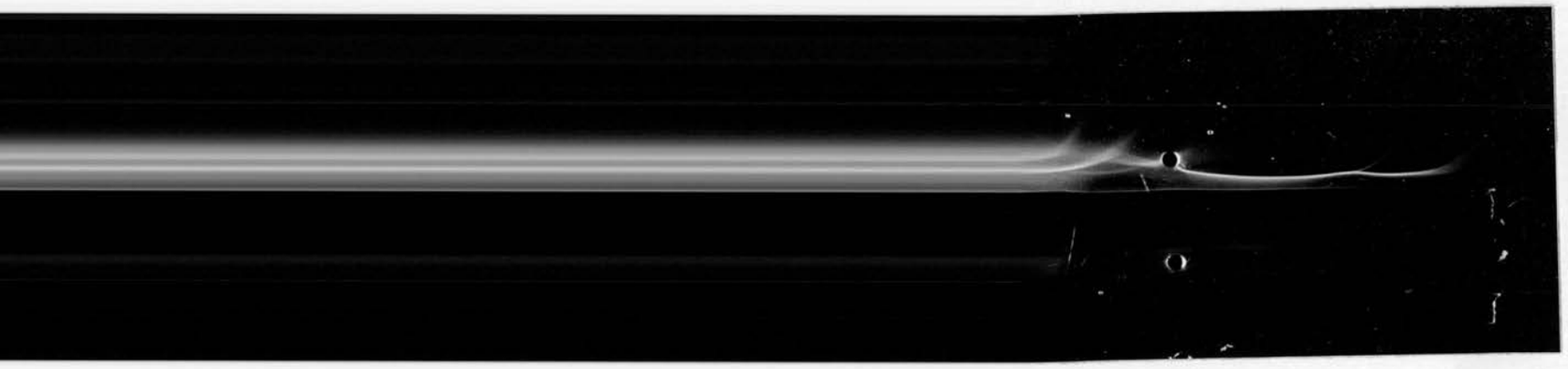
lower well, ovine serum IgM.

Troughs:

Upper trough, rabbit anti-sheep serum;

lower trough, rabbit anti-bovine IgM.


650
800
nt volume (ml)



(SRID) technique and also for raising anti-sheep IgM sera in rabbits.

IgA

See Figs. 4·5, 4·6 and 4·7. As described by Smith et al. (1975) but with some modifications, a pure IgA was prepared from sheep lung fluid gamma-globulin that had been precipitated by SAS. The isolation involved a stepwise elution method on a DE52 column, followed by sephadex gel-filtration. The salt precipitated gamma-globulin was dissolved in about 45 ml distilled water and dialysed against a starting buffer (0·01 M phosphate buffer, pH 7·6) for three days. This was the starting material to prepare pure IgA.

Five ml aliquots of this protein preparation were then applied to DE52 columns (bed size, 30 by 2·5 cm, and flow rate of 25 ml per hour) using a step-wise elution method. Five buffers, all at pH 7·6, were used. They were applied in the following order -

1. 0·01 M PO_4 buffer
2. 0·01 M PO_4 buffer, 0·05 NaCl
3. 0·01 M PO_4 buffer, 0·07 NaCl
4. 0·01 M PO_4 buffer, 0·1 NaCl
5. 0·01 M PO_4 buffer, 0·2 NaCl.

FIG. 4·5: IgA chromatogram.
Fractionation of lung fluid gamma-globulins on DE52; flow rate, 25 ml per hour.

Buffers:

B1, 0·01 M phosphate buffer;
B2, B3, B4 and B5 are 0·05, 0·07, 0·1 and 0·2 M NaCl respectively, all made up in 0·01 M phosphate buffer.
pH for the five buffers is 7·6.

FIG. 4·6: IgA chromatogram.
Purification of concentrated IgA rich fraction through Sephadex G200 column.

Buffer:

0·1 M Tris-NaCl; pH 8·0;
flow rate, 16 ml per hour.

Asc = Ascending part of the peak;

Des = Descending part of the peak.

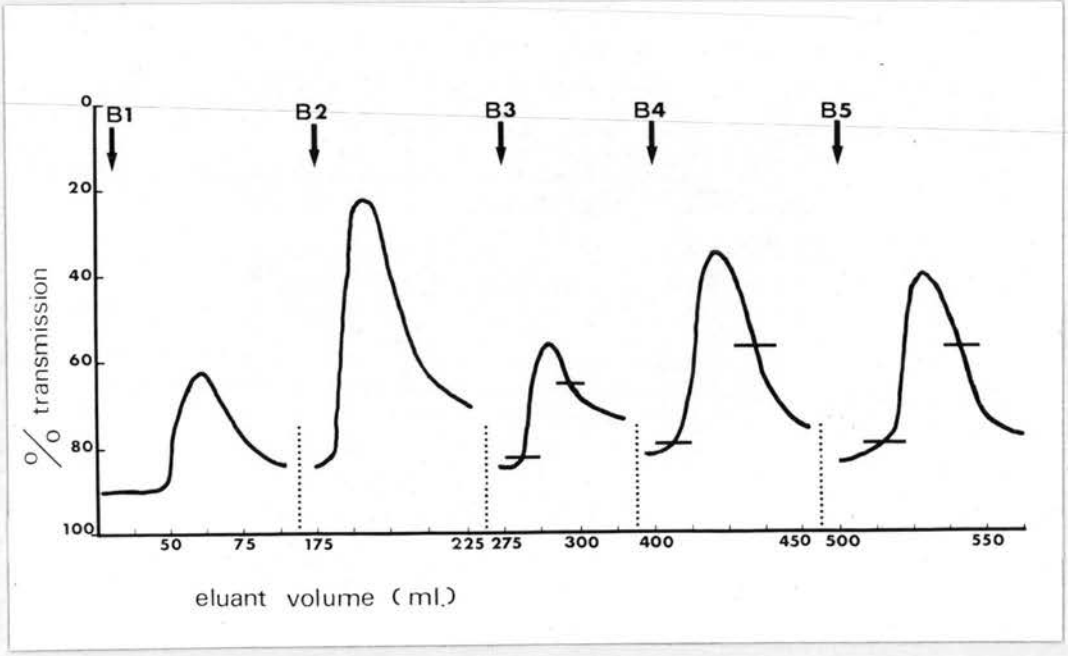
FIG. 4·7: Immunoelectrophoresis of IgA preparation.

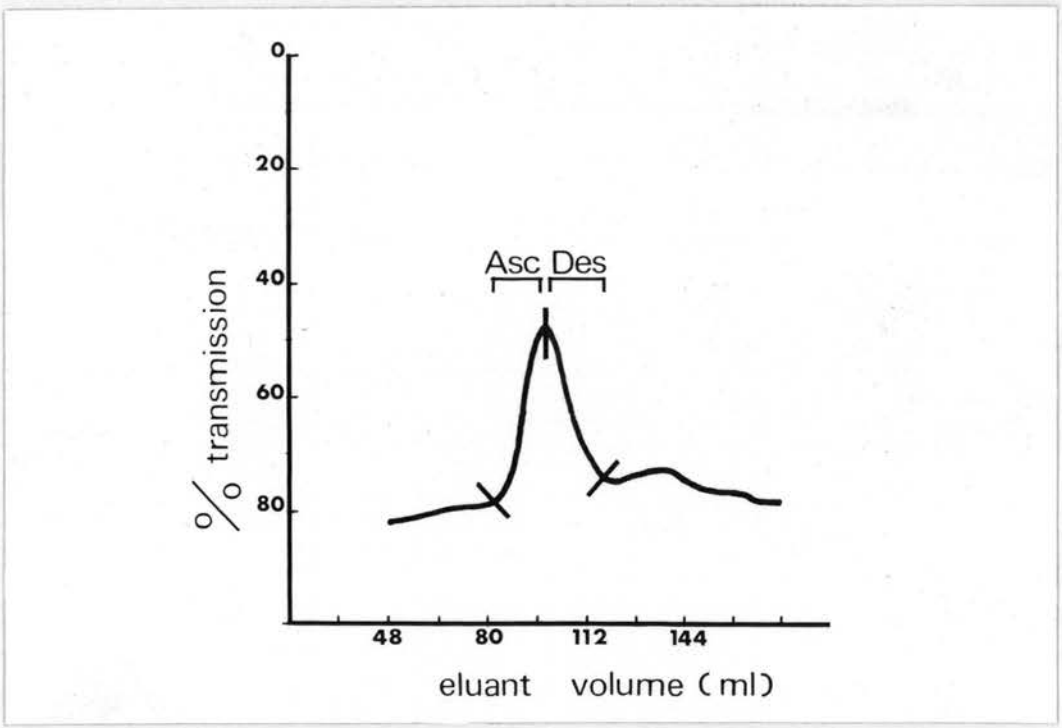
Wells:

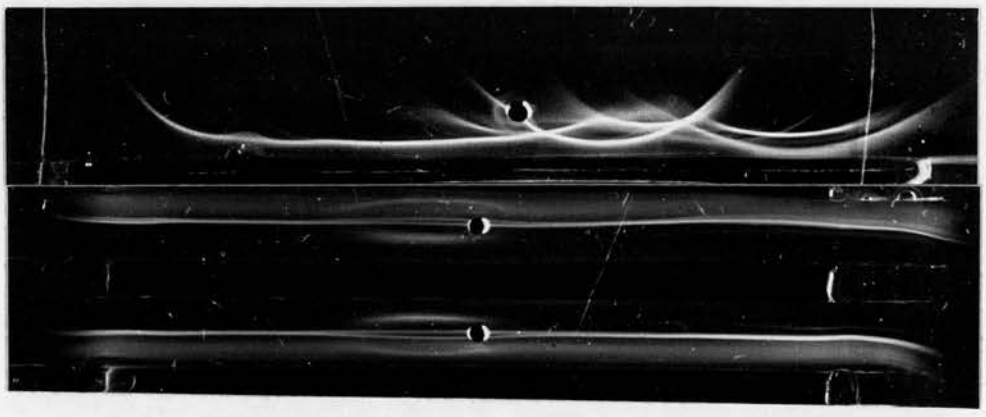
Upper well, sheep serum;
centre well, concentrated IgA from ascending part of the peak shown in Fig. 4·6;
lower well, concentrated IgA from the descending part of the same peak.

Troughs:

Upper and lower troughs, rabbit anti-sheep serum;
middle trough, pig anti-sheep IgA.


B1
B2
B3
B4
B5
0
20
40
60
80
100
% transmission
50
75
175
225
275
300
400
450
500
550
eluant volume (ml)


0
20
40
60
80
% transmission
Asc
Des
48
80
112
144
eluant volume (ml)



Buffer changes were performed immediately after achieving a major peak from the preceding buffer. After discarding the fractions representing the "fall through" and the second peaks, those of the last three peaks (Fig. 4·5), from the different runs of lung fluid gamma-globulins, were pooled together and concentrated to about 10 ml. Most of this IgA rich fraction was used to raise anti-sheep IgA in rabbits. Three ml of it was filtered through a sephadex G 200 column using a 0·1 M Tris-HCl, 1 M NaCl buffer, pH 8·0, at a flow rate of 16 ml per hour. Only one peak was achieved (Fig. 4·6) and eluates representing the ascending and descending parts of it were collected and concentrated separately. On immunoelectrophoresis, both collections showed similar single precipitation arcs with pig anti-IgA* identical to the arc produced by anti-sheep serum (Fig. 4·7). This preparation was used as IgA standard during the measurement of specific immunoglobulin concentrations by the SRID technique.

Protein estimation of different immunoglobulin standards

The purified preparations of IgG_1, IgG_2, IgM and IgA used as standards for the SRID technique

*Obtained from Dr. W. D. Smith, Moredun Institute, Edinburgh.

were analysed for their total protein content by the micro-Kjeldahl method. The protein contents, as measured by this method, were 2630, 500, 460 and 650 mg per 100 ml for IgG_1, IgG_2, IgM and IgA pure immunoglobulins respectively. Standard curves for each immunoglobulin were plotted on semilogarithmic graph papers. Pooled ovine whey and pooled serum were used as standards for SRID technique after calibrating them against these pure immunoglobulin preparations. The whey was calibrated against IgG_1 and the serum against IgG_2, IgM and IgA. Both were divided into aliquots of 0·3 ml each and stored at -20°C. Only one pool of each was used through the whole work.

Anti-sera production

With the exception of IgG_2, all other mono-specific antisera and also the anti-sheep sera were raised in mature New Zealand white or California rabbits. Anti-IgG_2 was raised in a goat. All antigens (sheep sera, or different ovine pure immunoglobulins) were firstly emulsified with an equal volume of Freund's Complete Adjuvant (FCA, Difco, England) using an electrical shaker (Griffin and George Ltd., Great Britain).

To prepare anti-IgG_2 serum, the goat received five intramuscular (i.m.) injections of one ml IgG_2 preparation (five mg per ml) at two-weekly intervals.

To prepare anti-whole sheep serum, anti-IgG_1 serum and anti-IgM serum, rabbits were given five i.m. injections at two-weekly intervals of either 0·5 ml whole sheep serum, 0·5 ml IgG_1 (five mg per ml) or 0·5 ml IgM (one mg per ml).

To prepare anti-IgA serum, rabbits were given five i.m. injections at two-weekly intervals of 0·5 ml of the IgA rich fraction derived from DE52 chromatography.

From the goat and from all rabbits that showed high antisera titres as checked by agar double diffusion, blood was collected 10 days after the fifth injection. The amount of blood collected was 100 ml from the jugular vein of the goat using 50 ml vacutainer and 20 G vacutainer needle, and 25 to 35 ml (depending on the size of the rabbit) from the rabbit ear vein by inflicting a small cut with the pointed end of a scalpel blade and leaving the blood to drip into test tubes. Blood was left overnight at $4^{o}C$, then the clot was removed and the samples were centrifuged at 2,000 rpm for 10 minutes. The resulting clear serum was then harvested and stored at $-20^{o}C$. Boosting injections were given six weeks after each serum collection, and this continued until the necessary amount of each antiserum required to cover all estimations was obtained.

Anti-sera purification

See Fig. 4·8. The anti-sheep serum was used in immunoelectrophoresis. The anti-sera to different immunoglobulins (Fig. 4·8) were rendered mono-specific as follows.

Anti-IgG_1 (Fig. 4·8c) was absorbed with pre-suckling lamb serum and ovine pure IgG_2. Goat anti-IgG_2 showed very slight anti-IgG_1 reaction, i.e. it was not completely pure, as previously claimed by Ciupercescu (1976), and was absorbed with ovine IgG_1 only until pure (Fig. 4·8d).

To make them mono-specific, anti-IgM and anti-IgA were absorbed with sheep IgG_1 and IgG_2 and also pre-suckling lamb serum. On immunoelectrophoresis, each of the anti-IgM and anti-IgA sera showed a main single precipitin arc corresponding to IgM and IgA immunoglobulins when reacted with sheep serum. However, even after repeated absorptions, a very faint precipitin line was visible (Fig. 4·8a, b). This was positioned at the cathode side of the main arc, and probably represents an $alpha_2$ macro-globulin activity. This faint line does not interfere with the estimation of immunoglobulins on SRID plates.

All antiserum absorption was carried out in a water bath (37°C) and the reaction was continued

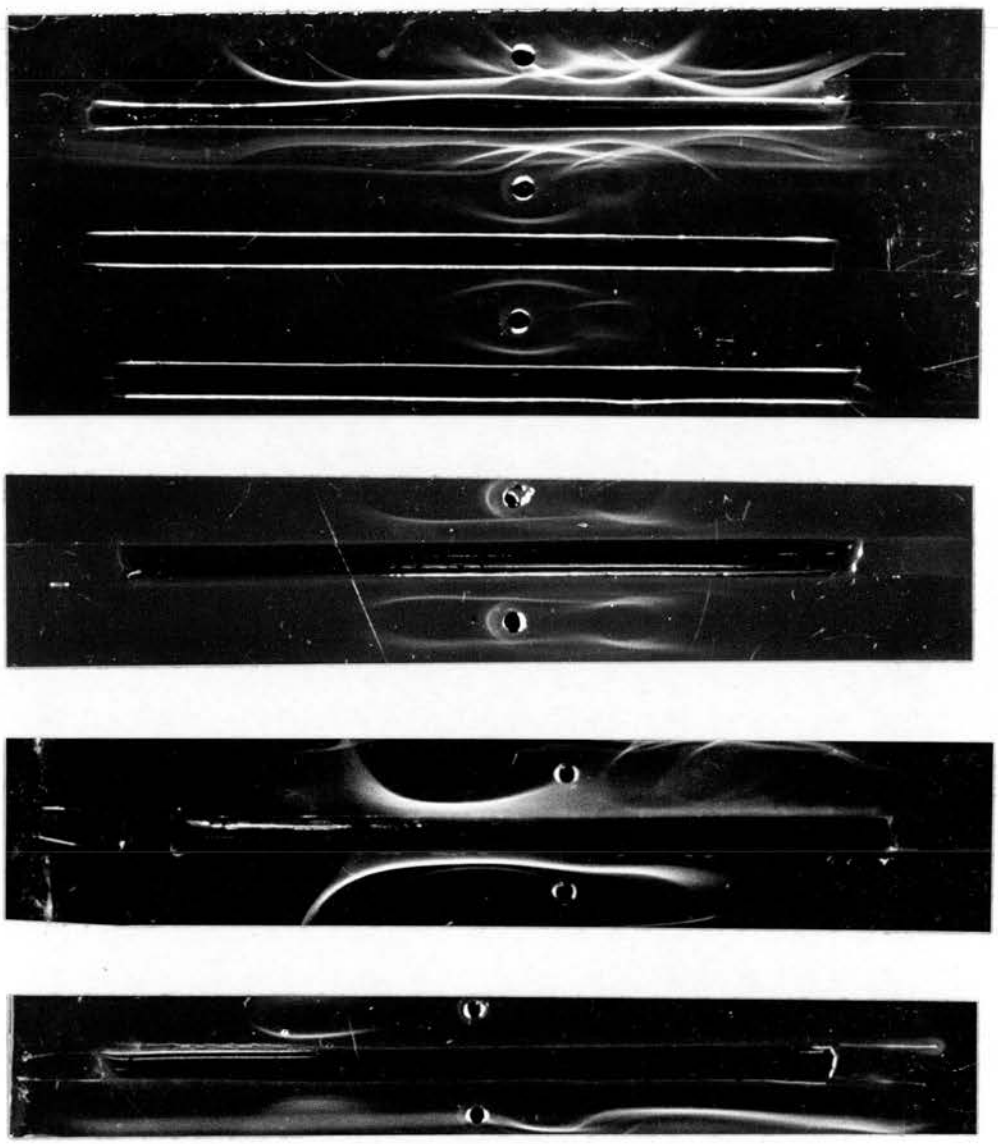
FIG. 4·8: Absorbed anti-sheep immunoglobulins.
All wells = sheep serum.
Troughs:

a) Upper trough, rabbit anti-sheep serum;
lower two troughs, anti-sheep IgA.

b) Rabbit anti-sheep IgM.

c) Rabbit anti-sheep IgG_1.

d) Goat anti-sheep IgG_2.



for three hours. The antiserum was then centrifuged (3,000 rpm for 10 minutes) and the supernatant was collected and checked by immunoelectrophoresis and agar gel-diffusion for specificity. After completing absorption, the different antisera were pooled separately and stored in two or four ml aliquots at -20°C.

METHODS USED FOR IDENTIFICATION OF PURE OVINE IMMUNOGLOBULINS AND THEIR SPECIFIC ANTI-SERA

Immunoelectrophoresis (IEP)

This was carried out according to the micro-method of Scheideggar (1955). The analysis was performed on 10 by 10 cm glass plates using one per cent ion agar No. 2 (Oxoid) with 0·1 M barbitone acetate buffer (Oxoid) at pH 8·6.

Agar gel-diffusion

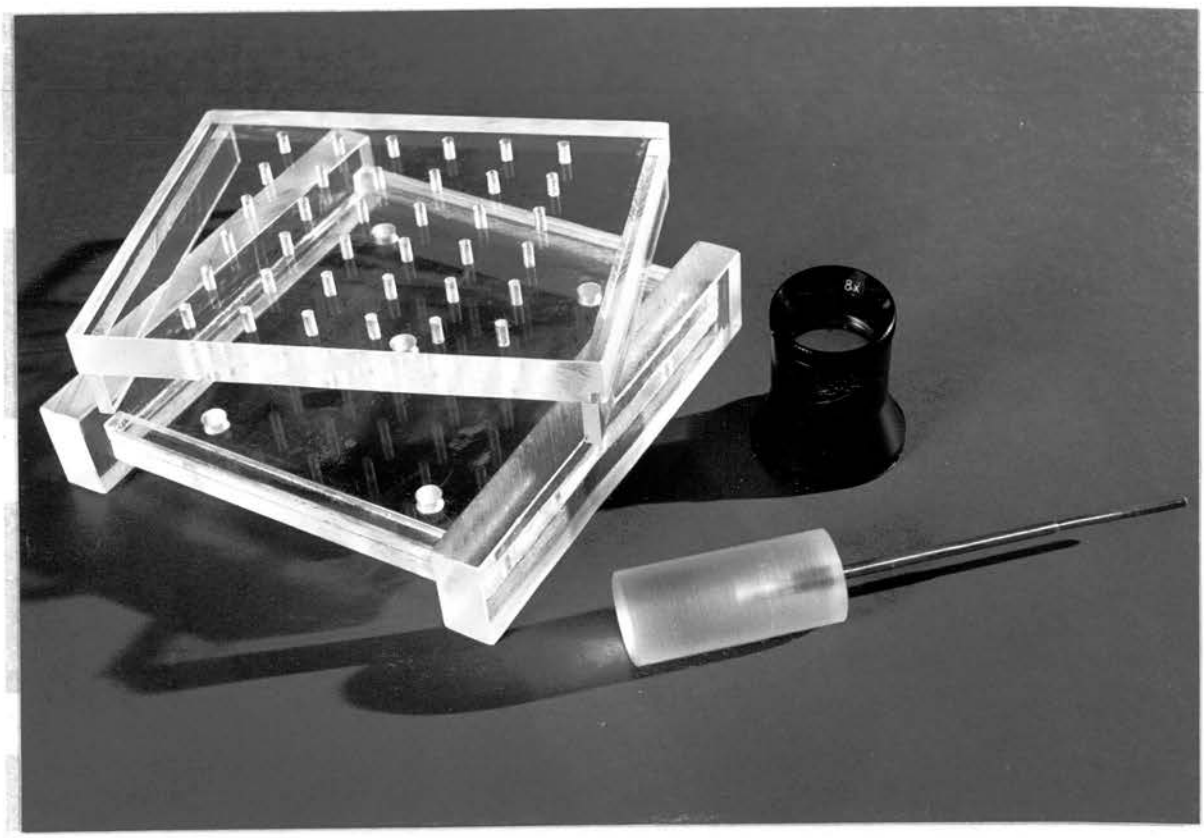
A double diffusion plate technique was used according to Ouchterlony (1953). The test was performed in 5 by 1·8 cm petri dishes (Sterlin Ltd.) using one per cent ion agar No. 2 (Oxoid) in 0·85 per cent phosphate buffer saline (PBS).

Quantitation of IgG_1, IgG_2, IgM and IgA concentrations

See Figs. 4·9a and 4·9b. The single radial immunodiffusion (SRID) technique of Mancini et al. (1965),

FIG. 4·9a: SRID unit consisting of plastic template, tubular well-cutter and calibrated viewer.

FIG. 4·9b: SRID plate (IgG_1).
Rabbit anti-sheep IgG_1 was diluted 1:20.
Standard wells: three top left.
Test samples which gave a larger or smaller ring than the neat or the most diluted standard respectively, were checked again at an appropriate dilution.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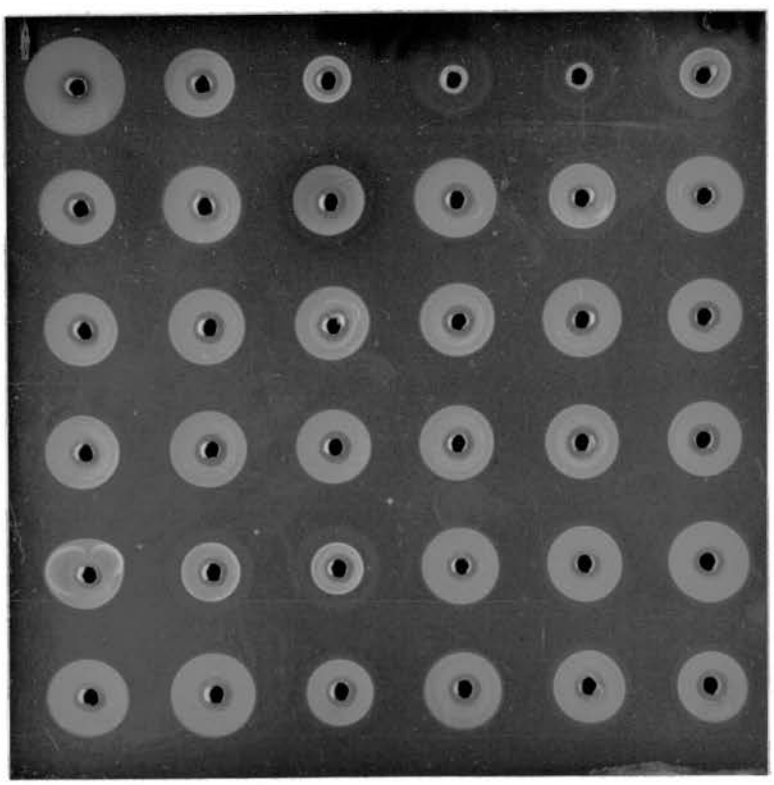


as modified by Fahey and McKelvey (1965), was employed to measure levels of the different classes of immunoglobulins in ovine sera and colostral whey.

Six dilutions of the standard pure preparations of IgG_1, IgG_2, IgM and IgA were used to calibrate pooled whey and pooled serum which were used as standards.

A three per cent ion agar No. 2 (Oxoid) in phosphate buffered saline (PBS) was melted and mixed with an equal volume of specific and optimally diluted antisera to give the agar a final dilution of 1·5 per cent. As checked by clarity and strength of precipitin rings in SRID plates, it was shown that antisera optimum dilution, using PBS, was one in 20 for anti-IgG_1 and anti-IgM, and one in 10 for anti-IgG_2 and anti-IgA. The antisera/agar mixture was then poured into 10 by 10 cm glass plates and left to solidify for two to three hours at $4^{\circ}C$ before the 36 antigen wells, of 2·7 mm diameter and 12 mm apart, were cut using a plastic template and tubular cutter (Fig. 4·9a). Capillary tubes were used to apply samples to wells. The standards, with known immunoglobulin contents, were always applied to the first three wells in the top left side of each plate, as neat fluid and one in eight and one in 32 dilutions. Thirty-three test samples were included in the remaining 33 wells. All test samples

were applied as neat sera but those which gave a larger ring than the neat standard were checked again at an appropriate dilution with PBS. For example, all the colostral whey samples were diluted one in 16 for IgG_1 estimation because of the high concentration of this particular immunoglobulin. The plates were then kept at $4^{\circ}C$ in a humid chamber and left to diffuse for 24 hours in the case of IgG_1, IgG_2 and IgA, and 48 hours in the case of IgM measurement (Wilson et al., 1972; Smith, 1975). The diameters of the concentric precipitin rings formed around the test well (Fig. 4·9b) were measured directly by a calibrated and magnified viewer (Matchless Machines Limited, England).

A graph was made for each plate and the immunoglobulin concentration of the test sample was calculated by comparing the ring diameter to the log of the immunoglobulin concentration of the standard.

OTHER LABORATORY ESTIMATION METHODS

Packed cell volume (PCV) percentage

This was estimated using a microhaematocrit centrifuge and reader (Gelman-Hawksley Limited, England). Capillary tubes were about three-quarters filled with blood, sealed and then centrifuged for five minutes at a rate of 12,000 rpm before the PCV percentage was read on the supplied special reader.

Blood glucose

Blood glucose was measured by a commercially produced kit (Boehringer, GOD-Perid-method). The method involves a glucose-oxidation reaction and the use of an ABTS oxygen accepter (2,2̀-azino-di(3)ethyl-benzothiazoline(6) sulphonic acid).

Total protein and gamma-globulin in serum

These were estimated in the sera of ewes and lambs by the Biuret reaction method as described by Henry (1964).

Serum albumin

Ewe serum albumin was estimated by a bromocresol green (BCG) dye binding technique as described by Doumas, Watson and Biggs (1971).

Blood urea

This was determined by the Urease Nesslerization technique based on Archer and Robb (1925). In this modified technique, a zinc sulphate/sodium hydroxide protein precipitant was used instead of the sodium tungstate/sulphuric acid precipitant, originally used.

Serum alkaline phosphatase

An "Optimised" three minutes colorimetric method (Boehringer kit) was employed to measure levels of alkaline phosphatase. In this method a 10 mM sodium p-nitrophenylphosphate substrate in 1 M diethanolamine,

0·5 mM $MgCl_2$ buffer, pH 9·8 was used. The reaction was carried out at 25°C.

Ketones and 3-hydroxybutyrate concentration

Blood plasma ketone levels were determined by the distillation method of Reid (1960). The 3-hydroxybutyrate concentration in plasma was measured by an automated colorimetric method of Zivin and Snarr (1973).

It must be pointed out that to carry out all estimations myself, was impossible. This was because

(1) I was very much engaged with the animal handling which involved ewe feeding, lambing and sampling, and

(2) some of the samples, like those for blood glucose and blood urea analysis, can not be stored at 4°C for longer than three days without undergoing some changes that will definitely affect the analysis results.

Consequently, the majority of the tests, other than those associated with immunoglobulin studies were undertaken by the technical staff of the Department of Veterinary Medicine, Royal (Dick) School of Veterinary Studies, Edinburgh, either on my behalf or with me.

All plasma ketones and serum 3-hydroxybutyrate estimations were undertaken by the Animal Nutrition Department of The Edinburgh School of Agriculture.

My work was part of a joint project conducted with Dr. W. J. M. Black of the Edinburgh School of Agriculture, whose prime interest was ewe nutrition during late pregnancy and these estimations were undertaken mainly for his benefit but will be used here to support my results when they are directly related to ewe nutrition.

All other estimations that appear in this thesis were carried out solely by me.

MANAGEMENT OF EXPERIMENTAL SHEEP

The chapters which follow will present all the work carried out during 1974 to 1976, to investigate the problem of PLM. Each of these chapters will describe in detail the work that has been carried out in each year. However, some criteria remained constant during the whole three years of study and it is convenient to devote the present section to a brief description of these common criteria.

Breed of ewes and rams

Three different breeds of ewes were used:

Scottish Halfbred ewes (Border Leicester cross Cheviot).

Greyface ewes (Border Leicester cross Blackface).

Finn Dorset ewes (Finnish Landrace cross Dorset Horn).

In all cases, these ewes were mated to a Suffolk tup (one tup was left with each 10 ewes during the mating time).

Standard management

All the sheep concerned in my work on PLM were kept at Woodhouselee farm (The Edinburgh School of Agriculture). The farm is a sheep orientated one, situated about seven miles south of Edinburgh, in an area which is about 700 feet above sea level. Only a small proportion of ewes were kept inside and these were ewes included in late pregnancy feeding studies.

On this farm it was a constant practice to synchronize ewes before mating. Synchronization was always achieved by the use of progesterone impregnated sponges (Syncro-Mate pessaries, G. D. Searle and Co. Ltd., England). The pessaries were applied in July, for the Christmas lambing and in October for the Easter lambing.

Before housing, all ewes were managed in more or less the same way. At mating and during early- and mid-pregnancy, they were kept on grass only. When the amount of grass was insufficient (e.g. toward the end of the year), hay was offered ad libitum.
Just before housing, all these ewes were pregnancy diagnosed, employing a Centaur Vetronaid fetometer (Sonicaid Ltd., England).

During late pregnancy, the management and feeding of the different groups of ewes that were included in the PLM study were carried out in one of the following three ways:

A. Individually-fed ewes

These ewes were included in some of the late pregnancy-feeding experiments. The ewes were housed during the last eight weeks of pregnancy in sheds and kept in individual pens (1·5 by 1·5 metres dimension), each supplied with a food box and a water-bucket. All food offered to these ewes was weighed and recorded prior to being placed in the feed containers. Twice weekly weighings of all refusals were carried out and the amount of food consumed by individual ewes was calculated by deducting refusals from the total amount of food offered. Hay boxes were filled on three occasions during the day or more often if demand was high.
Pelleted concentrates were offered in the morning feed only. Water was always available. Straw was used for bedding.

B. Group-fed ewes

Ewes on this system were housed in pens measuring six by six metres and there were normally

about 15 ewes per pen. Water was always available and food trough length was not less than 0·5 metre per ewe. The amount of food consumed was calculated as previously described, but on a group rather than on an individual basis. Straw bedding was used.

C. Commercial ewes

These animals were fed outside on hay and rising concentrate levels until about a week prior to anticipated lambing date. They were then housed inside in groups of 15 to 20 in pens measuring approximately six by six metres. No weighing of hay was undertaken and it was fed on an *ad libitum* basis. Concentrates were weighed and fed at rates of about 0·5 kg per head per day during the housed period. Water availability and bedding was as described for group-fed ewes.

Treatment of ewes after lambing

All after lambing treatment was identical irrespective of how the ewe had been housed prior to parturition. At lambing the ewes were housed with their lambs in individual straw-bedded pens for periods of between 24 and 48 hours. The diet consisted of hay *ad libitum*, 0·65 kg concentrates per head per day and as many turnips as the ewe required.

After experimental work on the lambs was completed (usually 48 hours after parturition) the ewe and her lamb(s) were either placed in larger pens with other ewes and progeny if the weather was bad, or when weather permitted they were turned out to grass where they were fed hay, concentrates and turnips. The quantities fed declined as grass became readily available.

Treatment of lambs

As soon as they had been cleaned by the ewe, all lambs were weighed using a spring balance (Slater, England) and their navel's were dipped in a 10 per cent (w/v) solution of iodine containing two per cent phenol. Each lamb was ear-tagged for identification purposes at this time. Castration and tail docking were undertaken within 24 hours of birth using elastic rings (Elastrator Co. Ltd., New Zealand). Natural sucking was allowed to occur although any lambs having difficulty were helped until they could feed themselves. Any lamb not able to be reared by its dam for any reason was transferred to a heated lamb bar where it was fed ad libitum on cold Lamblac milk substitute (Volac Ltd., England). This milk substitute is made basically of skimmed milk powder and contains 33 per cent fat. These lambs were taught to feed themselves via rubber teats attached to

a suspended bucket containing Lamblac and were later weaned on to a barley and protein pellet ration.

Post mortem examination

Autopsy of all dead lambs, during the whole period of investigation, was carried out by workers of the Veterinary Investigation Centre of The Edinburgh School of Agriculture. Carcases were collected in plastic bags and sent within 24 hours of death to the above-mentioned centre. Along with each carcase (identified by the ear-tag number), a card containing a brief history of the case was attached.

Post mortem examination was performed as soon as possible after death. Reports of the results of examination included a description of the state of the carcase and the possible cause of death.

Other constant criteria

Weighing

For ewes, this was regularly undertaken using a pig weigher (Geese, England). The same weighing machine was used to weigh lambs of 10 days of age or more. For weighing all types of food used during the study, a 1301 BCD scale (Avery, England) was employed.

Body condition scoring of ewes

This was undertaken using the following systems:

Score 1. Spinous processes of lumbar vertebrae sharp. Distinct gap between each process. Fingers will pass easily under transverse processes of lumbar vertebrae.

Score 2. Spinous processes prominant and smooth. Gap between spinous processes detectable. Fingers will pass under transverse processes which are smooth and rounded.

Score 3. Spinous processes smooth, rounded and slightly prominant. Moderate fat cover. Loin muscle full. Transverse processes smooth and detectable with firm pressure.

Score 4. Spinous processes just detectable as hard line. Thick fat cover. Loin muscle full. Transverse processes can not be felt.

Score 5. Spinous processes not detectable. Thick fat cover. Loin muscle full. Transverse processes can not be felt.

This technique was suggested by Jefferies (1961) and used by Russel, Doney and Gunn (1969).

Allocation of ewes to nutritional treatment

Studies on the effect of feeding levels during late pregnancy were carried out in the period extending between January and May of years 1974, 1975 and 1976. Ewes observed were of one breed (Scottish Halfbred) and were not markedly different in body weight at housing time.

Ewe body condition score and body weight were both taken into consideration prior to allocating the ewes to different nutritional treatments. This was done so as to equalize the effects of the above factors, both of which can affect ewe food intake (Foot, 1972; Ferguson, personal communication).

As it was impossible to get sufficient ewes of the same age at one particular time, this factor, i.e. age, was also taken into consideration and the different groups were equalized as near as possible for the age factor.

Data recording

All records were kept on specially designed, stencilled forms which helped ensure that a complete permanent record of results was obtained.

C H A P T E R F I V E

INVESTIGATIONS UNDERTAKEN IN 1974

INTRODUCTION

When this work on PLM was first envisaged in 1973 there was little published on an experimental approach to the subject and much of what was available referred to other sheep producing countries, such as Australia and New Zealand, where the climate and managemental methods varied considerably from those in Scotland. Literature referring to the British Isles was mainly of a survey nature and although valuable and interesting, it failed to yield any definite experimental results related to PLM under farm conditions.

For these reasons it was decided to undertake a general survey of the position as it affected low-ground sheep on The Edinburgh School of Agriculture estate at Bush, Midlothian. The arrangements for this were worked out in conjunction with Dr. W. J. M. Black, Senior Farms Manager, East of Scotland College of Agriculture (ESCA). Two factors became apparent: (1) It would be necessary, at this stage, to work generally within the framework provided by the various sheep projects already being conducted by ESCA. (2) I could decide on and investigate those parameters which were of interest to me or which previous literature suggested would be useful.

PARAMETERS INVESTIGATED

My main interest was in the field of immunoglobulins. In this connection the quantitative estimation of levels of different immunoglobulin classes in the sera of lambs was to be undertaken with the aim of ascertaining whether variations in these levels were related to PLM.

Previous literature indicated that factors such as lamb birth weight, litter size and managemental practices would prove important.

Besides some commercial groups of sheep, there was another group available from a nutritional trial and it was decided to investigate in a preliminary way what effect, if any, nutrition had on the factors mentioned previously.

These considerations were taken into account and the work was designed and recording methods set up to investigate the following parameters in relation to PLM.

a) Lamb birth weight in relation to lamb sex, litter size and the age and breed of the ewe.
b) Overall ewe performance and lamb mortality rates.
c) Packed cell volume (PCV), glucose, serum protein and gamma-globulin levels in lambs and ewes. Lambs were bled at birth and/or at 24 hours of age. Ewes were bled several times prior to lambing and at lambing (i.e. within one hour of parturition being completed). Ewes on nutritional study were bled

prior to lambing (at weekly intervals) for total ketone estimations (Reid, 1960).

d) The effect of lambing set-backs on subsequent lamb growth.

e) The effect of nutrition on some of the previous parameters.

f) Additional laboratory data on glucose, packed cell volume and ketones were also to be collected.

ANIMALS USED FOR THE INVESTIGATION

At the Easter lambing in March to April 1974, two groups of ewes were observed.

(a) Finn Dorset ewes:

This group consisted of 30 ewes of which four subsequently turned out to be barren. They were housed inside during the last eight weeks of pregnancy on a group-fed basis and fed hay ad libitum. During the first three weeks of housing, concentrates were replaced by sheep Mastermix pellets (Spencers feed supplements Ltd., Aberdeen) and whole barley in a ratio of one part pellets to nine parts barley. The pellets have a metabolizable energy of 8 MJ/kg dry matter, contain 36 per cent protein and in addition minerals, vitamins and trace elements were included. Barley has a metabolizable energy of 12·8 MJ/kg dry matter. This supplement was offered at the rate of 100, 225 and 330 g per ewe per day during the first, second

and third weeks of housing respectively. The supplement proved unpalatable to many ewes and was discontinued after three weeks and replaced by concentrates (12·3 MJ/kg dry matter) at rates varying from 330 g per ewe per day initially to 450 g per ewe per day at full term. This concentrate (processed at ESCA, Seafield Mill) contained barley, soya bean meal, molassine meal, vitamins and minerals, at a rate of 83·75, 10, 5 and 1·25 per cent respectively.

(b) Scottish Halfbred ewes:

This group consisted of 60 ewes (two later proved barren), individually penned and fed, and used in a nutritional trial designed for Dr. Black's work on the effects of hay quality. There were 15 different nutritional groups each containing four ewes and the trial was conducted during the last eight weeks of the ewes' pregnancy. Four hay qualities were used ad libitum. Their metabolizable energy values were as follows:-

Hay	1	: 6·69	MJ/kg	dry matter
"	2	: 7·11	"	" "
"	3	: 7·94	"	" "
"	4	: 8·36	"	" "

These hays were used alone or supplemented with concentrates (from Seafield Mill, as previously described) fed at varying rates during the last eight weeks of pregnancy. The concentrates were fed in an increasing amount toward

parturition. By this way, a ewe that received 150 g of concentrates per day at the start of concentrate feeding was offered 900 g per day during the last week of pregnancy. In addition, one group was kept, ad libitum, on Complete Ruminant Diet (CRD) only. This feed is an AA7-type (Animal Breeding Research Organization, Edinburgh) with an estimated metabolizable energy value of 9·08 MJ/kg dry matter, and processed in the same place as the concentrates.

All types of hay used were made on the farms of the Edinburgh School of Agriculture. The nutritive value of all types of foodstuff (i.e. hay, concentrates and CRD), as defined by their metabolizable energy content, were estimated regularly by analysis by the Animal Nutrition Department of the same School.

The whole experiment was set up as follows:-

Group No.	Hay No.	Concentrate supplements (kg) during last 8 weeks of pregnancy	Mean energy intake during the last 8 weeks of pregnancy (MJ/kg dry matter)
1	1	30	599·3
2	1	20	536·7
3	1	None	360·8
4	2	30	595·3
5	2	20	571·2
6	2	10	501·1
7	2	None	377·1
8	3	30	735·6
9	3	20	716·4
10	3	10	604·0
11	3	None	457·1
12	4	20	756·7
13	4	10	690·7
14	4	None	533·8
15	Complete Ruminant Diet only		1065·4.

Two further groups of ewes lambing in December 1974 and January 1975 were investigated.

a) Finn Dorset ewes:

This group consisted of 37 ewes managed commercially and brought inside one week prior to lambing. Hay was fed ad libitum and concentrates in increasing amounts as parturition approached, in accordance with good commercial practice.

b) Greyface ewes:

This group was commercially managed as described and contained 27 ewes.

RESULTS

FACTORS AFFECTING LAMB BIRTH WEIGHT

Sex and birth weight of lambs:

Reports concerning the effect of sex on lamb birth weight generally agree that males tend to be heavier than females. My observations showed the same trend (see Table 5·1). I calculated the mean birth weight and standard deviation for each group and compared them using student's 't' test. An illustration of the absolute differences in birth weight between male and female twin lambs are given in Table 5·1. The results of 't' test comparisons for all lambs in Experiment 1 is given in Table 5·2.

TABLE 5·1.

Birth weight of twins (kg)

Group of ewes	Males		Females	
	No. of lambs	Mean ± standard deviation	No. of lambs	Mean ± standard deviation
Scottish Halfbred	41	4·750 ± 0·720	29	4·150 ± 0·810
Greyface	17	4·479 ± 1·010	15	4·160 ± 0·726
Finn Dorset I	15	3·160 ± 1·151	7	3·121 ± 0·762
Finn Dorset II	14	3·630 ± 0·740	17	3·180 ± 0·690

TABLE 5·2.

Birth weight (kg) X sex comparison

(Experiment 1, 1974)

Breed	Nutri-tional bias	Singles		Twins		Triplets		Quadruplets	
		No. of lambs	t-test	No. of lambs	t-test	No. of lambs	t-test	No. of lambs	t-test
Finn x Dorset (Group 1)	-	Ins.	-	15 M 7 F	NS	10 M 8 F	NS	5 M 7 F	NS
Finn x Dorset (Group 2)	-	Ins.	-	14 M 17 F	NS	15 M 15 F	NS	7 M 9 F	NS
Greyface	-	Ins.	-	17 M 15 F	NS	6 M 3 F	NS	Ins.	-
Scottish Halfbred	+	3 M 7 F	NS	41 M 29 F	P < 0·001	22 M 17 F	P < 0·05	-	-

Ins. = Insufficient number of lambs available; P = Probability; NS = Comparison shows no significant difference.

M = Male F = Female

Although male lambs tended to be heavier than females the 't' test comparison revealed that apart from the Scottish Halfbred group these differences were not statistically significant. The Scottish Halfbred ewes were part of a nutritional experiment and to make certain that the differences illustrated were due to nutritional factors and not to sex, the results for these lambs were re-worked. Lambs born into each of the four nutritional groups (see page 173) were compared, i.e. male lambs in one nutritional group were compared with female lambs from the same nutritional group. This exercise revealed that out of the eight comparisons possible for twins and triplets only three showed statistically significant sex differences in birth weight.

To reinforce these observations, lambs from experiments done in 1975-1976 were compared for sex differences in birth weight and the 't' test results are shown in Table 5·3. In only one group was there a statistically significant difference and this was in a nutritionally biased experiment where re-working of the results again showed that the differences were mainly nutritional and not due solely to sex.

Litter size and birth weight of lambs:

The many authors who reported on the relation between litter size and lamb birth weight (see pages 10,11), all agreed that the bigger the litter size, the smaller the

TABLE 5·3.

Birth weight (kg) X sex comparison

(Experiments 2 - 5, 1975-1976)

Breed	Nutri-tional bias	Singles		Twins		Triplets		Quadruplets	
		No. of lambs	t-test	No. of lambs	t-test	No. of lambs	t-test	No. of lambs	t-test
Greyface (Easter 1975)	-	-	-	Ins.	-	-	-	-	-
Scottish Halfbred (a)*	-	-	-	21 M 21 F	NS	-	-	-	-
Scottish Halfbred (b)*	+	3 M 6 F	NS	17 M 13 F	NS	12 M 9 F	NS	6 M 6 F	NS
Scottish Halfbred (Easter 1976)	+	9 M 25 F	NS	20 M 25 F	P◁ 0·05	31 M 20 F	NS	-	-

*Scottish Halfbred groups (a) and (b) were two groups. Both lambed at Easter 1975 but were in two separate studies.

Ins. = Insufficient number of lambs available; P = Probability; NS = Comparison shows no significant difference.
M = Male F = Female

birth weight. My findings agree with these conclusions. Table 5·4 presents absolute figures (for 1974) which were calculated according to litter size. They represent normal lamb birth weight values for the different breeds of sheep that were included in the investigation of PLM. The above mentioned table and also the bar diagram (Fig. 5·1) indicates that single lambs are generally heavier than twins; twins heavier than triplets; triplets heavier than quadruplets and so on. Students 't' test was applied to these figures and the results (Table 5·5) proved that the difference in birth weight due to litter size was highly significant. Figures for later experiments conducted in years 1975 and 1976 supported this finding. These significant differences applied irrespective of whether the lambs originated from commercially fed ewes or ewes on nutritional studies. Only when litter size exceeded three did the 't' test fail to reveal significant birth weight differences between groups although the trend was still downwards. This is not surprising, as there must be a lower physiological birth weight limit for lambs surviving all or most of the gestation period.

Breed of ewe and lamb birth weight:

During my work on PLM, ewes from three different breeds, namely, Scottish Halfbred, Greyface and Finn Dorset were included and all were mated with Suffolk tups.

FIG. 5·1

Bar diagram showing the relationship between litter size and birth weight of lambs (means and standard deviations).

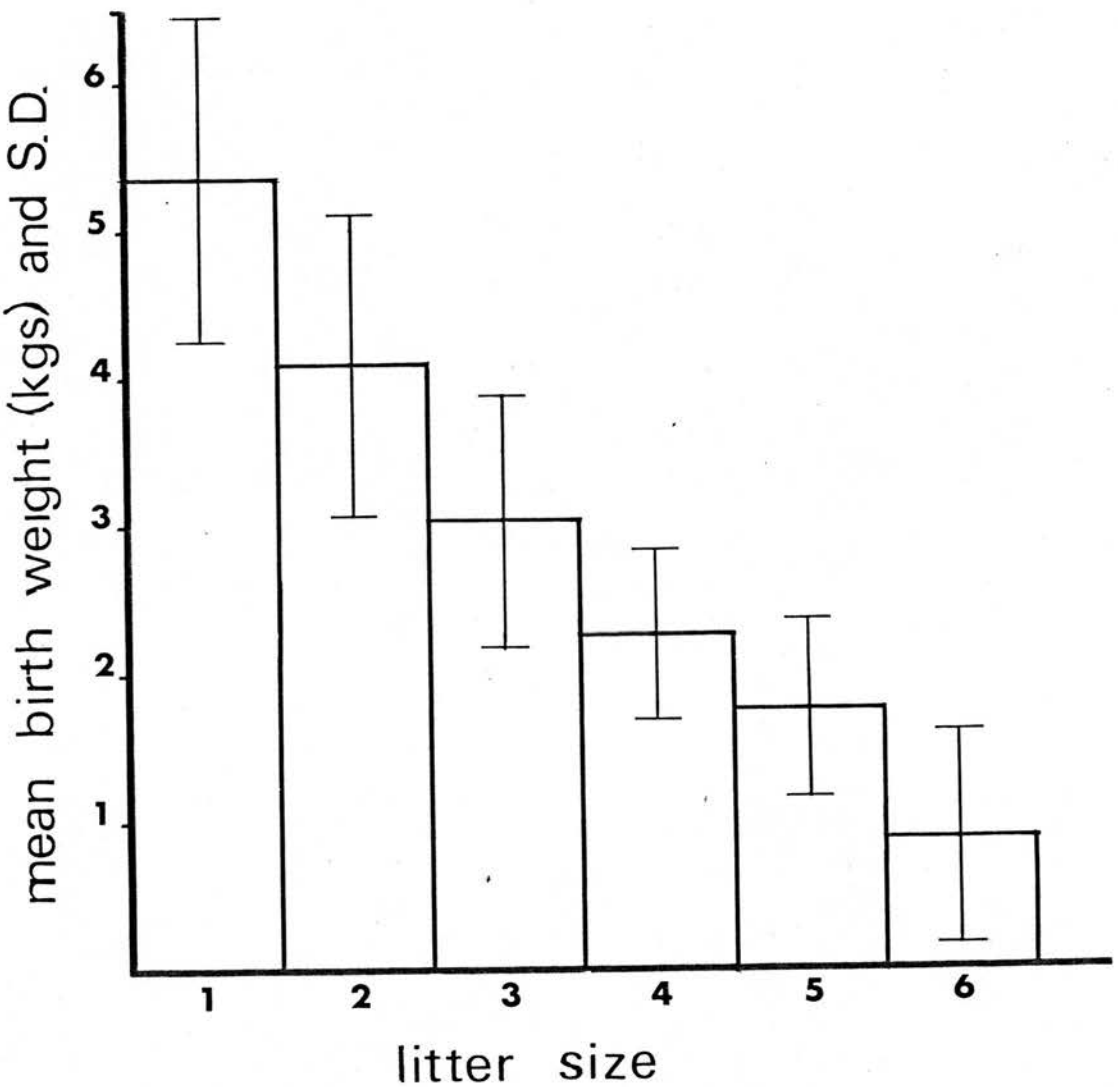


TABLE 5·4.

Lamb birth weight (kg) according to litter size

Breed		Singles	Twins	Triplets	Quadruplets
Scottish Halfbred	m.*	5·839	4·512	3·323	-
	s.d.**	0·751	0·804	0·901	
	n.***	10	70	39	
Greyface	m.	5·775	4·329	3·494	3·275
	s.d.	0·170	0·889	0·733	0·379
	n.	4	32	9	4
Finn Dorset	m.	4·600	3·290	2·690	2·330
	s.d.	1·140	0·890	0·720	0·550
	n.	9	53	48	28

*m. = mean s.d. = standard deviation n. = number of lambs

TABLE 5·5.

Birth weight (kg) X litter size 't' test comparison

Breed	Singles x Twins		Twins x Triplets		Triplets x Quadruplets	
	No. of lambs	t-test	No. of lambs	t-test	No. of lambs	t-test
Finn x Dorset (group 1)	3 s. 22 tw.	P<0·05	22 tw. 18 tr.	P<0·01	18 tr. 12 qu.	NS
Finn x Dorset (group 2)	6 s. 31 tw.	P<0·01	31 tw. 30 tr.	P<0·02	30 tr. 16 qu.	NS
Scottish Halfbred	10 s. 70 tw.	P<0·001	70 tw. 39 tr.	P<0·001	-	-
Greyface	4 s. 32 tw.	P<0·001	32 tw. 9 tr.	P<0·02	9 tr. 4 qu.	NS

Table 5·4 already referred to, gives information about the birth weight of lambs born to the three different ewe breeds. Table 5·6 shows the result of lamb birth weight comparisons as affected by breed of ewe. Lambs born to Scottish Halfbred or Greyface ewes are very comparable as far as lamb birth weight is concerned, although those of the first breed were in most instances slightly heavier (but never significantly) than those of the second breed. On the other hand, lambs born to Finn Dorset ewes were significantly smaller than those born to Greyface ewes. Sex differences in birth weight did not contribute to this difference (Table 5·2).

EWE AND LAMB PERFORMANCE

Overall ewe performance:

The performance of the four groups of ewes and the levels of PLM among each of them are summarised in Table 5·7.

From Table 5·7 the following facts emerge: (1) The number of lambs born is not always related to the number of lambs finally reared. (2) The number of ewes failing to rear lambs is variable. (3) Ewe milking ability has an important bearing on PLM.

Overall levels of PLM:

Total PLM for all groups observed during 1974 was 29·6 per cent (99 out of 334 lambs born). The distribution of these losses and the age at death showed a similar

TABLE 5·6.

Lamb birth weight (kg) - Ewe breed comparison

Breeds compared	t-test results			
	Singles	Twins	Triplets	Quadruplets
Scottish Halfbred and Greyface	NS	NS	NS	-
Greyface and Finn Dorset	NS	$P < 0.001$	$P < 0.01$	$P < 0.01$

TABLE 5·7.

Performance of the different groups of ewes

Group of ewes	No. of ewes	Average litter size	No. of lambs reared per ewe	PLM (%)	Ewes which lost one lamb or more (%)	% lamb deaths attributed to:	
						poor mothering instinct	lack of milk**
Scottish Halfbred	58	2·08	1·67	20·16	25·8	Nil	37·5
Greyface	27	2·11	1·63	22·80*	25·9	7·5	Nil
Finn Dorset 1	26	2·61	1·23	52·90	65·0	13·8	13·8
Finn Dorset 2	37	2·43	1·73	28·80	32·4	3·8	42·3
All groups	148	2·26	1·59	29·60	34·4	7·0	25·0

*Actual figure is 19·3 per cent as two of the lambs (3·5% of PLM) died at more than 28 days of age, i.e. outside the defined period for PLM.

**This was ascertained by the gross appearance and feel of the udder after lambing and by milk let down as either (1) poor: no milk or little milk available, or (2) good: adequate milk available.

pattern for all the groups of ewes. In general, very high losses occurred before or within the first few days of life and only a few lambs were lost after 10 days of age. Expressed as a percentage of all the lambs which died, 45·4 per cent were abortions/stillbirths, 35·3 per cent were lost between birth and 48 hours (only one out of the 35 lambs in this category died as a result of dystocia), 14·2 per cent were lost between 48 hours and 10 days of age and only 5·1 per cent died after 10 days of age.

These levels of PLM vary greatly with litter size. In each of the four groups of ewes, levels of lamb mortality increased with the increase in litter size. Pooled data for all the groups demonstrates the frequency of lamb losses in each litter size (Fig. 5·2). Within litter size, and calculated as a percentage of the lambs born, the highest losses occurred among quintuplets and sextuplets (with PLM levels of 90 per cent and 83·3 per cent respectively). Figures for the quadruplets, triplets, twins and singles were 58·3, 33·3, 8·75 and 4·34 per cent respectively.

Relying on recording, direct observation, post-mortem and bacteriological examinations, it was possible to identify the most likely cause of lamb deaths. Table 5·8 shows the number of lamb losses in relation to time and cause of death for each litter size.

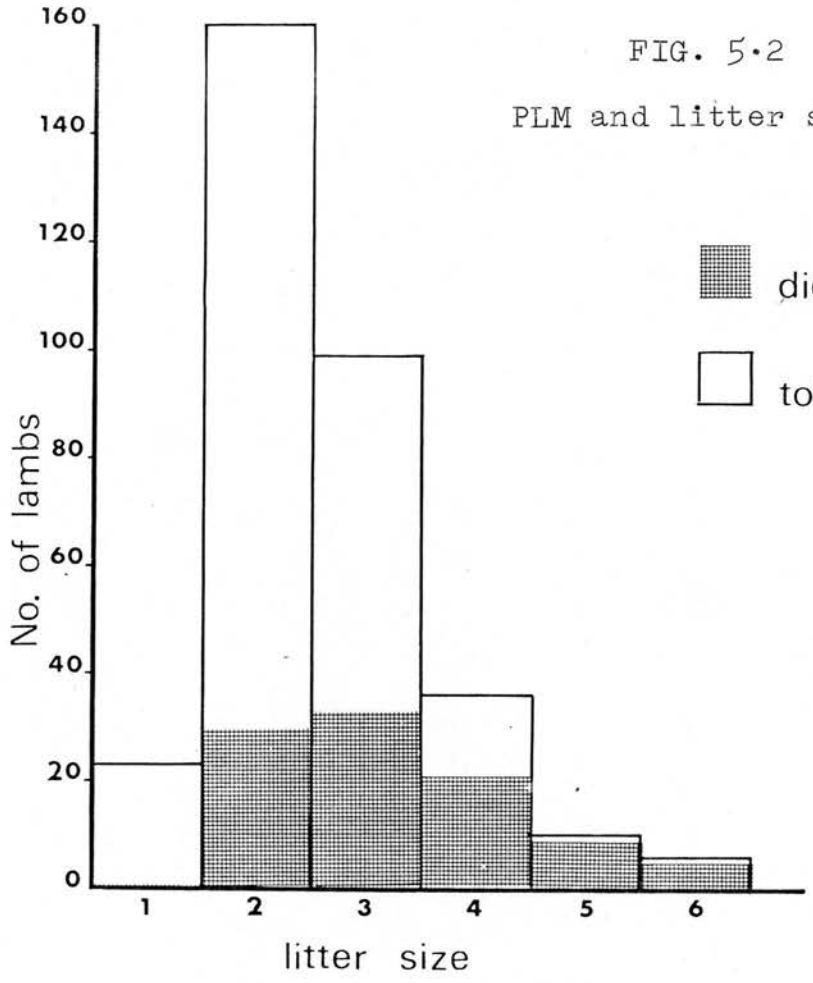


FIG. 5·2

PLM and litter size.

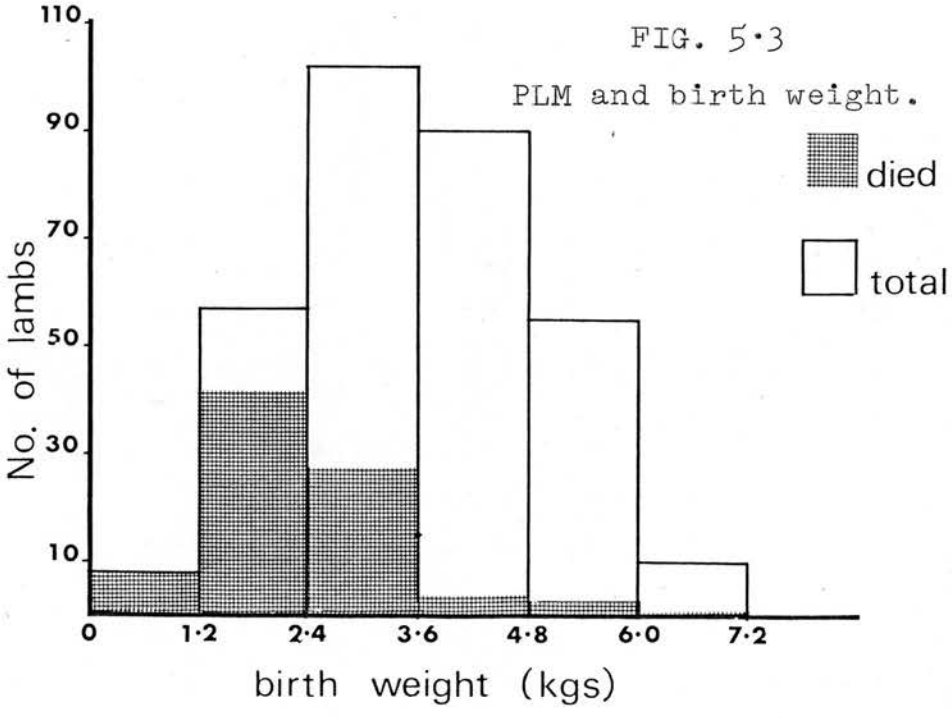


FIG. 5·3

PLM and birth weight.

TABLE 5.8.

Number of lamb deaths in relation to time and cause of death

Litter size	Time and cause of death	Abortion or stillbirth	Dystocia	0 - 48 hours				48 hours - 10 days		▸10 days	Total No. of lambs born
				Premature	Infection*	Starvation	Accidental	Infection*	Starvation	Various causes**	
	Singles	-	1	-	-	-	-	-	-	-	23
	Twins	6	-	7	1	-	3	4	5	4	160
	Triplets	14	-	3	6	4	2	2	2	-	99
	Quadruplets	11	-	3	3	1	1	1	-	1	36
	Quintuplets	8	-	1	-	-	-	-	-	-	10
	Sextuplets	5	-	-	-	-	-	-	-	-	6
	Total	44	1	14	10	5	6	7	7	5	334

*15 out of 17 of these cases were identified as E. coli septicaemia or E. coli enteritis and the first type was the more common. One lamb died of acute Pasteurella septicaemia and E. coli enteritis and one died of staphylococcal lung abscess complicated by E. coli enteric infection.

**Two died at 6 - 8 weeks of age as a result of early weaning on barley and concentrate pellets. On post-mortem, one showed chronic ruminitis and the other had fibrinous enteritis. A third lamb died at 12 days of age, as a result of umbilical infection and generalised peritonitis. The cause of death of the other two lambs was unknown.

In most postnatal deaths, these causes complicated each other, e.g. five out of the 15 lambs that died of E. coli infection revealed an absence of milk in their stomachs (starvation). E. coli infections, either in the septicaemic or enteric form were very common causes of neonatal lamb death.
Their were a few accidental cases, e.g. crushing, injuries. The lambs classed as premature were unable to cope with their new environment. The cause of death of these lambs was variable, i.e. starvation, crushing, E. coli, but in all cases the predisposing cause was prematurity.

FACTORS AFFECTING PLM

Lamb birth weight and PLM:

Data on the performance of ewes observed during the 1974 lambing and on the birth weight of live and dead lambs are summarised in Table 5·9. The comparison was conducted on the basis of litter size, whenever possible. The figures compared give clear evidence that the birth weight of dead lambs is significantly lower than that of the corresponding live lambs.

Among the four comparisons which showed no significant difference, two had insufficient numbers for the 't' test to be undertaken and in the other two cases the trend towards lighter birth weights in dead lambs was evident

TABLE 5·9.

Birth weight comparison between live and dead lambs

Group of ewes	Litter size	Lambs birth weight (kg)				Significance of differences between means ('t' test)
		live		dead		
		Mean	No.of lambs	Mean	No.of lambs	
Scottish Halfbred	Twins	4·59 ± 0·80	63	3·57 ± 0·92	7	P < 0·01
	Triplets	3·77 ± 0·86	23	2·68 ± 0·46	16	P < 0·001
Finn Dorset (group 1)	Twins	3·35 ± 0·60	15	2·48 ± 1·37	7	NS*
	Triplets	2·67 ± 0·40	11	1·77 ± 0·59	7	P < 0·01
	Quad-ruplets	2·50 ± 0·26	4	1·76 ± 0·25	8	P < 0·001
	Quin-tuplets	2·90	1	1·75 ± 0·53	9	Ins.*
Finn Dorset (group 2)	Twins	3·54 ± 0·72	26	2·54 ± 0·80	5	P < 0·01
	Triplets	3·14 ± 0·50	23	2·23 ± 0·64	7	P < 0·001
	Quad-ruplets	3·12 ± 0·52	8	2·05 ± 0·22	8	P < 0·001
	Sex-tuplets	2·00	1	0·64 ± 0·58	5	Ins.*
Greyface	Twins	4·41 ± 0·85	28	3·76 ± 1·06	4	NS*

*NS = Difference is not significant.

**Ins.= Insufficient number of lambs available for the comparison.

Single lambs and Greyface multiples omitted as no comparison was possible.

although not significant owing to the variable weights of the lambs which died.

It must be pointed out that, whether the lamb death was prenatal or postnatal, lambs in each of these two categories had significantly lower birth weight than their contemporary survivors, e.g. among Scottish Halfbred triplets, the mean birth weight (kg) and standard deviation for 23 surviving lambs was 3·77 ± 0·86, for nine aborted or stillborn lambs it was 2·57 ± 0·43 and for seven lambs which died after birth it was 2·66 ± 0·45. The first mean birth weight is significantly higher than the second ($P < 0.001$) and than the third ($P < 0.01$).

Although there was only one case of death due to dystocia (a single, born to a Finn Dorset group 1 ewe), this particular lamb had a very high birth weight (6·3 kg) compared to that of the two surviving singles of the same group (with mean birth weight of 3·92 kg).
The mean birth weight of all lambs observed was 3·975 kg. Among the 117 lambs which weighed over 4·00 kg at birth, PLM was only four per cent compared to 39 per cent among the remaining 205 lambs with birth weight less than 4·00 kg. The histogram (Fig. 5·3) shows levels of lamb losses as affected by birth weight.

Age of ewe and levels of PLM:

Ewes included in this analysis were divided into three age groups as follows: 1 to 2 years, 3 to 5 years

and ▷5 years of age (Table 5·10). Because Scottish Halfbred and Greyface ewes are two comparable breeds as far as ewe performance is concerned, their data concerning age of ewe and levels of PLM were pooled together. The same thing was done on the two groups of Finn Dorset ewes. As shown in the table below, ewe's age affects the levels of PLM among Scottish Halfbred and Greyface ewes. High lamb losses were recorded for both very young (1 to 2 years) and old (▷5 years) ewes, while those representing the middle group suffered the least in terms of PLM. For Finn Dorset ewes, levels of PLM were very high irrespective of the ewes age. None of the Finn Dorset ewes was over 5 years of age.

TABLE 5·10.

PLM per cent and age of ewe

Age of ewe (years)	Breed of ewe	
	Scottish Halfbred and Greyface	Finn Dorset
1 - 2	33·3 (9)	39·5 (17)
3 - 5	17·1 (39)	39·5 (46)
▷ 5	21·1 (39)	—

Numbers in brackets refer to the number of ewes in each comparison.

Ewes gestation length and PLM:

For ewes included in year 1974 work, the gestation length was calculated as the number of days between recorded mating and lambing date. Gestation length was calculated for five different groups of ewes according to their lambs survival data after all data for the three different breeds used in the study had been pooled. For ewes that gave birth to live lambs, the Scottish Halfbred and Greyface ewes had an average gestation length of 145·00 days and the Finn Dorset ewes had almost the same gestation length (145·03 days).

The groups and their calculated gestation length (mean ± standard deviation) were as follows:-

Group 1: 100 ewes with all lambs surviving
= 145·55 ± 2·80 days.

Group 2: 6 ewes that aborted
= 129·50 ± 6·18 days.

Group 3: 15 ewes with stillborn lambs
= 142·14 ± 2·98 days.

Group 4: 26 ewes with postnatal lamb death
= 143·37 ± 4·21 days.

Group 5: 1 ewe with lamb that died of dystocia
= 152·00 days.

Group 1 had significantly longer gestation length than group 2 ($P < 0·001$), group 3 ($P < 0·001$) and group 4 ($P < 0·01$) and a shorter gestation length than group 5.

Although only one case of lambing resulted in lamb death due to dystocia (0·7 per cent of total number of ewes lambed), 14 per cent of ewes in the three different breeds were actually offered different degrees of assistance at lambing (2 per cent needed considerable assistance and the other 12 per cent resulted in successful lambing after a slight degree of assistance only). More Finn cross Dorset ewes needed assistance at lambing (20 per cent of ewes lambed) than Scottish Halfbred and Greyface ewes (only 11 per cent of them needed slight assistance).

Although it is difficult to estimate time of death in utero for stillborn lambs, most of them showed a degree of development suggesting death just before delivery. Ewes that gave birth to stillborn lambs had a marginally shorter gestation length than those ewes with postnatal type of lamb death.

Haematological and biochemical parameters in lambs:

The following parameters were studied during my preliminary investigations into PLM:- Packed cell volume, blood glucose, total serum protein and serum gamma-globulin.

I used lambs from the four groups of ewes previously described, and calculated the mean value and standard deviation for each parameter according to litter size and

the breed of the ewe. It rapidly became obvious that the results were affected by litter size but were similar irrespective of whether the lambs were born to Finn Dorset, Greyface or Halfbred ewes. I consequently grouped all the results together on the basis of litter size and then did a comparison between lambs which survived and lambs which died.

The results for lambs which survived are presented in Table 5·11 and for lambs which died in Table 5·12. The samples collected at birth were from lambs born during April 1974, and those collected at 24 hours were from the same lambs and also from lambs born in December 1974. Student's 't' test comparisons were run and the results for lambs which subsequently died are shown in Table 5·13 which takes into account litter size and age. Similarly, Table 5·14 presents comparisons based on litter size and age, for both surviving lambs and those that died later.

PCV:

There was no significant difference between values in surviving newborn lambs at birth but in all instances the PCV fell during the first 24 hours of life. This is presumably as a result of fluid ingestion and absorption by the lamb. This trend was less marked or absent in lambs which died because they failed to suck adequately and, in some

TABLE 5·11.

Blood parameter values for all surviving lambs

(At birth and at 24 hours of age)

Litter size*	**	PCV (%)		Glucose (mg/100 ml)		Total protein (g/100 ml)		Gamma-globulin (g/100 ml)	
		Birth	24 hours	Birth	24 hours	Birth	24 hours	Birth	24 hours
Singles	m.	48·05	36·55	43·57	108·50	4·40	7·40	0·31	3·17
	s.d.	5·06	5·80	14·14	27·46	0·45	0·81	0·16	0·70
	n.	10	22	12	22	12	21	11	21
Twins	m.	46·63	36·78	45·44	95·57	4·30	6·70	0·20	2·48
	s.d.	7·75	5·36	21·77	28·82	0·57	1·33	0·11	0·98
	n.	68	123	67	125	68	131	69	131
Triplets	m.	46·24	37·50	51·79	97·28	4·34	6·33	0·15	2·36
	s.d.	4·70	5·65	33·55	27·78	0·98	1·46	0·11	1·12
	n.	29	54	29	54	29	55	29	55

* For bigger sets of litter size, i.e. quadruplets and more, insufficient numbers of lambs were available.

** m. = mean, s.d. = standard deviation, n. = number of lambs.

TABLE 5·12.

Blood parameter values for non-surviving lambs

(At birth and at 24 hours of age)

Litter size	*	PCV (%)		Glucose (mg/100 ml)		Total protein (g/100 ml)		Gamma-globulin (g/100 ml)	
		Birth	24 hours	Birth	24 hours	Birth	24 hours	Birth	24 hours
Twins	m.	37·60	34·30	52·90	85·50	4·03	6·55	0·14	2·06
	s.d.	7·68	7·70	31·50	30·38	0·21	1·65	0·04	0·98
	n.	3	10	3	8	3	10	3	10
Triplets	m.	41·50	42·30	45·20	74·60	3·91	5·18	0·18	1·12
	s.d.	5·29	8·65	20·30	37·44	0·42	1·02	0·11	0·76
	n.	10	10	10	11	10	13	10	13

* m. = mean, s.d. = standard deviation, n. = number of lambs involved.

TABLE 5·13.

Non-surviving lambs

Comparison of blood parameters ('t' test results) based on

(a) litter size, and (b) values at birth and 24 hours of age

Parameters	(a) Twins x Triplets comparison		(b) Birth x 24 hr values comparison	
	At birth	At 24 hours	Twins	Triplets
PCV	(3 T. x 10 Tr.)** NS*	(10 T. x 10 Tr.) P◁ 0·05	(3 x 10)*** NS	(10 x 10) NS
Glucose	(3 T. x 10 Tr.) NS	(8 T. x 11 Tr.) NS	(3 x 8) NS	(10 x 11) P◁ 0·05
Total protein	(3 T. x 10 Tr.) NS	(10 T. x 13 Tr.) P◁ 0·05	(3 x 10) P◁ 0·05	(10 x 13) P◁ 0·01
Gamma-globulin	(3 T. x 10 Tr.) NS	(10 T. x 13 Tr.) P◁ 0·02	(3 x 10) P◁ 0·01	(10 x 13) P◁0·001

*NS = Not significant

** = Number of lambs (T. = Twins and Tr. = Triplets)

*** = Number of lambs (No. sampled at birth x no. sampled at 24 hours of age).

TABLE 5·14.

Blood parameters, comparison between surviving and non-surviving lambs, at birth and at 24 hours of age

('t' test results)

Litter size	P C V		Glucose		Total protein		Gamma-globulin	
	At birth	At 24 hrs	At birth	At 24 hrs	At birth	At 24 hrs	At birth	At 24 hrs
Twins	*NS **(68 x 3)	NS (123 x 10)	NS (67 x 3)	NS (125 x 8)	NS (68 x 3)	NS (131 x 10)	NS (68 x 3)	NS (131 x 10)
Triplets	P < 0·02 (29 x 10)	P < 0·05 (54 x 10)	NS (29 x 10)	P < 0·05 (54 x 11)	NS (29 x 10)	P < 0·02 (55 x 13)	NS (29 x 10)	P < 0·001 (55 x 13)

*NS = Not significant

**Numbers in brackets represent number of samples taken from survivors x number taken from lambs that died.

cases, became dehydrated (Tables 5·12, 5·13).

Glucose:

No significant trend was apparent at birth but in all cases the blood glucose level rose rapidly after suckling. The trend was for single lambs to have the highest values and for lambs which died to have the lowest values at 24 hours, and this is probably an indication of food intake.

There was not, however, any clear cut significant difference between glucose values in lambs which died and in lambs which survived (Table 5·14), principally because a significant proportion of the lambs which died were healthy at the time of sampling. Only in lambs actually dying at the time of sampling were blood glucose levels significantly depressed.

Total protein:

This parameter behaved in much the same way as blood glucose with no significant trend at birth although there is the suggestion that lambs which died tended to have lower total protein levels at birth than lambs which survived. After suckling there was a marked increase in total serum protein levels with the highest values occurring in single lambs and the lowest in triplets. This is a

probable reflection on total food intake by the lamb. Triplets which died had significantly low serum proteins after suckling (Table 5·14).

Total gamma-globulin:

This parameter is also related to suckling and colostrum intake as was the total serum protein level. Gamma-globulins were uniformly low at birth although the tendency was for single lambs to have higher values than triplets. In all cases there was a marked and highly significant increase in gamma-globulin levels after suckling. This increase was related to litter size, with single lambs having the highest values. There was a lowering of serum gamma-globulin in lambs which died and in the case of triplets the values were significantly lower than those for surviving triplets (Table 5·14).

Immunoglobulin classes and subclasses (SRID values):

These values were measured separately from the total gamma-globulin values but produced a similar trend. There was no important variation in the sera levels of IgG_1, IgG_2, IgM and IgA in lambs born to the different groups of ewes observed so the values for all lambs (of equal litter size) were pooled together. These levels were measured in the

sera of lambs at 24 hours of age. At this age, the origin of almost all the serum immunoglobulin is the passively passed colostral immunoglobulins absorbed through the newborn's gut. Figures for the levels of the different immunoglobulins in sera, from surviving lambs at 24 hours of age, are shown in Table 5·15. These values are tabulated on a litter size basis.

IgG_1 forms the biggest part (78 per cent) of the lamb's serum immunoglobulins. IgM and IgA form 16 per cent and four per cent respectively, and IgG_2 is represented by a mere one per cent.

When a comparison of immunoglobulin values was made between surviving lambs from litters of different size there were the following significant variations:-

Triplets had lower IgG_2 levels than twins ($P < 0·01$) and than singles ($P < 0·001$), and less IgM than singles ($P < 0·02$). Generally, a trend existed in which lambs from big litters had lower immunoglobulins than those born to small litters (see Table 5·15). This trend persisted also among dead lambs (Table 5·16), with one exception where 12 dead triplets had an IgA mean of 0·154 g per 100 ml in comparison to 0·108 g per 100 ml shown by nine dead twins.

TABLE 5·15.

Serum mean immunoglobulin levels at 24 hours of age of surviving lambs

Litter size	No. of lambs	Immunoglobulin levels (g/100 ml)				
		IgG_1	IgG_2	IgM	IgA	Total
Singles	14	2·285 ± 0·639	0·041 ± 0·010	0·610 ± 0·236	0·128 ± 0·038	3·065 ± 0·668
Twins	107	2·231 ± 0·916	0·036 ± 0·017	0·427 ± 0·218	0·133 ± 0·076	2·828 ± 1·050
Triplets	55	2·072 ± 0·997	0·029 ± 0·012	0·408 ± 0·265	0·122 ± 0·039	2·632 ± 1·161

TABLE 5·16.

Serum mean immunoglobulin levels at 24 hours of age of lambs which died

Litter size	No. of lambs	Immunoglobulin levels (g/100 ml)				
		IgG_1	IgG_2	IgM	IgA	Total
Twins	9	1·717 ± 0·612	0·023 ± 0·004	0·349 ± 0·177	0·108 ± 0·041	2·200 ± 0·831
Triplets	12	0·967 ± 0·648	0·016 ± 0·010	0·215 ± 0·249	0·154 ± 0·046	1·351 ± 0 925

In general, dead lambs, twins or triplets, had lower immunoglobulin values than their corresponding survivors (see Tables 5·15 and 5·16). For total immunoglobulin values, dead triplets showed significantly lower values ($P < 0{\cdot}001$) than their corresponding survivors. Values for dead twins were lower (but not significantly) than those of surviving twins (d.f. = 114, 't' = 1·746). The difference in total immunoglobulin values between dead twins and surviving singles was statistically significant ($P < 0{\cdot}02$). Dead triplets had significantly lower IgG_1 and IgG_2 ($P < 0{\cdot}001$), lower IgM ($P < 0{\cdot}05$) and higher IgA ($P < 0{\cdot}05$) than their contemporary survivors. Dead twins also showed lower IgG_1, IgG_2, IgM and IgA than their contemporary survivors but the difference was only significant in the case of IgG_2 ($P < 0{\cdot}05$).

PERFORMANCE OF SURVIVING LAMBS

Lambs were weighed at intervals after birth in order to ascertain their rate of growth. Under the circumstances of this work, ewes and lambs were often outside in large groups which meant that all animals had to be brought inside in order to find and weigh a few lambs only. This was not a practical proposition and consequently lambs were brought in at weekly intervals and

all weighed at the same time. This meant that a lamb might for example have been 19 or 23 days old when the three weeks of age weighing was undertaken.

In order to bring the weights to a common denominator, I took the lamb's weight nearest to three or five weeks of age, calculated the average daily weight gain over the period and converted this to total gain at 21 or 35 days of age.

The mean increase in live weight calculated by this method over the first three and five weeks of life is presented in Table 5·17, together with the standard deviation. This table applies to single lambs and to twins, triplets and quadruplets reared as pairs of lambs by the ewe. Because of the existing sheep management system, there was no instance in which a complete litter of triplets or quadruplets was reared on the ewe. A Student 't' test comparison of this data indicated that the only significant growth rate variation was for single lambs in Finn Dorset 2, Scottish Halfbred and Greyface groups. In these instances single lambs always put on more weight than other lambs. Triplets and quadruplets reared as pairs grew just as fast as twin lambs of the same breed and in some cases slightly faster.

The considerable variation in lamb weight gain between the different ewe groups is caused by two factors, (a) breed and (b) time of year. Lambs born to the

TABLE 5.17.

Weight increase of lambs reared by the ewe

(Mean ± standard deviation)

Litter size	Weight increase by 3 weeks of age (kg)				Weight increase by 5 weeks of age (kg)			
	Finn Dorset 1	Finn Dorset 2	Scottish Halfbred	Greyface	Finn Dorset 1	Finn Dorset 2	Scottish Halfbred	Greyface
Singles	(2) 5·18 ± 1·45	(6) 5·81 ± 0·47	(10) 7·80 ± 0·96	(4) 6·98 ± 0·55	(2) 9·87 ± 1·91	(6) 9·97 ± 1·50	(10) 13·41 ± 1·73	(4) 10·89 ± 0·71
Twins	(10) 6·18 ± 1·26	(22) 3·88 ± 1·31	(58) 5·03 ± 1·44	(30) 4·93 ± 0·90	(10) 10·31 ± 2·00	(24) 6·65 ± 1·73	(55) 9·85 ± 2·33	(30) 7·65 ± 1·63
Triplets	(8) 5·00 ± 0·53	(16) 3·49 ± 0·74	(16) 5·13 ± 1·54	(6) 5·15 ± 1·61	(8) 8·88 ± 1·04	(16) 6·13 ± 1·63	(16) 10·10 ± 2·44	(6) 7·71 ± 1·84
Quadruplets	(2) 4·64 ± 0·25	(4) 3·26 ± 0·98	—	(2) 6·20 ± 0·44	(2) 7·87 ± 0·08	(4) 6·56 ± 0·66	—	(2) 8·58 ± 0·24

Number of lambs in brackets.

Scottish Halfbred ewes grew faster than most other lambs, those born to the Finn Dorsets tended to be slower and lambs born to the Greyfaces fell between the two. This appraisal is distorted by the season. Lambs born to the Scottish Halfbred and the first Finn Dorset group were reared at grass in the spring and summer, and had a considerable advantage as a result. Lambs born to the Greyface and second Finn Dorset group, on the other hand, were reared during the winter on poor grass, hay and concentrates. This system was less conclusive to milk production on this occasion than summer grazing.

These variations are highlighted in Table 5·18, which shows the absolute five week weight achieved by the lambs. In some cases the rate of growth is very rapid, i.e. Scottish Halfbred singles averaged a daily weight gain of 0·38 kg, but most of the five weeks weight differences in multiple litters are due to birth weight variations. In general terms, small lambs at birth grew rapidly (see Table 5·19) but failed to catch up on lambs with a greater birth weight, i.e. triplets and quadruplets rarely caught up with twins by five weeks of age.

I was interested to see if lambs fostered and fed artificial milk, lambs whose mothers had little milk at parturition, and lambs which were ill early in life suffered a setback in growth rate as a result. The relevant data is in Table 5·20.

TABLE 5·18.

Absolute weight (kg) of lambs at five weeks of age

(Mean ± standard deviation)

Litter size	Group of ewes			
	Finn Dorset 1	Finn Dorset 2	Scottish Halfbred	Greyface
Singles	(2) 13·80 ± 3·57	(6) 14·44 ± 2·44	(10) 19·14 ± 2·24	(4) 16·65 ± 0·85
Twins	(10) 13·96 ± 2·47	(24) 10·26 ± 1·82	(55) 14·51 ± 2·52	(30) 12·04 ± 2·28
Triplets	(8) 11·54 ± 1·41	(16) 9·32 ± 1·74	(16) 14·00 ± 3·03	(6) 11·32 ± 2·69
Quadruplets	(2) 10·32 ± 0·07	(4) 9·28 ± 0·34	—	(2) 12·00 ± 0

Number of lambs in brackets.

TABLE 5·19.

Body weight increase of lambs by five weeks of age

(Mean ± standard deviation),

expressed as percentage of their birth weight

Litter size	Group of ewes			
	Finn Dorset 1	Finn Dorset 2	Scottish Halfbred	Greyface
Twins	(10) 277·30 ± 34·55	(24) 189·05 ± 54·89	(55) 215·38 ± 57·73	(30) 175·57 ± 32·95
Triplets	(8) 339·25 ± 46·60	(16) 197·04 ± 61·02	(16) 261·94 ± 57·98	(6) 214·17 ± 19·58
Quadruplets	(2) 321·50 ± 12·02	(4) 253·00 ± 67·26	—	(2) 251·00 ± 25·46

Number of lambs in brackets.

TABLE 5·20.

Weight increase of other lambs

(Mean ± standard deviation)

Lamb's observed	Weight increase by 3 weeks of age (kg)				Weight increase by 5 weeks of age (kg)			
	Finn Dorset 1	Finn Dorset 2	Scottish Halfbred	Greyface	Finn Dorset 1	Finn Dorset 2	Scottish Halfbred	Greyface
Fostered triplets	(2) 4·48 ± 0·34	(4) 4·46 ± 1·52	(5) 3·72 ± 1·09	(3) 4·76 ± 0·99	(2) 8·04 ± 0·87	(6) 6·25 ± 2·06	(5) 6·55 ± 1·21	(3) 6·92 ± 1·38
Low milk twins*	—	(3) 3·85 ± 1·58	(6) 4·48 ± 2·51	—	—	(4) 5·86 ± 2·59	(6) 8·80 ± 3·60	—
ill twins**	—	—	—	(4) 4·62 ± 1·18	—	—	—	(4) 8·13 ± 1·84

* = Twins whose mothers had little milk at parturition; ** = ill shortly after birth.

Number of lambs in brackets.

Fostering of triplets produced variable weight gains, the Finn Dorset lambs keeping pace with their contemporaries and the Scottish Halfbreds falling well behind theirs. Fostering affected overall weight gain adversely but only slightly. The weight gains of the few twins, born to ewes with little milk were, as expected, slightly less than their contemporaries.

If lambs survived the first few days of life, either the ewe's milk supply improved and growth rate increased or the ewe was culled and the lambs fostered. This explains the apparent anomaly of lambs growing well on ewes with a supposed poor milk output.

Illness early in life, usually in the form of E. coli infections, appeared to have no effect on subsequent growth rate but the numbers involved were so small that it is impossible to draw definite conclusion from the data.

EFFECTS OF EWE NUTRITION IN LATE PREGNANCY

Monitoring of nutritional groups:

From the groups observed in 1974, only the Scottish Halfbreds fall into this part of the study. As has been stated previously (see page 138), there were 15 groups (with only four ewes in each) in this experiment, which had been designed for a hay quality study that was performed by other workers. The number of ewes in each group was too small to perform statistical comparisons

on the parameters observed in my part of the work and as my main interest in this experiment was to obtain as much information as possible about PLM, the 15 small groups were merged and regrouped as follows.

The mean energy intake expressed as metabolizable energy (MJ/kg dry matter) during the last eight weeks of pregnancy was calculated for each of these small groups according to the ewe's daily food intake and to the energy content of the feed stuff offered (see page 138). These mean energy intake values varied between 360·8 and 1065·4 MJ/kg dry matter. After ranking the 15 values in ascending order, they were allocated into four quartiles. The mean ewe metabolizable energy intake during the last eight weeks of pregnancy for each group was as follows:-

	Group 1	Group 2	Group 3	Group 4
Mean ME(MJ/kg DM):	424·55 ± 66·5	559·98 ± 29·4	653·43 ± 59·9	853·28 ± 184·2

By comparing the groups' ME values to each other (using Student's 't' test), it appeared that, ewes in group 1 received significantly lower energy than ewes in any of the other three groups (P was $< 0·01$ in each of the three comparisons). ME values for group 2 were significantly (but only at $P < 0·05$) less than those of group 3 and 4. Although group 3 had a lower energy intake than group 4, the difference was not statistically significant.

Mean levels of blood ketones during the last eight weeks of pregnancy are shown in Fig. 5·4a and Fig. 5·4b. This parameter was used at weekly intervals to monitor the nutritional status of ewes in the different nutritional groups. As early as the fifth week before lambing, ewes in group 1, which were kept on the lowest level of feeding, started showing blood ketone levels as high as 5·5 mg per cent. These levels increased gradually and a severe degree of hyperketonaemia was shown towards lambing. A mean blood ketone level of 13·8 mg per cent was observed in twin-bearing ewes in the last week of pregnancy. In Fig. 5·4a (group 1) the values for the last week of pregnancy failed to rise because two ewes with high ketone levels had lambed and were not sampled. None of the other three groups showed mean blood ketone values above 5 mg per cent except on one occasion where ewes in group 2 had blood ketone levels between 5·0 and 5·6 mg per cent during the last two weeks of pregnancy. Although groups 2, 3 and 4 did not show great variation in blood ketone levels, group 4 remained the lowest, throughout most of the period of observation.

Overall ewe performance:

This is summarised in Table 5·21. In this table two criteria were considered. These were (1) PLM levels and (2) ewe body weight loss or gain during pregnancy.

FIG. 5·4a

Ewe's plasma ketone levels during late pregnancy (all litters included).

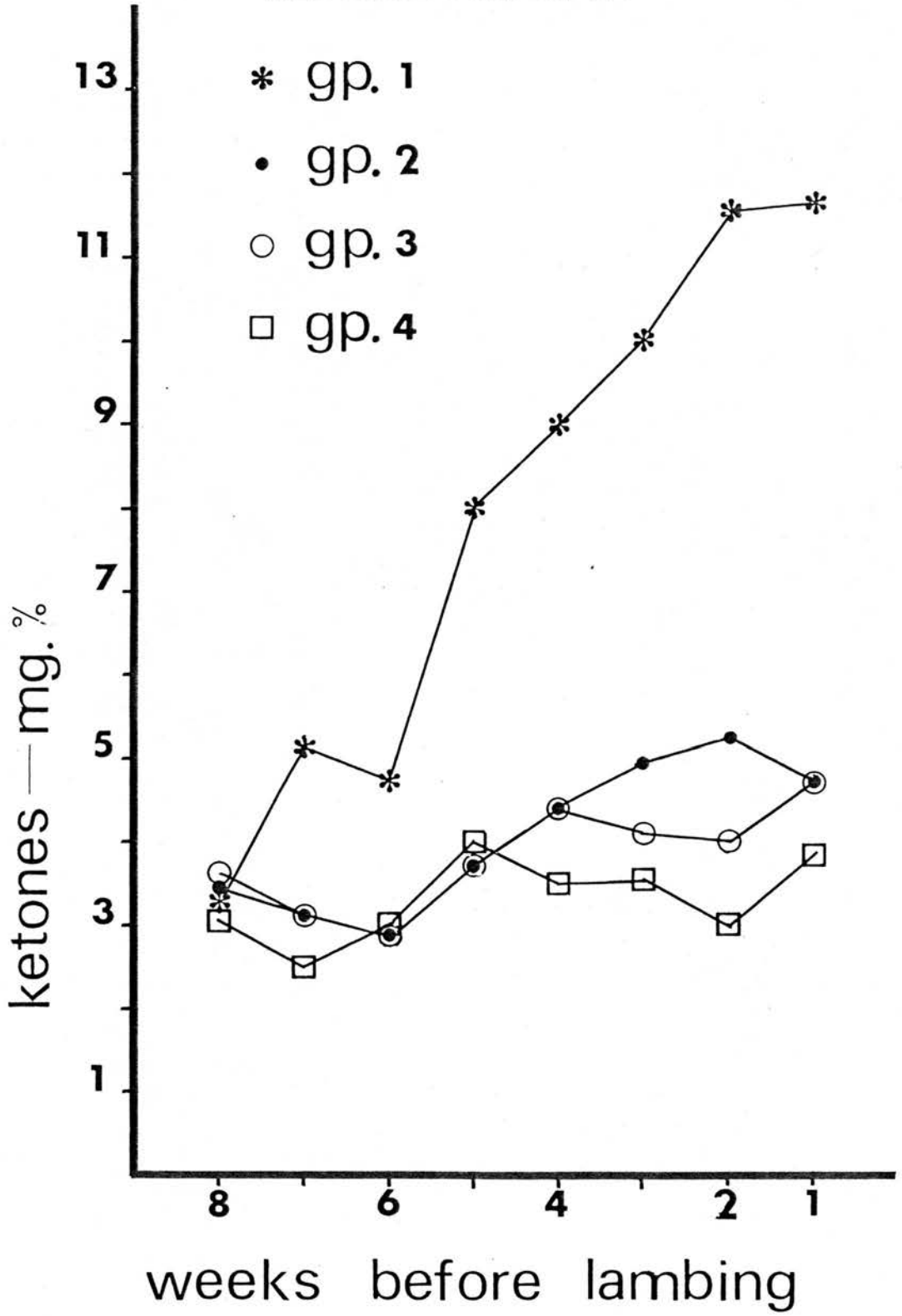


FIG. 5·4b

Ewe's plasma ketone levels during late pregnancy (ewes with twins only).

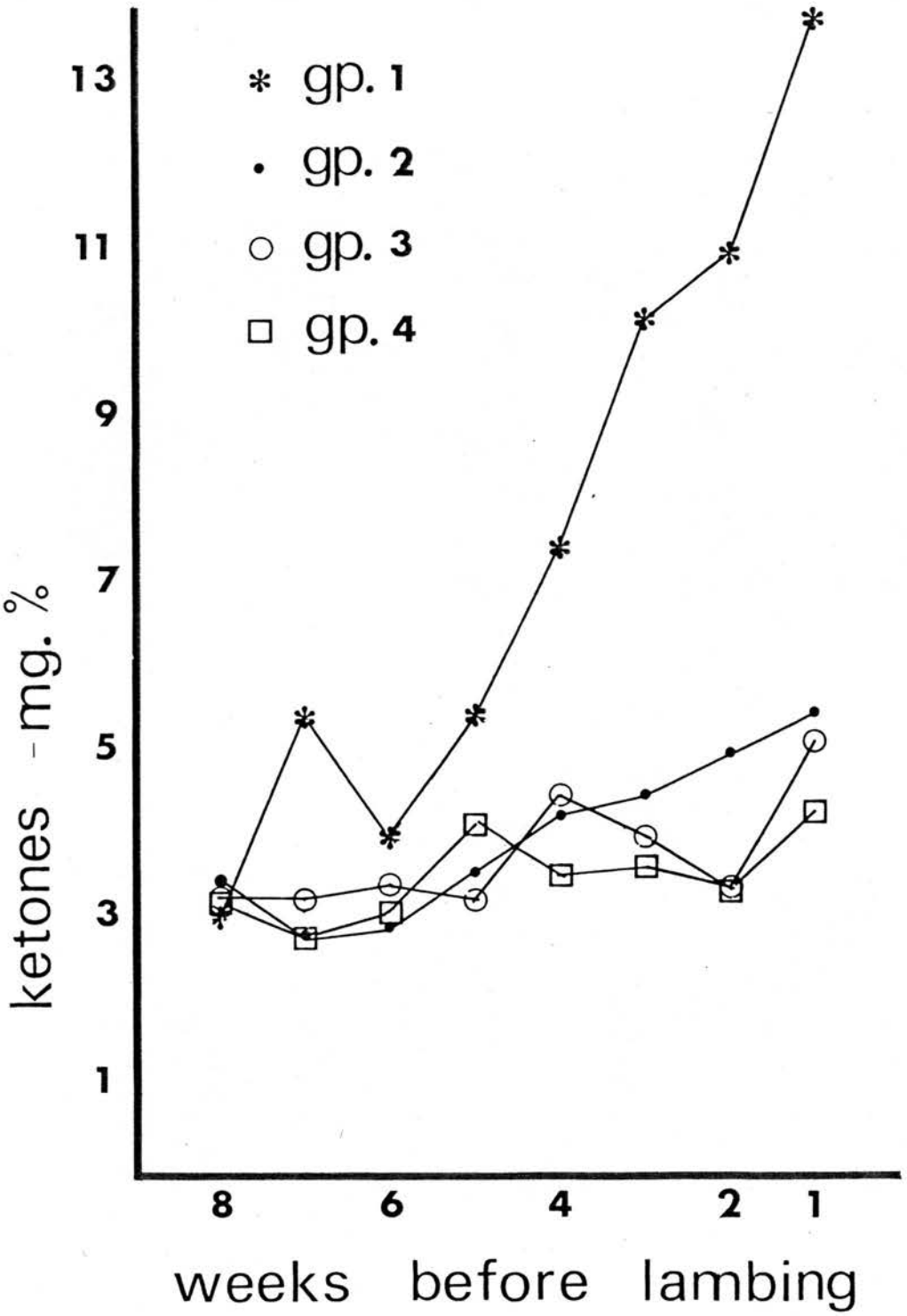


TABLE 5·21.

Nutrition and ewe performance

Nutritional group	Average ewe weight loss or gain (kg) between mating and lambing				No. of lambs born	PLM (%)	No. of aborted or stillborn lambs	No. of postnatal deaths
	All ewes		ewes with twins					
	No.	$\bar{x}$ ± s.d.	No.	$\bar{x}$ ± s.d.				
Group 1	16	-11·94 ± 6·90	11	-9·04 ± 7·10	31 (2 S,20 T & 9 Tr)*	48·3	11	4
Group 2	15	-5·80 ± 3·58	10	-5·60 ± 3·74	31 (2 S,20 T & 9 Tr)	6·45	—	2
Group 3	16	-3·25 ± 5·47	8	-2·11 ± 4·88	34 (3 S,16 T & 15 Tr)	11·76	3	1
Group 4	12	-0·25 ± 9·20	7	+0·35 ± 11·48	23 (3 S,14 T & 6 Tr)	13·00**	3	—

* S, T & Tr = Single, twin and triplet born lambs.

** = This percentage is represented by three lambs that were aborted by one ewe, 19 days before the expected day of lambing. Cause of abortion was unknown.

PLM:

In the different nutritional groups, the percentage of ewes that lost one or more lambs was 62·5, 6·6, 18·7 and 8·3 for groups 1, 2, 3 and 4 respectively. None of the four groups lost any of their single or twin born lambs with the exception of group 1 which lost 36·3 per cent of its twin born lambs. For triplets the picture was different - 77·3, 11, 26·6 and 50 per cent of the triplet born lambs were lost to groups 1, 2, 3 and 4 respectively. The unusual figure (50 per cent) for group 4 is represented by an aborted set of triplets (see Table 5·21).

The overall PLM level was significantly higher in group 1 than in any of the remaining three groups. This was confirmed by the chi-square (X^2) test which showed that the four groups are not homogeneous ($P < 0·001$) as a result of high lamb losses in group 1. When this group was excluded, the value for X^2 was not significant ($X^2_2 = 0·761$).

Ewe body weight loss:

Between mating and lambing, ewes in group 1 lost significantly more body weight than ewes in group 2 ($P < 0·01$), group 3 ($P < 0·001$) or group 4 ($P < 0·001$) when all ewes, irrespective of their litter size were included in the comparison (Table 5·21).

When this calculation was performed on ewes with twins only, almost the same picture emerged, i.e. ewes in group 1 lost more body weight than ewes in other groups. [The difference was statistically significant ($P < 0{\cdot}05$) between groups 1 and 3 and also 1 and 4, but not between 1 and 2 ('t' = 1·367, d.f. = 19)].

Although mean ewe body weight loss (whether calculated for all ewes, or ewes with twins only) tended to decrease with the increase in levels of energy intake, in groups 2, 3 and 4 only one of six comparisons conducted on ewe body weight loss gave a statistically significant difference. In this particular case, the difference in body weight loss between ewes in group 2 and those in group 4 was statistically significant ($P < 0{\cdot}05$).

A comparison between ewe body weight loss during pregnancy and lamb survivability was, in most cases, impossible because there were insufficient numbers of dead lambs available in the different nutritional groups. However, this type of comparison was possible in the following exceptional examples as a result of the high death rate among lambs born to group 1. In this particular nutritional group six ewes that lost one or more of their twin born lambs lost an average of 11·7 kg compared

to 8·0 kg lost by three ewes which had live sets of twins. Even this 8 kg loss is much greater than that observed in the corresponding ewes in the other nutritional groups, e.g. eight ewes with surviving twins in group 3 lost an average of only 1·5 kg during pregnancy. Three ewes (from group 1) which lost some of their triplet born lambs, showed an average body weight loss of 18·6 kg compared to only 8·5 kg lost by three corresponding ewes in group 3. In all groups where figures for comparison were available there was a tendency for ewes with triplets to lose more weight than ewes carrying twins.

Level of nutrition and lamb birth weight:

Birth weight of lambs born to the different nutritional groups and calculated on a litter size basis is presented in Table 5·22.

TABLE 5.22.

Lamb birth weight* (kg)

Litter size	Nutritional groups			
	Group 1	Group 2	Group 3	Group 4
Singles	(2) 5·10 ± 0·14	(2) 6·10 ± 0·35	(3) 6·01 ± 0·25	(3) 5·98±1·30
Twins	(20) 3·69 ± 0·71	(20) 4·63 ± 0·68	(16) 4·52 ± 0·63	(14) 5·18±0·73
Triplets	(9) 2·82 ± 0·74	(9) 3·43 ± 0·90	(15) 3·46 ± 0·88	(6) 3·55±1·09

* Values are presented as mean ± standard deviation.
Number of lambs in brackets.

Lambs born to group 1 tended to be lighter than those born to the other three groups but the difference was not significant statistically, with the following exceptions. In the case of twins, those born to group 1 ewes were significantly lighter than those born in any of the other three groups ($P < 0{\cdot}001$). Twins born to group 4 were significantly heavier than twins born to group 2 ($P < 0{\cdot}05$) or to group 3 ($P < 0{\cdot}02$).

Biochemical parameters for lambs:

As previously stated, some parameters were used for screening purposes during the work conducted in 1974. The levels of these parameters for lambs at birth and at 24 hours of age, were calculated on a nutritional and also on a litter size basis. No attempt will be made to list figures for all parameters here, as some of them did not show any important variation attributable to the factors considered. However, some examples will be presented to support any interesting findings and also to give information about the levels of the different parameters analysed.

The high level of lamb losses in group 1, mostly stillborn lambs or lambs which died soon after birth meant that I was unable to sample all the lambs at birth or at 24 hours of age. This of course affected the comparison results by reducing the number of lambs available.

Glucose:

Levels of blood glucose at birth for lambs of different litter size in the different nutritional groups varied inconclusively. Twins, for example, born to nutritional groups 1, 2, 3 and 4 had the following values respectively: 38·47 ± 12·71, 49·03 ± 27·98, 35·93 ± 12.41 and 46·32 ± 19·30 mg per 100 ml.

Total protein at birth:

The levels of total serum protein did not differ significantly among lambs as a result of litter size or nutritional status. The mean levels for singles, twins and triplets in the various nutritional groups varied from 4·28 to 4·88, 4·15 to 4·48 and 3·92 to 4·61 g per 100 ml respectively.

Total protein and total gamma-globulin at 24 hours of age:

Both of these parameters which increased greatly in the first day after birth, as a result of colostrum intake, showed the following trends:-

(1) In most cases, both values were lower (but not significantly) in lambs born to ewes in group 1.

(2) In the four nutritional groups, the levels of these two parameters showed a clear trend in relation to litter size. The bigger the litter size, the lower the values of total protein or gamma-globulin.

Example:-

Nutritional group 1

Litter size	Singles	Twins	Triplets
Total protein*	(2) 7·62 ± 0·02	(13) 6·29 ± 1·00	(4) 5·28 ± 2·14
Gamma-globulin*	(2) 3·25 ± 1·00	(14) 2·27 ± 1·06	(4) 1·38 ± 1·13

Nutritional group 4

Litter size	Singles	Twins	Triplets
Total protein*	(3) 8·24 ± 0·37	(14) 6·81 ± 1·52	(3) 6·60 ± 0·20
Gamma-globulin*	3·92 ± 0·72	2·35 ± 1·26	2·33 ± 0·36

*The values (g/100 ml) were calculated as mean ± standard deviation.

Number of lambs is shown in brackets.

Comparing total protein and gamma-globulin levels at 24 hours of age for lambs which later died with those for surviving lambs, in the different nutritional groups was in many cases impossible because of the number of lambs available. In many instances this number was either nil or too small to allow statistical analysis. In the following

examples lambs which died later always showed lower serum total protein and gamma-globulin than the corresponding survivors.

At 24 hours of age, mean serum total protein for triplets which died later from different nutritional groups varied between 3·79 ± 0·67 to 4·70 ± 0·28 g per 100 ml compared to 5·78 ± 0·83 to 6·78 ± 2·09 g per 100 ml for corresponding survivors. The mean serum gamma-globulin levels for dead triplets were 0·58 ± 0·14 to 0·80 ± 0·14 g per 100 ml compared to 2·19 ± 1·08 to 2·57 ± 1·25 g per 100 ml shown by surviving triplets of the different nutritional groups.

Biochemical parameters for ewes:

Parameters before lambing represent the average of the last two samples collected before lambing (one sample collected around three weeks and the other around one week before lambing). Total protein and gamma-globulin were measured in ewes sera before and at lambing.

Glucose before lambing:

The differences in glucose levels of the four nutritional groups were not statistically significant. However, ewes on the poorest nutritional diet had a mean blood glucose level of 40·16 ± 6·89mg per 100 ml in comparison to 44·27 ± 4·32 mg per 100 ml shown by ewes on the highest level of nutrition (i.e. group 4).

Total protein and gamma-globulin:

Levels of these parameters (calculated as mean ± standard deviation) are presented in Table 5·23. When the serum values for ewes carrying the same litter size were compared, no statistically significant difference (or trend) was noticed between the different nutritional groups. Within the same nutritional group, ewes serum values were compared to each other according to the litter size they produced (Table 5·23). Although none of the comparisons showed statistically significant differences, there was a noticeable trend in most of the values presented where ewes producing large litters tended to have lower serum total protein and gamma-globulin values than those producing small litters.

When parameters before and at lambing were compared it was noticed that in almost all the nutritional groups there was a tendency for ewe's serum total protein and gamma-globulin to be lower at lambing than before lambing. Some of these comparisons, particularly those in which sufficient ewes were available, showed a statistically significant difference (see Table 5·23).

TABLE 5·23.

Ewe's serum total protein and gamma-globulin (g/100 ml)

Nutritional groups:				Group 1			Group 2			Group 3			Group 4		
Litter size of ewes*:				S.	T.	Tr.	S.	T.	Tr.	S.	T.	Tr.	S.	T.	Tr.
Serum values	before lambing	Total protein	m.**	7·60	6·79	6·25	6·92	6·53	6·58	7·34	6·90	6·59	6·86	6·94	7·27
			s.d.	0·47	0·56	0·48	0·23	0·37	0·20	1·01	0·44	0·53	0·41	0·91	—
			n.	2	10	3	2	10	3	3	8	5	3	7	1
		Gamma-globulin	m.	2·56	2·15	1·89	2·15	1·95†	1·96	2·56†	2·97	2·03	2·22	1·97†	1·56
			s.d.	0·19	0·36	0·31	0·21	0·28	0·40	0·55	0·50	0·21	0·80	0·37	—
			n.	2	10	3	2	10	3	3	8	5	3	7	1
	at lambing	Total protein	m.	6·70	6·45	6·08	6·25	6·20	6·14	6·72	6·40	6·23	6·31	6·54	5·96
			s.d.	0·16	0·66	0·50	0·43	0·62	0·42	0·32	0·80	0·51	0·67	0·54	0·58
			n.	2	10	3	2	10	3	3	7	3	3	7	2
		Gamma-globulin	m.	2·27	1·66	1·60	1·50	1·57†	1·61	1·65†	1·71	1·59	1·50	1·53†	1·76
			s.d.	0·71	0·65	0·63	0·11	0·37	0·50	0·08	0·50	0·37	0·80	0·36	0·51
			n.	2	10	3	2	10	3	3	7	3	3	7	2

* S, T and Tr stands for Singles, Twins and Triplets respectively.

**m = mean; s.d. = standard deviation; n. = number of ewes.

† Student 't' test comparison between gamma-globulin levels before and at lambing showed statistically significant difference at $P < 0·05$ for groups 2 and 4, and at $P < 0·02$ for group 3.

DISCUSSION

Before the preliminary experiments started, I already knew from the literature that lamb birth weight was likely to be an important factor affecting mortality. If the sex of the lamb also affected birth weight then serious difficulties could arise in the interpretation of later results and in the planning of experiments. If male and female lambs had to be considered separately, the number of animals required to obtain statistically valid comparisons would exceed our resources.

Of the authors previously cited who indicated that male lambs were heavier than female lambs, only two (Moule, 1954; Mullaney, 1969) showed that the differences were statistically significant in the majority of instances and they were using Australian breeds under Australian conditions of management. While presenting his data concerning sex and birth weight of lambs, Moule (1954) did not take into consideration the effect of litter size and this could have been a source of bias. My results agreed with the majority of other workers, showing that males tended to be heavier than females but that in most instances the differences were statistically not significant. In these circumstances, it is unlikely that in future work, where mixed groups of male and female lambs are being compared, sex will have any marked

effect on the results especially as the allocation of lambs to the different treatments will be undertaken in a random manner. Consequently, sex need not be considered in later comparisons unless there is a marked discrepancy in sex representation between groups.

The second very important factor affecting birth weight is litter size and all the authors cited earlier, irrespective of the number of lambs involved in their experiments, showed that litter size had a statistically significant effect on lamb birth weight and that the two are inversely related. My results agreed with this conclusion and all future comparisons must clearly be made on the basis of litter size. This fact means that experimental numbers must in future be as high as our resources allow if valid results are to be obtained.

Regarding breed of the ewe and its effect on lamb birth weight, lambs born to Finn Dorset ewes were significantly lighter than lambs born to the other two breeds (Scottish Halfbred and Greyface) which did not differ significantly from each other.

The similarities and variations in lamb birth weight of different breeds of ewes were noticed also by other workers who used different breeds of ewes from those included in my study. Purser and Young (1959) reported an average birth weight of 7·16 and 6·49 kg for lambs born to Scottish Blackface and Welsh Mountain

respectively. Weiner (1967), while investigating the performance of five British breeds (Scottish Blackface, South Country Cheviot, Welsh Mountain, Lincoln Longwool and Southdown), found that singles and twins born to the Lincoln Longwool were the heaviest while twins born to the Welsh Mountain were the lightest. Apart from these two exceptions, lambs of similar litter size showed similar mean birth weight. Mullaney (1969) found that average birth weight of lambs born to Polwarth, Merino and Corriedale breeds was 7·3, 7·9 and 8·8 lb respectively. Donald and Russel (1970) analysed data for 13 breeds and reported that lamb birth weight varied from 2 kg and 1·75 kg for the Soay singles and twins respectively, to 6·9 kg and 5·3 kg for the heavy Lincoln singles and twins respectively. Their findings were based on data extracted from available flock records where in some cases lambs might have been at least a day old before the farmer had time to weigh them, while others could have been new-born. In the breeds I observed, all lambs were weighed immediately after the mothers had cleaned them. This was possible due to day and night observations at lambing.

Breed does affect lamb birth weight and the Finn Dorset cross lambs born in my study are lighter than those from the other ewes. Consequently, in order to minimise any breed variations, Finn Dorset ewes will be

omitted from future studies.

Concerning the subject of lamb survivability, my own observations showed that the most important factor limiting it is reduced birth weight and that the majority of lambs with low birth weights are usually born to big litters. These small lambs were either born dead, or died of starvation due to various reasons shortly after birth. On the other hand, the maximum survival rate in all lambs observed occurred at a birth weight of around 4 kg or more.

Insufficient numbers of surviving and non-surviving lambs (particularly of the latter) was a limiting factor when I performed statistical comparisons on lamb birth weight. Whenever the numbers were sufficient, the result of the comparisons showed that dead lambs were significantly smaller than corresponding survivors. This observation is in agreement with other authors although they used different breeds of sheep under different circumstances in various parts of the world (relevant references are listed in Chapter II).

One of the main factors causing a reduction in lamb birth weight is the increase in ewe prolificacy. The best example for ewes with high litter size among the groups I observed are the Finn Dorsets. During my work some of these Finn Dorset ewes gave birth to four lambs or more per litter. Some of their lambs weighed less

than 1 kg at birth. Lambs of this birth weight stood little chance of surviving due to weakness and the competition they faced from their heavier sibs for an adequate milk supply.

Robinson (1974), who observed the performance of 96 Finn Dorset ewes kept under ideal and costly experimental circumstances, reported a mortality rate of only 7·6 per cent. However, the average litter size reported by Robinson in this particular observation never exceeded 2·07 and the lambs born were heavier than the lambs born in my experiment. (He reported mean birth weight of about 4·7, 4·0 and 3·1 kg for singles, twins and triplets respectively.) Halliday (1976) in praising Finn Dorset lambs for their suckling performance, vigour and survivability did not specify the exact litter size he was working with although other data he presented could indicate a low figure. In one case where he used more than 400 lambs, only 45 of them were triplets and all the rest were singles or twins. Under these circumstances, it is not surprising that he observed a 7·2 per cent lamb mortality only. Both of the above mentioned authors performed their work in Scotland.

While it may be desirable to attempt to save the lives of as many lambs as possible, irrespective of how small they may be, most of the effort, in my opinion, should be directed towards breeding policies and ewe

management which lead to the production of lambs of optimum birth weight and vitality.

Some of the authors who discussed the relationship of birth weight and level of lamb mortality found that heavy lambs (usually singles) can suffer a high level of mortality, presumably due to dystocia (Watson and Elder, 1961; Gunn and Robinson, 1963; Hight and Jury, 1969; Singh and Singh, 1970; Harker, 1973, 1977). This picture is related to the level of management in general, and to the degree of shepherding at lambing in particular. The four groups of ewes included in my study were under continuous lambing surveillance and while 14 per cent of the ewes needed lambing assistance of different levels, only one case of dystocia, caused by the relatively large size of a single lamb, resulted in an unsuccessful lambing. Oversize dystocia can largely be prevented by good shepherding.

Another factor that seems to affect the level of lamb mortality is age of the ewe. Most authors who have studied PLM in relation to age and parity of the ewe tend to agree that PLM is higher among lambs of young ($\lhd 3$ years old) and aged ($\rhd 6$ years old) ewes than among those of intermediate age. This was the case among the Scottish Halfbred and Greyface ewes included in my study where both breeds had an average litter size of 2·1. In the Finn Dorset ewes, on the other hand, (with litter

size of 2·5) the ewe age effect was masked by the very high levels of lamb mortality. The only two age groups in this breed (⩽3 years and 3 to 5 years) both suffered equally high lamb losses.

As dystocia was not an important cause of lamb losses in the groups observed, the high losses reported for young ewes (⩽2 years old) could be related to a combination of many factors such as high litter size, small and weak lambs at birth, lack of mothering experience, and most likely due to low colostrum or milk production. The noticeably high losses among lambs born to 5 to 7 years old Scottish Halfbred and Greyface ewes were due to starvation, precipitated by the ewes' poor dental state ('broken mouth') and consequent feed intake and low milk production. Poor functioning udders mainly caused by chronic fibrosis and damaged teats can not be excluded as causes of PLM although in my study they accounted for only a small proportion of deaths.

The very high mortality rate among lambs born to all Finn Dorset I ewes was principally due to abortions or stillbirths. These ewes had not been on the farm long at this time and although the cause of death was not established in many cases, a random collection of blood samples at the end of the lambing season revealed a 15 per cent incidence of significantly high Toxoplasma haemagglutination titres. It seems probable that the

Finn Dorset ewes had less resistance than other ewes and were consequently badly affected by this organism. It is assumed that Toxoplasma caused intra-uterine deaths and reduced lamb viability which overrode the age effects seen in the other ewe breeds.

There is a relationship between gestation length and PLM. A shortening of the gestation period, even when it was marginal, seems to affect lamb survival. Important non-infectious predisposing factors which may shorten gestation length are litter size and the level of ewe nutrition (Thomson and Thomson, 1949; Alexander, 1956; Dawes and Parry, 1965).

The significantly shorter gestation period for ewes with stillbirths (10 per cent of all ewes observed) was represented by a 3·5 days difference from that calculated for ewes with surviving lambs only. Bearing this figure in mind, and as most of the stillborn lambs were well developed, the corticosteroid based methods of initiating parturition in ewes (Bassett and Thorburn, 1969) applied by Emady et al. (1974) and Shevah (1974) must be treated with caution as they involve a deliberate reduction in ewe gestation length that might result in high lamb losses (Halliday and Buttle, 1968). This will be particularly important when litter size is high.

As expected, the ewe that lost her big single lamb, as a direct result of dystocia, had a significantly longer

gestation period than the mean for her group. However, it is not possible to draw a firm conclusion about the relationship of gestation length, dystocia and lamb mortality from observations involving one ewe only, especially when there were many successful deliveries of large lambs (over 6 kg).

In the group of ewes I observed, there was a high percentage of ewes with no milk or little milk at lambing. Despite this, very few of them lacked a good mothering instinct but in cases where it did occur it was due to the failure of weak lambs to stimulate the ewe as has also been noted by Bareham (1976). The ewe's milking ability would appear to be more important in relation to lamb survival than mothering instinct as long as this is reasonably adequate. It is important to distinguish between these two factors.

During the investigation, the overall PLM levels remained very high. These levels varied greatly from one group of sheep to another and this was due to multiple factors associated particularly with litter size. As shown by most authors, half of the deaths occur between birth and 10 days of age as a result of starvation/E. coli infection. Immature or stillborn lambs form another important category.

Although the overall PLM among lambs I observed in 1974 can be described as very high (99 lambs representing 29·6

per cent of total lambs born), these levels are still within the range reported by other workers in Britain or other parts of the world (see Chapter I). This wastage can only be considered as very costly for the sheep industry.

The importance of lamb losses of this magnitude becomes clearer if related to the national flock of sheep. In Britain, there are about 12 million breeding ewes representing all sheep breeds (MLC, 1972). If only half of these ewes conceive and assuming that a litter size of 1·5 lamb per ewe will be achieved, a mortality of that level will mean the loss of about three million lambs. Each of these lambs, if reared successfully, will contribute to the total output by about £10 (Thomas, 1971; Barton and Blyth, 1977). Even if PLM cannot be avoided, halving the rate of loss would result in a saving of the order of £15 m. This sum would cover at least one-third of the total variable costs of all six million in-lamb ewes, considering that the total variable cost is about £7·70 per ewe (Barton and Blyth, 1977).

In the preliminary 1974 investigations, I estimated levels of PCV, glucose, total protein and immunoglobulins in newborn lambs, both at birth and 24 hours of age, to obtain quantitative values and to ascertain if they were related to PLM. Breed of ewe seems to have no effect on

them, i.e. values for lambs born to different ewe breeds were very similar. Consequently, data for all lambs were pooled so that a reasonable number of observations was obtained for surviving lambs. Despite this pooling of results, the number of analyses done on lambs which died remained small.

All the parameters showed rapid and very significant changes early in life as a result of colostrum ingestion and the lamb's new environment. A noticeable degree of variation was observed in relation to (1) litter size of lambs and (2) survivability. Of the four parameters included, most of the references mention total protein and gamma-globulin. A few refer to PCV or glucose values in lambs while studying topics that had no relation to the subject of lamb mortality.

The authors concerned used very small numbers of animals and they were working under pure experimental circumstances whereas, in my study, a relatively high number of lambs and ewes was used and the study was performed under field conditions. According to Comline and Silver (1972) a large increase in PCV (from 35 per cent to 40 per cent) occurred only 15 minutes before delivery and the levels fell back to about 35 per cent within 30 minutes after delivery. They and other workers assumed that these striking changes in the blood of lambs a few minutes after delivery occur as a result

of stimulation of the sympathetic system.

Upcott, Herbert and Robins (1971) reported PCV values of 36·4 ± 0·7 per cent for one-day-old normal lambs. Similar values were reported for two to three week old lambs (Gardner, 1973). McCance and Widdowson (1959) reported, in piglets, a fall in the haematocrit values associated with expansion of plasma volume following colostrum ingestion. Similar observations were made in calves as a result of colostrum or milk ingestion (McEwan, Fisher and Selman, 1968). In my study, very high PCV levels were recorded in lambs immediately after birth. These levels were affected by litter size and viability of lambs which were two important factors in deciding the amount of colostrum ingested. The marked reduction in the 24 hour PCV values of all surviving lambs as compared to birth values was more obvious in singles than triplets. This was presumably due to the fact that three small triplets for example, share nearly the same amount of colostrum as one single lamb has available. This was confirmed by the failure of survivors, born to large litters and also by non-surviving lambs of any litter, to show the striking reduction in PCV values at 24 hours of age. This failure could be related to one or more of the following factors:

1. Nutritional and maternal factors that resulted in low colostrum production.

2. Failure of lambs to ingest available colostrum.
3. Failure of the lamb's gut to absorb ingested colostrum.

It is possible that some of the dead lambs which still demonstrated high 24 hour PCV values suffered a critical state of dehydration. All these starvation factors seem to affect the ratio of the cellular constituents of the blood to that of the plasma volume.

With regard to blood glucose in lambs, Alexander and Peterson (1961) reported levels of 20 mg per 100 ml for 13 lambs just before death. These lambs had survived at least 15 hours after birth. Comline and Silver (1972) reported a marked but temporary glucose rise (80 mg per 100 ml) in lambs at delivery. The glucose values dropped to 40 mg per 100 ml just one hour after birth before it started to rise again following colostrum ingestion. Alexander, Bell and Hales (1972), who were interested in the physiology of the newborn, showed that by exposing newborn lambs to cold conditions that evoked a maximum response to cold, the plasma glucose levels increased from 100 to 200 mg per 100 ml within less than two hours of exposure.

In my study, the very high glucose levels measured for surviving lambs can only reflect the role of colostrum as a good source of energy, and also the efficiency

with which these lambs utilized it. In non-surviving lambs, glucose levels, although never as high as those of survivors, were by no means low. Cold exposure cannot account for the sharp increase in 24 hour glucose values as all lambs were kept under shelter for the first two or three days of life and environmental temperatures never reached those described by Alexander et al. (1972). Although the non-surviving lambs had lower 24 hour glucose values than corresponding survivors, these values never reached the low levels reported to dead lambs by Alexander and Peterson (1961). This is simply because these authors measured blood sugar very shortly before death while in my observations, the parameters were estimated at 24 hours of age at which time some lambs were still healthy and showed high glucose levels. However, lambs which died within one hour of sampling invariably had low glucose levels (<30 mg per 100 ml). PCV and blood glucose seem to give some indication of the early performance of newborn lambs and reflect their chances of survival. Type of litter, amount of colostrum produced by the ewe, and the ability of the lamb to suck available colostrum seem important related factors. It seems worthwhile to study both parameters in at least one later study to establish if they are useful indicators of performance.

In the last few years great emphasis has been put on the state of disease resistance of newborn lambs, as measured by their serum total protein, or more specifically, their serum immunoglobulin levels. During the neonatal period, lambs, like other newborn ruminants, are protected by the antibody provided by their mother through colostrum (passive immunity). My interest was to see how much this immunological status is affected by factors like breed of ewe and litter size, and the relation of that to lamb survival.

At birth, the serum total protein of lambs was reported by many authors to be very low and only traces of gamma-globulin were detected. My observations agreed with this and showed gamma-globulin levels too low at birth to be of any protective significance. These values showed striking increases following colostrum ingestion (McCarthy and McDougall, 1953; Kekwick, 1959; Georgiev, 1969; Knight and Leek, 1973; Healy and Falk, 1974; Larson et al., 1974). These levels did not differ in lambs born to the different breeds of ewes included in my study. Halliday (1968b, 1973), the only worker cited to report on this matter, also found no breed effect on lamb serum gamma-globulin levels at two to three days of age, as measured by electrophoresis. The breeds included in Halliday's study and observed in Scotland, included Scottish Blackface, Merino, Merino cross Cheviot, Soay,

Oxford-down and Southdown. The same author (1970, 1971a, 1976) claimed that lambs, pure or crosses, born to Finnish Landrace ewes showed superiority to other breeds in that they have higher serum gamma-globulin at one or two days of age. In my study, lambs born to Finn Dorset ewes (the nearest I have to pure Finnish Landrace) were in no case superior, at 24 hours of age, to those born to the other two breeds.

Regarding litter size, which in my opinion is very important in deciding the degree of sheep productive performance in general and that of the newborn lambs in particular, data presented by Ducker and Fraser (1976) showed that Greyface singles and twins did not differ as far as colostrum absorption was concerned (reflected by their serum gamma-globulin levels). This is not wholly surprising as Greyface twins are usually strong at birth and compare favourably with singles in regard to sucking ability. In my study, however, singles had an advantage over all other litters including twins. I ascribe this to the singles not having to compete for the available colostrum. Halliday (1970, 1971a, 1971b, 1976) presented conflicting results concerning litter size and lamb serum gamma-globulin. He singled out the pure Finnish Landrace and Finn Dorset lambs from the many other breeds he observed and stated that their serum gamma-globulin levels were high regardless of litter size.

In one of his investigations, Halliday (1976) presented mean serum gamma-globulin values of 3·05 g per 100 ml for quadruplets as compared to mean values of 2·95 g per 100 ml measured separately for singles, twins or triplets. He did not give details for levels of lamb losses in different litter sizes, or of the system under which these newborn lambs were managed, i.e. the involvement of fostering and colostrum feeding by bottles. Ciupercescu (1977) who briefly stated that he found no variation in serum immunoglobulins of Finn Dorset single, twin or triplet lambs, quoted no figures to support this statement.

The few workers who referred to the effect of large litter size on the serum gamma-globulin levels did not state if lambs from big litters were all healthy at the time of bleeding or died at a later date. An important possibility that must not be overlooked is that lambs born as triplets or quadruplets were suckled in pairs shortly after birth, because some of their sibs were either born dead or died immediately after birth. [This was the case in my work]. To prevent confusion, I have presented results for surviving and dying lambs separately. My data show a clear inverse trend for gamma-globulin levels in relation to litter size. One must distinguish between the number of lambs born in a litter and the number born alive and which suck, i.e. the

effective litter size. In general, lambs born in litter sizes of three or more are at disadvantage both in terms of survivability and serum antibody levels.

In relation to neonatal mortality, and as reported by the few other workers (Halliday, 1965b, 1976; Reid, 1972; Findlay, 1973; Harker, 1974) who share my interest in PLM, serum gamma-globulin levels and total protein were lower in lambs which died. The levels of total protein are, presumably, a reflection of the gamma-globulin levels as these show the biggest changes following colostrum ingestion. This was particularly the case when dead lambs were from large litters. Low serum protein and gamma-globulin levels may result from various factors which can affect the efficiency of colostrum intake. Because of the diversity of PLM causes and the relatively low number of screened dead lambs, it is very difficult to describe a clear relationship between lamb mortality and these parameters. However, the general impression suggests that most of the dead lambs have low serum antibody levels mainly due to starvation in the neonatal period.

Turning to specific immunoglobulins, i.e. IgG_1, IgG_2, IgM and IgA, in newborn lambs and the factors which may affect the circulating levels, a search through the literature failed to reveal data comparable to the results

I am presenting and, consequently, my preliminary work stands in isolation.

At 24 hours of age, the levels of lamb serum immunoglobulins (of colostral origin) reach their peaks (McCarthy and McDougall, 1953; Tumbleson, Littleton, Ticer, Komer and Bloomfield, 1968; Healy and Falk, 1974; Larson et al., 1974) then gradually decrease during the first few weeks of life until lambs start producing their endogenous immunoglobulins (Pearson and Brandon, 1976; Ciupercescu, 1977). My results concerning quantitation of the different immunoglobulins as measured by the SRID method, a specific and quantitative one for this purpose, showed that IgG_1 formed the major part of immunoglobulins in lambs at 24 hours of age. This is probably due to it being the main immunoglobulin in colostrum (Heimer, Jones and Maurer, 1969; Sullivan et al., 1969; Pearson and Brandon, 1976) as a result of it being efficiently secreted from ewe's serum into colostrum (MacKenzie and Lascelles, 1968; Watson, Brandon and Lascelles, 1972; Cripps and Lascelles, 1974).

The other major immunoglobulin in the sera of these lambs, at this critical stage, was IgM although its levels were only one-fifth of the IgG_1 levels. IgM might play an important role in protecting the newborn lamb against E. coli infection in a manner similar to that observed in

calves (Logan and Penhale, 1971; Logan, Stenhouse, Penhale and Armishaw, 1974).

Although in some individual cases the IgG_2 levels were nil, this immunoglobulin was available in detectable traces in the sera of the majority of lambs. These scarcely detectable levels of IgG_2 were recently observed by other workers (Varela-Diaz and Soulsby, 1972; Pearson and Brandon, 1976; Ciupercescu, 1977).

My results concerning IgG_1, IgG_2 and IgM were in general agreement with those reported by Ciupercescu (1977) who did not include IgA in his study. According to my observations, IgA is present in low levels in the sera of 24 hour old lambs.

All immunoglobulins showed a good deal of individual variation reflecting the multiple factors affecting their levels. The data also showed that the effects of factors like litter size and lamb vigour on levels of the specific immunoglobulins, IgG_1, IgG_2 and IgM, were similar to those on total gamma-globulins, i.e. the values were lower in lambs born to large litters and in dead lambs of any litter size. This might have been related to the amount of these immunoglobulins available in colostrum, the lambs ability to suck, and the amount of immunoglobulin absorbed through the intestine. Changes in IgA levels in relation to litter size and survivability, on the other hand, were inconclusive.

For example, at the time when dead twins showed lower IgA levels than surviving twins, the opposite was noticed in the case of triplets. It is possible that some of the dead triplets experienced an intra-uterine infection that caused this increase in their IgA levels.

It is difficult to draw a definite conclusion from the data discussed above but there may well be a link worthy of further investigation between circulating immunoglobulin levels, colostrum quality, lamb behaviour and disease.

So far, the discussion has covered the multiple factors that affect levels of lamb losses and also the biochemical parameters used in this relation. In my opinion, this discussion will not be complete without referring to the subsequent performance of the surviving lambs (i.e. their body growth) as affected by various factors.

My investigations indicate that breed of the ewe can affect growth rate of lambs. This finding is supported by recent observations made by Fogarty (1972) and Barker (1975). Both of these authors and also Robinson and Forbes (1968), Seth *et al.* (1972) and Robinson (1974) emphasised the effect of litter size on lamb growth rate. My results agreed with theirs in that lambs from multiple births (particularly from litters of three or more) weighed less at five weeks of age than most single born

lambs at that age. The poor performance of triplets and quadruplets was probably related to their low birth weight and low vigour at birth (Wallace, 1948a; Pout, 1973) and also to the level of ewe nutrition both during pregnancy and lactation, and its direct effect on milk production by the ewe (Robinson and Forbes, 1968; Pout, 1973; Singh, Tiwari, Singh and Honmode, 1973; Louca et al., 1974; Robinson and Ørskov, 1975). The effect of different types of ewe nutrition and climatic factors on lamb growth rate was clearly reflected by the variation in performance of lambs, included in my study, which were born during different lambing seasons. Grass shortage in the winter and cold weather and snowfall were associated with slow growth of lambs from the Finn Dorset 2 and Greyface groups of ewes, as compared to those born at Easter (Finn Dorset 1 and Scottish Halfbred) where milder weather conditions prevailed and good quality grass was available (see Table 5·17).

Robinson and Ørskov (1975) suggested what they call a "realistic target" of 400 g per day growth rate to be achieved by lambs during the first four to five weeks after birth. During my investigations, even singles from the best performing groups of ewe (Scottish Halfbred) did not succeed in beating this target. However, it must be pointed out that some groups of lambs I observed,

particularly singles and twins, showed growth rate levels of 300 to 350 g per day during their first five weeks after birth. This is reasonably good especially if consideration is given to the growth rate figures of 174 to 273 g per day between birth and weaning reported by Fogarty (1972) in Australia for Merino and Merino crosses, and the figures of 160, 125 and 110 g per day representing growth rate during the first 30 days of life of singles, twins and triplets respectively, reported by Seth et al. (1972) for lambs born to Indian breeds. Similar levels of growth rate to those reported by these authors were obtained for most of the triplets and quadruplets (all reared as pairs by their mothers) included in my study. The poor performance of lambs from large litters was exacerbated in some cases when triplets were reared in milk bars, a practice which saves the life of lambs, but is uneconomical (Lees, 1971) due to the high cost of labour and milk substitutes.

The overall poor performance of lambs from large litters, taking into account especially their high levels of mortality in early life, must raise doubts as to the wisdom of breeding for large litters, which otherwise may appear a convenient way of improving the production potential of sheep. The few twins on the other hand, that suffered a temporary set-back (see Table 5.20) caused either by illness in the neonatal period or by delay of

milk 'let down' managed to keep pace with their contemporary normal twins without the expense of additional feeding. The relatively high birth weight of these twins seems a very important factor in this connection.

Among the many factors that can modify the reproductive potential of sheep, which undoubtedly depends on genotype to a great extent, is nutrition. Several workers have shown that late pregnancy is an important stage during which levels of feeding of the pregnant ewe is vital both for ewes and their rapidly developing lambs.

From the 1974 work, I tried to extract as much information as possible concerning late pregnancy level of feeding and its effect on ewe production performance. This data from different nutritional groups of Scottish Halfbreds covered:

a) calculation and monitoring of levels of energy intake supplied by different levels of feeding.
b) PLM levels and ewe body weight loss as affected by various factors.
c) Variation in lambs birth weight.
d) Changes in some biochemical parameters estimated in ewes and lambs.

Reports regarding the above mentioned factors are very scarce and, when they are available for British sheep, a good proportion of them are concerned with hill breeds such as the Scottish Blackface.

As I pointed out previously, ewes included in this study were not under my full control. There were 15 groups of four ewes and each group received different levels of energy intake in the last eight weeks of pregnancy according to the quality of hay used and the amount of concentrates or complete ruminant diet offered. Because these ewes were primarily taking part in a hay quality study conducted by other workers, I was unable to design the work to conform with the aims of my project. The number of ewes and lambs in each of the 15 groups was too small to extract sufficient information concerning the factors relating to PLM. Accordingly, I regrouped them according to energy intake levels into the four groups described in the results. This system is not ideal as ewes with comparable energy intakes derived that energy from different food, at different rates during the last eight weeks of pregnancy, but the method is considered suitable for a preliminary study into the effects of nutrition on PLM.

In comparison to the suggested levels of energy intake for pregnant ewes (Robinson, Fraser and Bennett, 1971; Rutter, Laird and Broadbent, 1971; MLC, 1973; Robinson, 1974; Robinson and Ørskov, 1975), it can be said that of the four nutritional groups included in my investigation, only group 1 ewes were actually under serious nutritional stress. Group 2 and, to a certain

extent, Group 3 ewes might have been slightly undernourished, while group 4 ewes seemed to be on optimum levels of feeding in the last eight weeks of pregnancy. This was also confirmed when the nutritional status of these groups of ewes were monitored using plasma ketone levels. If, as described by Russel et al. (1967a), Russel (1971) and Davies and Ross (1973), plasma ketone levels of 8 to 10 mg per 100 ml represent hyperketonaemia in severely undernourished ewes, then ewes in group 1 were actually severely undernourished as early as four to five weeks before lambing. Russel et al. (1967a) suggested that plasma ketone levels of 2 mg and 4 mg per 100 ml reflect adequate feeding and moderate undernourishment respectively. This was based on observations made on pregnant Scottish Blackface ewes where most of them usually carry single lambs. Foot, Russel, Maxwell and Morris (1973) concluded that the number of lambs in the uterus is the most important factor contributing to differences in plasma ketone levels in pregnant ewes. As it is more common to get multiple births (twins and triplets) from the low ground Scottish Halfbred ewes with an average litter size of about 2·0 and, after considering the performance of ewes in nutritional groups other than group 1, it can be concluded that the levels of 3 mg or even 4 mg per 100 ml shown by pregnant ewes in group 4 will occur with adequate levels of feeding. Similarly,

levels from 4 to 5·6 mg per 100 ml would describe moderate undernourishment in this particular breed.

Until recently, the effect of level of ewe feeding in late pregnancy on PLM has been inadequately dealt with by most of the workers concerned with sheep production. In my opinion, the relationship is a very important one as has been clearly shown in the present preliminary work.

Very low levels of feeding of pregnant ewes resulted in serious mortality levels in lambs. Earlier work of a farm or an experimental nature, by Thomson and Fraser (1939), Underwood and Shier (1942), Underwood et al. (1943), Thomson and Thomson (1949), Blaxter (1957) and Robinson (1974) supports this finding. It must be pointed out that these workers carried out their work on different breeds of sheep and under different circumstances where investigation of PLM was not the main aim in their studies. At the same time, there were workers who reported no effect for level of late pregnancy feeding of ewes on PLM. Among these workers are Coop (1950) and Darroch et al. (1950) whose conclusions were based on differences resulting from either supplementing or not supplementing grazing ewes with just less than 250 g of concentrates per ewe per day during late pregnancy. As shown by mean lamb birth weight and/or post-partum ewe body weight, it can be said that the two nutritional

treatments these workers were referring to are very similar as far as level of feeding in late pregnancy is concerned.

Guyer and Dyer (1954) and Shevah et al. (1975) indicated that in their work there was no relationship between PLM rate and plane of ewe nutrition at late pregnancy. This unusual result merits comment. The report of Shevah et al. (1975) can be interesting on the basis that four out of five of their nutritional groups were by no means undernourished, i.e. that no meaningful difference existed between them, and that the fifth group of ewes while showing elevated ketone levels (i.e. 4·2 per 100 ml during the last six weeks of pregnancy) had only a minor reduction in weight gain. In short, their plane of nutrition was only marginally low in comparison with those fed to the other groups.
Although Guyer and Dyer (1954) did not explain causes of higher losses among high nutrition groups, it is possible that some of the losses resulted from dystocia cases or acidosis caused by over-liberal concentrates allowances.

The other important factor which can be affected by level of nutrition in late pregnancy is the ewe body weight change during pregnancy (i.e. ewe body weight loss or gain). This, by itself, can affect the lamb's chances of survival by affecting the ewe's mothering ability and readiness to support her newborn lamb.

These changes in ewe body weight can be influenced and controlled by the amount of food offered to the ewe during late pregnancy (Alexander and Peterson, 1961; Treacher, 1970; Robinson et al., 1973). Of those who claimed that the level of feeding during late pregnancy does not affect ewe body weight are Coop (1950) and Shevah et al. (1975). Rutter et al. (1971), while performing some husbandry studies on Greyface ewes that were housed in the last 16 weeks of pregnancy, also reported similar findings. Although they used different feeding regimes, the amount of food offered and its nutritive value indicate that the level of energy intake was similar for all groups. The 20 kg body weight loss for both high and low nutrition groups is a good indication of this. Working with Scottish Blackface ewes, McClelland and Forbes (1973) showed that level of feeding in the last six weeks of pregnancy did not have a significant effect on ewe body weight change. However, the four nutritional groups they observed were on similarly low levels of feeding (daily energy intake varying between 6·5 and 8·2 MJ per day), and all these groups suffered ewe body weight loss during pregnancy, varying between 9·5 and 12·5 kg.

My data showed that ewe body weight can be influenced markedly by the level of energy intake in the last eight weeks of pregnancy. In the poorly fed group of ewes the

loss of body weight is associated with high levels of lamb mortality. This finding is supported by workers like Thomson and Thomson (1949) and Louca et al. (1974).

There are particularly high mortality rates among lambs born in large litters and my data showed also that, within nutritional groups, ewe body weight loss varied with litter size and that ewes with multiple births, particularly those with triplets, showed significant body weight loss during pregnancy. The importance of litter size in these situations has also been emphasised recently by Foot et al. (1973) and Robinson and Ørskov (1975).

One of the factors important for lamb vitality and survival is lamb birth weight. My data show that under-nourishment of the ewe during pregnancy clearly affected birth weight of lambs, irrespective of their litter size, although the effect was more significant among lambs from multiple types of birth.

The few authors who claimed no effect of level of feeding in the last six to eight weeks of pregnancy on lamb birth weight are Coop (1950), Darroch et al. (1950), Guyer and Dyer (1954), Rutter et al. (1971), McClelland and Forbes (1973) and Shevah et al. (1975). The type of criticism I presented earlier concerning the above mentioned authors' approach to one or more of the criteria used in their nutritional study can also be repeated here. On the other hand, other authors like Thomson and Thomson

(1949) and Russel et al. (1967a) have stated that even moderate degrees of undernourishment in late pregnancy can cause marked reductions in lamb birth weight. In relation to this, it is important to state that in my work which indicated that ewes in nutritional group 2 might have been under a moderate degree of undernourishment, only a marginal reduction in lamb birth weight occurred as compared to that of group 4 which represented high levels of feeding at late pregnancy.

However, my finding that very low levels of feeding in late pregnancy can markedly reduce the birth weight and thus the vitality of lambs is in keeping with the views expressed by many others (Thomson and Fraser, 1939; Underwood et al., 1943; Wallace, 1948a; Thomson and Thomson, 1949, 1953; Russel et al., 1967a, b; Treacher, 1970; Russel, 1971; Robinson et al., 1973; Louca et al., 1974; Bareham, 1976).

The life of the newborn lambs is at jeopardy in these circumstances. In the work I performed on Scottish Halfbreds, a 25 per cent reduction in lamb birth weight occurred in the case of the ewes severely undernourished during late pregnancy. Similar findings were also reported in hill sheep by Russel (1971).

Poor levels of feeding during late pregnancy, which can be aggravated by other factors like bad weather conditions, poor management and low level of feeding at other

important stages in the ewe breeding cycle, seem to have great influence in deciding the size of lamb losses and is a topic worthy of further planned study.

Only a few biochemical parameters were included in my study concerning ewe nutrition and PLM but previous workers in the same field have ignored these parameters almost completely.

In relation to the influence of late pregnancy level of feeding on PLM, blood parameters in lambs in the neonatal period have not been studied at all.

As the main aim of the study is lamb survivability, it is necessary to get sufficient numbers of observations in the different nutritional groups in order to make useful comparisons. These observations should be made on both surviving and non-surviving lambs. Unfortunately, the number of dead lambs for which parameters were estimated was very low and in many cases it was nil. However, the estimations achieved showed that as far as lambs are concerned, at birth, blood glucose or serum protein levels showed no important variation in relation to the level of feeding of their mothers in the last eight weeks of pregnancy. This could mean that until this stage (i.e. delivery), any expected variation will be reflected by other criteria like birth weight and vitality of lambs. Blood parameter levels seem to show their importance only after the start of suckling, i.e. following colostrum ingestion.

Poor levels of feeding during late pregnancy appear to be related to lower serum total protein and gamma-globulin levels in lambs at 24 hours of age, especially in lambs from large litters.

Lambs which died, irrespective of their nutritional group, tended to have low gamma-globulin levels at 24 hours of age. As most of the lamb deaths occurred in the severely undernourished group of ewes, the low level of feeding in late pregnancy should be held responsible, at least partially, for these changes in gamma-globulin levels. These changes are possibly a reflection of both the low colostrum production by the mothers and the low vitality of the lambs.

Regarding biochemical parameters estimated in ewes' sera, in my experiments the level of feeding in late pregnancy seemed to have no clear-cut effect on blood glucose. This was perhaps due to the fact that either glycolysis or glyconeogenesis was in action as a response to a coinciding glucose abundance or scarcity dictated by the amount of energy supplied by the ewe diet.

Shevah *et al.* (1975) observed the effect of different levels of feeding during late pregnancy on blood glucose levels of groups of Finn Dorset ewes. They reported that the groups on the lower planes of nutrition had lower blood glucose levels. Unfortunately, their data may be somewhat misleading as the levels of glucose they reported

start after the ewes had been on the experimental diets for one week, i.e. the actual base lines are not given by them.

Ewes' serum total protein and gamma-globulin levels during late pregnancy were not affected by level of late pregnancy feeding in the groups included in my study. However, two trends were observed, regardless of the ewes' nutritional group:

1. The higher the litter size, the lower the level of total protein and gamma-globulin in the ewes' sera. This was possibly due to the high demand by the uterus and its heavy load of fetuses, on the food offered.

2. These parameters were lower at lambing than before lambing. This could be a result of the coinciding high colostrum production and the transfer of protein (particularly gamma-globulin) from the ewes' sera to lacteal secretion.

From the circumstances that surrounded this preliminary nutritional study, it is difficult to draw definite conclusions from the behaviour of parameters analysed in both the ewes and their lambs. These parameters will be considered in greater detail in the work which follows, concerning levels of nutrition in late pregnancy, in experiments designed for the purpose.

Conclusion

The work so far described was a preliminary study required to establish some basic data in relation to PLM. The information obtained is in some cases definite and not in dispute but in several instances the results cannot be wholly relied on because of insufficient data or experimental design factors which were outside my control.

Litter size is without doubt an important factor in relation to PLM and to associated topics such as lamb birth weight and circulating immunoglobulin levels. Lamb sex is not closely enough associated with birth weight to make it an important aspect of future work, but ewe breed can affect lamb birth weights and future work should be confined to one or two closely related ewe breeds. Ewe milk production is a topic worth pursuing in relation to PLM and additional data on the causes and times of death in young lambs would help establish the present data. In further work, allowances must be made to mitigate any effects caused by the age of the ewe on PLM levels.

The various biochemical parameters already studied are not always of obvious value in relation to PLM but trends have emerged. Blood glucose and PCV have not been established as clear indications of a lamb's likely early performance but would be worth some further study

to clarify the position. Total protein and associated immunoglobulins in lambs appear to be related to deaths in lambs although in some cases the connection is tenuous. More detailed study on this aspect would be desirable, particularly in relation to the connection between the delay of colostrum intake by the newborn lamb, the immunoglobulin content of colostrum and the circulating immunoglobulin levels seen in lambs after sucking.

The work completed on nutritional studies has shown encouraging results and a connection between PLM, ewe body weight loss and ewe nutrition in late pregnancy seems to exist. The experimental design of this work was not, however, ideal and further work based on fewer nutritional groups will have to be undertaken to confirm the trends already referred to. The methods of monitoring ewe nutritional status to date have been the established and useful techniques of energy intake and plasma ketone levels. In future work additional monitors such as serum albumin and blood urea might yield further information on this topic.

C H A P T E R S I X

COLOSTRUM DEPRIVATION

INTRODUCTION

Two separate experiments were conducted during the Easter lambing of 1975. The first concerned the effects of colostrum deprivation on newborn lambs and the results will be presented here. The second was concerned with the effects of levels of feeding during the last eight weeks of pregnancy on ewe performance and the results of this work will be presented in Chapter VII.

EXPERIMENTAL DESIGN

This experiment was designed to study the effect of total colostrum deprivation, for varying periods after birth, on a lamb's subsequent ability to absorb immunoglobulin, and to investigate the effect of deprivation on PLM and growth rate.

In order to avoid any effect due to variable litter size, or lamb fostering, this work was undertaken only on ewes producing live twin lambs.

Both Greyface and Halfbred ewes were available for this experiment and previous work (see Chapter V) had indicated that between 50 and 60 per cent of these ewes could be expected to produce twins. Consequently, in order to obtain sufficient twins for the planned experiment, a mixed group of 64 ewes was assembled after mating

and kept together throughout pregnancy. They were managed according to good commercial practice (see Chapters IV and V). One week prior to lambing they were brought inside and fed a diet of hay, concentrates and turnips on a group fed basis.

The proposed deprivation study was planned as follows with the object of having at least 10 sets of twins in each experimental group. Any sets of twins born would be allocated on a random basis, by reference to a table of random numbers (Snedecor and Cochran, 1971) into one of three groups.

(a) Control group. Lambs were allowed and encouraged to suck their dams as soon after birth as possible.

(b) Five hours deprivation group. Lambs were deprived of all food for a period of five hours after birth.

(c) Nine hours deprivation group. Lambs were deprived for nine hours after birth.

The process of deprivation was carried out by placing the ewe and her lambs in an individual pen, covering the ewe's udder with a specially designed cloth cover to prevent the lambs sucking, and as a further precaution keeping a close watch to ensure that no lamb gained access to a teat. At the end of the deprivation period the cover was removed and the lambs allowed to suck in the normal way. No difficulties were encountered with

this procedure although a few lambs required initial encouragement before they would suck properly.

In the event only thirty-one ewes (18 Halfbred and 13 Greyface) produced live twins and they were allocated in the following way.

Control group - 10 ewes

Five hours deprivation group - 11 ewes

Nine hours deprivation group - 10 ewes.

It must be pointed out that 15 of the 31 ewes were in lamb for the first time (two year old gimmers). These gimmers were allocated to deprivation treatments separately from the other ewes whose age varied from two to five years.

Ewes and lambs were kept in individual pens for a period of not less than 48 hours and then, when the weather was suitable, allowed outside.

SAMPLES COLLECTED AND PARAMETERS OBSERVED

Lambs were weighed at birth, 24 and 48 hours of age, and at regular periods thereafter. Ewes were weighed 24 hours after lambing. Lambs were bled at 24 and 48 hours of age and the samples analysed for PCV, glucose, total protein and immunoglobulin content. Ewe colostrum samples, collected prior to the lambs first suck, were analysed for whey protein and immunoglobulins.

RESULTS

The effects of colostrum deprivation on lambs performance and on the levels of some blood parameters will be presented below.
Any differences between groups were analysed using Students 't' test.

LAMB PERFORMANCE

Illness and losses of lambs (see Table 6·1):

In the control group none of the lambs showed any sign of illness. PLM in this group was nil. In the five hour group, four lambs (18·1 per cent) developed "watery mouth" syndrome*. One of these lambs (4·5 per cent of total lambs involved) died of coli septicaemia and joint infection, eight days after birth. Another lamb developed "joint ill" 14 days after birth. This lamb did not show any signs of improvement in spite of treatment and was culled at six weeks of age.

Among lambs in the group deprived of colostrum for nine hours, eight (40 per cent) showed the "watery mouth" syndrome. None of the lambs were treated and yet only one of them (5 per cent of total) died at three days of age. The cause of death was coli septicaemia.

*Lambs with "watery mouth" syndrome were sluggish and not interested in sucking. They showed signs of abdominal pain. Copious and frothy salivation was noticed. These signs usually continued for one day only, after which lambs either recovered or died as a result of terminal coli septicaemia.

Body weight increase of lambs:

Table 6·1 shows the body weight of lambs at birth, 24 hours and 48 hours of age. The table also presents mean gross increase in lamb weight at three and five weeks of age.

Weight gain during the first 48 hours of life was, as expected, reduced in the deprivation groups but this disadvantage appeared to be overcome by three weeks of age.

BIOCHEMICAL PARAMETERS

Blood glucose (Table 6·2) showed no significant variation but PCV (Table 6·2) tended to indicate that deprived lambs were still suffering from a degree of dehydration at 24 hours of age but that fluid balance appeared to have been fully restored by 48 hours of age.

Immunoglobulin values are presented in Table 6·3. Total protein and gamma-globulin values (Biuret) showed a declining trend as the period of deprivation increased but only the differences between the control group and the nine hour deprivation group were statistically significant ($P < 0·05$). Individual immunoglobulins (SRID) indicated a similar trend with IgG_1 and IgM being principally affected. At 24 hours the control group had significantly more IgG_1 than the deprived groups ($P < 0·02$) but the difference was less significant at 48 hours of age ($P < 0·05$) or not significant.

TABLE 6·1.

Lamb body weight change (calculated as mean ± standard deviation)

Deprivation group	No.* of lambs	PLM %	Body weight (kg) at:			Body weight increase (kg) at:	
			Birth	24 hours of age	48 hours of age	3 weeks of age	5 weeks of age
Control	20	Nil	5·35 ± 0·68	5·50 ± 0·78	5·67 ± 0·89 (18)	5·93 ± 1·05 (18)	11·12 ± 2·35
Five hours	22	9	4·87 ± 0·69	4·96 ± 0·69	5·12 ± 0·67	6·18 ± 2·02 (19)	11·70 ± 2·23 (18)
Nine hours	20	5	4·76 ± 0·77	4·75 ± 0·75	4·70 ± 0·80	5·47 ± 1·53 (13)	10·77 ± 1·68 (16)

* If different from these, it is noted in brackets.

TABLE 6·2.

Blood glucose (mg/100 ml) and PCV values (%) for lambs at 24 and 48 hours of age

(Mean ± standard deviation)

Deprivation group	Mean values at 24 hours of age		Mean values at 48 hours of age	
	Blood glucose	P C V	Blood glucose	P C V
Control	(20) 105·88 ± 26·0	(20) 38·48 ± 3·9	(17) 99·52 ± 19·9	(18) 36·72 ± 4·4
Five hours	(20) 108·87 ± 26·8	(22) 42·84 ± 5·9	(20) 105·03 ± 24·4	(18) 39·92 ± 5·2
Nine hours	(18) 110·29 ± 47·0	(18) 40·39 ± 6·1	(20) 102·52 ± 26·2	(20) 37·65 ± 6·1

Number of lambs in brackets.

TABLE 6·3.

Lamb's serum total protein and gamma-globulin values at 24 and 48 hours of age (g/100 ml)

(Mean ± standard deviation)

Deprivation group	No.* of lambs	Mean values at 24 hours of age						
		Total protein	Gamma-globulin	IgG_1	IgG_2	IgM	IgA	Total (SRID)
Control	18	(20) 7·01 ± 1·57	(20) 2·70 ± 1·01	2·86 ± 0·81	0·05 ± 0·05	0·75 ± 0·51	0·17 ± 0·10	3·83 ± 1·08
Five hours	22	6·57 ± 1·14	2·30 ± 0·82	2·14 ± 0·87	0·04 ± 0·04	0·56 ± 0·32	0·152 ± 0·075	2·89 ± 1·14
Nine hours	20	6·07 ± 0·90	2·00 ± 0·83	1·93 ± 0·85	0·056 ± 0·056	0·35 ± 0·23	0·126 ± 0·038	2·40 ± 1·02

Deprivation group	No.* of lambs	Mean values at 48 hours of age						
		Total protein	Gamma-globulin	IgG_1	IgG_2	IgM	IgA	Total (SRID)
Control	18	6·43 ± 1·13	2·35 ± 0·82	2·02 ± 0·77	0·035 ± 0·01	0·507 ± 0·254	0·135 ± 0·05	2·69 ± 0·93
Five hours	22	(20) 5·98 ± 1·19	(20) 2·05 ± 0·78	(20) 2·02 ± 0·81	(20) 0·035 ± 0·01	(20) 0·51 ± 0·34	(20) 0·218 ± 0·26	(20) 2·78 ± 1·18
Nine hours	20	5·91 ± 1·07	1·77 ± 0·76	1·516 ± 0·57	0·036 ± 0·017	0·361 ± 0·228	0·144 ± 0·07	2·06 ± 0·81

*If different from below, it is shown in brackets in corresponding box.

Findings were similar for IgM and total immunoglobulins with the most marked variations occurring between the control and nine hour deprived groups at 24 hours of age ($P < 0.02$ to < 0.001).

Colostral whey protein and immunoglobulins were similar for all three groups of ewes (no significant variation) and so the results have been pooled and were as follows:

No. of whey samples	Total protein	Gamma-globulin	IgG_1	IgG_2	IgM	IgA	Total (SRID)
29	17·483 ± 5·46	9·024 ± 3·63	5·455 ± 2·24	0·079 ± 0·036	1·071 ±0·55	0·308 ±0·13	6·910 ±2·72

There is a marked similarity between the ratio of individual immunoglobulins (calculated as percentage of total immunoglobulins) in colostral whey and those in the lambs sera for each group as shown below:-

Deprivation group	Number of samples	Colostral whey immunoglobulin %				Number of samples	Lamb serum immunoglobulin % at 24 hours of age			
		IgG_1	IgG_2	IgM	IgA		IgG_1	IgG_2	IgM	IgA
Control	9	78·03	1·40	15·54	5·02	18	74·67	1·30	19·58	4·43
Five hours	11	80·20	0·90	14·75	4·15	22	73·92	1·38	19·44	5·25
Nine hours	9	78·19	1·12	16·46	4·23	20	78·49	2·28	14·09	5·13

The discrepancies between the total gamma-globulin results as measured by the Biuret technique and the total immunoglobulin as measured by the SRID technique will be discussed later in Chapter VIII.

DISCUSSION

During this study into the influence of colostrum deprivation on mortality and growth rate in lambs, and on the degree of absorption of different immunoglobulins from ewe's colostrum, only ewes with healthy twins and ample amounts of colostrum were used. The twins were usually strong at birth and weighed 4 to 6 kg each. This procedure was followed in order to avoid any complications which would undoubtedly arise if lambs from different litter sizes were compared. It is important to point out, however, that this initial selection procedure introduces a degree of bias into the study, particularly in relation to mortality as it effectively excludes several important causes of death such as abortions, prematurity and probably starvation.

It may be argued that as mortality is highest in small lambs from large litters this work should have been conducted using triplets. This course was not taken because previous experience indicated that early mortality in the smallest of a set of triplets was a likely event even without colostrum deprivation. Accordingly, there would have been difficulty in obtaining sufficient intact triplet litters for this work.

Working on a purely experimental basis, Shaw (1971) found that by letting lambs suck colostrum for two hours

after birth, before dosing them orally with E. coli suspension, lower death rates occurred than when lambs were dosed immediately after birth and were not allowed to suck colostrum until 12 hours after birth. Similarly, Campbell (1974) reported that when lambs were allowed to suck colostrum freely, few losses resulted from challenging them with a very pathogenic strain of E. coli. On the other hand, lambs that were deprived of colostrum, suffered very high losses from the same bacterial challenge even if they were fed as much milk as they were able to suck from foster mothers. Very recently, when my work was progressing, Ducker and Fraser (1976), who applied intensive care and management on their housed ewes and lambs, found no effect of six or 18 hours of colostrum deprivation on levels of mortality among Greyface lambs. Out of 39 lambs (singles and twins only), in each of three groups, one was lost in their control non-deprived group, two in the six hour deprivation group and three in their 18 hour deprivation group. The cause of death of these lambs was not ascertained.

During my observations, lamb mortality did not show any clear relationship with the length of deprivation although the three deaths did occur in deprived groups. Overall mortality in the deprived groups was 7·1 per cent and this is comparable with the 6·2 per cent mortality

rate observed in non-deprived twin lambs which died from infections or accidents during the 1974 lambing period (see Table 5·8). Under the circumstances of my work it cannot be said that the deprivation as such increased the mortality rate appreciably.

The deaths occurring in the deprived groups resulted from a terminal E. coli infection and emphasised the importance of this micro-organism in causing losses among young lambs. This was also observed in the field work performed in New Zealand by Hughes (1971 - 1972b), in Australia by Dennis (1974c), in Sweden by Gunnarsson et al. (1972) and is also reported in reviews published by Watt (1965), Stamp (1967) and Owen (1976). Levels of management can decide the degree of contamination of the lambing shed into which lambs are born with no protective antibody. No attempt was made to judge the degree of contamination in the lambing pens used in my work but they were comparable with those found on most commercial farms.

The effect of deprivation has been clearly reflected in terms of lamb activity early in the life. The longer the deprivation period the higher the number of lambs that were lethargic and showed the "watery mouth" syndrome. By ensuring a reasonable degree of husbandry, good shelter and also immediate colostrum sucking after the end of the deprivation period, the majority of these

inactive lambs managed to overcome this temporary illness.

The watery mouth symptoms rarely occurred before suckling had taken place and in most cases developed a few hours after the first feed. It seems probable that it was associated with indigestion. The lambs deprived for the longest period were the hungriest and the most likely to gorge themselves and the symptoms appeared mainly in this group. Continued feeding after symptoms developed seemed to aid recovery presumably by preventing any explosive growth of E. coli in the intestine.

Before discussing the subsequent performance of deprived lambs, mention should be made of the birth weights shown in Table 6·1. It seems a purely chance occurrence that the lambs assigned to the colostrum deprived groups had lower birth weights than the control non-deprived lambs and that those lambs which were longest deprived had the lowest mean birth weight. Ewes and their lambs were randomly allocated to the various groups, as previously described, with the intention of preventing this type of bias.

As expected, lambs on the longer period of deprivation failed to gain weight over the first 48 hours of life. This initial set-back meant that the mean weight of the nine hour deprived group was 0·97 kg less than the control group at 48 hours of age. This mean difference increased to 1·01 kg at three weeks of age but by five

weeks of age had stabilized at 0·94 kg. The five hour deprived group showed no depression of growth rate when compared to the controls. In fact, in the five week period, the lambs which had been subjected to five hours deprivation of colostrum gained 0·58 kg more than the controls.

As it is possible that either the deprivation of colostrum or the difference in mean birth weight of the nine hour deprived group may have accounted for the variations in weight gain, it is necessary to attempt to compare the deprived lambs with non-deprived lambs of similar birth type, mean birth weight and range of birth weights. Records of Scottish Halfbred twins from the 1974 experiment were available. These lambs had not been deprived of colostrum. From the records, the 1974 lambs were allocated to two groups to ensure similar mean birth weights and weight ranges to those of the control and nine hour deprived groups in the colostrum deprivation experiment. This gave two groups of 1974 lambs described as "higher" and "lower" birth weight. Both groups were strong and healthy and their weights fell within the range usual to such lambs. Their body weight increases were as follows (Table 6·4).

TABLE 6·4.

Group of twins	No. of lambs	Birth weight (kg)	Mean body weight increase (kg) at	
			3 weeks of age	5 weeks of age
Control	20	5·35 ± 0·68	5·93	11·12
74 H*	13	5·41 ± 0·26	5·59	10·54
9-Hour Deprived	20	4·76 ± 0·77	5·47	10·77
74 L*	16	4·77 ± 0·14	5·51	10·26

*74 H = "higher" birth weight lambs;
74 L = "lower" birth weight lambs, in 1974.

Table 6·4 sets out data enabling a comparison to be made between the two groups of lambs in the deprivation experiment (Control and nine hour deprived), the two groups of lambs in the 1974 experiment ("higher" and "lower" birth weight) and between groups from the two experiments.

At three weeks of age there was practically no difference in weight gain between nine hour deprived lambs and lambs (group 1974 "L") of similar mean weight and weight range at birth which were not deprived. The deprived lambs having gained 0·04 kg less than the others. During the next two weeks, the deprived lambs gained a

mean 5·30 kg compared with the 4·75 kg mean gain of the 1974 "L" group. It would seem that deprivation of colostrum for the first nine hours of life had conferred no growth rate disadvantage on the deprived lambs compared with undeprived lambs of similar birth weight. Thus, in the colostrum deprivation experiment, any difference in total weight gain to week five between the control lambs and the deprived lambs cannot be ascribed to the deprivation of colostrum for nine hours. It may be ascribed to the difference in mean birth weight of the two groups. However, it should be emphasised that all of the lambs under discussion, whether controls or deprived in the deprivation experiment, or of lower or heavier weight in the 1974 experiment, had birth weights well within the range regarded as good, and predictive of viability. Further, the birth weight differences between the groups were small.

Lambs of reasonable vitality and birth weight, i.e. singles and twins, only suffer marginal set-backs in their growth rate in the first few weeks of life as a result of colostrum deprivation. This is in agreement with the findings reported by Ermekov et al. (1973) and Ducker and Fraser (1976).

In other circumstances, e.g. where birth weights are low, litter size larger, and where the environment is adverse to the lambs, colostrum deprivation may have a

deleterious effect on subsequent weight gain.

Twenty-four hour blood glucose values appeared to have no correlation with periods of deprivation. However, the PCV of the deprived lambs did show a downward trend similar to that of the controls between 24 and 48 hours of age. The initial degree of dehydration appeared to have been overcome by 48 hours of age in the deprived groups. Of these two parameters, glucose particularly failed to yield useful answers in relation to early lamb performance.

Although colostrum deprivation did not affect total protein levels greatly, and by no means stopped immunoglobulin absorption when colostrum did become available, deprived lambs had lower levels of these parameters, particularly when the deprivation was as long as nine hours. McCarthy and McDougall (1953), who used one lamb only in each deprivation group, noticed that deprivation, longer than 29 hours, stopped globulin absorption but deprivation of less than 12 hours had no effect on the absorption rate. However, they presented extremely low globulin levels even for the control non-deprived lambs (only 0·28 to 0·48 g per 100 ml, at two days of age). Bem and Popescu (1973) reported that a mere six hours of colostrum deprivation will markedly reduce the 24 hour levels of lamb gamma-globulin, and that these first six hours of the newborn's life are very critical for gamma-

globulin absorption. Lecce and Morgan (1962) using small numbers of lambs (only five in the whole study) reported that starvation of lambs for the first 24 to 48 hours of life did not stop the subsequent absorption of colostral protein. They suggested that the feeding of colostrum not only provided a source of absorbable gamma-globulins but also engendered a rapid "closure" of the gut to gamma-globulins fed later. As well, the closed or non-permeable gut is resistant, they state, to invasion by normal flora micro-organisms. They did not quantitate gamma-globulin levels but instead, they used electrophoresis to see if the major gamma-globulin fractions existed or not. Colostrum deprivation of lambs undertaken by Ducker and Fraser (1976) did not affect serum gamma-globulin levels. These authors used the ZST test of McEwan et al. (1970) which does not differentiate between the different classes of immunoglobulins.

The reduction in circulating immunoglobulin levels in deprived lambs occurs as a result of the gut closure to immunoglobulin absorption but even nine hours deprivation failed to affect the normal course of events sufficiently to reduce immunoglobulin values to the levels associated in the 1974 work with increased lamb mortality. In such circumstances it appears that if a newborn lamb fails to feed overnight it will still be capable of absorbing sufficient immunoglobulin from colostrum when

it is fed in the morning provided dehydration and hypothermia have not become serious threats to life.

The reductions in immunoglobulin levels are mainly due to falls in IgG_1 and IgM, but not IgG_2 and IgA which are the minor immunoglobulins in lamb sera at 24 hours of age. By 48 hours of age, immunoglobulin absorption appears to have ceased or the immunoglobulin content of colostrum has fallen to negligible levels. (This latter factor was not investigated).

The nine hours deprivation put the lambs into a situation where they had about 30 to 40 per cent less IgG_1 and IgM at 48 hours of age, than non-deprived lambs. At this age, the serum IgA level seems not to be affected by the deprivation, i.e. the level of serum IgA is similar in deprived and non-deprived lambs. The absence of an effect on the serum levels may reflect the possible retention of IgA in the gut where it has a local protective function, at a time when the local gut cells (mostly IgA-specific) had not started producing their own immunoglobulin (Lee and Lascelles, 1970; Beh and Lascelles, 1974). Levels of IgG_2 were very low in the sera of lambs whether they were deprived or not.

In the case of newborn calves, the effect of delaying colostrum intake, on immunoglobulin absorption was such that absorption could be reduced by half if colostrum feeding was delayed from two to 20 hours (Kruse, 1970).

Penhale et al. (1973), after depriving various groups of calves of colostrum for 0, 5 and 9 hours after birth, went even further and postulated that a complete deficiency of serum IgG, IgM and IgA might occur if calves were deprived of colostrum for 27, 16 and 22 hours respectively. (These figures were calculated from the relationship of values at different times of deprivation and the extrapolation of results). The above mentioned authors (Kruse, 1970; Penhale et al., 1973) offered restricted amounts of pooled colostrum (usually in one feed only) at the end of the deprivation period. These workers ignored the importance of the mothering behaviour of the dam and of allowing the calves to suck freely when the period of deprivation was over, matters to which Selman et al. (1971) have referred. It is known also that numerous feeds of colostrum ensure higher levels of circulating gamma-globulin in newborn lambs than one feed only (Halliday and Williams, 1976). Accordingly, the possibility is that the conclusions of Kruse (1970) and Penhale et al. (1973), regarding the time at which colostral immunoglobulins cease to be absorbed by calves, may require to be reviewed to take account of these factors. It is evident that their conclusions, while applicable to calves in the special circumstances of their experiments do not apply to deprived lambs. In my opinion, delay of colostrum intake will, no doubt, put the lambs at a slight

disadvantage with regard to its passive immune status but the situation will only be critical if lambs are small and weak at birth and are born under adverse weather conditions.

The profile and distribution of the four different immunoglobulins in lambs' sera at 24 hours of age is similar to that of their mothers' colostrum. My data showed that most of the immunoglobulin in the colostrum consists of IgG_1 (78 per cent) followed by IgM (about 16 per cent). IgA levels were only 4 to 5 per cent while IgG_2 was less than 2 per cent.
In this connection, Pahud and Mach (1970) reported mean ovine colostrum IgG, IgM and IgA values of 6·00, 0·41 and 0·20 g per 100 ml respectively. Though the trend is similar to the one I have presented, these authors did not differentiate IgG into its two sub-classes. They also reported much lower IgM values in the colostra they examined. Smith et al. (1975), who also did not measure IgG_1 and IgG_2 separately, reported colostral IgG, IgM and IgA values of 10·12, 0·29 and 0·62 g per 100 ml respectively. While reporting very low IgM levels, the IgA levels were somewhat higher than the levels I presented. They based their findings on only 19 colostral samples collected from various breeds of ewes.

Values presented by the above mentioned authors, and by me, tended to show great individual variation.

There could be more than one factor responsible for this but, in my opinion, the amount of colostrum produced by the ewe might have a major effect on the colostrum immunoglobulin concentration (dilution factor). Although all the authors named used the same method for the estimation, i.e. the SRID technique, the various complicated steps of the method could have added to some of the variation in the levels of the different immunoglobulins.

Conclusion

This study was performed to ascertain the influence of delaying colostrum intake on the survivability and performance of strong, healthy twin lambs born to mothers with adequate amounts of colostrum at lambing. In these circumstances, temporary deprivation resulted in no really significant upset to lamb performance in terms of survivability or future performance.

In relation to this type of study, the only biochemical parameters which proved to be useful indicators for lamb vitality and performance in the neonatal period, were serum total protein and the different immunoglobulin concentrations. It is important that these parameters should be included in any future work concerning more detailed study of the importance of colostrum for newborn lambs.

Colostrum deprivation of lambs immediately after birth, particularly when it was as long as nine hours, slightly lowered the immunoglobulin levels during the first two days of life, a very critical period for lamb survival. From the management point of view, and in situations where continuous, direct surveillance at lambing is not possible, it can be postulated that, at least in flocks where triplets and quadruplets are expected, if lambs do not manage to suck within nine hours after birth (example: lambs born during the night which do not manage to suck by the morning) they are still capable of absorbing enough colostral immunoglobulin providing they can obtain sufficient colostrum.

As colostrum deprivation on its own has not markedly affected either mortality or twin lamb growth rates, it seems desirable to investigate deprivation simultaneously with other important factors, for example the level of feeding which ewes receive in late pregnancy, which has an important effect on the amount of colostrum they produce, and on the birth weights and vitality of their lambs. This, in my opinion, will bring the study nearer to natural farm circumstances and will help bring into perspective the significance of colostrum in lamb survivability.

C H A P T E R S E V E N

EFFECTS OF LEVELS OF FEEDING IN THE LAST EIGHT WEEKS OF PREGNANCY ON EWE PERFORMANCE

INTRODUCTION

As stated previously, the work presented in Chapter V concerning levels of feeding in late pregnancy was designed by other workers for other aims and was not ideally suited to my research aims. At the beginning of 1975, a follow up experiment was designed and its main aim was to study the effects of different levels of feeding during the last eight weeks of pregnancy on the following:

a) Levels of PLM.

b) Subsequent lamb performance.

c) Biochemical parameters, particularly immunoglobulins, in serum and colostrum.

EXPERIMENTAL DESIGN

Forty Scottish Halfbred ewes, all previously diagnosed as pregnant, were allocated to four nutritional groups on the basis of ewe body weight and condition score. Age of the ewe (varying between three and seven years) was also taken into consideration during the allocation so that each group contained ewes of the same age and weight range. They were all managed commercially until eight weeks before the expected date of

lambing when they were brought inside and housed in individual pens for the experiment. Methods of management and feed recording after housing were the same as those described for the 1974 nutritional study.

The experiment was designed to compare the effects of feeding poor quality and good quality hay on ewe performance, to see if supplementation of the poor hay with concentrates had beneficial effects and to compare these results with those from a high plane of nutrition provided by feeding complete ruminant diet (CRD). It was considered that the energy intake from good hay and from poor hay supplemented with concentrates would be similar but that the effects of these diets on ewe performance might be variable.

The experiment was designed on the following basis, to encompass as wide a range of food quality and type as possible.

Group 1 - Eleven ewes fed poor quality hay 'C' only. This hay had an ME value of 7·99 MJ/kg DM.

Group 2 - Eight ewes fed good quality hay 'A' only, (ME value 10 MJ/kg DM).

Group 3 - Ten ewes fed poor quality hay 'C' plus 30 kg concentrates per ewe, (ME value 12·3 MJ/kg DM).

Group 4 - Eleven ewes fed complete ruminant diet (CRD) only, (ME value 9·08 MJ/kg DM).

Both types of hay and also the CRD were offered ad libitum. Group 2 was also used for nutritional studies by other workers hence the smaller number of ewes involved.

The experiment did not run according to plan, mainly because some ewes refused to eat after four to six weeks on the experiment and the following exclusions had to be made.

Group 1 - one ewe refused food.

Group 2 - one ewe was barren.

Group 3 - two ewes refused food.

Group 4 - two ewes refused food and another two were barren.

Hence in the final analysis fewer ewes were available than had been calculated and the group sizes were reduced to:-

Group 1 - Ten ewes on poor hay.

Group 2 - Seven ewes on good hay.

Group 3 - Eight ewes on poor hay and concentrates.

Group 4 - Seven ewes on CRD.

SAMPLES COLLECTED AND PARAMETERS OBSERVED

Lambs were weighed at birth, 24 and 48 hours of age, and at regular periods thereafter. Ewes were weighed 24 hours after lambing. Lambs were bled at 24 and 48 hours of age and the samples analysed for PCV, glucose, total protein, and immunoglobulin content. Ewes were bled several times before and also just after lambing and the

samples were analysed for 3-hydroxybutyrate, urea and albumin as an indication of the ewes nutritional status, and also for total protein, gamma-globulin (Biuret) and immunoglobulin fractions (SRID). Colostrum samples collected from ewes just after lambing, and prior to the lambs first suck, were analysed for whey protein and immunoglobulins.

RESULTS

LEVELS OF FOOD INTAKE DURING LATE PREGNANCY

By calculating the daily food intake of each ewe in the four nutritional groups and knowing the nutritional values (expressed in MJ/kg DM, metabolizable energy) of the different nutrients offered, the mean energy intake of each group during the last eight weeks of pregnancy could be calculated. They were as follows.

Group 1 = 465·4 ± 57·0 MJ/kg DM.
Group 2 = 845·8 ± 112·2 " "
Group 3 = 837·6 ± 85·0 " "
Group 4 = 1221·0 ± 175·6 " "

Values for group 1 were significantly lower than those for groups 2 and 3 ($P < 0·001$). Values for groups 2 and 3 were almost identical and both were significantly lower ($P < 0·001$) than the group 4 value.

To ascertain if nutrient requirements were being met each ewe was monitored at weekly intervals during the last

eight weeks of pregnancy by estimating levels of plasma 3-hydroxybutyrate (3-HB) using an enzyme method of Zivin and Snarr (1973). This is an automated method used instead of the total ketone bodies method (Reid, 1960) that was employed during the 1974 work on PLM. This change in method occurred because the Reid technique had been discontinued by ESCA in favour of the faster automated 3-HB technique and was consequently no longer available to us.

The corresponding 3-HB values for the four nutritional groups of ewes are shown in Figures 7·1 and 7·2. As shown in these figures, ewes in group 1 (all, or only those carrying twins) began to show increasing 3-HB levels by the sixth week before lambing and thereafter this group had the highest 3-HB levels among all the four groups observed. As early as the fifth week before lambing, ewes in this group showed values of 1·25 millimole per litre (mM/L) while at two weeks before lambing the average value was over 2 mM/L. This was in spite of the group's relatively low litter size (1·9 lambs per ewe lambing).

Corresponding values for ewes in groups 2, 3 and 4 were always lower than 1 mM/L, with very few exceptions, for example, ewes in group 2, perhaps because of their relatively very high litter size (2·43 lambs per ewe lambing)

FIG. 7·1

Ewe's plasma 3-HB levels during late pregnancy (all litters included).

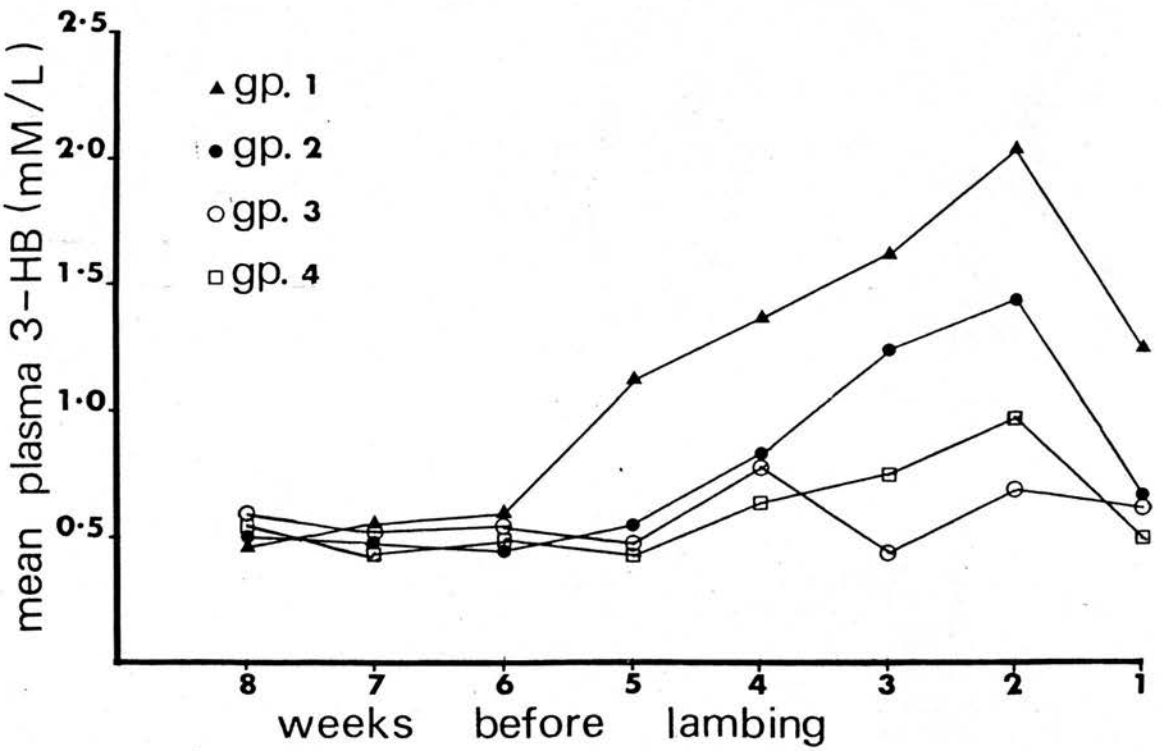
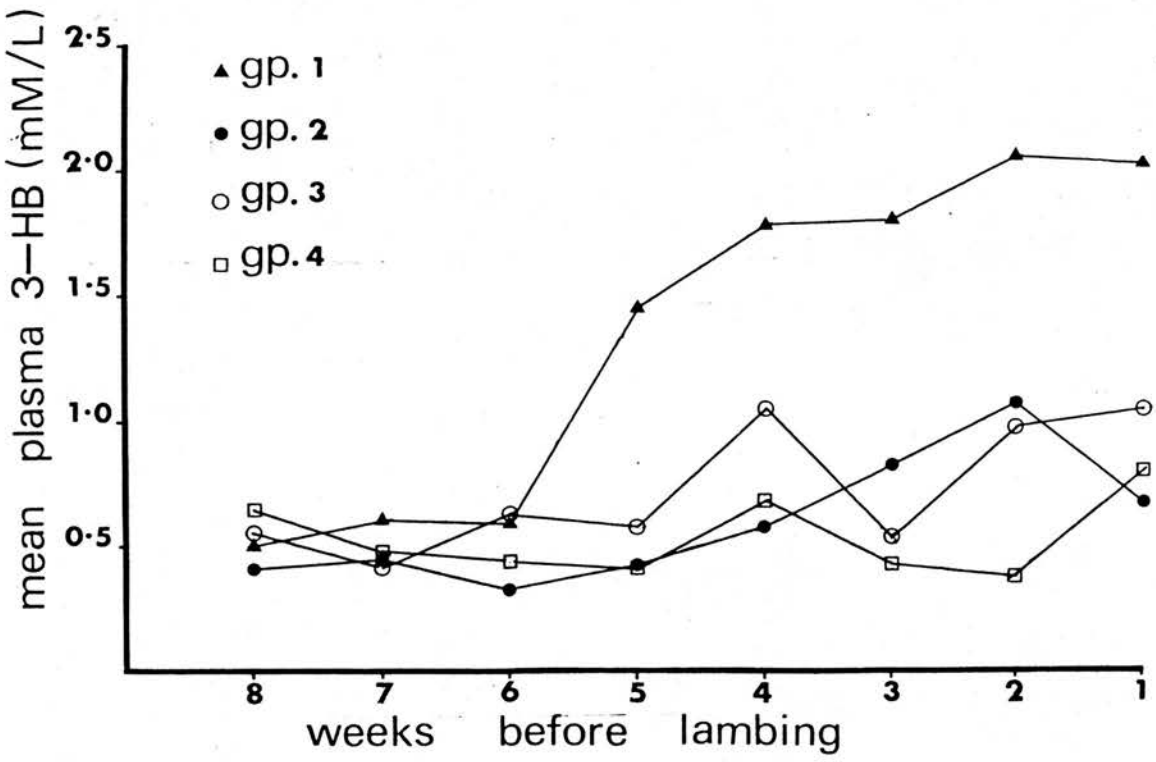


FIG. 7·2

Ewe's plasma 3-HB levels during late pregnancy
(ewes with twins only).



showed 3-HB values of 1·27 and 1·44 mM/L during the third and second weeks before lambing respectively. When values were calculated for ewes carrying twins only, to equalize the litter size effect on the readings, group 4 which was kept on very high levels of feeding during pregnancy, showed the lowest values. Groups 2 and 3 overlapped each other but remained relatively low, while group 1 stayed uniquely the highest in plasma 3-HB levels throughout the period of observation.

EWES PERFORMANCE

The performance of ewes in the different nutritional groups is shown in Table 7·1. Ewes on low levels of nutrition (group 1) showed the highest values in: ewe weight loss during pregnancy, poor milk supply at lambing, and number of ewes losing lambs.

Comparison of ewe body weight loss between the different nutritional groups (when 't' test application was possible) showed that ewes with twins in group 1 lost significantly more body weight during pregnancy than the corresponding ewes in groups 2 and 4 ($P < 0·05$) and in group 3 (d.f. = 6, 't' = 2·04). For ewes carrying singles, the loss of those in group 1 was significantly higher ($P < 0·001$) than the corresponding ewes in group 3. These weight losses correspond with the mean ewe body score measured 24 hours after lambing (Table 7·1).

TABLE 7·1.

Performance of ewes in the different nutritional groups

Nutritional groups		Group 1	Group 2	Group 3	Group 4
No. of ewes in each group		10	7	8	7
No. of ewes dying		1	1	Nil	Nil
No. of ewes with no milk at lambing		5	2	Nil	1
No. of ewes that lost one or more lambs		4	1	3	1
*Ewe weight loss or gain during pregnancy (Mean ± standard deviation)	Ewes with singles	(3) −13·5 ± 1·32	(1) 3·0	(3) 0·66 ± 1·55	(1) 10·5
	Ewes with twins	(5) −14·4 ± 10·5	(4) −0·5 ± 5·0	(3) −1·5 ± 2·3	(2) 11·0 ± 4·9
	Ewes with triplets	(2) −17·5 ± 17·7	—	(2) 3·5 ± 0·7	(2) 6·0 ± 0·0
	Ewes with quadruplets	—	(2) −7·5 ± 9·2	—	(1) −8·0
Mean ewe body score		(9) 1·58 ± 0·47	(6) 2·1 ± 0·52	(8) 2·3 ± 0·33	(5) 2·7 ± 0·27
Average litter size		1·9	2·43	1·88	2·29
PLM %		21	11·7	26·6	18·7
**No. of lambs lost in each litter size	Singles	Nil (3)	Nil (1)	1 (3)	Nil (2)
	Twins	2 (10)	Nil (8)	Nil (6)	Nil (4)
	Triplets	2 (6)	—	3 (6)	Nil (6)
	Quadruplets	—	2 (8)	—	3 (4)

*Number of ewes in brackets. **Number of lambs in brackets.

In spite of the relatively low litter size in group 1, four out of the 19 lambs born to this group were lost. Two lambs died within two days of birth as a result of starvation/coli septicaemia. A third one showed signs of watery mouth at three days of age and later developed a generalized joint-ill. This lamb later died when 25 days old. The fourth lamb was very weak at birth and its mother had no milk at lambing. This lamb remained ill-thriven until its death at four weeks of age. Both of the last two lambs represent a late postnatal type of death.

Levels of PLM in groups 3 and 4 appear high, but are greatly distorted by relatively high litter size. In group 4 for example, which represents the highest level of feeding during late pregnancy, the average litter size was 2·29 lamb per ewe. The 18·7 per cent of PLM in this group was represented by three lambs from the only set of quadruplets in the group. One of them was a stillborn. The other two died within four days of birth. One was born weak and died accidentally (crushed by the mother) and the other died as a result of starvation/E. coli infection. The mother of these three dead lambs had also relatively very large teats, a factor that was at least partially responsible for the inability of some of these lambs to suckle.

In group 2 (with an average litter size of 2·43 lambs

per ewe), the 11·7 per cent PLM is represented by two lambs from one set of quadruplets. One was stillborn and the other died at four days of age from starvation/ E. coli infection. The mother of these two lambs died 17 days after lambing as a result of hypocalcaemia.

Four of the 15 lambs born to group 3 were lost. One of them was a heavy single (weighing 6·5 kg at birth) that died due to dystocia. The other three were all triplets from separate litters. One of them died accidentally, and the other two failed to make use of their mother's available milk. They died three days after birth as a result of starvation/coli septicaemia.

With the exception of group 1, none of the groups lost any of their twin born lambs.

The overall PLM for all ewes included in this study was 19·2 per cent, with the triplets and quadruplets suffering the highest percentage of losses (28 and 42 per cent respectively).

BIRTH WEIGHT AND GROWTH RATE OF LAMBS

Average weights of lambs at birth and at three weeks of age are presented in Table 7·2. Ewes in group 1 had the smallest lambs at birth. In three out of six possible birth weight comparison (within the same litter size) between lambs born to group 1 and those born to any of the other three groups, the difference was statistically significant ($P < 0·01$ or $< 0·001$).

TABLE 7·2.

Body weight of lambs at birth and at three weeks of age

(Mean ± standard deviation)

Nutritional groups		Group 1	Group 2	Group 3	Group 4
Average lamb birth weight (kg) for:	Singles	(3) 5·46 ± 0·76	(1) 6·6	(3) 6·08 ± 0·81	(2) 6·35 ± 0·49
	Twins	(10) 3·74 ± 0·54	(8) 4·89 ± 0·68	(6) 4·71 ± 0·71	(4) 5·06 ± 0·59
	Triplets	(6) 2·57 ± 0·54	—	(6) 3·88 ± 1·09	(4) 4·30 ± 0·53
Average lamb body weight increase (kg) at three weeks of age for:	Singles	(3) 6·53 ± 0·88	—	(2) 6·90 ± 1·62	(2) 8·37 ± 0·05
	Twins	(6) 2·90 ± 2·05	(8) 6·75 ± 0·69	(6) 6·98 ± 1·42	(4) 6·88 ± 0·05
	Triplets reared as twins	(4) 3·59 ± 1·35	—	(2) 6·34 ± 0·87	(4) 7·10 ± 0·95

Number of lambs in brackets. No quadruplets were included as they were not represented in groups 1 and 3, and also only two of the 12 quadruplet lambs born to groups 2 and 4 survived to three weeks of age.

As an expression of ewe milk availability and also lamb viability, twins born to group 1 lost 0·09 kg of their body weight between birth and 48 hours of age compared to a body weight gain of 0·24, 0·64 and 0·49 kg achieved by twins born to groups 2, 3 and 4 respectively. The figures for lambs born to groups 3 and 4, but not of group 2, were significantly higher than that of group 1 ($P < 0·01$ and $< 0·05$ respectively).

The same pattern emerged when the subsequent performance of lambs (expressed as kg body weight increase at three weeks of age) was observed. At three weeks of age, twins born to group 1, in spite of their small birth weight, recorded an increase in body weight (expressed as percentage of their birth weight) of only 77·6 compared to 140·4, 137·5 and 149·8 recorded by twins born to groups 2, 3 and 4 respectively.

As far as birth weight and subsequent increase in body weight of lambs, and also other criteria used to monitor ewe performance (see Table 7·1), ewes in groups 2, 3 and 4 performed more or less similarly but in most cases, those of group 4 (with the highest nutritional level at late pregnancy) remained the best of the three.

EWE BLOOD PARAMETERS BEFORE AND AT LAMBING

Parameters estimated on the ewe's blood before and at lambing included the following: urea, total protein, albumin, gamma-globulin and also the different types of

immunoglobulins. Mean values for the first four are shown in Table 7·3. For the different immunoglobulins (as analysed by the SRID technique) and their totals, the mean values are presented in Table 7·4.

Before lambing, the parameters were estimated at four, two and one week before lambing for nutritional groups 1, 3 and 4. Ewes in group 2, however, were expected to show values similar to those of group 3. For this reason and also because of assistance and facility limitations, both in the farm and the laboratory, bleeding of these ewes before lambing was not attempted.

Parameters at lambing (week 0 in the above-mentioned two tables) were estimated on samples collected from ewes within one hour of the lambing process being completed.

Blood urea:

At all observed stages before lambing, ewes in group 1 had the lowest blood urea values. These values were significantly lower than the corresponding ones for group 4 ($P < 0.001$ always). They also had lower urea values than ewes in group 3 but the difference was statistically significant only between values measured at four weeks before lambing ($P < 0.05$).

Ewes in group 3 also had significantly lower urea values at four and two weeks ($P < 0.001$) and one week ($P < 0.02$) before lambing, than those in

TABLE 7·3.

Ewe's blood parameters before and at lambing

(Mean ± standard deviation)

Nutritional groups	Weeks before lambing*	Urea (mg/100 ml)				Total protein (g/100 ml)				Albumin (g/100 ml)				Gamma-globulin (g/100 ml)			
		4	2	1	0	4	2	1	0	4	2	1	0	4	2	1	0
	Group 1	12·91 ± 4·08 (10)	12·00 ± 3·34 (10)	13·81 ± 4·73 (9)	15·90 ± 10·10 (10)	6·54 ± 0·34 (10)	6·25 ± 0·36 (9)	6·46 ± 0·36 (9)	6·21 ± 0·26 (10)	2·29 ± 0·22 (10)	2·23 ± 0·17 (10)	2·14 ± 0·15 (9)	2·17 ± 0·24 (10)	2·08 ± 0·21 (10)	1·91 ± 0·18 (10)	1·43 ± 0·17 (9)	1·76 ± 0·15 (10)
	Group 3	17·00 ± 2·46 (8)	15·23 ± 3·43 (7)	17·25 ± 5·43 (6)	15·60 ± 11·50 (7)	6·75 ± 0·46 (8)	6·62 ± 1·13 (8)	6·60 ± 0·74 (6)	6·40 ± 0·88 (7)	2·22 ± 0·16 (8)	2·00 ± 0·29 (8)	1·93 ± 0·43 (6)	2·11 ± 0·15 (7)	2·07 ± 0·41 (8)	1·90 ± 0·69 (8)	1·50 ± 0·43 (6)	1·48 ± 0·28 (7)
	Group 4	38·08 ± 6·41 (6)	33·44 ± 7·07 (7)	27·86 ± 6·84 (5)	21·61 ± 7·40 (7)	6·25 ± 0·33 (6)	5·91 ± 0·34 (7)	6·12 ± 0·18 (5)	6·10 ± 0·45 (6)	2·21 ± 0·17 (6)	2·25 ± 0·13 (7)	2·10 ± 0·12 (5)	2·23 ± 0·15 (6)	1·69 ± 0·15 (6)	1·35 ± 0·22 (7)	1·12 ± 0·19 (5)	1·11 ± 0·21 (6)

*Week 0 represents samples taken at lambing. Number of ewes in brackets.

TABLE 7·4.

Levels of the different immunoglobulins in ewe's sera before and at lambing, as measured by the SRID technique (g/100 ml)

(Mean ± standard deviation)

Nutritional groups	Weeks before lambing*	IgG_1				IgG_2				IgM				IgA				Total			
		4	2	1	0	4	2	1	0	4	2	1	0	4	2	1	0	4	2	1	0
	Group 1	1·55 ± 0·54 (10)	1·42 ± 0·66 (10)	1·31 ± 0·29 (10)	1·50 ± 0·61 (10)	0·70 ± 0·26 (10)	0·65 ± 0·20 (10)	0·62 ± 0·27 (10)	0·66 ± 0·26 (10)	0·50 ± 0·27 (10)	0·40 ± 0·10 (10)	0·56 ± 0·33 (10)	0·40 ± 0·15 (10)	0·42 ± 0·13 (10)	0·46 ± 0·16 (10)	0·47 ± 0·06 (10)	0·41 ± 0·13 (10)	3·04 ± 0·89 (10)	2·93 ± 0·73 (10)	2·97 ± 0·62 (10)	2·97 ± 0·83 (10)
	Group 3	1·69 ± 0·52 (8)	1·46 ± 0·73 (8)	1·02 ± 0·38 (8)	1·44 ± 0·79 (7)	0·53 ± 0·17 (8)	0·53 ± 0·19 (8)	0·47 ± 0·17 (8)	0·52 ± 0·19 (7)	0·48 ± 0·15 (8)	0·39 ± 0·18 (8)	0·58 ± 0·32 (8)	0·51 ± 0·34 (7)	0·55 ± 0·14 (8)	0·63 ± 0·17 (8)	0·69 ± 0·07 (8)	0·62 ± 0·24 (7)	3·26 ± 0·68 (8)	2·99 ± 0·83 (8)	2·75 ± 0·73 (8)	3·09 ± 0·70 (7)
	Group 4	1·47 ± 0·48 (6)	1·17 ± 0·45 (7)	0·87 ± 0·32 (7)	1·07 ± 0·54 (6)	0·66 ± 0·26 (6)	0·58 ± 0·19 (7)	0·48 ± 0·17 (7)	0·64 ± 0·24 (6)	0·38 ± 0·11 (6)	0·31 ± 0·05 (7)	0·34 ± 0·10 (7)	0·35 ± 0·10 (6)	0·61 ± 0·07 (6)	0·62 ± 0·17 (7)	0·64 ± 0·08 (7)	0·59 ± 0·11 (6)	3·12 ± 0·48 (6)	2·68 ± 0·61 (7)	2·33 ± 0·45 (7)	2·65 ± 0·47 (6)

*Week 0 represents samples taken at lambing. Number of ewes in brackets.

group 4. Toward lambing, urea values for group 4 were reduced and this actually coincided with a marked reduction in CRD consumption during the last week of pregnancy.

At lambing, urea values show considerable individual variations as reflected by the standard deviation figures calculated for means in the different nutritional groups (see Table 7·3). Urea values for group 4 were higher than the corresponding values for groups 1 and 3 but the difference was not statistically significant.

Total protein and albumin:

Serum total protein levels for ewes in the different nutritional groups did not show any great variation. Ewes in group 4, however, tended to show slightly lower values than ewes in the other groups.

Albumin values in nutritional groups 1, 3 and 4 before and at lambing overlapped each other and did not show any clear variation.

Gamma-globulin:

This parameter showed interesting variations, particularly among ewes at both extremes of the nutritional range. Ewe's serum levels in groups 1 and 3 are not very different from each other but

both of them were consistently higher than the corresponding values for group 4. Serum gamma-globulin values for ewes in group 1 were significantly higher than those in group 4 at four weeks ($P < 0.01$), two weeks ($P < 0.001$) and one week ($P < 0.01$) before lambing.

Values for individual serum immunoglobulins (as measured by SRID technique) and their totals are shown in Table 7.4. The picture observed above for gamma-globulin was also repeated here by total immunoglobulins and IgG_1 which forms around 50 per cent of this total. Those parameters decreased toward lambing. In very late pregnancy IgG_1 and total immunoglobulins were lower in ewes on very high nutrition levels (group 4) than the corresponding levels in ewes that were kept on very low levels of nutrition at late pregnancy (i.e. group 1). This picture became most prominant one week before lambing where group 4 showed significantly lower IgG_1 ($P < 0.01$) and lower total immunoglobulins ($P < 0.05$) than group 1. Serum IgG_2 and IgM were also lower (but not significantly) in group 4 than in group 1. Levels of serum IgA before and at lambing, on the other hand, were consistently lower in group 1 than in group 4. In the last week of pregnancy and at lambing, for

example, the difference in IgA levels of these two groups was statistically significant at $P < 0{\cdot}001$ and $P < 0{\cdot}02$ respectively.

Colostral whey parameters:

These are presented in Table 7·5. Ewes in nutritional groups 3 and 4 showed only slightly lower total protein concentrations than those in group 1. This picture was greatly magnified in the case of gamma-globulin. Group 4 for example, had significantly lower gamma-globulin ($P < 0{\cdot}01$) than group 1.

In all groups colostrum was viscid in consistency and yellow in colour but there was a marked difference in the amount secreted by the ewes in each group and those ewes with low levels of secretion produced exceptionally viscid and highly coloured colostrum. The amount of colostrum produced by the three groups was reflected by their lambs body weight at 48 hours of age (see page 254).

When the different types of colostral immunoglobulins and their totals were estimated, the same picture re-occurred. Group 4 had significantly lower IgG_1 ($P < 0{\cdot}01$) and IgM ($P < 0{\cdot}05$), and lower (but not significantly lower) IgG_2 and IgA than group 1. Corresponding values for group 3 fell in between.

TABLE 7·5.

Ewe's colostral whey parameters (Mean ± standard deviation)

Nutritional groups	No. of samples	Total protein (g/100 ml)	Gamma-globulin (g/100 ml)	SRID values (g/100 ml)				
				IgG_1	IgG_2	IgM	IgA	Total
Group 1	8	21·75 ± 3·56	11·94 ± 2·64	7·82 ± 2·35	0·13 ± 0·04	1·33 ± 0·60	0·53 ± 0·27	9·81 ± 2·70
Group 3	7	18·59 ± 4·28	9·68 ± 2·46	6·52 ± 2·17	0·11 ± 0·04	1·11 ± 0·48	0·43 ± 0·12	8·16 ± 2·20
Group 4	6	19·60 ± 3·77	7·83 ± 1·80	3·88 ± 1·28	0·11 ± 0·03	0·72 ± 0·30	0·41 ± 0·16	5·12 ± 1·36

BLOOD PARAMETERS FOR LAMBS

Nutritional groups comparison:

Blood PCV and glucose levels for surviving twins and triplets (only a few singles and quadruplets were available in some of the nutritional groups) at 24 and 48 hours of age are presented in Table 7·6. There were no important differences in the values of the above-mentioned two parameters, in relation to the nutritional treatment of the ewe. Lamb's 48 hour PCV values, irrespective of litter size or nutritional treatment were lower (but not significantly) than their 24 hour values. Blood glucose values measured both at 24 and 48 hours of age tended to be very high in lambs born to all nutritional groups. These levels showed big individual variation as reflected by the standard deviation values (see Table 7·6).

Levels of serum total protein, gamma-globulin and the different types of immunoglobulins in lambs of the four nutritional groups were calculated on a litter size basis. Table 7·7 presents the 24 hour levels for most of the surviving lambs in groups 1 to 4.

None of the values for surviving lambs were extremely low and when the 't' test was employed to compare corresponding values of different nutritional groups almost all these comparisons showed no statistically significant difference. However, a trend was

TABLE 7·6.

PCV and glucose values in the sera of surviving lambs at 24 and 48 hours of age

(Mean ± standard deviation)

Nutritional groups	Litter size	No.* of lambs	P C V (%)		Glucose (mg/100 ml)	
			24 hours	48 hours	24 hours	48 hours
Group 1	Twins	8	37·4 ± 4·7	(7) 35·0 ± 4·6	113·9 ± 16·4	(7) 110·1 ± 18·3
	Triplets	4	(3) 39·7 ± 5·7	34·5 ± 5·8	110·2 ± 34·6	90·7 ± 29·2
Group 2	Twins	8	39·5 ± 4·1	39·4 ± 3·1	96·8 ± 16·7	(7) 113·4 ± 6·9
Group 3	Twins	6	34·7 ± 1·3	30·2 ± 3·1	115·7 ± 18·2	106·6 ± 24·1
	Triplets	3	40·2 ± 1·9	36·6 ± 3·2	86·7 ± 32·5	113·6 ± 22·6
Group 4	Twins	4	37·7 ± 4·2	30·4 ± 5·6	88·6 ± 35·5	(3) 118·9 ± 4·9
	Triplets	6	36·9 ± 3·9	33·5 ± 2·6	107·4 ± 35·6	109·2 ± 15·3

*If different from these it is noted in brackets.

TABLE

24 Hour serum parameters for surviving

Nutritional groups	Litter size	Number* of samples	Total protein (g/100 ml)	Gamma-globulin (g/100 ml)
Group 1	Singles	3	5·57 ± 1·52	1·46 ± 1·04
	Twins	6	(7) 5·14 ± 1·13	(7) 1·50 ± 0·98
	Triplets	4	6·20 ± 0·99	2·45 ± 1·12
Group 2	Singles	1	6·80	2·85
	Twins	8	6·73 ± 1·15	2·53 ± 0·88
	Quadruplets	6	5·36 ± 1·59	1·32 ± 0·91
Group 3	Singles	2	6·69 ± 0·13	2·94 ± 0·56
	Twins	6	6·90 ± 0·65	2·52 ± 0·58
	Triplets	3	6·57 ± 0·58	2·88 ± 0·35
Group 4	Singles	2	7·41 ± 1·82	3·25 ± 1·39
	Twins	4	5·98 ± 1·24	2·28 ± 0·36
	Triplets	6	(5) 5·44 ± 1·20	(5) 1·50 ± 1·04
	Quadruplets	1	5·60	1·40

*If different from these it is noted in brackets.

7·7.

lambs (Mean ± standard deviation)

SRID values (g/100 ml)				
IgG_1	IgG_2	IgM	IgA	Total
1·80 ± 1·64	0·05 ± 0·02	0·28 ± 0·27	0·13 ± 0·07	2·26 ± 1·93
1·01 ± 0·64	0·03 ± 0·02	0·29 ± 0·20	0·16 ± 0·13	1·50 ± 0·84
1·77 ± 0·48	0·03 ± 0·01	0·35 ± 0·14	0·23 ± 0·12	2·39 ± 0·54
2·05	0·03	0·60	0·39	3·07
2·13 ± 0·53	0·05 ± 0·23	0·63 ± 0·19	0·18 ± 0·07	2·99 ± 0·65
1·22 ± 1·19	0·03 ± 0·01	0·17 ± 0·17	0·16 ± 0·09	1·58 ± 1·33
2·30 ± 0·56	0·05 ± 0·01	0·60 ± 0·08	0·26 ± 0·06	3·22 ± 0·56
2·49 ± 0·56	0·04 ± 0·01	0·54 ± 0·29	0·21 ± 0·12	3·28 ± 0·82
1·73 ± 0·11	0·03 ± 0·00	1·00 ± 0·50	0·24 ± 0·16	3·00 ± 0·57
2·40 ± 0·71	0·03 ± 0·01	0·64 ± 0·51	0·11 ± 0·02	3·19 ± 1·20
2·87 ± 1·12	0·04 ± 0·01	0·51 ± 0·06	0·12 ± 0·02	3·54 ± 1·08
2·41 ± 1·83	0·06 ± 0·03	0·41 ± 0·22	0·20 ± 0·12	3·08 ± 1·98
1·10	0·02	0·48	0·17	1·77

noticed in some of the values measured, particularly when nutritional groups 1 and 4, representing the very low and very high levels of nutrition at late pregnancy respectively, were compared. Singles and twins in group 4 showed higher total protein and gamma-globulin values than their contemporaries in group 1. Values for triplets born to group 1 were unexpectedly high. This was possibly due to the high percentage of weak triplets (two out of six) born to this group. These weak lambs (both of them died later) seemed unable to compete with their sibs so that the survivors had no more competition for colostrum than would a twin lamb.

The IgG_1, IgM and total SRID values were higher in surviving group 4 lambs than those in group 1. However, only in the case of IgG_1 and total values for twins did a 't' test comparison reveal significant differences ($P < 0{\cdot}001$ and $P < 0{\cdot}01$ respectively). IgG_2 and IgM values showed no differences between lambs in group 1 and those in group 4. As might be expected, the total protein, gamma-globulin and SRID values for groups 2 and 3 tended to fall between those of groups 1 and 4 and had the closest correlation with group 4 results.

At 48 hours of age, the levels of almost all parameters observed tended to be markedly lower than their

corresponding levels measured at 24 hours. This was almost consistently the case in lambs of all litter sizes in nutritional groups 2, 3 and 4 and is illustrated by the 48 hour results for twins shown below (g/100 ml) -

	IgG_1	IgG_2	IgM	IgA	Total
Group 2	1·62	0·04	0·53	0·10	2·29
Group 3	2·13	0·04	0·34	0·12	2·63
Group 4	1·61	0·22	0·45	0·11	2·39

Lambs in group 1, on the other hand, showed only slight declines or rising values at 48 hours. The mean total SRID values for 24 and 48 hours of age are shown here, -

		Group 1	Group 2	Group 3	Group 4
Total	24 hours	2·05	2·54	3·168	3·27
(g/100 ml)	48 hours	1·90	2·04	2·600	1·88

These differential rates of fall may be accounted for by rapidity of initial colostrum intake. In group 1 colostrum secretion and intake was initially slow and in group 4 very rapid, hence the antibody absorption mechanism would cease to operate sooner in group 4 lambs and their circulating antibody levels would fall first.

Dead/live lambs comparison:

PCV and glucose:

The values for survivors, at 24 and 48 hours of age, have already been shown in Table 7·6. Because

the number of blood samples collected from dead lambs was very small, it was impossible to make any valid comparison between live and dead lambs according to litter size. Values were therefore pooled and illustrate the following points.

Levels of blood glucose for dead lambs were not different from those of survivors - Example: Nine non-surviving lambs (all multiple born) had a 24 hour mean glucose value of 104·9 ± 44·5 mg/100 ml, compared to 101·6 ± 26·8 mg/100 ml value showed by 46 multiple born surviving lambs.

PCV, on the other hand, stayed higher (perhaps because of the lack of colostrum intake and/or dehydration) in non-surviving lambs.

The nine dead lambs mentioned above had a 24 hour mean PCV value of 40·9 ± 7·4 per cent compared to 37·0 ± 4·6 per cent shown by the multiple born survivors. The difference between the two values was just statistically significant ($P < 0·05$).

Total protein and immunoglobulins:

Values for the different biochemical parameters dealt with are presented in Table 7·7. Corresponding values for dead lambs are presented in Table 7·8.

The number of lambs that were bled at 24 hours of age is smaller than the corresponding number of

TABLE 7·8.

24 Hour serum parameters for lambs which died

(Mean ± standard deviation)

Nutritional groups	Litter size	No. of samples	Total protein (g/100 ml)	Gamma-globulin (g/100 ml)	SRID values (g/100 ml)				
					IgG_1	IgG_2	IgM	IgA	Total
Group 1	Twins	2	4·27 ± 0·38	0·18 ± 0·10	0·30 ± 0·08	0·03 ± 0·001	0·04 ± 0·03	0·10 ± 0·001	0·46 ± 0·12
	Triplets	2	5·20 ± 0·98	0·73 ± 0·88	0·83 ± 0·94	0·04 ± 0·001	0·26 ± 0·29	0·15 ± 0·05	1·29 ± 1·29
Group 2	Quad-ruplets	1	5·8	0·3	0·10	0·02	0·06	0·13	0·32
Group 3	Triplets	3	3·86 ± 0·60	0·16 ± 0·15	0·35 ± 0·39	0·03 ± 0·006	0·04 ± 0·01	0·13 ± 0·05	0·55 ± 0·33
Group 4	Quad-ruplets	2	4·00 ± 0·56	0·45 ± 0·07	0·17 ± 0·09	0·015 ± 0·004	0·11 ± 0·07	0·09 ± 0·03	0·39 ± 0·13

dead lambs. This is because some of the lambs died at or shortly after birth.

The consistent pattern which emerges is that total protein, gamma-globulin and the various immunoglobulins are lower in lambs which died than in those which survived. Many of these differences were not statistically significant principally because the number of dead lambs was too small for a valid comparison, e.g. group 1. Despite this disadvantage, the results for the group 3 triplets were significantly different ($P < 0{\cdot}01$) when surviving and non-surviving lambs were compared for total protein, gamma-globulin, IgG_1, IgM and total SRID values.

Statistical comparisons for quadruplets in groups 2 and 4 were not possible but the same trend was apparent in these lambs (Tables 7·7 and 7·8).

DISCUSSION

In this nutritional study, plasma 3-hydroxybutyrate was used as an indicator of energy intake during late pregnancy. A search of the literature failed to reveal any published evidence of this parameter being used as a monitor for this purpose although it is used successfully by workers of the Hill Farming Research Organisation, Edinburgh (HFRO).

Russel (Personal communication), a worker in HFRO, is about to publish results of his investigations on values of plasma 3-HB. He associated values of about 0·7, 1·1 and 1·6 mM/L with adequately nourished, moderately undernourished and severely undernourished Greyface ewes respectively, during the last six weeks of pregnancy.

3-HB levels measured by the method of Zivin and Snarr (1973) appear to be a good indicator of energy intake although the quantitative values are at variance with these reported in my preliminary nutritional work, which refers to plasma total ketones as measured by the methods of Reid (1960). Regarding the two values, i.e. 3-HB and total ketones, the Hill Farming Research Organisation, Edinburgh, suggested the following formula which can be used to change 3-HB values to total ketones and vice versa:

Total ketones (mg/100 ml) = [3-HB value X 6·3137] - 1·90396.

Plasma ketone levels of 8 to 10 mg per 100 ml were described by Russel *et al.* (1967a), Russel (1971) and Davies and Ross (1973) as indicative of undernourishment in pregnant ewes. If the above formula is used, these levels will be equivalent to 1·57 to 1·88 mM/L in terms of 3-HB. After taking litter size into consideration, ewes with twins in group 1 showed levels between 1·5 and 2·0 mM/L during the last five weeks of pregnancy. Ewes

in group 2 and 3 showed much lower levels and only on a few occasions in the last three weeks before lambing were their levels slightly higher than 1·0 mM/L (about 4·4 mg per 100 ml total plasma ketones). My previous results indicated that at this level a slight degree of nutritional stress exists. Ewes in group 4 on the other hand showed very low values until the last week before lambing where, in spite of a noticeable increase in 3-HB value, the readings never reached 1 mM/L. For ewes with twins, it was 0·8 mM/L at the greatest, and this is equivalent to 3·14 mg per 100 ml total plasma ketones.

This variation in 3-HB values measured for ewes with twins clearly reflected the nutritional status of ewes in the different groups. Bearing in mind the levels of energy intake by ewes during late pregnancy, it seems that 3-HB values up to 1 mM/L, or slightly higher, could mean that ewes are not under serious nutritional stress. It is only when these levels exceed 1·5 mM/L that the ewes can be described as severely undernourished. On this basis, only the ewes in group 1 could be described as severely undernourished.

The other techniques which I used to monitor energy intake in pregnant ewes were blood urea and serum albumin levels. These methods have to date been used in cattle on a large scale since the introduction of the metabolic profile test by Payne, Dew, Manston and Faulks (1970).

Since then, further work done by Payne, Rowlands, Manston and Dew (1973), Rowlands, Payne, Dew and Manston (1973) and Manston, Russell, Dew and Payne (1975) on dairy cows, mainly during the lactation period, has had the aim of ascertaining possible factors that could help in the selection of superior cattle.

There were no clear-cut findings. Interpretation of results concerning the effect of feeding levels on the blood chemical constituents of these cows was difficult because of a complex of factors such as type and energy content of the food offered, activity of rumen micoflora and amount of ammonia produced in the rumen which also affects digestion and metabolism. This work proved of some use regarding parameters such as urea, albumin and haemoglobin. Payne and his colleagues have urged that a more accurate study of the influencing factors and of the blood indicators should be undertaken.

Urea and albumen levels were employed by Blowey (1972) and Parker and Blowey (1976) in their "mini-profile test" used to ascertain the nutritional status of dairy cows. Relatively little work of this kind has been carried out on sheep.

In my work, blood urea levels appeared to be related to the levels of feeding during late pregnancy particularly when the extremes of these levels were compared, i.e. group 1 and group 4. This relationship

existed throughout the observed period but not at lambing, possibly due to the noticeable and not unexpected reduction of food intake a few days before lambing, by all ewes regardless of their nutritional treatment. Blood urea levels, however, only show clear variations in very well nourished ewes and this technique, although worthy of further study, would not appear to be as sensitive as the 3-HB method for detecting levels of undernourishment.

In humans, a reduction in the serum protein (due to reduction of albumin alone) was reported in association with malnutrition many years ago by Bruckman, D'Esopo and Peters (1930). Serum albumin levels of the ewes I observed were not affected by the levels of feeding in late pregnancy even when very low or very high levels of feeding were used. Although this parameter was of no value as an indicator of energy intake in the context of my work it may have some value in relation to the overall protein status of both ewes and lambs.

Litter size was an important factor affecting the levels of energy requirement by ewes in late pregnancy. The low nutrition group (group 1) showed a considerable degree of undernourishment, as indicated by their high 3-HB values, in spite of the group's relatively low litter size (1·9 lambs per ewe).

Although ewes of groups 2 and 3 had similar levels of

energy intake in the last eight weeks of pregnancy, those in group 2 showed a higher degree of nutritional stress. This may be accounted for by the fact that group 2 ewes averaged 2·43 lambs per litter while the corresponding figure for group 3 ewes was only 1·88. Ewes of group 4 had 3-HB values in late pregnancy higher, in most instances, than those of group 3 ewes. Again, the likely reason is the larger mean litter size of group 4 ewes, i.e. 2·29.

The type of food offered during late pregnancy, irrespective of its energy content, had a bearing on the results obtained in this work. Ewes which refused food during the experiment, were excluded and consequently reduced the number of ewes and lambs available for further study. If such ewes had been retained, as they would have been in commercial circumstances, then the incidence of early lambings and PLM would in all probability have increased. Although this effect was eliminated from the experiment it is noteworthy that refusals occurred in nine per cent of group 1 ewes, 20 per cent of group 3 ewes, 18 per cent of group 4 ewes and not at all in group 2 ewes. Very high energy diets would appear to be particularly liable to refusal and could be a significant factor in increasing PLM. This opinion is strengthened by the fact that the majority of ewes excluded for food refusal, did not eat well even after their diet was

changed and they produced weak early lambs.

The reduction in experimental animals, caused by food refusal, affected the overall PLM figures for ewes which completed the experiment. With such small numbers of animals left in each group, a single lamb death could increase the PLM figure by as much as seven per cent and the poor performance of one ewe could upset the figures for the whole group. For example, when one ewe in the highest nutritional group lost three lambs from a set of quadruplets the poor performance of this single animal raised the group's PLM figure to the level of group 1.

Litter size also had a bearing, with PLM being highest in the multiple litters, most of the multiple litters occurring in the higher nutrition groups.

Besides all the factors referred to above, which might have affected the interpretation of my findings in relation to PLM, the diversity of causes of lamb deaths added to the difficulties. In nutritional group 3 for example, the death of one lamb due to an accident unrelated to nutrition during late pregnancy made PLM look very high and the appropriate figure is 20·0 per cent instead of 26·6 per cent if that death is excluded.

The PLM figures are misleading for the reasons already stated but the level of ewe nutrition does not only affect PLM it also affects factors such as the ewe's body weight and condition scoring, her milking

ability, the number of ewes which lost lambs, the number of ewes which died and the birth weight and early growth rate of the lambs born. It is wiser, in the context of this experiment, to see how the nutritional regimens affected these factors as a whole in order to draw more realistic conclusions than those available from the PLM figures alone.

Ewe weight loss or gain during pregnancy followed the pattern established in 1974 and it was clear that ewes with large litters lost more weight, or gained less, than ewes carrying small litters. It was also clear that group 1 ewes lost considerably more weight than ewes in any other group. Ewes in group 1 lost 20 per cent or more of their body weight during pregnancy and this could only adversely affect the animals' productive performance after lambing.

The ewe death rate was nil in the high nutrition groups but ewes were lost in the hay only groups. However, both deaths occurred well after lambing and were due to metabolic causes. It would be unwise to ascribe either death directly to the effects of late pregnancy feeding.

It may also be unwise to connect the number of ewes which lost lambs directly to the nutritional regimen. The results in this connection appear unclear with both group 1 and group 3 having 40 per cent of their ewes losing

lambs. However, in group 1, all the ewes which lost lambs did so as a direct result of their own inadequate milk production, while in group 3 one ewe lost her lamb accidentally and in another case Toxoplasma rather than nutrition may have been the cause of weak lambs being born. Only in the case of group 1 ewes can the loss of lambs be clearly ascribed to poor milk production resulting from poor late pregnancy feeding.

Lamb birth weights are affected, as they were in 1974, by litter size with the smallest lambs occurring in the largest litters. In addition there is a clear distinction between group 1 lambs and all other groups. Lamb birth weights were adversely affected, irrespective of litter size, by the poor group 1 nutrition. In the better nourished groups, lamb birth weight was slightly affected by levels of nutrition. Birth weights of lambs from groups 2 and 3 only decreased noticeably when litter size exceeded two.
The heaviest lambs occurred in group 4, the group with the highest energy intake.

The initial colostrum production by the ewe, low lamb birth weights and the subsequent growth rate of lambs are factors which may well be linked. Group 1 ewes showed a 50 per cent incidence of poor early colostrum production, lamb birth weights were low and the growth rate of twins and triplets was seriously impaired

up to three weeks of age. This pattern does not occur in any of the other groups. Even group 2 ewes, which had an unacceptably high incidence of poor colostrum production after parturition, appear to have come into milk within 48 hours of parturition and there was no detrimental effect on lamb growth rates.

It may be stated that although PLM levels appeared high in all groups, in reality it was only in group 1 that nutrition had a serious overall effect on ewe and lamb performance. Ewe body weights, ewe milk production, lamb birth weights and subsequent growth rates were all adversely affected in group 1. In no other group did this pattern of events occur. Undernourishment of ewes at this level, which is seen in some commercial farms in Scotland, will have a serious effect on lamb production on such farms.

As the 1974 and 1975 results indicate that the feeding of very poor hay only during pregnancy does have adverse effects, it would be profitable in future work to increase ewe nutrition to a level comparable with that found in most Scottish farms to ascertain if any adverse effects occur at this level when lambs are deprived of colostrum.

Of the biochemical parameters measured in ewe sera, prior to or at parturition, total protein levels appeared to be only slightly affected by nutrition. The levels

declined in all groups as parturition approached. The levels in group 4 ewes were consistently lower than those for ewes in groups 1 and 3. This reduction was accounted for by the lower gamma-globulin levels in the sera of group 4 ewes and most of this was accounted for by a reduction in the level of IgG_1.

Because level of feeding in late pregnancy can affect the amount of colostrum or milk produced (Wallace, 1948 a, c; Thomson and Thomson, 1953; Guyer and Dyer, 1954; Robinson and Forbes, 1968; Treacher, 1970; Louca et al., 1974), and as IgG_1 is the major immunoglobulin of the colostrum, one can draw the conclusion that the lower levels of serum IgG_1 in group 4 ewes is related to their greater colostrum production and hence greater transfer of IgG_1 to the udder. It is possible that the slight changes in serum IgM and IgG_2 could be attributed to the same reason.

Serum IgA remained unchanged in the last four weeks of pregnancy but was significantly higher in higher nutritional groups than in the extremely low nutrition group. This would have been due to an increased local antibody production of IgA by more numerous IgA synthesizing plasma cells (Lee and Lascelles, 1970) in the mammary glands as a result of their stimulation for more colostrum secretion in the very high nutrition group. Some of the IgA might have been transferred into the circulation,

hence the higher levels of IgA in the sera of these ewes. Relationships of this kind between levels of circulating IgA and the activity of the local immune system have been noticed among pregnant cows in relation to local stimulation of the udder by E. coli vaccine (Wilson et al., 1972) and also in pregnant ewes' udders that have been infused three weeks before lambing with killed Brucella abortus organisms (Lascelles and McDowell, 1970; Watson and Lascelles, 1973a).

In the initial stages of this work I considered that low levels of ewe nutrition might adversely affect the concentrations of colostral immunoglobulins. Both the literature referred to, and the results presented here, dispute this assumption, in so far as it relates to immunoglobulin concentrations in colostrum. The absolute amounts are another matter.

As I stated previously, there were many workers who reported a high correlation between levels of ewe feeding in late pregnancy and the quantities of colostrum or milk produced. However, with the exception of Treacher (1970), these workers did not refer to the effects of these feeding levels on the quality of the lacteal secretions. Treacher (1970) reported high concentrations of total protein in colostrum produced by ewes that were undernourished during late pregnancy and attributed this to the small amounts of colostrum produced by them. In my

study although the colostrum total protein and all the major immunoglobulins were measured in the different nutritional treatments, no attempt was made to measure the volume of colostrum produced.

The protein and immunoglobulin measurements made on colostrum samples taken just after parturition, did not indicate that ewes on good nutrition had higher concentrations than ewes on poor nutrition, in fact the reverse was true. The protein content of colostrum is related to the amount of colostrum produced and hence indirectly to the nutritional status of the ewe.

The gross examinations of the udders and also the changes in lamb's body weight at 48 hours of age (discussed previously) indicated that the amount of colostrum produced by ewes in the very low nutrition group, i.e. group 1, was extremely low. The colostrum produced by ewes in the group showed very high concentrations of total protein and of the different immunoglobulins, particularly IgG_1 and IgM, the two major immunoglobulins of colostrum. The better performance of group 3 ewes in terms of colostrum production was reflected by their intermediate immunoglobulin values. In the very high nutrition group (group 4), large amounts of colostrum were secreted with total protein and immunoglobulin concentrations lower than those of group 1. Clearly, considerable dilution occurred. Nevertheless,

these values were by no means low and were more than compensated for by the volume produced.

Severe undernourishment of ewes in late pregnancy will either stop colostrum production, delay its "let down" or result in the production of small quantities of highly concentrated secretion. In the last situation, although this colostrum will have very high concentrations of immunoglobulins, its physical characters (i.e. volume and consistency) will greatly reduce its usefulness for the newborn lamb.

Unfortunately, owing to the difficulties in the production of specific antisera for the SRID technique, it was not possible to process the colostrum samples from the 1975 work before the end of the year. The results were not available for perusal until early 1976 by which time it was not possible to organise a study of colostrum production by ewes in the spring of 1976. Such an experiment directed towards establishing the total quantities of the various immunoglobulins in colostrum, and not simply their levels at a given time in unquantified volumes of colostrum, is essential if a fuller understanding of the effect of nutrition in late pregnancy is to be acquired.

Among the parameters analysed in the lambs' sera, both PCV and glucose tended to be poor indicators of the levels of late pregnancy feeding. Glucose values showed

great individual variations making interpretation very difficult, particularly in my experimental circumstances which involved only a few lambs in each nutritional group. PCV values at 48 hours were much lower than their values at 24 hours, and this might have been an affect of the plasma volume increase following colostrum ingestion. Similar findings were observed in piglets (McCance and Widdowson, 1959) and calves (McEwan et al., 1968).

In group 1, the continuing high PCV readings in lambs at 48 hours coincided with the high percentage of ewes with no milk at lambing and reflects the low rate of colostrum ingestion and absorption with corresponding modest expansion of blood volume in these lambs. In group 4, on the other hand, where very high levels of feeding were offered during late pregnancy, heavier and stronger lambs were produced, and more colostrum was secreted, the lamb's PCV had declined considerably from 24 to 48 hours. It is possible that, had dead twins and triplets in group 1 survived until sampling at 48 hours, these differences in PCV levels of the very high and very low nutrition groups of lambs would have been more obvious. This is on the assumption that each lamb would have ingested even less colostrum.

It was interesting to notice that PCV levels of twins born to group 2 were similar at 24 and 48 hours.

These lambs were born to ewes which received only good quality hay in late pregnancy. From the changes in PCV values, and also the record of relatively poor milk production performance as indicated by its lack at lambing (see Table 7·1), it seems that hay alone, even of good quality, in the absence of concentrate supplementation had put the ewes at some disadvantage regarding the amount of milk produced immediately after lambing.

Turning to more important parameters like total protein and immunoglobulins, and their levels in lambs' sera, as affected by the ewes' nutritional treatment, the comparisons were performed on surviving lambs only. When corresponding values for singles or twins were compared on a nutritional treatment basis, lambs born to the poor nutrition group had less total gamma-globulin and especially IgG_1 and IgM. This seems to be an indirect effect of level of ewe feeding in late pregnancy through limiting the amount of colostrum produced in which IgG_1 and IgM are the two major immunoglobulins. When comparisons on lambs from bigger litters (three or more) were performed the whole picture was clearly distorted by the litter size itself (due to weakness or death of some of the lambs born to these litters). An example of that is the case of triplets in groups 1 and 4 (see Table 7·7).

Only at the extremes of the levels of feeding used during the last eight weeks of pregnancy did the immunoglobulin levels in sera of surviving lambs show marked variations.

The lambs born to ewes on the good quality hay (group 2) had serum immunoglobulin levels higher than those whose mothers had been on the poor quality hay alone (group 1) and no doubt, in certain circumstances, this would be an advantage to them. The lambs of group 2 ewes had immunoglobulin levels comparable to those of lambs from group 3 mothers which had been on poor hay plus concentrate supplementation in late pregnancy. Concentrate supplementation seemed to increase colostrum availability at lambing time as did also good quality hay in the circumstances of this experiment in comparison with the results of feeding poor hay only. As lambs in groups 2 and 3 had comparable 24 and 48 hour levels of serum immunoglobulins, it seems that the vitality at birth of lambs in group 2 (as expressed by their high birth weight) helped them to compensate for their mother's moderately poor milking performance at lambing.

It was noticed that in circumstances where ewes were fed to requirement (groups 2 and 3) or more than requirement (group 4), the lambs' serum IgG_1 and IgM levels reached a maximum at 24 hours of age. These values were significantly reduced at the age of 48 hours. IgG_2 and

IgA did not show great changes following colostrum ingestion. This shows the importance of ensuring adequate colostrum intake in these early critical hours of the newborn lamb's life where the gut immunoglobulin absorption seems to be at its highest rate. The lower levels of immunoglobulin in the lamb's sera at 48 hours (in lambs born to the above mentioned three nutritional groups) coincided with the rapidly falling levels of immunoglobulins in the colostrum secreted at that time Ignatēva, 1971; Porter, 1972). At the same time, it could be postulated that the sucking by lambs, of optimum amount of colostrum might have enhanced the "shut down" mechanism of the newborn gut against all particles whether they are useful immunoglobulin molecules or harmful invading micro-organisms.

Lambs born to the low nutrition group, on the other hand, showed lower immunoglobulin levels at 24 hours of age. At 48 hours of age, these levels were fractionally reduced and this might have indicated a slower rate of immunoglobulin absorption compared to that shown by lambs born to the other three nutrition groups.

In spite of these noticeable changes, levels of immunoglobulins in surviving lambs born to the poor nutrition group were not very low and it seems likely that this situation was achieved partially at the expense of lambs which failed to get adequate colostrum and

eventually died. The levels were, however, consistently less than those of lambs from the other groups and this factor allied to others, such as low birth weight and poor milk supplies, contributed to the poor production performance of group 1 lambs.

When comparisons are made between lambs which died and those which survived, changes in the blood parameters are not easy to interpret. This is mainly due to the small number of lambs available in each group. However, where comparisons could be made, both PCV and glucose levels followed similar patterns to those observed previously in the preliminary nutritional study of 1974 and the colostrum deprivation study of 1975. That is to say, glucose measured in non-surviving lambs did not differ from that of surviving ones, but in both groups of lambs, high individual variations were noticed. As I stated previously, this parameter is very low only at or shortly before death. Twenty-four hour PCV, on the other hand, was still high for non-surviving lambs, possibly due to their lower rate of colostrum intake and/or absorption, and to some degree of dehydration. Neither of these parameters will be included by me in future investigations on lamb losses.

In relation to serum protein levels of surviving and non-surviving lambs, in spite of the very small number of lambs that were bled before death, my data

showed very clearly that lambs which died had extremely low serum total protein and gamma-globulins at 24 hours of age. In the sera of lambs which died, IgG_1 and IgM levels were markedly low compared with those of surviving lambs. These lower levels clearly reflect the very inadequate intake or insufficient utilisation of colostrum by these lambs. Serum IgG_2 and IgA levels of lambs which died did not differ from those of survivors. In relation to IgG_2 my previous work (see Chapter VI) and the present study show that only traces of this immunoglobulin existed in ewe colostrum, hence the very minute amount of it in the newborn lambs' sera. The extremely high levels of IgG_2 in the ewes' sera, observed by other workers (Watson and Lascelles, 1973b; Ciupercescu, 1977) and by myself, in comparison to its levels in ewes' colostrum and in the sera of their 24 and 48 hour old lambs suggests that very little transfer from serum to colostrum occurs during pregnancy (unlike the case of IgG_1 and IgM). Further, the very low levels of IgG_2 in colostrum and in the sera of young lambs might indicate its insignificant role in the protection of the newborn lambs against infection.

At a time when serum levels of the different immunoglobulins in non-surviving lambs indicated that they were at a disadvantage regarding systemic antibody status, the absence of colostrum or milk from the majority of these lambs at post-mortem examination could suggest that

they might have been at a similar disadvantageous situation regarding the role of local immunity, generally exerted by IgA, i.e. these non-surviving lambs had been starved both in terms of colostrum ingestion and passively achieved immunity.

For non-surviving lambs, serum immunoglobulins were low regardless of their litter size, but the fact that most of the deaths occurred among triplets and quadruplets emphasises that the relationship between litter size and PLM should always be treated seriously.

Conclusion

Forty ewes were included in the design of the experiment. Food refusals and barrenness reduced the number in each nutritional group below that desirable and this has, to some extent, affected the conclusions. However, certain conclusions can be reached and some assumptions made.

1. Plasma 3-HB is a useful indicator of the nutritional status of pregnant ewes, especially when very different levels of feeding are compared. In ewes similarly fed, the effect of litter size can be monitored by plasma 3-HB estimations. As expected, large litters impose additional stress on pregnant ewes.

2. Urea levels in ewe blood are also related to the level of feeding in the last eight weeks of pregnancy, but are a less sensitive indicator of nutritional status than 3-HB levels.

3. Blood albumin levels were not affected by nutritional status of pregnant ewes.

4. The levels of nutrition used in this experiment affected ewe productive performance. Very high energy diets caused food refusal in some ewes. Low levels of nutrition clearly affected ewe and lamb weights, ewe milk production and consequently lamb growth rates. PLM figures were misleading in this work.

5. There is a relationship between levels of feeding during late pregnancy, ewes serum immunoglobulins particularly IgG_1 and to a lesser extent IgM and IgG_2, and levels of colostrum production. IgA did not show any significant changes apart from its increase in the sera of well fed ewes which produced larger amounts of colostrum.

6. Colostrum quantity rather than quality is affected by low levels of nutrition, and future work measuring total colostrum secretion as well as immunoglobulin levels is indicated.

7. IgG_1 and then IgM seem to be the important immunoglobulins (at least quantitatively) in the sera of 24 and 48 hour old lambs. These levels are related to the distribution of the different immunoglobulins in the colostrum, and also to the quantities of colostrum ingested by the lambs. Again there is a correlation between serum immunoglobulins and PLM. Levels of ewe nutrition in late pregnancy are related to starvation in newborn lambs as nutritional levels affect ewe colostrum production. As both factors, i.e. nutrition and colostrum shortage, seem to be major causes of poor ewe and lamb performance and of PLM, as well as studying their joint effects on these matters, it is necessary to have a clearer understanding of their operation separately. The necessary investigations will require larger groups of ewes on different nutritional planes than were available in the present work. Further, such a study would be a major piece of work in its own right.

8. Low levels of ewe feeding in late pregnancy, by itself (reported in this chapter and also in Chapter V) and colostrum deprivation of lambs, as such (reported in Chapter VI), seem to affect, but not necessary significantly, the ewes

and lambs performances, and also most of the biochemical parameters studied. As both of these factors are related, and they seem to represent major causes of the general set-back in lamb performance and of PLM, it is important at this stage to try studying the effects of the two factors simultaneously, i.e. the interaction between the two factors and their overall effect on PLM and ewe performance. When this is attempted, larger nutritional groups will have to be employed.

C H A P T E R E I G H T

OBSERVATIONS ON THE EFFECTS OF LATE PREGNANCY FEEDING AND COLOSTRUM DEPRIVATION AND THEIR COMBINED EFFECTS ON EWE AND LAMB PERFORMANCE

INTRODUCTION

In the previous three chapters the results of separate studies made into the effects of various nutritional regimens on PLM and other ewe performance criteria, and the effect of colostrum deprivation, as such, on the performance of newborn lambs have been recorded. To put the whole matter in perspective, I decided to study the combined effects of the above mentioned two factors, i.e. ewe late pregnancy level of feeding and colostrum deprivation of lambs, on the following.

1. Changes in ewe body weight and condition scoring between mating and lambing.
2. PLM and the consequent performance of surviving lambs.
3. Biochemical parameters in ewes and their lambs.

This work was conducted during the Easter lambing of 1976.

EXPERIMENTAL DESIGN

A flock of 57 pregnant Scottish Halfbred ewes were brought inside eight weeks before the expected day of lambing. Before this time, the ewes were managed exactly in the same way as those groups of Scottish Halfbred ewes observed in the Easter lambings of 1974 and 1975.

At the beginning of the experiment, the ewes were allocated to two nutritional groups. During the allocation, ewe body weight, condition scoring and age were taken into consideration. The two nutritional groups were:-

1. Low-nutrition: The 29 ewes in this group were fed ad libitum hay during the last eight weeks of pregnancy. The nutritive value of this hay, as measured by its ME content was 9·15 MJ per kg DM.

2. High-nutrition: This group included 28 ewes which were offered the same hay as that given to ewes in the low nutrition group ad libitum. Ewes in this group were also supplemented with concentrates. The concentrate which was of the same origin and nutritive value as that used in 1974 and 1975 work, was offered in increasing amounts toward lambing so that each ewe received about 20 kg of it during the last eight weeks of pregnancy.

During housing, the two groups of ewes were kept separately in two big pens (approximately 8 x 8 metres). They were managed exactly in the same way as the group-fed sheep previously described in Chapter IV.

Two of the ewes in the low-nutrition group and one

of the high-nutrition group were later deleted as they proved to be barren. One ewe in each of the two nutritional groups were also later excluded as they lambed very late. Because of these exclusions, only 26 ewes were left in each of the nutritional groups.

At lambing, the management of the ewes and their lambs was the same as that described for other nutritional groups observed during the previous two years of study on PLM.

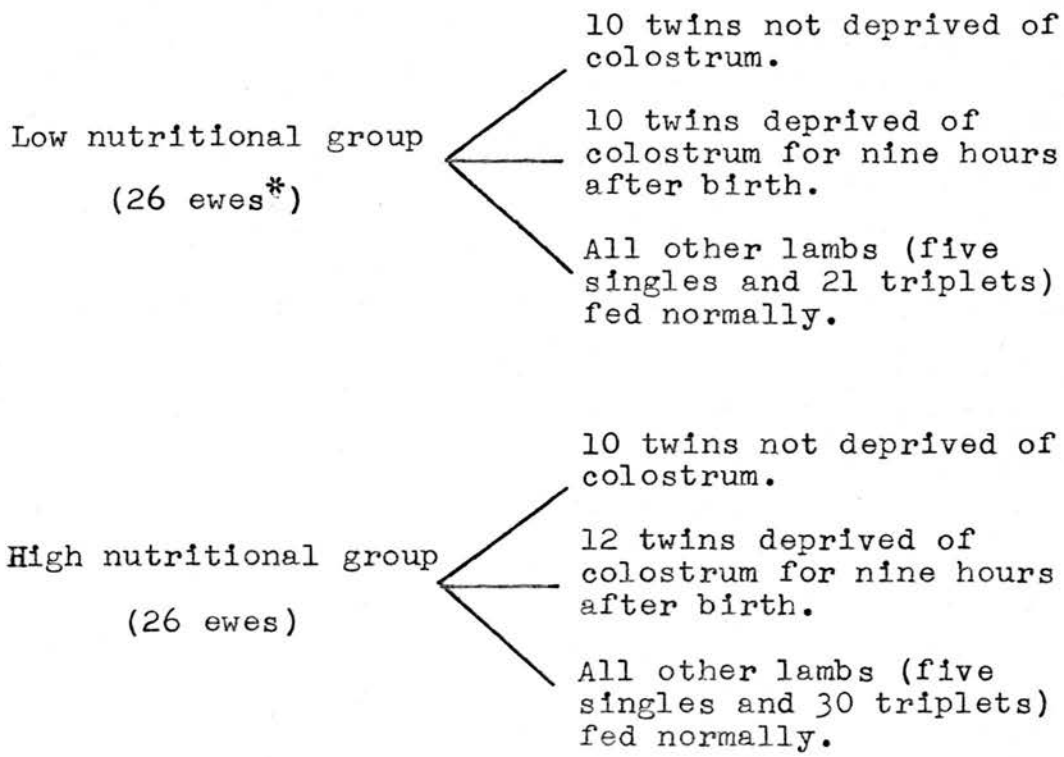
All ewes, regardless of their litter size were included in the nutritional study. Simultaneously, a colostrum deprivation study was undertaken and for this purpose only ewes that gave birth to surviving twin lambs were used. From each nutritional group, ewes and their twins were randomly allocated, using a table of random numbers (Snedecor and Cochran, 1971) into one of the following two groups:

a. Control group: Twins in this group were reared by their mothers naturally. They were allowed to suck as early as possible.

b. Deprived group: Twins in this group were deprived completely of colostrum for nine hours after birth. A suitable cloth cover, neatly placed on the udder, and careful observation were used to ensure a complete colostrum deprivation. At the end of the

deprivation period the cloth was removed and lambs were allowed to suck their mother freely and naturally.

The experimental design can be briefly described as follows:



SAMPLES COLLECTED AND PARAMETERS OBSERVED

All ewes were weighed and body condition scored at mating and at 24 hours after lambing. They were all bled several times during the last eight weeks of pregnancy and also shortly after lambing, and the following

*Three of these ewes aborted (all with twins), 10 to 15 days before expected day of lambing. A fourth one lambed to term with twins, one of which died very shortly after birth. These four ewes were excluded from the experiment.

parameters were observed: plasma 3-hydroxybutyrate (before lambing only), blood urea and serum albumin for monitoring the ewe's nutritional status. The sera were also analysed for total protein, gamma-globulin (Biuret) and the different types of immunoglobulins (SRID). Colostrum samples collected shortly after lambing (before lamb sucking) were analysed for whey protein and immunoglobulin levels.

Lambs were weighed at birth, 24 and 48 hours of age and also around three weeks of age. All lambs were bled at birth, 24 hours, 48 hours and also once at four to six weeks of age. Samples collected at birth were analysed for serum alkaline phosphatase (SAP) and immunoglobulins (SRID). Those collected at 24 and 48 hours were analysed for alkaline phosphatase, total protein, gamma-globulin (Biuret) and immunoglobulins (SRID).

A parameter not included in the previous two years of observations on perinatal lamb mortality is SAP. This parameter has been badly ignored in sheep in relation to performance of normal sheep or clinically ill sheep. Healy (1971a) postulated a relationship between SAP and growth rate of newborn lambs. The same author (1971b) also reported an increase in SAP activity following feeding but colostrum was not essential to manifest this increase. In this study, this parameter will be related to the intra-uterine growth of

the fetuses (as assessed by lamb birth weight) and also to the consequent growth rate of lambs in both nutritional groups. The effect of sex and litter size of newborn lambs on SAP will be studied.

The age of the lambs at the last sampling varied from four to six weeks because lambs were left to graze with their mothers in different fields covering a wide grazing area. This made it practically impossible to bleed all lambs at one fixed age. The samples collected from lambs at four to six weeks of age were analysed for alkaline phosphatase levels and also for levels of the different types of immunoglobulins as measured by the SRID technique.

RESULTS

NUTRITIONAL STUDIES

Levels of feeding during late pregnancy:

Total energy intake was calculated from the ewe total food intake and the nutritive value of the food offered. In the low nutrition group, the mean ewe total energy intake during the last eight weeks of pregnancy, expressed in terms of metabolizable energy (ME) was 689·4 ± 41·2 MJ per kg DM. The corresponding value for the high nutrition group was 906·06 ± 40·0 MJ per kg DM. Although the difference between the two values was statistically highly significant ($P < 0{\cdot}001$), the nutritional level calculated for the low nutrition groups can

not be described as a very low one particularly if it is compared to the low levels used in the previous years of study. This was, in part deliberate (see Chapter VII) and in part because following a good hay crop the poorest quality hay available for my study in quantities sufficient to cover the whole work conducted in 1976 was one with an ME content of 9·15 MJ per kg DM. As shown by the following figures, and supported by the findings of Ferguson (1975), the better the quality of hay the greater the intake.

Year and type of hay	Hay ME content (MJ/kg DM)	Average ewe food intake in the last eight weeks of pregnancy	
		kg	MJ/kg DM
Hay I (1974)	6·99	54	360·8
Hay C (1975)	7·99	58·3	465·6
Hay A (1975)	10·00	84·6	845·8
Hay (1976)	9·15	75·3	689·4.

If the first two types of hay are described as 'poor' and the third one as 'good', depending on the performance of ewes that were kept on them (see previous chapters), then the hay of 1976 can be at least described as a 'medium/good' type of hay. Although ewes included in nutritional trials of 1974 and 1975 were individually fed, and those observed in 1976 were group fed, the two systems of feeding did not have any effect on the level of feed intake (Ferguson, 1975). The above mentioned

figures offer an explanation for the relatively high total energy intake value of the poor nutrition group of 1976.

Levels of feeding in the two nutritional groups were monitored several times during late pregnancy by measuring plasma 3-HB levels (Fig. 8·1). Ewes in the low nutrition group always had higher 3-HB levels than those in the higher nutrition group whether the levels were calculated for all ewes irrespective of their litter size, or for ewes carrying twins only. Ewes in the low nutrition group were not actually under severe nutritional stress. This is shown by their 3-HB values (1·0 mM/L) for ewes with twins, as late as the last week of pregnancy, a moderate value compared to those noticed in the 1975 work (see Fig. 7·2). Nevertheless, the 3-HB levels reflected the levels of nutrition and indicated that the two groups of sheep represented two distinct nutritional groups.

Other parameters used in monitoring levels of nutrition during late pregnancy are urea and albumin. Levels of urea and albumin in ewes before lambing are shown in Table 8·1. The estimations of these parameters and others were performed on three occasions during the last eight weeks of pregnancy. At the second bleeding (two to four weeks before lambing) it was not possible to bleed all the ewes.

When Student's 't' test comparison, based on level of nutrition or type of litter size, was carried out on

FIG. 8·1

Ewe's plasma 3-HB levels during late pregnancy.

(Average litter size: High group = 2·19, Low group = 2·08)

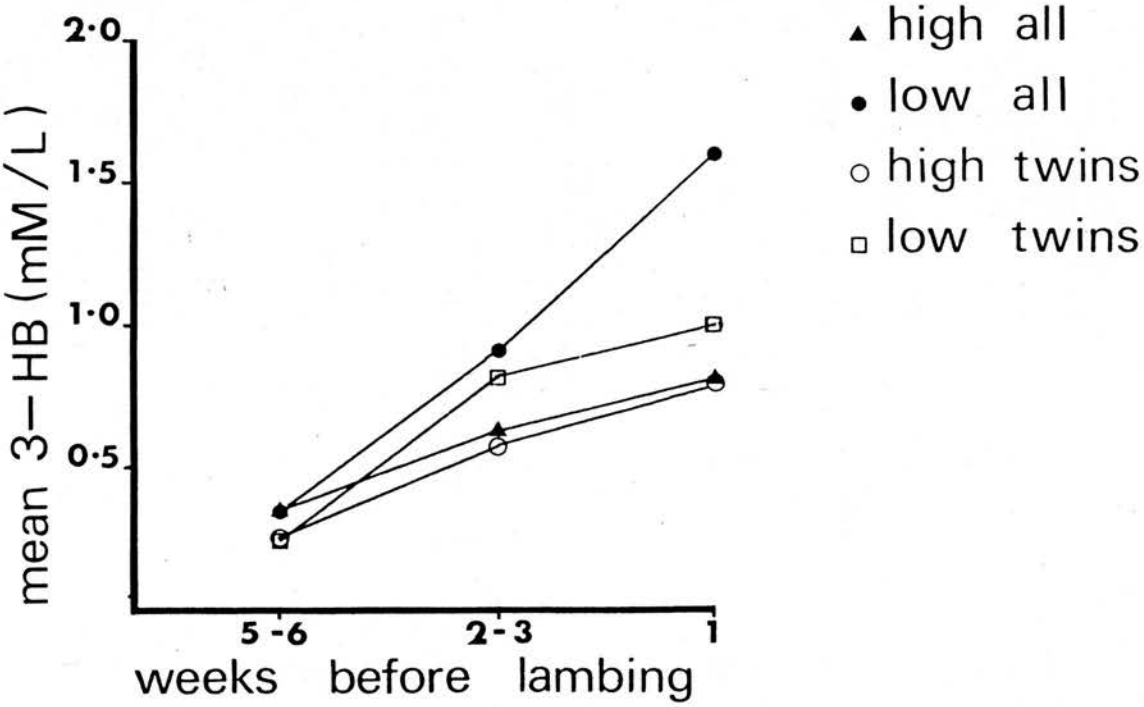


TABLE 8·1.

Levels of urea (mg/100 ml) and albumin (g/100 ml) in ewes sera before lambing (Mean ± standard deviation)

Ewe's litter size	Weeks before lambing	No.* of samples	High nutrition group		Low nutrition group	
			Urea	Albumin	Urea	Albumin
Singles	<2	5	19·6±6·3	2·54±0·18	13·3±1·8	2·44±0·15
	2 - 4	3	25·4±2·7	2·45±0·10	(2) 13·0±1·6	(2) 2·48±0·11
	5 - 7	5	19·5±3·3	2·45±0·36	15·7±4·9	2·68±0·35
Twins	<2	11	17·9±3·7	2·49±0·18	(10) 14·6±4·2	(10) 2·32±0·23
	2 - 4	5	20·4±3·6	2·38±0·11	19·0±4·7	2·26±0·25
	5 - 7	10	19·3±2·8	2·29±0·07	17·8±4·4	2·43±0·39
Triplets	<2	10	20·9±8·2	2·47±0·20	(8) 16·8±4·3	(8) 2·33±0·20
	2 - 4	4	23·7±7·5	2·31±0·08	(5) 15·7±2·7	(5) 2·33±0·12
	5 - 7	10	19·5±3·3	2·25±0·26	(8) 17·0±2·6	(8) 2·57±0·26

*If different from these it is noted in brackets.

data in Table 8·1, none of the comparisons showed statistically significant differences. However, ewes in the high nutrition group showed higher albumin levels at < 2 weeks before lambing as compared to levels measured at five to seven weeks before lambing. The opposite was shown by ewes of the low nutrition group, i.e. their albumin levels decreased toward lambing.

Urea levels showed a very clear trend as the mean urea levels for all types of litter size, and at any stage before lambing, were always higher in the high nutrition group than in the low nutrition group.

Biochemical parameters in ewes sera before lambing:

Serum total protein and gamma-globulin (Biuret):

Mean levels of these two parameters measured at different occasions during the last eight weeks of pregnancy are shown in Table 8·2. Student's 't' test comparisons performed on this data showed that in most cases (11 out of 14 comparisons), the differences due to type of litter size or the levels of nutrition in late pregnancy were statistically not significant. However, figures in this table show the following.

1. There is a reduction in the levels of gamma-globulin and also total protein at two to four weeks before lambing. The levels at

TABLE 8·2.

Levels of total protein and gamma-globulin (g/100 ml) in ewes before lambing (Mean ± standard deviation)

Ewe's litter size	Weeks before lambing	No.* of samples	High nutrition group		Low nutrition group	
			Total protein	Gamma-globulin	Total protein	Gamma-globulin
Singles	< 2	5	6·17 ± 0·93	1·74 ± 0·42	5·96 ± 1·10	1·66 ± 0·55
	2 - 4	3	6·23 ± 0·11	1·74 ± 0·12	(2) 6·40 ± 0·14	(2) 1·69 ± 0·30
	5 - 7	5	6·84 ± 0·66	2·12 ± 0·30	6·88 ± 0·96	1·88 ± 0·39
Twins	< 2	11	5·89 ± 0·38	1·39 ± 0·27	(10) 6·39 ± 0·50	(10) 1·93 ± 0·44
	2 - 4	5	5·86 ± 0·22	1·35 ± 0·17	6·04 ± 0·61	1·79 ± 0·20
	5 - 7	10	6·53 ± 0·51	1·91 ± 0·31	6·92 ± 0·74	2·08 ± 0·23
Triplets	< 2	10	6·25 ± 0·71	1·98 ± 0·75	(8) 5·87 ± 0·26	(8) 1·69 ± 0·26
	2 - 4	4	5·80 + 0·26	1·46 + 0·23	(5) 6·10 + 0·24	(5) 1·68 + 0·22
	5 - 7	10	6·77 ± 0·63	2·06 ± 0·40	(8) 6·77 ± 0·26	(8) 1·88 ± 0·25

*If different from these it is noted in brackets.

this stage were not usually different from those estimated at 0 to 2 weeks before lambing.

2. In most cases of multiple births the serum levels of the above mentioned parameters were lower at advanced stages of pregnancy in the high nutrition group than in the low nutrition group. In this connection, the difference in the levels of serum gamma-globulin, in ewes with twins for example, was statistically significant ($P < 0{\cdot}01$) at 2 to 4 and 0 to 2 weeks before lambing as compared to levels measured at 5 to 7 weeks before lambing.

These changes could be related to the start and the efficiency of colostrum production by the ewe.

Ewes serum immunoglobulins (SRID):

Like other parameters estimated for ewes in late pregnancy, the levels of IgG_1, IgG_2, IgM and IgA were not affected by the type of litter size. In relation to this, none of the comparisons conducted on litter size basis within a nutritional group was statistically significant. For this reason, data for these immunoglobulins were re-calculated for ewes irrespective of their litter

size, and their mean values at different stages in late pregnancy, for the two nutritional groups, are presented in Table 8·3. The table shows the following.

1. IgG_1 levels were significantly reduced toward lambing. This reduction was very obvious only in ewes kept on high level of nutrition where the difference between IgG_1 levels at 5 to 7 weeks and at 2 to 4 or 0 to 2 weeks before lambing was highly significant (P was $< 0{\cdot}001$ in both comparisons). Although this difference was not statistically significant in the case of the low nutrition group, the IgG_1 levels were reduced toward lambing, and at 0 to 2 weeks these levels were significantly lower ($P < 0{\cdot}05$ only) than the corresponding levels at 5 to 7 weeks before lambing. At no stage during the observation period were the IgG_1 levels in the low nutrition group as low as the corresponding levels in the high nutrition group.
2. IgM is the only other immunoglobulin which showed decreasing levels toward lambing, but at a lower rate than that of IgG_1.

At 0 to 2 weeks before lambing (in the high nutrition group only) the difference

TABLE 8·3.

Levels of serum immunoglobulin (g/100 ml) in ewes before lambing as measured by the SRID technique (Mean ± standard deviation)

Nutritional group	High			Low		
Weeks before lambing	5 - 7	2 - 4	0 - 2	5 - 7	2 - 4	0 - 2
Number of samples	23	10	25	19	12	22
IgG_1	1·51 ± 0·30	1·01 ± 0·20	1·08 ± 0·26	1·59 ± 0·23	1·36 ± 0·32	1·26 ± 0·35
IgG_2	0·53 ± 0·15	0·51 ± 0·11	0·57 ± 0·18	0·54 ± 0·16	0·62 ± 0·17	0·59 ± 0·15
IgM	0·46 ± 0·12	0·42 ± 0·09	0·33 ± 0·16	0·44 ± 0·12	0·44 ± 0·12	0·38 ± 0·15
IgA	0·60 ± 0·09	0·64 ± 0·09	0·73 ± 0·14	0·59 ± 0·16	0·62 ± 0·16	0·58 ± 0·13
Total	3·09 ± 0·39	2·58 ± 0·21	2·70 ± 0·43	3·14 ± 0·49	3·05 ± 0·42	2·79 ± 0·49

from levels estimated at 5 to 7 weeks before lambing became statistically significant ($P < 0.01$).

3. No significant changes in the level of IgG_2 were noticed during the observation period, in either low or high nutrition groups. The same thing applies to IgA levels in the low nutrition group. In the high nutrition group, however, the IgA levels, contrary to what was noticed for IgG_1 and IgM, increased toward lambing. At 0 to 2 weeks before lambing, these levels were significantly higher ($P < 0.001$) than those measured at 5 to 7 weeks before lambing.

4. Probably due to the behaviour of IgG_1, the total immunoglobulin levels in the high nutrition group were significantly reduced at 2 to 4 weeks ($P < 0.01$) and 0 to 2 weeks ($P < 0.01$) before lambing as compared to values estimated at 5 to 7 weeks before lambing. This was not the case in the low nutrition group where the reduction was only noticeable at 0 to 2 weeks before lambing ($P < 0.05$) when compared to levels measured at 5 to 7 weeks before lambing.

5. Comparing immunoglobulin levels between the high and low nutritional groups revealed very few statistically significant differences. The few significant ones are the following: The high nutrition group had significantly lower IgG_1 levels at 2 to 4 and 0 to 2 weeks before lambing (P was $< 0{\cdot}01$ and $< 0{\cdot}05$ respectively), higher IgA at 0 to 2 weeks before lambing ($P < 0{\cdot}001$) and lower total immunoglobulins at 2 to 4 weeks before lambing ($P < 0{\cdot}01$) than the low nutrition group.

Biochemical parameters for ewes at lambing:

See Tables 8·4 and 8·5. Blood and colostrum samples were collected from ewes immediately after lambing (before suckling took place) and were analysed for the following: total protein, gamma-globulin (Biuret) and the different types of immunoglobulin (SRID). Blood was also analysed for urea and albumin.

Values for the blood and colostral whey parameters are presented in Tables 8·4 and 8·5 respectively. In both tables the results were calculated on a litter size basis.

Blood parameters estimated at lambing (Table 8·4) showed the following.

1. On the basis of levels of nutrition in late pregnancy, none of the parameters compared showed statistically

TABLE 8·4.

Ewe blood biochemical parameters at lambing

(Mean ± standard deviation)

Nutritional group	Litter size	No.* of ewes	Urea (mg/100 ml)	Total protein (g/100 ml)	Albumin (g/100 ml)	Gamma-globulin (g/100 ml)	Immunoglobulins (SRID) (g/100 ml)				
							IgG_1	IgG_2	IgM	IgA	Total
High	Singles	5	19·94±6·10	6·38±0·61	2·77±0·14	1·98±0·98	1·03±0·28	0·58±0·11	0·32±0·08	0·55±0·12	2·48±0·42
	Twins	11	18·75±5·92	6·13±0·40	2·53±0·29	1·21±0·40	1·17±0·41	0·62±0·17	0·25±0·11	0·66±0·19	2·70±0·58
	Triplets	10	24·53±9·63	6·96±0·97	2·48±0·24	1·72±0·69	(9) 1·32±0·44	(9) 0·73±0·22	(9) 0·32±0·12	(9) 0·65±0·15	(9) 3·04±0·74
Low	Singles	5	16·56±5·91	6·42±0·47	2·61±0·24	1·28±0·35	0·93±0·41	0·50±0·13	0·28±0·05	0·56±0·25	2·27±0·72
	Twins	10	18·12±6·98	6·48±0·87	2·40±0·24	1·57±0·39	1·31±0·36	0·70±0·19	0·34±0·10	0·55±0·12	2·90±0·47
	Triplets	10	19·63±7·54	6·49±0·70	2·41±0·23	1·52±0·24	1·32±0·37	0·60±0·18	0·30±0·14	0·46±0·23	2·69±0·65

*If different from these it is noted in brackets.

TABLE 8·5.

Total protein and immunoglobulins (g/100 ml) in the colostral whey of ewes at lambing

(Mean ± standard deviation)

Nutritional group	Litter size	No. of ewes	Total protein	Gamma-globulins	Immunoglobulins (SRID)				
					IgG_1	IgG_2	IgM	IgA	Total
High	Singles	5	23·68 ± 6·45	13·12 ± 1·83	6·03 ± 0·88	0·10 ± 0·01	1·42 ± 0·08	0·41 ± 0·09	7·96 ± 0·87
	Twins	11	25·33 ± 4·48	13·92 ± 3·40	6·42 ± 2·04	0·10 ± 0·03	1·30 ± 0·57	0·67 ± 0·30	8·49 ± 2·32
	Triplets	9	22·91 ± 6·94	12·70 ± 5·23	5·91 ± 2·22	0·13 ± 0·02	1·29 ± 0·43	0·64 ± 0·12	7·98 ± 2·47
Low	Singles	5	24·54 ± 8·69	11·88 ± 3·95	7·76 ± 6·24	0·10 ± 0·02	1·33 ± 0·81	0·52 ± 0·19	9·71 ± 6·91
	Twins	8	24·21 ± 4·65	15·56 ± 3·15	6·68 ± 1·49	0·13 ± 0·02	1·32 ± 0·58	0·77 ± 0·36	8·90 ± 1·73
	Triplets	10	26·72 ± 9·83	15·34 ± 7·00	7·31 ± 4·27	0·13 ± 0·04	1·33 ± 0·72	0·74 ± 0·40	9·51 ± 4·81

significant differences with one exception in which ewes with twins in the high nutrition group had significantly lower gamma-globulin levels ($P < 0.05$) than their contemporaries in the low nutrition group. This was also noticed when IgG_1 levels were measured.

2. In most cases, urea levels remained higher in the high nutrition group than in the low nutrition group.
3. Levels of the immunoglobulin fractions (i.e. IgG_1, IgG_2, IgM and IgA) measured at lambing were not different from those estimated during the last two weeks of pregnancy (presented in Table 8·3). IgG_1 and IgM remained significantly lower than their values at 5 to 7 weeks before lambing (P was < 0.01 for IgG_1 and < 0.001 for IgM in both nutrition groups). In this connection, levels of IgG_2 and IgA remained unchanged.
4. It was noticed that at lambing, ewes with singles tended to have lower IgG_1, IgG_2 and total immunoglobulin than those with multiples (twins or triplets), in both nutritional groups.

In colostrum (Table 8·5), the difference between corresponding values for the high and low nutrition groups were not statistically significant for any of the parameters observed. In most cases the values in the

low nutrition group were either similar or slightly higher than the corresponding values measured in the high nutrition group. This slight difference may be related to the volume of colostrum secreted (see Table 8·6).

Performance of ewes and their lambs:

See Table 8·6. Because of the nature and design of the 1976 work, half of the surviving twins were deprived of colostrum. The effect of colostrum deprivation on twin lamb performance and biochemical parameters will be presented and discussed separately from those of single and triplet lambs. Information about twins presented in this section refers only to birth data for all twins and to data from control non-deprived twins.

In Table 8·6, ewe and lamb performance data is presented. Whenever it was possible, Student's 't' test was applied to this data and the results are as follows.

Ewe body weight change during pregnancy:

Ewes in the high nutrition group either showed some body weight gain, or a much smaller weight loss in comparison to those in the low nutrition group where ewes, irrespective of their litter size, always showed a high mean body weight loss. Comparisons between the two nutritional groups showed statistically significant differences (P was $< 0{\cdot}01$ for ewes with singles, and $< 0{\cdot}05$ for ewes

TABLE

Overall performance of

(Some data are presented as

Nutritional group	Litter size	No. of ewes	No. of lambs born	Ewe body weight loss or gain during pregnancy (kg)	Ewe body condition scoring 24 hours after lambing	Ewes with no milk or little milk at lambing (% & No.)	No.* of lambs showing watery-mouth syndrome	No. of lambs dying (PLM)
High	1	5	5	(5) +15·20 ± 7·22	(5) 2·90 ± 0·65	Nil	Nil	Nil
	2	11	22	(11) +1·09 ± 6·89	(11) 2·54 ± 0·47	Nil	2	Nil
	3	10	30	(9) −0·44 ± 8·75	(10) 2·40 ± 0·46	10% (1)	2	8
Low	1	5	5	(5) −1·10 ± 5·62	(4) 2·87 ± 0·25	20% (1)	Nil	Nil
	2	14	28	(11) −5·95 ± 5·50	(8) 2·31 ± 0·37	50% (7)	1	11†
	3	7	21	(7) −9·21 ± 3·96	(7) 2·08 ± 0·20	57% (4)	2	3

Number of ewes or lambs written in brackets.

*In the case of twins, deprived ones were not included.

†Three of these lambs were deprived of colostrum for nine hours after birth.

8·6.

ewes and their lambs

mean ± standard deviation)

Birth weight of all lambs born (kg)	Birth weight of survivors only (kg)	Lambs body* weight increase at 48 hours of age (kg)	Lambs body* weight increase at 3 weeks of age (kg)	Overall PLM (%)
(5) 5·75 ± 0·41	(5) 5·75 ± 0·41	(5) 0·79 ± 0·18	(5) 9·00 ± 1·36	
(22) 5·13 ± 0·64	(22) 5·13 ± 0·64	(10) 0·47 ± 0·35	(10) 6·99 ± 0·47	14·0
(30) 4·02 ± 0·68	(22) 4·13 ± 0·60	(21) 0·24 ± 0·34	** (16) 6·38 ± 1·34	
(5) 6·18 ± 0·63	(5) 6·18 ± 0·63	(5) 0·77 ± 0·64	(5) 7·95 ± 1·87	
(26) 4·13 ± 0·88	(17) 4·36 ± 0·73	(9) 0·33 ± 0·18	(9) 5·81 ± 1·46	25·9
(21) 3·59 ± 0·65	(18) 3·67 ± 0·65	(17) 0·15 ± 0·16	** (14) 5·48 ± 1·49	

**Triplets reared in pairs.

with twins or triplets). When figures for ewe body weight gain or loss were compared according to litter size, but within the same nutritional group, the difference was only significant when data for ewes with singles were compared with those of ewes with triplets. The difference between corresponding figures for ewes with singles and ewes with triplets was statistically significant ($P < 0.01$ and < 0.02 in the high and low nutrition groups respectively). In the high nutrition group, ewes with singles gained significantly more body weight than those carrying twins ($P < 0.01$) but in the low nutrition group the difference was not great enough to be statistically significant. Although ewes with triplets tended to lose more body weight than those carrying twins, the difference between the two, in both nutritional groups was statistically not significant.

Ewe body condition scoring:

See Table 8·6. There was a persistent and obvious trend in the mean body condition scoring values in relation to level of nutrition in late pregnancy and also in relation to litter size (see Table 8·6). In spite of this, none of the comparisons performed on the data in relation to nutritional treatment or litter size showed

statistically significant variations.

Milk availability at lambing:

See Table 8·6. Only one out of 26 ewes observed in the high nutrition group showed a scarcity of milk at lambing. On the other hand, 45 per cent of the ewes in the low nutrition group had very little milk or no milk at all at lambing.

Illness and mortality of lambs:

See Table 8·6. Although a large number of lambs were lost, particularly to the low nutrition group, only very few lambs showed the 'watery mouth' syndrome, a commonly observed condition among new-born lambs in Scotland. This is perhaps due to the fact that most of the dead lambs were either stillborn or died within a few hours after birth giving little time for symptoms to develop.

The over-all mortality figures were much higher in the low nutrition group than in the high nutrition group. None of the singles born to either of the nutritional groups died. Although the high nutrition group lost none of their twins, eight of their triplets died (four stillborns; three died within two days of birth as a result of starvation/*E. coli* infection and one died accidentally). Of the 11 twins dying in the low nutrition

group, six were aborted to three ewes 10 to 16 days before term; one died of unknown causes immediately after birth; and four died of starvation/*E. coli* infection at 24 hours of age (three of them were among lambs deprived of colostrum for nine hours after birth).

Two of the abortion cases were diagnosed as EAE (Enzootic Abortion of Ewes) and the third was diagnosed as toxoplasmosis. After excluding the abortion cases, 50 per cent of the PLM cases had a positive Toxoplasma fluorescent antibody test when lambs spleen smears were made. (This aspect will be discussed later.)

Lamb birth weight:

With the exception of singles, lambs born to the high nutrition group tended to be heavier than those born to the low nutrition group (see Table 8·6). When calculated for all lambs born (i.e. survivors and non-survivors), the difference in mean birth weight, due to nutritional treatments was statistically significant for twins ($P < 0{\cdot}001$) and triplets ($P < 0{\cdot}05$). The same picture emerged when the mean birth weight of survivors was calculated.

Dead lambs in the high nutrition group (eight triplets) had a mean birth weight of $3{\cdot}72 \pm 0{\cdot}82$ kg,

and those which died in the low nutrition group (nine twins and three triplets) had a mean birth weight of $3 \cdot 54 \pm 0 \cdot 91$ kg. In both cases, these dead lambs weighed less (but not significantly) than the corresponding surviving triplets.

Growth rate of lambs:

This was calculated as lambs body weight increase (kg) at 48 hours and three weeks of age (see Table 8·6). In all instances, the increase was higher in lambs born to the high nutrition group than in corresponding lambs born to the low nutrition group. This is in part due to the amount of colostrum and milk produced by the ewes and to the efficiency with which the lambs made use of them. However, the difference was statistically not significant except when three weeks body weight increase of twins in both groups were compared ($P < 0 \cdot 02$).

Biochemical parameters for lambs:

Lambs were bled on four occasions, at birth, 24 hours, 48 hours and four to six weeks of age. Values for the different parameters analysed are presented in Tables 8·7 and 8·8. All these values, in the two nutritional groups, were calculated for surviving lambs on a litter size basis. It must be pointed out that from three days of age triplets were split up, two lambs

TABLE 8·7.

Biochemical parameters for surviving lambs

(Mean ± standard deviation)

Nutritional group		High nutrition group			Low nutrition group		
Litter size		Singles	Twins**	Triplets	Singles	Twins**	Triplets
Number of lambs*		5	10	21	5	9	18
Alkaline phosphatase (I.U./L) at:	Birth	1031·0 ± 236·0	(9) 1177·8 ± 432·4	717·0 ± 446·0	(4) 856·0 ± 509·0	(10) 550·5 ± 188·8	445·0 ± 205·0
	24 hours	1468·0 ± 507·0	1587·5 ± 546·4	(20) 1062·0 ± 590·0	(3) 1260·0 ± 651·0	922·8 ± 375·6	(17) 787·0 ± 458·0
	48 hours	(4) 1255·0 ± 434·0	912·5 ± 172·6	(17) 764·0 ± 304·0	800·0 ± 390·0	760·5 ± 255·7	524·0 ± 237·0
	4–6 weeks	1095·0 ± 242·5	(8) 986·2 ± 197·4	(16) 893·4 ± 349·7	923·0 ± 267·2	(8) 676·9 ± 180·1	(10) 885·5 ± 401·8
Total protein (g/100 ml) at:	24 hours	7·36 ± 1·34	6·83 ± 0·73	6·37 ± 1·35	6·60 ± 1·52	6·60 ± 0·95	5·67 ± 0·75
	48 hours	7·58 ± 0·99	6·52 ± 0·97	6·13 ± 1·17	6·29 ± 0·75	6·20 ± 1·40	5·39 ± 0·80
Gamma-globulin (g/100 ml) at:	24 hours	3·69 ± 0·48	2·59 ± 0·85	2·34 ± 1·28	2·52 ± 0·87	2·34 ± 1·28	1·70 ± 0·91
	48 hours	3·23 ± 0·99	2·19 ± 0·65	(19) 2·01 ± 1·00	(4) 2·33 ± 0·94	2·19 ± 1·05	1·40 ± 0·72

*If different from these, it is noted in brackets.

**Only non-deprived lambs were included.

TABLE

Levels of immunoglobulins in

(Mean ± standard

Nutritional group		High nutrition group		
Litter size		Singles	Twins**	Triplets
Number of samples*		5	10	21
IgG_1 at:	Birth	0·006 ± 0·005	0·017 ± 0·04	0·001 ± 0·004
	24 hours	2·90 ± 0·24	2·28 ± 0·71	1·79 ± 1·10
	48 hours	2·71 ± 0·54	1·98 ± 0·61	1·45 ± 0·93
	4–6 weeks	0·53 ± 0·10	(9) 0·59 ± 0·19	0·50 ± 0·25
IgG_2 at:	Birth	Nil	0·007 ± 0·01	0·003 ± 0·010
	24 hours	0·07 ± 0·01	0·06 ± 0·02	0·05 ± 0·02
	48 hours	0·06 ± 0·01	0·06 ± 0·01	0·05 ± 0·02
	4–6 weeks	0·06 ± 0·04	(9) 0·07 ± 0·03	0·09 ± 0·06
IgM at:	Birth	0·020 ± 0·018	0·007 ± 0·02	0·014 ± 0·026
	24 hours	0·98 ± 0·39	0·68 ± 0·54	0·44 ± 0·31
	48 hours	0·77 ± 0·41	0·58 ± 0·32	0·48 ± 0·40
	4–6 weeks	0·10 ± 0·04	(9) 0·11 ± 0·04	0·12 ± 0·04
IgA at:	Birth	0·133 ± 0·049	0·113 ± 0·04	0·125 ± 0·043
	24 hours	0·10 ± 0·03	0·10 ± 0·03	0·13 ± 0·13
	48 hours	0·10 ± 0·02	0·11 ± 0·06	0·12 ± 0·03
	4–6 weeks	0·48 ± 0·08	(9) 0·50 ± 0·10	0·52 ± 0·10
Total immuno-globulins at:	Birth	0·158 ± 0·051	0·144 ± 0·07	0·143 ± 0·08
	24 hours	4·04 ± 0·50	3·13 ± 1·03	2·42 ± 1·36
	48 hours	3·64 ± 0·95	2·73 ± 0·84	2·09 ± 1·35
	4–6 weeks	1·18 ± 0·14	(9) 1·28 ± 0·25	1·23 ± 0·35

*If different from these, it is noted in brackets.

8·8.

surviving lambs (g/100 ml)
deviation)

Low nutrition group		
Singles	Twins**	Triplets
5	9	18
(4) Nil	(10) 0·005 ± 0·01	(17) 0·001 ± 0·004
2·10 ± 0·81	2·12 ± 1·19	1·45 ± 0·73
2·07 ± 0·93	1·87 ± 0·78	1·07 ± 0·67
0·59 ± 0·29	0·64 ± 0·47	(12) 0·50 ± 0·15
(4) Nil	(10) Nil	(17) 0·005 ± 0·01
0·06 ± 0·01	0·05 ± 0·02	0·04 ± 0·03
0·05 ± 0·01	0·05 ± 0·01	0·03 ± 0·02
0·14 ± 0·15	0·07 ± 0·02	(12) 0·07 ± 0·04
(4) 0·028 ± 0·024	(10) 0·023 ± 0·02	(17) 0·020 ± 0·027
0·60 ± 0·26	0·50 ± 0·30	0·36 ± 0·32
0·54 ± 0·20	0·48 ± 0·34	0·29 ± 0·21
0·10 ± 0·04	0·12 ± 0·02	(12) 0·12 ± 0·04
(4) 0·140 ± 0·029	(10) 0·129 ± 0·03	(17) 0·109 ± 0·028
0·11 ± 0·02	0·12 ± 0·06	0·10 ± 0·03
0·14 ± 0·05	0·13 ± 0·04	0·12 ± 0·06
0·57 ± 0·09	0·48 ± 0·09	(12) 0·51 ± 0·09
(4) 0·168 ± 0·043	(10) 0·157 ± 0·05	(17) 0·136 ± 0·05
2·87 ± 1·02	2·79 ± 1·51	1·95 ± 1·05
2·80 ± 1·01	2·53 ± 1·08	1·51 ± 0·84
1·46 ± 0·46	1·31 ± 0·49	(12) 1·20 ± 0·18

**Only non-deprived lambs were included.

staying with the ewe and one being fostered.

Alkaline phosphatase (AP):

See Table 8·7. Levels of AP measured in the sera of lambs at birth, 24 hours, 48 hours and at four to six weeks of age showed the following in relation to nutritional treatments:

At birth, multiple born lambs in the high nutrition group had significantly higher AP levels than their contemporaries in the low nutrition group ($P < 0{\cdot}02$ and $< 0{\cdot}05$ for twins and triplets respectively). In singles, although the difference was not statistically significant, those born to the high nutrition group had noticeably higher AP mean values than singles in the low nutrition group.

On the other three sampling occasions, although the difference in levels of AP due to nutritional treatment was only significant in the case of twins at 24 hours and four to six weeks of age ($P < 0{\cdot}01$) and in the case of triplets at 48 hours of age ($P < 0{\cdot}02$), the mean levels for those in the high nutritional group were consistently and markedly higher than the corresponding levels in the low nutritional group.

Sex of lamb seems to have no effect on SAP. Twins at birth, for example, showed the following SAP levels (I.U./L).

	High nutrition group		Low nutrition group	
	Males	Females	Males	Females
Mean	1242·6	1021·7	656·9	607·9
Standard deviation	513·6	532·8	348·8	495·7
Number of lambs	12	9	8	12

In both nutritional groups, comparisons performed on a sex basis showed statistically no significant differences. However, as shown in the above example, males tended to have slightly higher SAP than females. This is in agreement with previous results which indicated that male lambs at birth tended to be slightly heavier (but not significantly) than females.

When comparisons were made between different litter sizes within the same nutritional group it was found that at birth singles in the low nutrition group had significantly higher levels than triplets ($P < 0{\cdot}02$). At birth and at 24 hours of age, twins born to the high nutrition group had significantly higher levels than triplets ($P < 0{\cdot}02$ and $< 0{\cdot}05$ respectively). At 48 hours of age, singles born to the high nutrition group had significantly higher levels than twins ($P < 0{\cdot}05$), and in the low nutrition group, twins had significantly higher levels than triplets ($P < 0{\cdot}05$).

As shown in Table 8·7, with only few exceptions, AP

levels decreased with the increase in litter size.

The nine triplet born lambs that were reared in the milk bar had a mean AP value of 473·3 ± 116·3 I.U./L (International Unit per Litre) at the age of four to six weeks. These levels were significantly lower than the levels shown by triplets, reared as pairs by their mothers, in the low and the high nutrition groups ($P < 0·01$ in both comparisons). In this relation it was noticed that these nine lambs, which had been reared on milk substitute since the age of three days, achieved a three week increase in body weight amounting to only 4·47 ± 1·75 kg. This was markedly lower than the corresponding figures for triplets reared by their mothers (see Table 8·6) in the high nutrition group ($P < 0·01$) and the low nutrition group (d.f.= 21, 't' = 1·475).

Total protein and gamma-globulin:

As affected by nutritional treatments, both of these parameters were higher in lambs born to high nutrition group than in corresponding lambs born to the low nutrition group (see Table 8·7). However, the Student 't' test comparison applied on these values showed statistically significant difference only in the following: total protein levels measured for singles and triplets at 48 hours of age ($P < 0·05$) and gamma-globulin values measured for

singles at 24 hours of age and for triplets at 48 hours of age (P was < 0.05 in both cases).

When litter size comparisons within a nutrition group were made it was found that total protein levels tended to decrease with increase in the litter size (see Table 8·7). The differences were only significant in one comparison in which twins born to the low nutrition group had significantly higher levels than low nutrition triplets ($P < 0.01$).

The litter size variations within a nutritional group were even more apparent with gamma-globulin values. At 24 hours of age, singles born to the high nutrition group showed significantly higher levels than twins ($P < 0.02$) and triplets ($P < 0.05$). At 48 hours of age, singles in the high nutrition group had significantly higher levels than twins ($P < 0.05$) while twins in the low nutrition group had significantly higher levels than low nutrition triplets ($P < 0.05$).

In other comparisons that failed to show statistically significant difference gamma-globulin levels always fell as litter size increased (see Table 8·7).

Immunoglobulin levels (SRID):

Data concerning lamb immunoglobulins presented in Table 8·8 have shown the following.

1. At birth: Only traces of immunoglobulins were detected although singles showed slightly higher levels than multiples. IgA forms the largest part of the circulating immunoglobulins but at this stage there was no noticeable nutritional effect on the immunoglobulin distribution.
2. At 24 hours: As a result of colostrum ingestion, the levels of both IgG_1 and IgM were greatly increased. IgG_2 levels, although they were easily detected by the SRID technique, showed very low values. IgA levels were similar to those estimated at birth. Student's 't' test showed no statistically significant difference when corresponding values of the four immunoglobulins were compared for nutritional treatment effect. However, with the exception of IgA, the immunoglobulin mean values tended to be higher in the high nutrition group than in the low nutrition group. This is best shown when total immunoglobulin values were calculated (Table 8·8). In this relation, the difference between the two nutritional groups was statistically significant in the case of singles ($P < 0.05$).

3. At 48 hours of age, immunoglobulin levels tended to be noticeably lower than the corresponding levels measured at 24 hours of age and again although there was no statistically significant difference between nutritional treatments, those levels measured in the high nutrition group tended to be higher than the corresponding levels measured in the low nutrition group (IgA was an exception).
4. At four to six weeks of age, the comparisons performed on the levels of different immunoglobulins, on a nutritional treatment basis, were inconclusive. In both nutritional groups, these levels as compared to their 24 and 48 hour levels showed that IgG_1 and IgM were markedly reduced, IgG_2 slightly increased, and IgA increased by as much as four-fold. Total immunoglobulins for surviving lambs in the two nutritional groups tended to be comparable at this age.
5. When comparisons between different litter sizes within one nutritional group were made all the values at birth were too low to show variations of any significance. At 24 hours of age, singles in the high nutrition group

had significantly higher IgG_1, IgM and total immunoglobulin than triplets ($P < 0.05$, < 0.01 and < 0.02 respectively). At 48 hours of age, singles in the high nutrition group showed significantly higher IgG_1 levels than twins ($P < 0.05$). They also had significantly higher IgG_1 and total immunoglobulin levels than triplets ($P < 0.01$ and < 0.05 respectively). Singles born to the low nutrition group had significantly higher IgG_1 and total immunoglobulin values than twins ($P < 0.02$ in both cases). They also had significantly higher IgG_1, IgM and total immunoglobulin levels than triplets ($P < 0.02$, < 0.05 and < 0.01 respectively). None of the comparisons performed on four to six week values showed statistically significant differences.

In the main, the SRID values at 24 and 48 hours tended to be higher in lambs in the smaller litters. This seems to be most obvious with the levels of IgG_1 and to a certain extent, IgM, something similar to the patterns seen in ewe colostral whey (see Table 8·5). In this relation, IgG_2 and IgA seem to behave inconclusively. At four to six weeks of age, the difference in the levels of the different immunoglobulins in surviving lambs, in relation to litter size, seems to diminish.

The triplets reared in the milk bar, when four to six weeks old, showed IgG_1, IgG_2, IgM, IgA and total immunoglobulin values of 0·40 ± 0·30, 0·145 ± 0·097, 0·10 ± 0·04, 0·49 ± 0·11 and 1·16 ± 0·40 g per 100 ml respectively. With the exception of IgG_2, these values were not significantly different from corresponding ones measured for triplets that were reared in pairs by their mothers. In this connection, lambs in the milk bar had markedly higher IgG_2 levels than triplets in the low nutrition group ($P < 0·05$) and the high nutrition group (d.f. = 28, 't' = 1·94).

Biochemical parameters for dead lambs:

Of the few lambs which died and had biochemical parameters estimated, four were twins referred to later under the colostrum deprivation part of this chapter. Of the remainder, only two triplets in the high nutrition group had their AP and immunoglobulin values estimated. Both of these lambs died on the second day after birth. They had mean AP values of 377·0 ± 208·0 and 535·0 ± 176·0 I.U./L, at birth and at 24 hours of age respectively. Both of these values were much lower than corresponding levels estimated for live triplets born to the same nutritional group (see Table 8·7). The two lambs had 24 hour mean IgG_1, IgG_2, IgM, IgA and total immunoglobulin values of 0·12 ± 0·12, 0·02 ± 0·02, 0·05 ± 0·07, 0·12 ± 0·08 and 0·31 ± 0·27 g per 100 ml respectively.

With the exception of IgA, all these levels were much lower than the corresponding ones measured for surviving triplets born to the same nutritional group (see Table 8·8).

COLOSTRUM DEPRIVATION STUDIES

In the following part of this chapter, the effect of colostrum deprivation for nine hours after birth, on mortality and subsequent performance of twins born to the two nutritional groups, and also on their biochemical parameters will be presented.

In the high and low nutrition groups, lambs were either completely deprived for nine hours (hence HD and LD groups of lambs) or left to suck their mothers naturally, i.e. no colostrum deprivation was imposed and these will represent the two control groups (HC and LC).

Mortality and performance of lambs:

These are briefly presented in Table 8·9. From this table the following were noticed.

1. Ewes with no milk or little milk at lambing were only recorded among ewes that were kept on low level of feeding during the last eight weeks of pregnancy.
2. As far as early lamb illness is concerned, deprivation of lambs of colostrum early in their life, resulted in very high percentage of lambs

TABLE 8·9.

Performance of colostrum deprived and non-deprived lambs (all twins)

Nutritional group	High		Low	
Deprivation treatment	Control	Deprived	Control	Deprived
Number of lambs involved*	10	12	10	10
Number (and percentage) of ewes lacking milk at lambing	Nil	Nil	2 (40%)	2 (40%)
Number (and percentage) of lambs with 'watery mouth' syndrome	2 (20%)	6 (50%)	1 (10%)	5 (50%)
Number (and percentage) of lambs died	Nil	Nil	1 (10%)	3 (30·0%)
Lamb birth weight (kg)	4·89 ± 0·50	5·32 ± 0·70	4·35 ± 0·55	4·25 ± 0·93
Lamb body weight increase at 48 hours of age (kg)	0·47 ± 0·35	0·14 ± 0·36	0·33 ± 0·18	0·15 ± 0·27
Lamb body weight increase at three weeks of age (kg)	6·99 ± 0·47	6·36 ± 1·62	(9) 5·81 ± 1·46	(6) 6·45 ± 2·11
Three weeks total body weight increase of surviving lambs (kg)	69·93	63·58**	52·29	39·29

*If different from these, it is noted in brackets.

**Corrected for 10 lambs.

(50 per cent in both deprived groups) showing the 'watery mouth' syndrome. This syndrome was also noticed in two lambs in the HC group, but disappeared shortly after colostrum sucking. The only ill lambs in the LC group died of starvation/ E. coli infection at 24 hours of age. All ill lambs in the HD group survived without treatment, while three out of the five ill lambs in the LD group died of starvation/coli-enteritis, all at one to two days of age.

All dead lambs were among lambs born to the low nutrition group of ewes and their mothers had very little milk at lambing. The LD group had the highest percentage of lamb mortality (30 per cent).

3. A clear effect of late pregnancy level of feeding on lamb birth weight in the four groups was noticed.

4. When the increase in surviving lambs body weight at 48 hours and three weeks of age was compared between the nutritional groups, only the following showed statistically significant differences:

HC and HD at 48 hours, and HC and LC at three weeks of age ($P < 0.05$ in both cases).

Table 8.9 showed that colostrum deprivation affected the body weight increase of lambs at 48 hours

of age in both nutritional groups. The increase in body weight of lambs at three weeks of age showed that level of maternal nutrition and, indirectly, the amount of milk produced have an effect on growth rate of lambs in the first three weeks of life. When lamb body weight increase from birth to three weeks of age was calculated for every 10 lambs born live in a nutritional group (Table 8·9), lambs in the HC group had the highest increase in body weight followed by HD and then LC group. Lambs in the LD group performed very badly and showed a three week body weight increase of about half of that shown by lambs in the HC group. This is a reflection of the slow growth rates and substantial death rates of the groups performing badly.

At three weeks of age, surviving lambs in both deprived groups performed just as well as those in the HC group. Possible reasons for this are that the HD lambs were big enough to overcome any deprivation setback while in the LD group the weakest lambs died leaving only those with vigour and good mothers. In the LC group, poor growth may be because the weakest lambs did not die - they survived but pulled down the group's average growth figure.

The effect of colostrum deprivation on biochemical parameters:

Table 8·10 presents levels of the different parameters estimated in deprived lambs of both nutritional groups (i.e. HD and LD). The corresponding values for control non-deprived lambs (i.e. those included in the HC and LC groups) are previously listed (see Tables 8·7 and 8·8). Parameters measured for all lambs, at birth, and also later for control lambs in both nutritional groups were already dealt with, as in these circumstances, they were affected by the nutritional treatment only and not by colostrum deprivation.

As a combined effect of ewes late pregnancy levels of feeding, and colostrum deprivation of lambs for nine hours after birth, the corresponding values for lambs in the HD and LD group will be compared. For the effect of colostrum deprivation only, values for lambs in the control and deprived groups but within the same nutritional treatment will be compared.

Alkaline phosphatase:

AP values for deprived lambs in the LD group were much lower than the corresponding values measured for the HD group of lambs at 24 hours ($P < 0{\cdot}05$) and 48 hours (d.f. = 16, $t = 1{\cdot}85$) of age. After losing 30 per cent of their lambs, the LD group showed high AP levels in the remainder (but

TABLE 8·10.

Biochemical parameters for surviving deprived twins

(Mean ± standard deviation)

Nutritional group	Age at sampling	No.* of lambs	Alkaline phosphatase (I.U./L)	Total protein (g/100 ml)	Gamma-globulin (g/100 ml)	Immunoglobulins (g/100 ml)				
						IgG_1	IgG_2	IgM	IgA	Total
High	Birth	12	1125·5±596·5	—	—	0·014 ±0·026	0·007 ±0·017	0·012 ±0·023	0·124 ±0·033	0·157 ±0·075
	24 hours	12	1485·5±516·8	6·76±0·84	2·60±0·62 (11)	2·18 ±0·61	0·06 ±0·01	0·57 ±0·34	0·10 ±0·02	2·92 ±0·87
	48 hours	11	912·3±478·0	6·26±0·65 (12)	2·24±0·70 (9)	1·73 ±0·50	0·05 ±0·01	0·46 ±0·23	0·14 ±0·02	2·39 ±0·69
	4-6 weeks	12	875·2±166·7	—	—	0·54 ±0·26	0·08 ±0·06	0·14 ±0·05	0·49 ±0·09	1·25 ±0·33
Low	Birth	10	704·5±589·4	—	—	0·002 ±0·007	0·009 ±0·015	0·040 ±0·090	0·118 ±0·014	0·168 ±0·097
	24 hours	8	906·9±393·9	5·21±1·07 (10)	1·27±1·01 (10)	1·36 ±0·90	0·05 ±0·02	0·27 ±0·27	0·12 ±0·02	1·80 ±1·14
	48 hours	7	566·4±124·8	5·50±0·95	1·46±1·01	1·42 ±0·84	0·04 ±0·02	0·24 ±0·19	0·14 ±0·04	1·84 ±1·00
	4-6 weeks	7	967·5±524·6	—	—	0·36 ±0·17	0·05 ±0·01	0·15 ±0·06	0·52 ±0·07	1·08 ±0·23

*If different from these, it is noted in brackets.

with noticeable individual variation as shown by the standard deviation figure) at four to six weeks of age.

In the high nutrition group, deprived lambs tended to have similar or slightly lower AP than non-deprived lambs at 24 hours, 48 hours and four to six weeks of age. In the low nutrition group on the other hand, deprived lambs had significantly lower AP levels ($P < 0.02$) than non-deprived lambs at 48 hours of age. This difference was not noticeable at four to six weeks of age, perhaps due to the death in the first few days of life, of three weak lambs in the deprived group.

Total protein and gamma-globulin (Biuret):

At 24 hours of age, lambs in the LD group had significantly lower total protein and gamma-globulin than lambs in the HD group (P was < 0.01 for both parameters). At 48 hours, this difference was still marked but not statistically significant (for total protein: $d.f. = 17$, $t = 2.04$, and for gamma-globulin: $d.f. = 14$, $t = 1.82$).

There was a similar trend when the two parameters were compared between the LD and LC groups at 24 hours ($P < 0.02$ for total protein). It was only in the low nutrition group that deprived lambs

had always markedly lower total protein and gamma-globulin values than their contemporary control lambs (see Tables 8·7 and 8·10).

Immunoglobulins (SRID):

See also Table 8·8. As far as the simultaneous effect of level of ewe feeding during late pregnancy and colostrum deprivation of lambs, on lamb's biochemical parameters, the same picture observed for total protein and gamma-globulin emerged here but only in relation to IgG_1, IgM and total immunoglobulin levels. IgG_2 and IgA levels were not affected by the above mentioned factors. At 24 hours of age, lambs in the LD group had significantly lower IgG_1, IgM and total immunoglobulin than their contemporaries in the HD group (P was $< 0{\cdot}01$, $< 0{\cdot}05$ and $< 0{\cdot}05$ respectively). At 48 hours of age, this difference was only significant in the case of IgM ($P < 0{\cdot}05$) but was also quite noticeable in the case of IgG_1 and total immunoglobulin (see Table 8·10).

In the high nutrition group, deprived lambs at 24 and 48 hours of age always had lower IgG_1, IgM, total immunoglobulin and to a lesser extent IgG_2 levels than lambs in the control group. This is more pronounced in the case of lambs born to the low nutrition group (see Table 8·10).

In both of these sampling occasions, IgG_2 and IgA showed no noticeable variation.

At four to six weeks of age, the effect of colostrum deprivation on immunoglobulin values had largely been overcome although lambs in the LD group were still showing markedly lower IgG_1 and total immunoglobulin values than their contemporaries in the LC group (see Tables 8·8 and 8·10).

Biochemical parameters for dead twins:

Three deprived twins died on the second day after birth and all of them belonged to the LD group. A fourth lamb died in the LC group. All deaths were diagnosed as starvation/coli-enteritis. Levels of the different parameters estimated for these lambs (some data are missing) at 24 hours of age are as follows:-

Lamb ear-tag No.	Nutritional/ deprivation treatment	A P (I.U./L)	Total protein (g/100 ml)	Gamma-globulin (g/100 ml)	Immunoglobulins (g/100 ml)				
					IgG_1	IgG_2	IgM	IgA	Total
465	L C	1120	7·4	0·74	0·68	0·04	Nil	0·10	0·82
455	L D	1420	4·4	0·21	0·01	Nil	0·06	0·13	0·21
782	L D	430	4·0	0·33	-	-	-	-	-
783	L D	330	4·0	0·33	-	-	-	-	-

As appears from above, all these lambs were hypogammaglobulinaemic. Alkaline phosphatase was very low, at least in half of them.

The three dead lambs in the LD group showed a mean birth weight of 3·7 kg as compared to 4·5 kg for the seven survivors in the same group. These dead lambs were born to two ewes that had very little colostrum at lambing. This is a good example of the kind of relationship that exists between the ewes level of feeding in late pregnancy, the amount of colostrum (or milk) they produced at lambing, and the birth weight of lambs, in deciding the fate and future performance of the newborn lambs.

DISCUSSION

In the study presented in this chapter, the number of nutrition groups was reduced to two, so that more ewes and lambs could be included in each group. One type of hay was used but concentrates were offered to one group only, hence the difference in the levels of energy intake calculated for the two nutritional groups. The feeding levels offered are similar to those commonly used in lowland British sheep farms, i.e. the experimental nutritional groups simulate farming practice.

By depriving some lambs from both nutritional groups of colostrum, it was possible to observe the effect of this deprivation on lamb performance as affected by the nutritional state of the mother. By observing the performance of all lambs born to the two nutritional treatments,

the effect of nutrition alone on the performance of lambs of different litter size was observed independently, and additional data to that obtained in previous years on important factors like litter size, birth weight, ewe body weight loss and milk production were obtained.

Group feeding of ewes seems to have an advantage over individual-pen feeding. During the 1975 nutritional study, food refusal was a problem but no such difficulties occurred during the present study. This could be partly due to less confinement, i.e. free movement and better exercise stimulated the ewes' appetite, and partly because of the better quality hay and the optimum amounts of concentrates offered.

The figures presented for the ewes in the low nutrition group concerning total hay intake (kg), energy intake (MJ per kg DM) and plasma 3-HB levels during the last eight weeks of pregnancy indicate that this group was under nutritional stress during some stages of this period but that the stress was never severe. This is mainly due to the quality of hay used which, if compared to the poorest hay used in my previous years of investigation, can be described as of medium/good quality. Hay quality affected the food intake of pregnant ewes and accounted for the variations in levels of energy intake. Similar findings were reported by Blaxter and Wilson (1963). Their work showed that feeding Welsh Mountain wethers ad

libitum hay of ME of 10·24, 9·45 and 9·03 MJ per kg DM resulted in corresponding daily voluntary hay intakes of 70, 62 and 57 g per kg ewe body weight. They and other workers (Crabtree and Williams, 1971; Ferguson, 1975) are some of the many who reported extensively on the relationship between hay quality and hay intake as affected by levels of concentrates supplementation. Offering poor quality hay only to ewes in late pregnancy put the ewes in a critical situation that led generally to poor performance on their part (see results presented in previous chapters).

The behaviour of plasma 3-HB as a parameter for monitoring nutritional status confirmed the findings observed in the 1975 nutritional study. This parameter reflected the levels of feeding during late pregnancy, even when these levels were only moderately low. The levels of energy intake, and the plasma 3-HB values, indicated that there were two distinct ewe nutritional groups in this experiment. This forms the basis for my comparative studies of the combined effects on lambs of levels of ewe feeding and colostrum deprivation of lambs.

Blood urea and serum albumin were the other two parameters estimated in relation to the ewes level of feeding in late pregnancy. Urea values were directly related to the levels of feeding regardless of the ewes' litter size. From the values measured for nutritional groups included

in this study, and also the 1975 nutritional study where more extreme levels of nutrition were employed, it seems that adequate nutrition can be expected to raise the blood urea levels in the last four to seven weeks of pregnancy to a mean of about 20 mg per 100 ml. Severe undernourishment, on the other hand, might be associated with levels well below 15 mg per 100 ml. These values are postulated for normal ewes in relation to levels of food offered in late pregnancy. They must not be confused with the very high urea levels (>50 mg per 100 ml) that might be observed in clinically ill ewes.

Although serum albumin levels did not show marked variations, the different behaviour of this parameter in the two nutritional groups seems interesting and might have reflected the nutritional status of ewes. During the observation period, the albumin levels in the high nutrition group rose as parturition approached and this corresponded with the pooled results for group 4 ewes in 1975. Adequate nutrition appears to raise ewe serum albumin levels slightly until just prior to lambing. The values in the low nutrition group fell as lambing approached and this corresponds with the results for group 3 ewes in 1975, which had a higher but still comparable energy intake. Lower nutrition levels appear to marginally reduce the serum albumin levels. The extent of this effect will depend not only on energy intake

but also on hay quality and concentrates offered.

At lambing, levels of blood urea and serum albumin in ewes presented a shift in the trends seen during late pregnancy. In the high nutrition group, albumin levels continued to rise and urea levels remained stationary but in the low nutrition group both the albumin and urea levels rose. The results for the high nutrition group are compatible with those expected in well fed ewes. The low nutrition group results have no obvious explanation but may be connected with a reduction in appetite prior to lambing and the ewe's need to draw on her body resources to maintain lamb growth.

In relation to levels of ewe feeding in late pregnancy ewe total serum protein levels were not a good indicator of energy intake. However, levels of gamma-globulins, total or fractions, seem to be related to feeding levels. The reduction in total immunoglobulins, especially IgG_1 and, to a certain extent, IgM, was more marked in the case of the high nutrition group. It is interesting to note that the major changes in ewes' serum immunoglobulin levels occurred at two to four weeks before lambing. At this time, for example, the high nutrition group experienced a serum IgG_1 reduction of about 33 per cent as compared to the levels measured at five to seven weeks before lambing, whereas there was only a 15 per cent reduction in the case of the low nutrition group. The

other immunoglobulin which showed similar changes is IgM but in this case, although the reduction was more noticeable in the high nutrition group it occurred less than two weeks prior to lambing. These changes in IgG_1 and IgM appear to be related to udder development. The high nutrition group showed the greatest changes in immunoglobulin levels and they also showed earlier udder development and produced more colostrum than the low nutrition ewes.

As was noticed in the previous nutritional study, IgG_2 did not show any important changes, and this coincided with its very minute levels in the colostrum. Serum IgA levels did not decrease toward lambing. Instead, they were elevated particularly less than two weeks before lambing in the high nutrition group, where more udder activity and then higher colostrum secretion was noticed. This might have contributed to the increased IgA levels in the circulation.

It is important to note that most of these changes observed in the serum immunoglobulins of the well fed ewes occurred at two to four weeks before lambing. This could indicate an important turning point in the immunoglobulin kinetics which might have been related to levels of feeding offered to the ewes in the last few weeks of pregnancy, and also to the amount of colostrum secreted. All these events seem to impose some quantitative changes in the

ewes' serum IgG_1, IgM and IgA but not IgG_2. At lambing, the IgG_1 levels in ewe sera began to increase when compared to the levels less than two weeks before lambing. This change occurred in both nutrition groups and coincided with active production of colostrum and the start of the change-over by the lacteal tissue to the production of milk. This increase may be related to litter size with the largest increase occurring in ewes with multiple births. IgM showed a marginal decrease at this time but IgG_2 and IgA did not show significant changes.

Regarding the composition of the colostrum secreted by the ewe at lambing, Perrin (1958) and Treacher (1970) contradict each other when referring to the fat and total protein content. (Neither author referred to protein fractions.) The first author claimed a reduction in the concentrations of these two parameters, associated with low levels of feeding during late pregnancy while Treacher reported the opposite effect.

In my study the quality of colostrum produced by ewes in the two nutritional groups, as measured by their total protein and, more specifically, immunoglobulin concentrations, does not reflect the level of feeding during late pregnancy. This confirms the findings I observed in the nutritional studies of 1975. However, the ewes' milking ability, particularly of those with twins and triplets,

was affected by nutrition. Only one of the adequately fed ewes had impaired milking ability but more than half of the ewes in the inadequately fed group had poor milking ability at lambing, associated with a colostrum of high protein content. This was mainly expressed in terms of IgG_1 which is the major immunoglobulin in colostrum. This is in agreement with the findings of Treacher (1970).

In the high nutrition group, even when large amounts of colostrum were produced, the immunoglobulin concentrations remained very high. This further supports my previous conclusion that it is the volume of colostrum available in the udder at lambing, which is important, rather than the mere gamma-globulin concentrations, which tended to be high in all the colostrum samples examined.

After discussing the effect of level of late pregnancy feeding on parameters related to the subject of nutritional monitoring, and also to the variations in immunoglobulins, it might be convenient at this stage to refer to the ewes' and lambs' performance as described in general production terms. This might make it easier to understand the changes in the other group of biochemical parameters measured for lambs, which will be discussed later.

The low level of feeding used in this study affected the performance of ewes (with multiple births only) in terms of ewe body weight loss and condition scoring. This was more pronounced in the case of large litter sizes,

i.e. triplets. Ewe body weight change and condition scoring could prove very useful parameters in describing levels of feeding required by the ewe during late pregnancy but variations imposed by litter size could complicate the conclusions. From the figures presented in this study, it seems that in order to ensure good ewe performance, ewes should maintain a condition score of 2·5 or more at lambing, regardless of litter size.

Low levels of feeding adversely affect ewe performance, but at the same time unnecessary over-feeding of ewes with single lambs should also be avoided. This can only be done if cheap and feasible methods of litter size identification which, until now, has been confined to the use of x-rays, are available. This identification would be performed at the start of the last third of the gestation period, i.e. when concentrate supplementation is recommended. This last remark is made because ewes with singles, which constituted up to 10 per cent of all ewes observed, gained unnecessary body weight in the high nutrition group, while corresponding ewes in the low nutrition group lost very little body weight and produced just as heavy or even heavier lambs at birth.

The availability of colostrum at lambing has been mentioned previously but is included again because it is an important criterion for assessing ewe performance in relation to level of feeding in late pregnancy. The feed

levels used in this experiment did not result in any severe undernourishment and yet almost half of the poorly fed ewes lacked colostrum or demonstrated poor milk "let-down". This will, undoubtedly, endanger the lambs' chance of survival particularly in the case of multiple births.

All the criteria used in describing lamb performance were clearly affected by litter size regardless of the nutritional group. Singles in both groups were heavy at birth, showed no sign of illness, suffered no losses and expressed optimal suckling performance as shown by their great increase in body weight at 48 hours of age.

The effect of ewe level of feeding in the last eight weeks of pregnancy on lamb performance was more profound when multiple births were observed. In terms of early illness, judged by the number of lambs showing the "watery mouth" syndrome, both groups of lambs showed similar morbidity rates. However, it is also important to stress that most of the lamb deaths occurred either at or a few hours after birth and this gave little time for "watery mouth" syndrome or colibacillosis signs to appear.

The variation in lamb birth weight in relation to the ewes' level of feeding during late pregnancy and the survivability of lambs observed in this study confirmed my previous findings in 1974 and 1975 and agreed with the findings of many authors whom I quoted previously. That

is, the birth weight of multiple lambs, such as twins and triplets, is directly related to levels of feeding during late pregnancy. Further, all dead lambs, observed in this experiment, were much smaller than surviving lambs, again possibly indicating the relationship between birth weight and survivability or PLM.

Regarding PLM, there were almost twice as many losses in the low nutrition group as in the high nutrition group. The fact that all twins born to the high nutrition group were reared successfully as compared to a 40 per cent loss in the low nutrition group, and that all the losses in the high nutrition group was confined to triplets, suggests that levels of feeding in late pregnancy are an important factor in deciding the pattern and size of PLM.

Terminal E. coli infection following starvation in the first few days of life was a major cause of neonatal mortality. In all my experimental investigations on PLM, almost all the losses occurred just before, during, or shortly after lambing, while late postnatal mortality was very low.

In 1976, in particular, abortion cases were relatively high (about six per cent of all ewes observed) and were the result of Enzootic Abortion of Ewes (EAE) or toxoplasmosis. All ewes were from one flock and, during housing, they were all kept in one shed and offered the same type of hay. The levels of feeding in late pregnancy were the

only source of difference in management between the two nutritional groups. While it is true that ewes in the low level group were not severely undernourished, the large number of abortion cases in the last two weeks of pregnancy, about 12 per cent in this group, could indicate that low levels of feeding during late pregnancy predispose the ewes to the effects of certain infectious forms of abortion. Thus, the possibility exists that good levels of feeding in areas where toxoplasmosis or EAE is common may reduce the level of loss by enabling the ewes to carry their lambs to term. This is, of course, an unproven speculation which requires experimental investigation.

Toxoplasmosis was diagnosed in 50 per cent of the lambs that died shortly before or after birth. This disease can cause great economic losses by either killing lambs shortly before or at lambing, or by reducing the vitality of live newborn lambs.

The subject of ovine abortion did not form a significant part of my study, but deserves further comment because of its important role in reducing lamb survivability. Among the many causes of ovine abortion, EAE and Toxoplasma gondii occur commonly in many parts of Britain (Beverley and MacKay, 1962; Beverley and Watson, 1962; Watt, 1965; Stamp, 1967). In EAE, an abortion incidence of five per cent has been suggested by Watt (1965) to occur

in chronically affected flocks while in recently affected flocks the figure could be as high as 20 to 30 per cent. The ewes I observed had experienced previous infection, hence the very low incidence (two per cent only) of EAE among them.

Vaccination against EAE is undertaken on the ESCA farms and this would have reduced disease incidence. It is possible that ewes which aborted may have been missed during the vaccination programme.

Toxoplasmosis, on the other hand, played an important role in deciding levels of lamb losses as did also other factors such as ewe nutritional state, colostrum starvation and neonatal infections. However, its role as a major cause of lamb losses is confined to certain flocks.

Toxoplasmosis in sheep was briefly but comprehensively reviewed by Watson (1971). Although this disease has been diagnosed as a cause of abortion in Britain on a farm basis (Beverley and MacKay, 1962) and on an experimental basis (Watson and Beverley, 1971) the whole epidemiological and control picture of the disease is still not clear. Apart from the ovine source of infection, e.g. an infected ewe, and aborted fetuses or placentae, non-ovine factors such as cats, mice and even birds or insects could play an important role in introducing infection to disease free farms. The lack of an effective vaccine against the disease continues to be an unsolved problem.

Many diagnostic methods have been used including pathological (gross and microscopic) examinations of aborted material, isolation of the sporozoa by mouse inoculation (a very slow and time-consuming process), serological tests such as the dye test with rising titres on paired ewe's sera, and the direct and indirect fluorescent antibody tests. Although there is a wide range of diagnostic methods, it seems that a single and quick confirmatory test does not exist for this disease. A combination of history of fresh sheep introduction, or movement of sheep to new farms, and histological and serological examinations are required to establish an accurate diagnosis of this disease. Regarding serology, workers of the ESCA (1976) have recently reported that the indirect fluorescent antibody test (IFAT) performed on spleen smears from aborted fetuses or lambs which died in the neonatal period was the most reliable test.

The relationship between this protozoon as a cause of ovine abortion and other predisposing factors (e.g. nutritional or husbandry) are far from being crystalized.

Returning to the subject of PLM, it is important at this stage to suggest that efforts to lower the levels of these losses should be directed towards reducing levels of stillbirths, in which poor maternal nourishment in late pregnancy and abortion (caused in my work mainly by Toxoplasma gondii) are major factors, and also to reducing

levels of neonatal losses for which efficient ingestion and absorption of adequate amounts of colostrum seems very vital.

In the case of surviving lambs, and similarly to the findings of the previous nutritional studies performed in 1974 and 1975, high levels of feeding during late pregnancy put the lambs in an advantageous state in terms of body weight increase during the first few weeks of life. At 48 hours of age, for example, twins and triplets born to the high nutrition group were at about 0·15 and 0·10 kg advantage in body weight increase to corresponding lambs in the low nutrition group (singles of both nutritional groups showed similar increase). This advantageous state persisted even when lambs were three weeks of age, and at this time the figures for differences in body weight increase were 1·05, 1·20 and 0·90 kg in the case of singles, non-deprived twins and triplets respectively. This, in my opinion, was due to two main reasons:

1. The more highly fed mothers produced adequate amounts of colostrum/milk,

and

2. the heavier and stronger lambs which were born to them could use the available milk efficiently.

As was the case in observations I made during the 1975 nutritional study, levels of ewe feeding during the

last eight weeks of pregnancy seem to have a marked effect on ewe and lamb performance in terms of: ewe body weight and condition score changes during pregnancy, levels of milk production, extent of PLM and then the future growth rate of lambs during the first three weeks of life. The extent of these effects seems to vary with the type of litter size.

Biochemical parameters were again analysed in lambs. One of these, serum alkaline phosphatase (SAP), has not been used in my previous studies.

Enzymology has been used for a long time as a diagnostic aid mainly in human medicine and to a lesser extent, in veterinary medicine. Serum alkaline phosphatase (SAP) is an important parameter in the case of liver damage and biliary obstruction. Normally, however, SAP values are very high in growing children and young animals, and this is mostly related to excessive bone activity and bone development. There are different circulating isoenzymes that can be identified by electrophoretic analysis. The enzyme exists in almost every body organ. In young lambs at birth, most of the circulating alkaline phosphatase is of skeletal origin but, after a day or two of suckling, SAP activity starts increasing in the lambs' sera as a result of increasing intestinal alkaline phosphatase activity (Healy, 1971b and 1975 a, b). This author reported values of about 750, 1450, 1000 and 740 I.U./L

for some Finn Dorset cross Suffolk lambs at birth, 24 hours, 48 hours and four weeks of age respectively. In adult sheep, all three isoenzymes, i.e. liver, skeletal and intestinal, were identified (Healy, 1971a and 1975a,b) but the total SAP levels were extremely low (example: 50 to 100 I.U./L in >2 year old ewes) as compared to that of young lambs.

During my investigations, total SAP was analysed in the sera of young lambs only. The aim was to try to pin-point changes, if any, in the levels of this enzyme in relation to lamb growth rate in utero (i.e. at birth) and shortly afterwards. It is important to emphasise that the values I am referring to were estimated for normal young lambs only and not for adult or clinically ill sheep.

SAP values measured for young lambs showed great individual variations but they are comparable to those reported by Healy (1971b and 1975a,b). However, the values he reported for the Finn Dorset cross Suffolk lambs at birth and four weeks of age were only comparable to those of my Scottish Halfbred triplets and were much lower than those of singles or twins included in my investigations. This is possibly because of the bigger litter size and then the slower skeletal development of Finn Dorset cross Suffolk lambs.

Apart from the findings reported by Healy (1971b) which showed no relationship between SAP activities and

birth weight, sex and litter size of lambs (although he did not present any figures to support his findings), there has been no other report dealing with the effect on SAP levels of these factors or of ewe plane of nutrition.

My work shows a clear and direct effect of late pregnancy plane of nutrition on SAP levels of lambs at birth. Lambs born to the high nutrition group have significantly higher SAP at birth than the corresponding lambs born to the low nutrition group. This finding is comparable to the effect of these feeding levels on lamb birth weight. High levels of feeding have improved the rate of fetal bone growth and resulted in the birth of heavier and physically stronger lambs. This could have an important bearing on the behaviour of newborn lambs in terms of early lamb activity and better suckling performance, all very necessary for better lamb survivability (Moule, 1954; Alexander and Peterson, 1961; Smith, 1964; Bareham, 1976).

SAP levels have significantly increased at 24 hours of age as a result of intestinal isoenzyme activity (Healy, 1971b, 1975b) following colostrum ingestion. At 48 hours the levels have fallen to levels similar to those measured at birth. This might have coincided with changes in the activity of gut cells, the rate of colostrum absorption and the quality of the ingested colostrum. Although it is interesting to observe a similar increasing

and then decreasing levels of immunoglobulins in the lambs' sera during the first two days of life, these changes in SAP seem not to be related to colostrum feeding only. Indeed, Healy (1971b) reported similar change in SAP following the feeding of ewe's or cow's milk to newborn lambs.

The direct relationship between the ewes' level of feeding during late pregnancy and SAP in lambs existed in most cases not only at 24 or 48 hours of age but even as late as four to six weeks of age. The higher SAP in lambs born to the high nutrition group coincided with a better performance of these lambs in terms of growth rate which has been referred to previously. The generally high SAP activity noticed in four to six week old lambs of both nutritional groups could reflect fast growth caused by an improved ewe milking ability. This improvement in milking ability was stimulated in part by the availability of adequate amounts of grass during these weeks of lactation.

If one accepts SAP as a good indicator of skeletal development, litter size appeared again as an important factor inversely affecting this development. In the adequately fed group, singles and twins had comparable SAP levels while triplets were at great disadvantage, as shown by values measured at birth (reflecting the intrauterine fetal growth) or at 24 hours, 48 hours and four

to six weeks, reflecting future lamb performance as affected by the birth weight, state of development of lambs after delivery, and the standard of ewe milking ability and lamb sucking. In the low nutrition group, the poorer development and growth of lambs was not restricted to triplets only. Even twins, which are normally quite comparable to singles in birth weight and strength, showed reduced SAP levels in all stages of development. This relationship between nutrition of the mother, litter size, and the development of the newborn lambs could help in explaining the catastrophic performance of some groups of ewes observed in different Scottish farms (Barton, personal communication).

Regarding sex of lambs, SAP at birth was higher, but not significantly, in males than in females. This is in line with the similar findings I presented in 1974 (see Chapter V) concerning the relation between birth weight and sex of lambs. The fact that males are heavier, but not significantly, than females at birth suggests a direct relationship between level of intra-uterine growth of fetuses and their weight at birth on the one hand, and their skeletally derived SAP levels (Healy, 1971b, 1975b) on the other.

Another support to the relationship between SAP and growth of lambs is supplied by surviving sets of triplets in this work, which were reared in milk bars using milk

replacer. These lambs performed badly in terms of body weight increase in the first few weeks of life. This coincided with significantly low SAP as compared to triplets reared naturally by their mothers.

From the above-mentioned findings, I conclude that SAP is a valid biochemical parameter in screening the intra-uterine and postnatal growth of lambs, in relation to ewe nutrition, litter size and lamb birth weight.

Total protein and total immunoglobulin levels in lambs followed the patterns established in 1975. Values fell as litter size increased and there was a further reduction in lambs born to ewes on the low plane of nutrition. Gamma-globulin levels in the low nutrition triplets consequently approached a value in some lambs close to that associated with increased mortality. In the lambs which died, and for which values are available, it was again clear that the total immunoglobulin levels were much lower than those in surviving lambs.

In brief, my findings indicated a relationship between levels of ewe feeding during late pregnancy, litter size, lamb survivability and total protein or gamma-globulins. Of the immunoglobulin fractions, IgG_1 and IgM were the main ones to be affected by nutritional treatments and litter size, while concentrations of IgG_2 and IgA failed to show any significant changes.

In previous experiments, I have concentrated on

immunoglobulin levels in the critical first 24 to 48 hours of life. In this experiment, I also investigated levels in lambs of four to six weeks of age. Only Pearson and Brandon (1976) appear to have reported on this aspect before. Using five Merino lambs, they presented comparable values to mine regarding IgG_1, IgG_2 and IgM, but much lower IgA values.

By four to six weeks after birth, the level of the maternal antibodies in the lamb's serum has greatly declined. By this time, most surviving lambs have passed the main dangers of the perinatal period.

At four to six weeks, the serum immunoglobulin levels of IgG_1 and IgM were greatly reduced when compared to 24 hour values. Serum IgG_2 levels marginally increased while IgA levels were increased noticeably. It is possible that the large amounts of maternal IgG_1 and IgM which passed to the lambs, following colostrum sucking, actually interfered with the endogenous synthesis of IgG_1 and IgM by lambs which, as shown by Piercy (1973), are immunologically competent at birth. IgG_2, which is very deficient both in colostrum and in the serum of 24 and 48 hour old lambs, and IgA, which seems not to be affected by colostrum ingestion, are produced earlier by the lymphoid tissue of young lambs, hence their increase at four to six weeks of age. The very high initial levels of IgG_1 and IgM and their possible relatively slow degradation might

have also masked their even slower endogenous production.

The behaviour of IgG_2 mentioned above, was more manifest in the case of lambs (from sets of triplets) that were kept in a milk bar from the age of two to three days. Under milk bar conditions where bacterial challenges are expected to be heavier than those of the open grazing fields, there was a significant increase in lambs serum IgG_2 by the age of four to six weeks. This increase took IgG_2 values closer to those I reported in the sera of adult ewes and those reported by Watson and Lascelles (1973a), Cripps and Lascelles (1974) and Ciupercescu (1977). This could suggest that, as IgG_2 is very low in lamb sera during the first days of life, its protective role is limited during this period but that this role increases in importance as the animal matures.

The markedly high levels of IgA in the sera of four to six week old lambs might reflect its importance in the neonatal period, when intestinal infections, particularly those associated with E. coli, are very common. The local protection role described for this immunoglobulin by Lee and Lascelles (1970) and Beh and Lascelles (1974) could support the validity of this suggestion.

The nutritional study just discussed, included larger numbers of animals in each group than the 1975 work which made comparison easier. The nutritional factors were not exactly the same as in 1975 and yet the overall results reached showed the same trends. Nutrition does affect

both ewes and lambs, and can have a serious effect on the mortality, morbidity and antibody status of lambs. It was clear that the lower plane of nutrition already affected the early milking ability of ewes as it had in the 1975 work, and I wished to see if colostrum deprivation had any significant additional effect on the lambs. Before discussing the deprivation study, mention must again be made of the high levels of mortality encountered in the nutritional work (see Table 8·6). Of the eleven twin lambs dying on this experiment, six deaths resulted from abortions due to EAE or Toxoplasma. Nutritional factors were also probably associated with these abortions and consequently the mortality rate for twins born in the low nutrition group is high.

The colostrum deprivation experiment was done only on ewes producing live twins and the abortion element was thereby excluded from this work. It is therefore essential, if I am to ascertain whether colostrum deprivation exacerbated the mortality caused solely by low nutrition, to rearrange the figures quoted in Table 8·6. Five twin lambs died of causes other than abortion in the low nutrition group. One of the deaths occurred immediately after birth and the dam was consequently excluded from the deprivation experiment. This means that the number of twin lambs which were born live but died as a result of low nutrition numbered two out of a total of 12 lambs born.

The mortality rate, excluding abortions, was consequently just under 17 per cent. In the deprived section of the low nutrition group, the mortality rate was 30 per cent (see Table 8·9). On the other hand, no lambs died in the high nutrition group, even if colostrum deprived. It appears that colostrum deprivation alone of lambs from adequately nourished ewes (see Chapter VI) or from well nourished ewes (this chapter) does not increase lamb mortality but when it occurs in association with low ewe nutrition it does increase lamb mortality. This increase is probably caused by the stress of deprivation followed by the subsequent inadequate colostrum production in less well nourished ewes.

As was to be expected the growth rate of deprived lambs was curtailed over the first 48 hours of life, irrespective of the dams' nutritional status. This effect was similar in both deprived groups, on the basis of absolute weight gain. However, the discrepancy between the growth rate of control and deprived lambs was greatest in the high nutrition group because of the higher body weight increase of the high nutrition control group over this 48 hour period.

The early depression of growth rate in deprived lambs which survived was rapidly overcome and the growth rates up to three weeks of age indicated that deprived lambs grew almost as well as the controls in the high nutrition

group and apparently better than the controls in the low nutrition group. These figures are somewhat misleading in the low nutrition group because three lambs died in the deprivation group and left their sibs to be reared as singles. Consequently, these lambs grew rapidly as they had no competition for the milk available and this increased the mean weight gain figures for the group. This difficulty can be resolved by calculating the difference between the birth and three week weight for each lamb and thus finding the total weight gain in each group. Lambs which died would have a nil figure. This illustrates clearly (Table 8·9) that the high nutrition controls outstripped the low nutrition controls in total weight of lamb produced, due to differences in mortality and growth rate. The deprived high nutrition group performed better than the low controls and the worst group of all was the low deprived group. The low control group produced only 75 per cent of the weight gain of the high controls and this difference is due to mortality and nutritional differences. When colostrum deprivation is added to this the figure drops to 56 per cent. Such differences would have profound economic effects if they occurred on a commercial farm although such a state of affairs could only arise if lambing supervision was extremely poor.

The variation in growth rate between lambs in the

different groups is mirrored by the alkaline phosphatase levels (Tables 8·7 and 8·10). Both poor ewe nutrition and colostrum deprivation reduced the lambs' levels of alkaline phosphatase in the first 48 hours of life. Again this parameter seems to be a useful measure of growth rate *in utero* and shortly after birth.

In addition to the depression of early growth rate, deprivation also had a marked affect on the incidence of the "watery mouth" syndrome. The syndrome was commoner and more pronounced in both deprived groups when compared to their controls. In the high nutrition group, adequate colostrum was produced and "watery mouth" was associated with the indigestion which resulted from hungry lambs gorging themselves after the period of deprivation. In the low nutrition group, only small amounts of colostrum were produced by some ewes and "watery mouth" in these cases was associated with the retention of meconium which is normally removed by the mechanical stimulation and lubricant action of colostrum. The relationship between meconium retention and colostrum was suggested by Watt (1965). In no case were lambs treated and the majority recovered within 24 hours of the onset of symptoms. Those which died did so as a result of *E. coli* infections. All had low immunoglobulin levels which suggests that there is a relationship between immunoglobulin levels in lambs and the incidence of *E. coli* disease early in life.

This is in agreement with workers such as Shaw (1971) and Campbell (1974).

Total protein values at 24 hours of age were only depressed in the low nutrition deprived group and the same pattern emerged for gamma-globulins (Biuret). In previous deprivation work with twin lambs, significant depression of gamma-globulin levels was not a feature. The marked depression to mean levels of 1·27 g per 100 ml in the deprived low nutrition group must reflect the combined effects of deprivation and low nutrition. Exactly the same pattern is evident in the SRID results with total immunoglobulin values being in excess of 2·8 g per 100 ml except for the deprived low nutrition group where they fell to 1·8 g per 100 ml. This fall was due to depression in the levels of IgG_1 and IgM, the two major immunoglobulins a lamb derives from colostrum, and the same was true for low nutrition lambs which died. In these cases, the immunoglobulin levels were very low. This reinforces previous observations that lamb mortality and low immunoglobulin levels are closely related. This does not necessarily mean that the lamb dies because it has no antibodies to protect it against bacterial attack, but it does imply that colostrum is an important factor in lamb survival. This importance may derive from the transfer of antibodies, the non-specific factors in colostrum said to be important in development of the newborn animals'

defence mechanism (Piercy, 1973, 1974), or because colostrum is the lamb's only early external source of energy. In practice a combination of these factors probably operates.

Conclusion

1) Of the parameters used to monitor the energy intake of ewes, 3-HB estimations again proved superior to the measurement of either urea or albumin levels.

2) The levels of energy intake used in this experiment were less extreme than those used previously and as a result the problems of food refusal on high energy diets were avoided.

3) Feeding levels in late pregnancy affected the ewes' levels of serum immunoglobulins and this was related to colostrum production. Those ewes producing most colostrum had the lower levels of circulating immunoglobulin.

4) Low levels of ewe nutrition during the last eight weeks of pregnancy did not affect the protein or immunoglobulin concentrations of the colostrum secreted but they adversely affected the amount of colostrum produced.

5) Low levels of ewe feeding caused ewes to lose weight during pregnancy. This weight loss was also related to the number of lambs the ewe was carrying.

6) Low levels of ewe feeding adversely affected lamb birth weights, lamb viability and subsequent lamb growth rates in both twins and triplets. In the high nutrition group only triplets did less well than the single lambs.

7) Both toxoplasmosis and EAE caused lamb deaths. There appears to be a connection between the level of ewe nutrition and the effects exerted by Toxoplasma in that the lower level of nutrition may predispose to a more severe effect of the infection.

8) Low maternal nutrition had adverse affects on the lambs' alkaline phosphatase levels early in life. This parameter is useful in assessing intra-uterine growth rates, retrospectively.

9) The lambs' serum levels of total protein and gamma-globulin (particularly IgG_1 and IgM) were adversely affected by a low level of maternal nutrition and by litter size. Lambs which died had extremely low levels of protein and immunoglobulin.

10) Colostrum deprivation for nine hours after birth increased the incidence of the "watery mouth" syndrome.

11) Colostrum deprivation reduced lamb growth rate from birth to 48 hours of age. It did not have any other adverse effects in lambs born to the high nutrition group.

12) Colostrum deprivation, when it occurred in conjunction with low levels of ewe nutrition, increased the lamb mortality rate, reduced circulating levels of protein and immunoglobulin and badly affected the lambs' productive performance.

OVERALL DISCUSSION AND CONCLUSIONS 1974 - 1976

The 1974 work, with the exception of the nutritional study, was preliminary work and, as such, must stand on its own as previously described. There are, however, one or two points which emerged from this work which were corroborated by, or used in, subsequent studies, for instance lamb birth weights and the various parameters used to measure lamb performance were all affected by litter size. The larger the litter the more likely these factors were to be adversely affected. This statement is, in general, true for PLM, lamb growth rates and the lamb's immunoglobulin status.

At a time when increased lamb production is called for, mainly in the form of greater numbers of lambs per ewe, these basic facts are of great importance. It is of little value to produce more lambs if the result is that the mortality and morbidity rates keep pace with increased production. The fostering of lambs in "milk bars", in my work, was not a particularly successful or economically viable procedure. The rearing of complete sets of triplets by a single ewe has been attempted only by the more successful members of the farming community and has met with variable degrees of success. In a few cases all the lambs were reared but in one trial of which I am aware, (FitzSimons, personal communication), only one-third of the ewes managed to rear three lambs successfully

to weaning. In these circumstances it may be prudent to think of increasing productivity by rearing a greater proportion of lambs born rather than trying to increase the number of lambs born per ewe at each parturition.

The nutritional studies undertaken in this work indicated that ewe nutrition has a bearing on the birth weight and subsequent performance of lambs as well as affecting the ewe herself. For reasons which have already been explained, the nutritional projects of 1974 and 1975 had drawbacks which either reduced the number of animals available or meant that small numbers of animals on different nutritional regimes were compared. It seems desirable therefore to assess the nutritional work over the three years to see if the trends emerging in each year hold true over the whole period. As the hay quality and amount of concentrate fed varied from year to year and as some ewes were fed individually and others were fed on a group basis it seems best to undertake any comparative work on the basis of energy intake (MJ per kg DM) during the last eight weeks of pregnancy. Three nutritional groups emerge from the combined results.

1) A low group with an energy intake of between 400 - 560 MJ per kg DM, made up of animals from groups 1 and 2 in 1974 and group 1 in 1975.

2) A medium group with an energy intake of 650 - 860 MJ per kg DM, made up from groups 3 and 4 in 1974, groups 2

and 3 in 1975 and the low group in 1976.

3) A high group with an energy intake of 900 - 1220 MJ per kg DM, derived from group 4 in 1975 and the high group in 1976.

The most important mean figures obtained from the combined data are given in Table 8·11 and illustrate that several overall trends appear throughout the three year period. Ewe weight loss is markedly affected by the plane of nutrition. Energy intakes of less than 600 MJ per kg DM cause serious weight loss, particularly in ewes carrying multiple litters. Very high levels of energy intake allow ewes to put on excessive, unnecessary weight.

Diet also affects the ewe's ability to secrete adequate amounts of colostrum immediately after parturition. In the low nutrition group this is carried over into the post partum period and seriously affects subsequent lamb development. PLM increases as the ewe's energy intake declines and lamb birth weights are also affected. The effect on birth weight is particularly pronounced when litter size exceeds two and is a major factor in increasing the mortality rates which escalate rapidly as litter size increases. Even in the high nutrition group the mortality rate in triplets is unacceptably high.

In lambs which survived, growth rate to three weeks of age is related to pre-partum ewe nutrition when litter

TABLE 8·11.

Overall ewe and lamb performance

Levels of energy intake			LOW	MEDIUM	HIGH
Number of ewes			41	69	32
Mean ewe body weight, loss or gain in kg	All ewes		-10·29 (40)	-1·09 (64)	+ 4·20 (30)
	Ewes with singles		-13·50 (3)	-0·20 (9)	+14·30 (6)
	Ewes with twins		-14·40 (5)	-3·98 (18)	+ 2·53 (13)
	Ewes with triplets		-17·50 (2)	-6·37 (9)	+ 0·87 (11)
Percentage of ewes with insufficient colostrum			32·5 (40)	23·1 (69)	6·0 (32)
Mean lamb birth weight in kg		Singles	5·54 (7)	6·08 (15)	5·87 (7)
		Twins	4·07 (49)	4·55 (70)	5·08 (26)
		Triplets	2·98 (24)	3·56 (48)	4·03 (33)
Overall percentage levels of PLM			24 (81)	18·5 (135)	*11·5 (69)
Percentage levels of PLM among:		Singles	Nil (7)	6·6 (15)	Nil (7)
		Twins	18·0 (50)	15·4 (72)	Nil (26)
		Triplets	41·6 (24)	27·0 (48)	22·2 (36)
3 weeks mean body weight increase of survivors in kg		Singles	7·18 (7)	7·63 (13)	8·81 (7)
		Twins	4·64 (38)	5·56 (53)	6·95 (14)
		Triplets	4·52 (11)	5·43 (27)	6·52 (20)
24 hour biuret gamma-globulin levels g/100 ml		Singles	2·46 (7)	3·01 (14)	3·55 (7)
		Twins	2·08 (33)	2·32 (49)	2·50 (14)
		Triplets	1·72 (14)	1·93 (33)	2·17 (26)

Number of animals included in each calculation in brackets.

*Quadruplets excluded.

size exceeds one, i.e. the poorer the ewes' nutrition the slower their lambs' growth rate. It must also be pointed out that these figures are for survivors and in the groups with the slowest growth rate there was also the highest incidence of mortality. The figures do not allow an accurate comparison of productive performance between the groups but groups with low growth rates and high mortality will obviously be much less productive than groups with low mortality and high growth rates.

Poor colostrum production and low birth weights combined to affect the lambs' circulating gamma-globulin levels. These levels declined as ewe nutrition levels fell and as litter size increased. The reduction in level was accounted for by falls in IgG_1 and IgM. Low immunoglobulin levels were associated with increased mortality. In this work no attempt has been made to equate low serum immunoglobulin levels in lambs with poor resistance to bacterial disease, i.e. in the context of this work immunoglobulin levels are really a measure of colostrum ingestion and hence of energy intake by the lamb. Future work could usefully be directed towards assessing the precise role of energy intake and resistance to bacterial disease in relation to lamb mortality. Further work is called for to measure the amount of colostrum produced by ewes in different nutritional circumstances and to investigate whether relationships exist

between the colostral volume and the immunoglobulin and other content of colostrum.

The colostrum deprivation studies cannot be amalgamated and must stand as previously described. Deprivation in association with adequate ewe nutrition has little detrimental effect on lambs but when combined with low nutrition it increases PLM.

SUPPLEMENT

DISCUSSION ON TECHNIQUES USED TO MEASURE GAMMA-GLOBULINS:

INTRODUCTION

The different techniques, the biuret and the SRID, were used to measure gamma-globulins in my work. The first was used as a rapid screening test and the second as a more precise method of detecting specific immunoglobulins. During my investigations, noticeable discrepancies between total gamma-globulin values measured by the above two techniques were observed and this, in my opinion, is worth considering. It is hoped that in this additional discussion a preliminary, but by no means complete, comparison of the two values will be performed and possible sources of variations will be discussed.

Widescale comparative studies on the different methods used to estimate gamma-globulins for routine clinical work or for research purposes have been performed in humans. In so far as farm animals are concerned, such comparisons, although of a more limited nature, were performed on calf sera by workers who were trying to check the validity of the method they were using (McEwan *et al.*, 1970; McBeath, Penhale and Logan, 1971; Vior, Toma, Grigore, Constantinescu, Secaşiu and Contora, 1973; Reid and Clifford, 1974). The main methods compared on calves'

sera are the zinc sulphate turbidity test (ZSTT), the refractometer method, electrophoresis which has usually been standardized against total protein measured by biuret or micro-Kejldahl methods, and the SRID technique. These methods were employed, either as such or with slight modifications, by many authors. In this connection, the comprehensive review presented by Vior et al. (1973) is worth consultation.

Although much research attention has been paid recently to ovine immunoglobulins and lamb survival, a comparative investigation into the different laboratory methods used for measuring these parameters has been almost completely ignored. A search through the literature revealed only one brief reference by Ducker and McEwan (1972) who reported a high correlation between serum gamma-globulin levels of three to seven day old lambs when measured by biuret/electrophoresis and by the ZSTT of McEwan et al. (1970).

Henry (1964), while reviewing the work of others, reported large variations in the level of gamma-globulins estimated by different methods and stated that gamma-globulin levels could be twice as high by immunologic test as by electrophoresis. The same author suggested that gamma-globulins could appear in the beta or alpha-2 electrophoretic fractions due to incomplete separation of the different globulin fractions.

During the different stages of my work, gamma-globulins in lamb's serum and in ewe's serum and colostrum were analysed by two methods, the chemical biuret method of Henry (1964), and the more specific immunodiffusion method (SRID) of Mancini et al. (1965) as modified by Fahey and McKelvey (1965). Total gamma-globulin values measured by the two methods can be seen in the following previously presented tables: 6·3, 7·3, 7·4, 7·5, 7·7, 8·2, 8·3, 8·4, 8·5, 8·7, 8·8 and 8·10. Data from these tables are summarised in the accompanying table which also includes data from experiments which have not so far been tabulated. Both sources reveal consistent differences between the biuret and SRID results. In ewe sera, the SRID tests gave considerably higher results than biuret, in lamb sera the same trend emerged but the differences were less obvious, while in colostrum there was a reversal of this pattern with biuret giving the higher results.

Investigations and results:

Firstly, the data given in the table were assessed for the significance of correlation (as measured by 't' test) and the results are given in the table. There was a highly significant correlation between the two methods. Having ascertained this fact, I now undertook a small additional experiment to look at the discrepancies already noted.

Mean gamma-globulin values as measured by biuret or SRID methods

Type of sample		Year of study	No. of samples	Mean biuret values (g/100 ml)	Mean SRID* values (g/100 ml)	Significance of correlation "p"
Ewes' sera	Before lambing	1975 (nutritional study)	86	1·74	2·94	$< 0·01$
		1976	142	1·86	2·97	$< 0·01$
	Immediately after lambing	1975 (nutritional study)	27	1·53	2·88	$< 0·05$
		1976	49	1·54	2·74	$< 0·01$
Lambs' sera at 24 or 48 hours of age		1974	345	1·64	1·85	$< 0·01$
		1975 (deprivation study)	116	2·22	2·77	$< 0·01$
		1975 (nutritional study)	125	1·68	2·09	$< 0·01$
		1976	169	2·12	2·42	$< 0·01$
Colostrum		1975 (deprivation study)	26	9·26	7·05	$< 0·05$
		1975 (nutritional study)	21	10·30	8·96	$< 0·01$
		1976	44	14·37	8·98	$< 0·01$

*Sum of IgG_1, IgG_2, IgM and IgA values.

Firstly, eight colostrum samples were analysed for their gamma-globulin contents in order to ascertain the reproducibility of the two methods. Duplicate results were subjected to paired 't' test analysis and differences between duplicates were found to be statistically not significant (t_7 = 0·492 and 1·68 for biuret and SRID methods respectively). As anticipated, the two methods gave highly reproducible results.

Now I analysed fresh samples to make certain that the discrepancies previously described were reproducible. Eight colostrum samples, eight ewes' sera and eight lambs' sera (from 24 to 48 hour old lambs), were randomly collected from animals available in ESCA farms. They were examined for their gamma-globulin contents using biuret and SRID methods (total values measured by the second method represents the sum of IgG_1, IgG_2, IgM and IgA values estimated separately). These samples, i.e. ewes' sera, lambs' sera and ewes' colostrum, showed mean biuret values of 1·33, 2·38 and 8·49 g per 100 ml respectively, as compared to corresponding SRID mean values of 2·65, 2·57 and 5·22 g per 100 ml. These figures confirmed that the previously observed pattern in relation to the methods used and the type of sample analysed had been reproduced. In these samples, mean biuret readings were as much as 102 per cent lower in the case of ewes' sera, 3·4 per cent lower in the case of lambs' sera and 61·4

per cent higher in the case of ewes' colostra, as compared to the corresponding SRID values. Henry (1964), who described the biuret method in use during my work, reported an 85 per cent recovery by this method. He also suggested that only 80 to 90 per cent of these values are gamma-globulins while the remainder are alpha- and beta-globulins, i.e. gamma-globulin levels were underestimated by about 23·5 to 32·0 per cent.

I now applied agarose gel electrophoresis (Corning EEL, England) on the 24 colostrum and serum samples, previously referred to (see Figs. 1·a, b, c), at 100 v for 15 minutes in the case of colostrum and 19 minutes in the case of serum. It appeared that the lambs' serum gamma-globulin region is a major and distinct one from the rest of the protein fractions (Fig. 1·a), and the beta to gamma-globulin mean ratio was 1:4·2. Albumen was present in quantity. In ewes' sera (Fig. 1·b), where the main globulin fractions seem to interfere with each other, the beta- and gamma-globulins were at a mean ratio of 1:1·5. Again albumin was present in quantity.

It seems possible that during the salting out procedure for the biuret technique the relative amounts of alpha, beta and gamma-globulin present affect the results. 'Immunologic' gamma-globulins are found in the alpha and beta electrophoretic fractions and may be left behind during salting out so if the relative amount of

AGAROSE GEL ELECTROPHORETIC PATTERNS

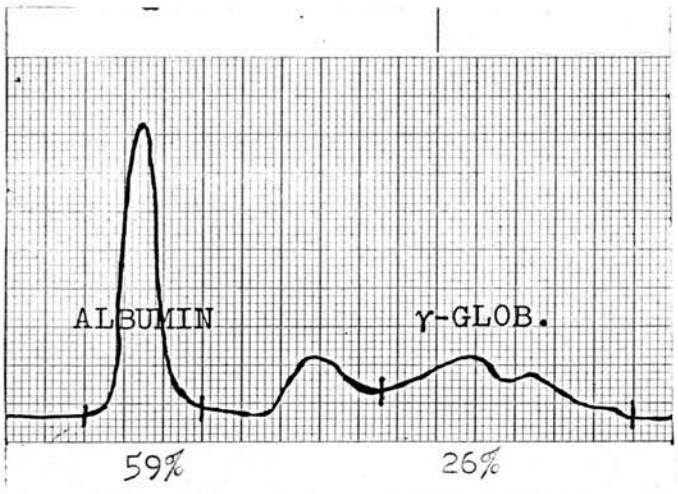


FIG. 1·a: Electrophoretic pattern of lambs' serum.

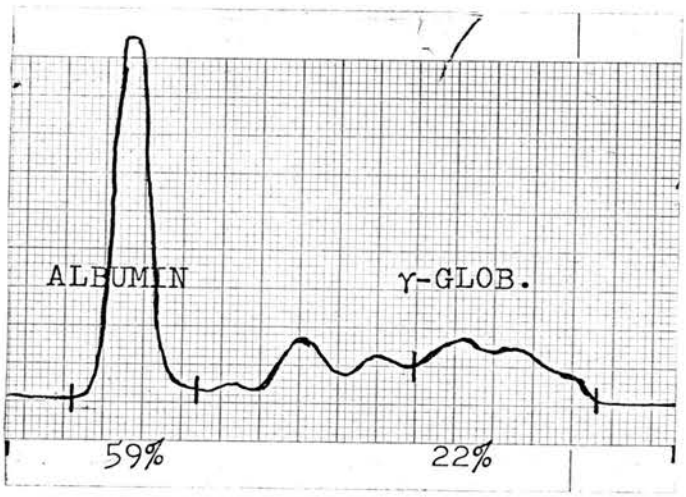


FIG. 1·b: Electrophoretic pattern of ewes' serum.

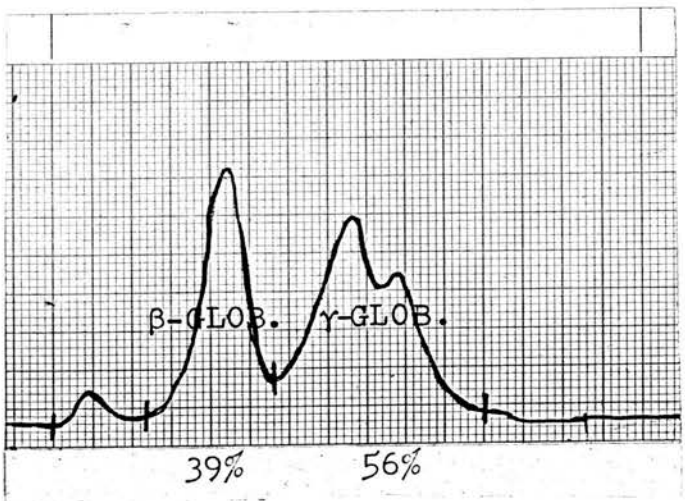


FIG. 1·c: Electrophoretic pattern of ewes' colostrum.

beta-globulin is high (as in ewes' sera), the biuret test will give low values compared to the SRID technique. If, as in lambs' sera, the beta-globulin component is relatively small then the two methods will give comparable values.

In colostrum, however, (Fig. 1·c), the beta-globulin fraction is high and the reverse trend occurred so the previous argument either applies to serum only, or is erroneous. In the colostrum samples the discrepancy between biuret and SRID measurements decreased with the increase in gamma-globulin levels: at $<4{\cdot}0$, 4 to 6 and >6 g per 100 ml biuret values, the discrepancy from corresponding SRID values was 137·3, 85·6 and 8·2 per cent respectively. This may have been directly related to the relative size of beta-globulin interference. That is, the beta-globulins, including 'non-immunologic' globulins, were salted out by the biuret technique and the biuret results were correspondingly higher than those measured by SRID. This argument is in direct conflict to the one propounded for serum and must remain speculation.

In order to find out what was present in the salt precipitation used in the biuret technique, I obtained precipitates from ewe and lamb sera and from colostrum, and performed immunoelectrophoresis on them against anti-sheep sera (Fig. 2). It was noticed that all the

FIG. 2:

Wells:

First upper and first lower, sheep serum;

second upper, salt precipitated lamb serum gamma-globulins;

third upper, salt precipitated whey gamma-globulins;

rest, salt precipitated ewe serum gamma-globulins.

Troughs:

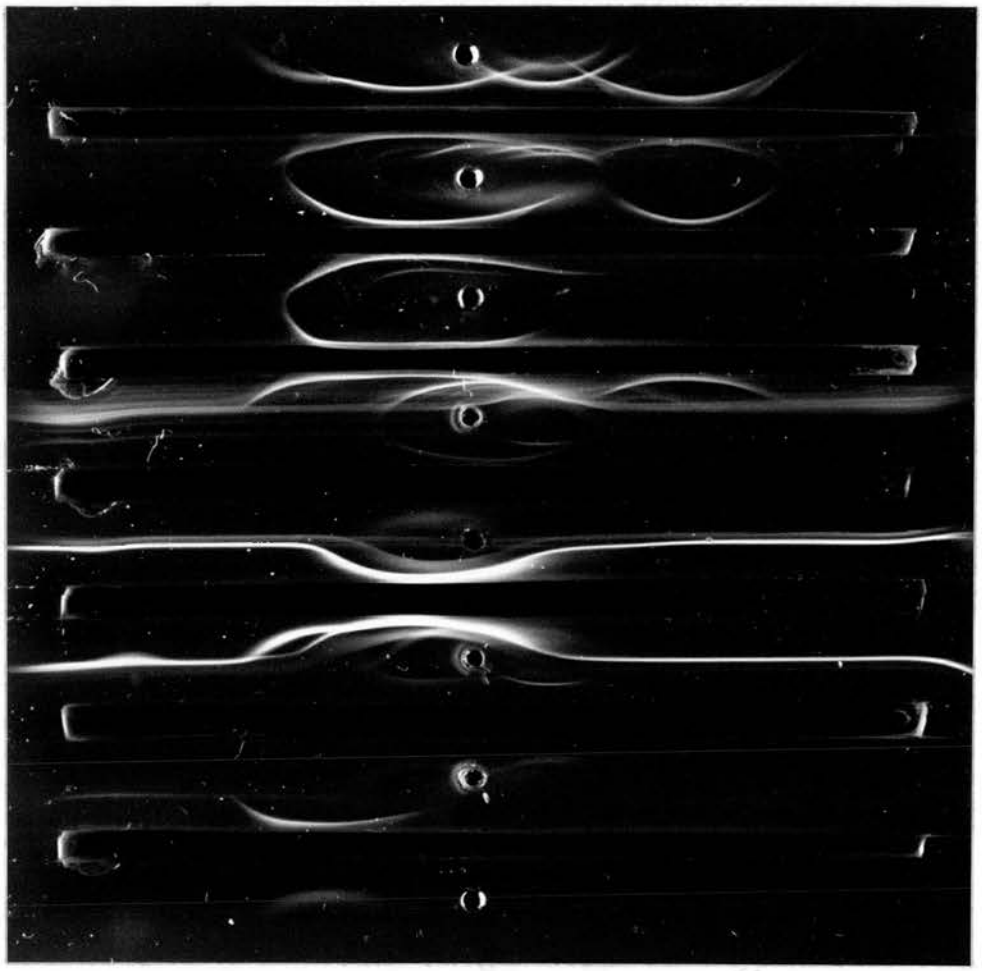
Top three, anti-sheep serum;

fourth upper, anti-rich IgA fraction antiserum;

fifth upper, anti-rich IgG_1 fraction antiserum;

sixth upper, anti-rich IgM fraction antiserum;

lower trough, anti-rich IgG_2 fraction antiserum.



major immunoglobulins existed in these samples. In ewes' sera, where SRID readings tended to be markedly higher than the corresponding biuret readings, immuno-electrophoresis against anti-sheep IgG_1, IgG_2, IgM and IgA rich fraction anti-sera (Fig. 2), suggested clearly noticeable levels of all the major immunoglobulins. This does not, of course, mean that all 'immunologic' alpha or beta-globulin fractions were present. The technique does not detect beta-globulins, as the necessary anti-sera was unavailable, so it fails to confirm or refute the previous hypothesis.

During the immunoelectrophoresis examination, it was interesting to observe that albumin, which exists in high levels in the sera and only in negligible levels in colostrum, was still present in noticeable quantities in both ewes' and lambs' sera precipitates (see Fig. 2). For this reason gamma-globulins from eight ewes' sera were precipitated (separately) as described for the biuret method of Henry (1964). When examined for total protein content (i.e. total gamma-globulins), the eight sera showed a mean level of 1·33 g per 100 ml. They were later subjected to albumin estimation using the BCG method of Doumas et al. (1971) and showed a mean value of 0·168 g per 100 ml. Quantitatively these albumin levels constitute 12·6 per cent of the levels assigned for the gamma-globulins. These measurable quantities

of contaminating albumin do not necessary cause weight for weight changes in the gamma-globulin results but the fact that they do exist in the gamma-globulin precipitate could suggest some degree of interference in the final gamma-globulin values. This last point was worth investigating. To this end the albumin content of a sample of pure ovine albumin was estimated using the BCG method, and the gamma-globulin precipitate from eight colostral whey samples was estimated for protein content by the biuret technique. Albumin and whey precipitate were then mixed 1:1 and the gamma-globulin content of the mixture estimated by the biuret technique. If albumin interfered with the method the results should have been at variance with the estimated content of the mixture. In fact, there was no conclusive evidence of marked interference by the albumin with half the samples giving readings equal to or in excess of the calculated values.

It appears that the discrepancies in results reported here are real but that this initial investigation has failed to yield any definite evidence to account for the differences.

Data regarding the application of the SRID technique in the animal fields are lacking. Even in the human field, where good facilities allow an intensive use of this test, problems are still widely associated with it. The structural and functional complexity of the immuno-

globulins themselves, specificity of antisera produced, accuracy of the standards used especially the absence of a common reference standard, all contribute to variations in the results. Nevertheless, the technique is a very specific and quantitative one, and its importance becomes greater when determination of the different classes of immunoglobulins is required. This was one of the main reasons for its inclusion in my study.

This simple comparative example reported here, concerning the two methods employed to measure immunoglobulins, demonstrates the need for a comprehensive investigation and comparison of the available methods, and a thorough review of what has been achieved so far regarding immunoglobulin measurements. A standardized method for this purpose is necessary not only in sheep but in all farm animals. This, undoubtedly will help in achieving more accurate interpretations with the likelihood that the findings can be applied in a meaningful manner.

REFERENCES

AALUND, O., OSEBOLD, J. and MURPHY, F. A. (1965). Isolation and characterization of ovine gamma-globulins. Archs. Biochem. Biophys., 109, 142-149.

ALEXANDER, G. (1956). Influence of nutrition upon duration of gestation in sheep. Nature, Lond., 178, 1058-1059.

ALEXANDER, G. (1964). Lamb survival: Physiological considerations. Proc. Aust. Soc. Anim. Prod., 5, 113-122.

ALEXANDER, G (1971 - 1972a). Neonatal survival in sheep, cattle and pigs: A general approach. Victorian Vet. Proc., 30, 70-73.

ALEXANDER, G. (1971 - 1972b). Survival of the newborn: Nutritional factors, environmental and behavioural hazards. Victorian Vet. Proc., 30, 73-77.

ALEXANDER, G. and PETERSON, J. E. (1961). Neonatal mortality in lambs. Aust. vet. J., 37, 371-381.

ALEXANDER, G., BELL, A. W. and HALES, J. R. S. (1972). The effect of cold exposure on the plasma levels of glucose, lactate, free fatty acids and glycerol and on the blood gas and acid-base status in young lambs. Biol. Neonate, 20, 9-21.

ARCHER, H. E. and ROBB, G. D. (1925). The tolerance

of the body for urea in health and disease. Q. Jl. Med., 18, 274-287.

BAREHAM, J. R. (1976). The behaviour of lambs on the first day after birth. Br. vet. J., 132, 152-162.

BARKER, J. D. (1975). A field trial of Finnish Landrace rams as sires of crossbred ewes. Anim. Prod., 20, 371-380.

BARNICOAT, C. R., LOGAN, A. G. and GRANT, H. I. (1949a). Milk secretion studies with New Zealand Romney ewes. Part I and II, J. agric. Sci., Camb., 39, 44-55.

BARNICOAT, C. R., LOGAN, A. G. and GRANT, H. I. (1949b). Part III and IV, J. agric. Sci., Camb., 39, 237-248.

BARTON, G. M. Personal communication (1974).

BARTON, G. M. and BLYTH, A. (1977). In "Perinatal losses in lambs". East of Scotland College of Agriculture, Edinburgh, 52-55.

BASSETT, G. D. and THORBURN, G. D. (1969). Foetal plasma corticosteroids and the initiation of parturition in sheep. J. Endocr., 44, 285-286.

BEH, K. J. and LASCELLES, A. K. (1974). Class specifity of intracellular and surface immunoglobulin of cells in popliteal and intestinal lymph from sheep. Aust. J. exp. Biol. med. Sci., 52, 505-514.

BELL, D. S. (1944). Infant mortality in relation to nutrition of the ewe. J. Anim. Sci., 3, 451.

BEM, Si. and POPESCU, Ar. (1973). Application of non-specific homologous gamma-globulins for reducing the losses of newborn lambs. LUCRĂRILE INSTITUTULUI DE CERCETĂRI VETERINARE ŞI BIOPREPARATE "PASTEUR", 10, 13-27.

BEVERLEY, J. K. A. and MACKAY, R. R. (1962). Ovine abortion and Toxoplasmosis in the East Midlands. Vet. Rec., 74, 499-501.

BEVERLEY, J. K. A. and WATSON, W. A. (1962). Further studies on Toxoplasmosis and ovine abortion in Yorkshire. Vet. Rec., 74, 548-552.

BLAXTER, K. L. (1957). The effect of defective nutrition during pregnancy in farm livestock. Proc. Nutr. Soc., 16, 52-58.

BLAXTER, K. L. and WILSON, R. S. (1963). The assessment of a crop husbandry technique in terms of animal production. Anim. Prod., 5, 27-42.

BLOWEY, R. W. (1972). Metabolic profiles - Some aspects of their interpretation and use in the field. Vet. A., 13, 21-30.

BOOTH, P. (1972). Lambing losses are too high. Pastoral Rev. Graz. Rec., 82 (10), 457.

BOURNE, F. J. (1969). IgA immunoglobulin from porcine serum. Biochem. biophys. Res. Commun., 36, 138-145.

BOURNE, F. J. (1971a). Pig Immunoglobulins. Ph.D. Thesis, University of Bristol.

BOURNE, F. J. (1971b). Porcine immunoglobulins. Vet. A., 12, 74-85.

BOURNE, F. J. (1973). In: Symposium on nutrition of the young farm animals. The immunoglobulin system of the suckling pig. Proc. Nutr. Soc., 32, 205-215.

BOWDEN, D. M. (1971). Non-estrified fatty acids and ketone bodies in blood as indicators of nutritional status in ruminants: A review. Can. J. Anim. Sci., 51, 1-13.

BRANDON, M. R., WATSON, D. L. and LASCELLES, A. K. (1971). The mechanism of transfer of immunoglobulin into mammary secretion of cows. Aust. J. exp. Biol. med. Sci., 49, 613-623.

BROADBENT, D. W. (1971). Some causes of ovine perinatal mortality in Victoria. Victorian Vet. Proc., 29, 45.

BROADBENT, D. W. (1975). Infections associated with ovine perinatal mortality in Victoria. Aust. vet. J., 51, 71-74.

BRUCKMAN, F. C., D'ESOPO, L. M. and PETERS, J. P. (1930). The plasma proteins in relation to blood hydration. IV. Malnutrition and serum proteins. J. clin. Invest., 8, 577-590.

BURNET, F. M. (1967). The impact on ideas of immunology. Cold Spring Harb. Symp. quant. Biol., 32, 1-8.

BUTLER, J. E. (1971). Review of the bovine immunoglobulin. J. Dairy Sci., 54, 1315-1317.

BUTLER, J. E. and MAXWELL, C. F. (1972). Preparation of bovine immunoglobulins and free secretory component and their specific antisera. J. Dairy Sci., 55, 151-164.

CAMPBELL, S. G. (1974). Experimental colostrum deprivation in lambs. Br. vet. J., 130, 538-543.

CHAUVEAU, M. A. (1888). Sur le mécanisme de l'immunité. Annls Inst. Pasteur, 2, 66-74.

CIUPERCESCU, D. D. (1975). Personal communication.

CIUPERCESCU, D. D. (1976). Production of mono-specific goat antisera to sheep IgG_1 and IgG_2 immunoglobulins. Immunology, 30, 59-61.

CIUPERCESCU, D. D. (1977). Dynamics of serum immunoglobulin concentrations in sheep during pregnancy and lactation. Res. vet. Sci., 22, 23-27.

CLINE, M. J. (1975). The white cell. 1st ed., 290-302, Cambridge. Massachusetts: Harvard Univ. press.

CLOETE, J. G. (1972). Nutrition and reproduction in sheep. Jl. S. Afr. vet. med. Ass., 43, 147-153.

COMLINE, R. S. and SILVER, M. (1972). The composition of foetal and maternal blood during parturition in the ewe. J. Physiol., 222, 233-256.

COOP, I. E. (1950). The effect of level of nutrition during pregnancy and during lactation on lamb and wool production of grazing sheep. J. agric. Sci., Camb., 40, 311-340.

COOP, I. E. and CLARK, V. R. (1969). The influence of nutritional level in early pregnancy of the ewe. J. agric. Sci., Camb., 73, 387-394.

CRABTREE, J. R. and WILLIAMS, G. L. (1971). The voluntary intake and utilisation of roughage concentrate diets by sheep. 1. Concentrate supplements for hay and straw. Anim. Prod., 13, 71-82.

CRIPPS, A. W., HUSBAND, A. J. and LASCELLES, A. K. (1974). The origin of immunoglobulins in intestinal secretion of sheep. Aust. J. exp. Biol. med. Sci., 52, 711-716.

CRIPPS, A. W. and LASCELLES, A. K. (1974). The biological 'half-lives' of IgG_1 and IgG_2 in young milk-fed lambs and in non-pregnant and colostrum forming sheep. Aust. J. exp. Biol. med. Sci., 52, 717-719.

CUMMING, I. A. (1972a). The effect of increasing and decreasing live weight on ovulation and embryonic survival in the Border Leicester and Merino ewes. Proc. Aust. Soc. Anim. Prod., 9, 192-198.

CUMMING, I. A. (1972b). The effect of nutritional restriction on embryonic survival during the first three weeks of pregnancy in the Perendale ewe. Proc. Aust. Soc. Anim. Prod., 9, 199-203.

CURSON, H. H. and MALAN, A. P. (1935). The changing proportions of the Merino lamb from the second to the fifth month of prenatal life. Onderstepoort J. vet. Sci. Anim. Ind., 4, 481-492.

CURTAIN, C. C. (1969). A new immunoglobulin sub-class in the sheep. Immunology, 16, 373-380.

CURTAIN, C. C. (1975). The blood of sheep. 1st ed., 185-195. Berlin: Springer - Velag.

CURTAIN, C. C. and ANDERSON, N. (1971). Immunocytochemical localization of the ovine immunoglobulins IgA, IgG_1, IgG_{1A} and IgG_2: Effect of gastrointestinal parasitism in the sheep. Clin. Exp. Immunol., 8, 151-162.

CURTIS, J. and BOURNE, F. J. (1971). Immunoglobulin quantitation in sow serum, colostrum and milk and the serum of young pigs. Biochim. biophys. Acta, 236, 319-332.

DALY, P. J. (1973). Mortality and disease in intensive sheep management systems. Res. Rep. - An Foras Taluntais. Anim. Prod., 179-182.

DARROCH, J. G., NORDSKOG, A. W. and VAN HORN, J. L. (1950). The effect of feeding concentrates to range ewes on lamb and wool productivity. J. Anim. Sci., 9, 431-445.

DAVIES, H. L. (1964). Lamb losses in South-Western Australia. Proc. Aust. Soc. Anim. Prod., 5, 107-112.

DAVIES, P. J. and ROSS, D. B. (1973). Plasma glucose and acetone concentrations in pregnant and lactating ewes. Proc. Nutr. Soc., 32, 13A.

DAWES, G. S. and PARRY, H. B. (1965). Premature delivery and survival in lambs. Nature, Lond., 207, 330.

DENNIS, S. M. (1964). Tale from dead lambs. J. Agric. West. Aust., 5, 200-205.

DENNIS, S. M. (1965a). Dead lambs - a dead loss. J. Agric. West. Aust., 6, 216-219.

DENNIS, S. M. (1965b). Why did these lambs die? J. Agric. West. Aust., 6, 228-233.

DENNIS, S. M. (1965c). "Predators", lamb killers or scavengers? J. Agric. West. Aust., 6, 249-250.

DENNIS, S. M. (1965d). Congenital abnormalities in sheep. J. Agric. West. Aust., 6, 235-240.

DENNIS, S. M. (1966). Perinatal lamb mortality in Western Australia. R.C.V.S. Thesis, London.

DENNIS, S. M. (1970). Perinatal lamb mortality in a pure-bred Southdown flock. J. Anim. Sci., 31, 76-79.

DENNIS, S. M. (1972). Perinatal lamb mortality. Cornell Vet., 62, 263-273.

DENNIS, S. M. (1974a). Perinatal lamb mortality in Western Australia. 1. General procedures and results. Aust. vet. J., 50, 443-449.

DENNIS, S. M. (1974b). Perinatal lamb mortality in Western Australia. 3. Congenital infections. Aust. vet. J., 50, 507-510.

DENNIS, S. M. (1974c). Perinatal lamb mortality in Western Australia. 4. Neonatal infections. Aust. vet. J., 50, 511-514.

DENNIS, S. M. (1975a). Perinatal lamb mortality in Western Australia. 7. Congenital defects. Aust. vet. J., 51, 80-82.

DENNIS, S. M. (1975b). Embryonic duplications in sheep. Aust. vet. J., 51, 83-87.

DENNIS, S. M. (1975c). Congenital respiratory tract defects in lambs. Aust. vet. J., 51, 347-350.

DENNIS, S. M. (1975d). Congenital defects of the nervous system of lambs. Aust. vet. J., 51, 385-388.

DENNIS, S. M. (1975e). Perinatal lamb mortality in Western Australia. 5. Vibrionic infections. Aust. vet. J., 51, 11-13.

DENNIS, S. M. and LEIPOLD, H. W. (1968). Congenital cardiac defects in lambs. Am. J. vet. Res., 29, 2337-2340.

DENNIS, S. M. and NAIRN, M. E. (1970). Perinatal lamb mortality in a Merino flock in Western Australia. Aust. vet. J., 46, 272-276.

DICKSON, I. A. (1977). In "Perinatal losses in lambs". East of Scotland College of Agriculture, Edinburgh. 50-51.

DONALD, H. P. and RUSSELL, W. S. (1970). The relationship between live weight of ewe at mating and weight of newborn lamb. Anim. Prod., 12, 273-280.

DONALD, L. G. (1977). In "Perinatal losses in lambs". East of Scotland College of Agriculture, Edinburgh. 38-40.

DONEY, J. M. and GUNN, R. G. (1976). Factors affecting reproductive performance in sheep. Vet. A., 16, 70-74.

DOUMAS, B. T., WATSON, W. A. and BIGGS, H. G. (1971). Albumin standards and the measurement of serum albumin with Bromo Cresol Green. Clinica chim. Acta, 31, 87-96.

DUCKER, M. J. and McEWAN, A. D. (1972). The estimation of immune globulin levels in neonatal lamb serum using a turbidity test. Br. vet. J., 128, lix-lxi.

DUCKER, M. J. and FRASER, J. (1976). A note on the effect of time of uptake of colostrum on blood gamma-globulin levels, mortality and subsequent performance in housed lambs. Anim. Prod., 22, 411-414.

EAST OF SCOTLAND COLLEGE OF AGRICULTURE, Edinburgh Veterinary Investigation Centre. Annual Report (1976).

EDEY, T. N. (1969). A review: Prenatal mortality in sheep. Anim. Breed. Abstr., 37, 173-190.

EDIN. SCHOOL OF AGRIC., Annual Report (1973).

EMADY, M., NOAKES, D. E., HADLEY, J. C. and ARTHUR, G. H. (1974). Corticosteroid induced lambing in the ewe. Vet. Rec., 95, 281-285.

ERMEKOV, M. A., TEN, V. M., SADYKOV, T. S. and NURMAGAMBETOV, D. N. (1973). Early ingestion of colostrum improves viability of lambs. Ovtsevodstvo, 8, 21-22.

FAHEY, J. L. and McKELVEY, E. M. (1965). Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Immun., 94, 84-90.

FEINSTEIN, A. and HOBART, M. J. (1969). Structural relationship and complement fixing activity of sheep and other ruminant immunoglobulin G sub-classes. Nature, Lond., 223, 950-952.

FERGUSON, J. A. Personal communication (1973).

FERGUSON, J. A. (1975). Hay quality and the pregnant ewe. Ph.D. Thesis, Univ. of Edinburgh.

FINDLAY, C. R. (1973). Serum immunoglobulin levels in lambs under a week old. Vet. Rec., 92, 530-532.

FISHER, E. W. and MARTINEZ, A. A. (1975). Serum immune globulins in calves receiving E. coli endotoxin. Vet. Rec., 96, 527-528.

FITZSIMONS, J. Personal communication (1977).

FOGARTY, N. M. (1972). Cross breeding for lamb production. 1. Survival and growth of first cross lambs. Aust. J. exp. Agric. Anim. Husb., 12, 234-239.

FOOT, J. Z. (1972). A note on the effect of body condition on the voluntary intake of dried grass

wafers by Scottish Blackface ewes. Anim. Prod., 14, 131-134.

FOOT, J. Z., RUSSEL, A. J. F., MAXWELL, T. J. and MORRIS, P. (1973). Variation in intake among group-fed pregnant Blackface ewes given restricted amounts of food. Anim. Prod., 17, 169-177.

FRANKLIN, E. C. (1975). Structure and function of immunoglobulins. Acta endocr., Copenh., 78 (Suppl. 194), 77-95.

GARDNER, D. E. (1973). Values for certain blood and urine constituents of normal young lambs. N. Z. vet. J., 21, 70-73.

GEORGE, J. M. (1973). Dystocia in ewes. In "Wld. Conf. Anim. Prod.", Melbourne, 2(c)4 - 2(c)5.

GEORGIEV, S. (1969). Age changes in the protein fractions of the blood serum of lambs. Veterinarnomed. Nauk., 6, 53-60.

GLEICH, G. J., BIEGER, R. C. and STANKIEVIC, R. (1969). Antigen combining activity associated with immunoglobulin D. Science, New York, 165, 606.

GLIMP, H. A. (1972). Effect of diet composition on performance of lambs reared from birth on milk replacer. J. Anim. Sci., 34, 1085-1088.

GUNN, R. G. and ROBINSON, J. F. (1963). Lamb mortality in Scottish hill flocks. Anim. Prod., 5, 67-76.

GUNN, R. G., DONEY, J. M. and RUSSEL, A. J. F. (1969). Fertility in Scottish Blackface ewes as influenced by nutrition and body condition at mating. J. agric. Sci., Camb., 73, 289-294.

GUNN, R. G. and DONEY, J. M. (1973). The effect of nutrition and rainfall at the time of mating on the reproductive performance of ewes. J. Reprod. Fert., Suppl. 19, 253-258.

GUNNARSSON, K., JACOBSSON, S. O., LINBERG, P. and MÖLLERBERG, L. (1970). Mortality in lambs and its causes. Svensk Vet Tidn., 22, 649-654.

GUNNARSSON, K., JACOBSSON, S. O. and MÖLLERBERG, L. (1972). Mortality and findings at P.M. examination of lambs from Swedish sheep farms. Nord. Vet Med., 24, 473-483.

GUYER, P. Q. and DYER, A. J. (1954). Study of factors affecting sheep production. Missouri Agricultural Experiment Station. Res. Bull. No. 558.

HALLIDAY, R. (1965a). The transfer of antibodies from ewes to their lambs. J. Immun., 95, 510-516.

HALLIDAY, R. (1965b). Failure of some hill lambs to absorb maternal gamma-globulin. Nature, Lond., 205, 614.

HALLIDAY, R. (1968a). Serum gamma-globulin levels in dead lambs from hill flocks. Anim. Prod., 10, 177-182.

HALLIDAY, R. (1968b). Serum protein concentrations in 2-day-old Finnish Landrace, Scottish Blackface, Merino and Merino cross Cheviot lambs. J. agric. Sci., Camb., 71, 41-46.

HALLIDAY, R. (1970). Protein concentrations in colostra from Finnish Landrace cross Scottish Blackface ewes during the first week of lactation and in sera from the ewes and their lambs on the third day of lactation. J. agric. Sci., Camb., 74, 103-106.

HALLIDAY, R. (1971a). Total serum protein and immunoglobulin concentrations in Scottish Blackface and Merino lambs at birth and during the first two days of suckling. J. agric. Sci., Camb., 77, 463-466.

HALLIDAY, R. (1971b). Total protein and immunoglobulin concentrations in sera from 2-day-old Finnish Landrace cross Dorset Horn lambs. J. agric. Sci., Camb., 77, 209-211.

HALLIDAY, R. (1973). Serum immunoglobulin concentration at two days of age in lambs born after ova transfer between ewes of different breed. J. agric. Sci., Camb., 81, 29-32.

HALLIDAY, R. (1976). Variations in immunoglobulin concentrations in Finnish Dorset Horn lambs. Res. vet. Sci., 21, 331-334.

HALLIDAY, R. and BUTTLE, H. R. L. (1968). The effects of Cortisone acetate on length of the gestation period and the survival of foetal Scottish Blackface lambs. J. Endocr., 41, 447-448.

HALLIDAY, R. and WILLIAMS, M. R. (1976). Passive immunity in the lamb. Effects of a second feed of colostrum on antibody absorption from the first feed. Res. vet. Sci., 21, 173-175.

HALPERN, H. S. and KOSHLAND, M. E. (1970). Novel subunit in secretory IgA. Nature, Lond., 228, 1276-1278.

HARKER, D. B. (1973). Serum immunoglobulin in lambs under a week old. Vet. Rec., 93, 431.

HARKER, D. B. (1974). Serum immunoglobulin levels in artificially reared lambs. Vet. Rec., 95, 229-231.

HARKER, D. B. (1977). In "Perinatal losses in lambs". East of Scotland College of Agriculture, Edinburgh. 20-24.

HARRISON, E. T. and MAGE, M. G. (1967). Isolation and characterization of sheep $gamma_1$- and $gamma_2$-immunoglobulins and their polypeptide chains. Biochim. biophys. Acta, 147, 52-59.

HARTLEY, W. J. and BOYES, B. W. (1964). Incidence of ovine perinatal mortality in New Zealand with particular reference to intra-uterine infections. N. Z. vet. J., 12, 33-36.

HARTLEY, W. J. and KATER, J. C. (1964). Perinatal disease conditions of sheep in New Zealand. N.Z. vet. J., 12, 49-57.

HAUGHEY, K. G. (1967). The occurrence of congenital infections associated with perinatal lamb mortality. Aust. vet. J., 43, 413-420.

HAUGHEY, K. G. (1973a). Abnormalities in the Central Nervous System associated with perinatal lamb mortality. 2. Association of the abnormalities with recognised lesions. Aust. vet. J., 49, 9-15.

HAUGHEY, K. G. (1973b). Birth injury to the Central Nervous System as a component of perinatal lamb mortality. N.S.W. vet. Proc., 9, 29-30.

HEALY, P. J. (1971a). Serum alkaline phosphatase in sheep. Clinica chim. Acta, 33, 431-436.

HEALY, P. J. (1971b). Serum alkaline phosphatase in the newborn lamb. Clinica chim. Acta, 33, 437-441.

HEALY, P. J. (1975a). Isoenzymes of alkaline phosphatase in serum of lambs and ewes. Res. vet. Sci., 19, 120-126.

HEALY, P. J. (1975b). Isoenzymes of alkaline phosphatase in serum of newly born lambs. Res. vet. Sci., 19, 127-130.

HEALY, P. J. and FALK, R. H. (1974). Values of some biochemical constituents in the serum of clinically-normal sheep. Aust. vet. J., 50, 302-305.

HEIMER, R., CLARK, L. G. and MAURER, P. H. (1969). Immunoglobulins of sheep. Archs. Biochem. Biophys., 131, 9-17.

HEIMER, R., JONES, D. W. and MAURER, P. H. (1969). The immunoglobulins of sheep colostrum. Biochemistry, 8, 3937-3944.

HENRY, R. J. (1964). Clinical Chemistry: Principles and technics., 1st ed., 182-209. New York: Hoeber and Row.

HIGHT, G. K. and JURY, K. E. (1969). Lamb mortality in hill country flocks. Proc. N.Z. Soc. Anim. Prod., 29, 219-232.

HILL, I. R. and PORTER, P. (1974). Studies of bactericidal activity to E. coli of porcine serum and colostral immunoglobulins and the role of lysozyme with secretory IgA. Immunology, 26, 1239-1250.

HOBBS, J. R. (1971). Immunoglobulins in clinical chemistry. Adv. clin. Chem., 14, 219-317.

HOLMES, R. J. (1975). Relationship of maternal behaviour to neonatal loss in prolific sheep. N. Z. vet. J., 23, 219.

HOUSTON, D. C. (1972 - 1973). A report on Hoodie Crows in Argyll. Department of Forestry and Natural Resources, Edinburgh University.

HOUSTON, D. C. and MADDOX, J. C. (1974). Causes of mortality among young Scottish Blackface lambs. Vet. Rec., 95, 575.

HUDSON, R. J., BANDY, P. J. and KITTS, W. D. (1970). Immunochemical quantitation of ovine immunoglobulins. Am. J. vet. Res., 31, 1231-1236.

HUGHES, K. L. (1971 - 1972a). Congenital developmental abnormalities in neonates. Victorian Vet. Proc., 30, 77-83.

HUGHES, K. L. (1971 - 1972b). Infections affecting survival of neonates. Victorian Vet. Proc., 30, 83-86.

HUGHES, K. L. (1975). Listeria as a cause of abortion and neonatal mortality in sheep. Aust. vet. J., 51, 97-99.

HUGHES, K. L., HARTLEY, W. J., HAUGHEY, K. G. and McFARLANE, D. (1964). A study of perinatal mortality of lambs from Oberon, Orange and Merino districts of New South Wales. Proc. Aust. Soc. Anim. Prod., 5, 92-99.

HUGHES, K. L., HAUGHEY, K. G. and HARTLEY, W. J. (1971). Perinatal lamb mortality: infections occurring among lambs dying after parturition. Aust. vet. J., 47, 472-476.

HUSBAND, A. J. and LASCELLES, A. K. (1974). The origin of antibody in intestinal secretion of sheep. Aust. J. exp. Biol. med. Sci., 52, 791-799.

IGNATĒVA, E. N. (1971). Importance of colostrum for rearing lambs. Ovtsevodstvo, 4, 27.

ISHIZAKA, K., ISHIZAKA, T. and HORNBECK, M. (1966). Physico-chemical properties of human reaginic

antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. J. Immun., 97, 75-85.

JANUSZ, M., GODZŃSKA, H. and LISOWSKI, J. (1973). Immunoglobulins of colostrum. 1. Preparation and identification of ovine colostral immunoglobulins. Arch. Immunol. Ther. Exp., 21, 435-444.

JEFFERIES, B. C. (1961). Body condition scoring and its use in management. Tasm. J. Agric., 32, 19-21.

JOHNSTON, W. S. (1977). In "Perinatal losses in lambs". East of Scotland College of Agriculture, Edinburgh. 10-13.

JONAS, W. E. (1968). The immunoelectrophoretic pattern of some body fluids of sheep. Res. vet. Sci., 9, 324-330.

JONAS, W. E. (1969a). The distribution and properties of IgM in some body fluid of sheep. Res. vet. Sci., 10, 83-92.

JONAS, W. E. (1969b). Determination of sheep anti-Salmonella typhimurium immunoglobulin class by antiglobulin testing. Res. vet. Sci., 10, 311-320.

JUMA, K. H., ELIYA, J., AL-RAWI, A. A. and ABUMAALY, H. N. (1974). Survival of lambs in Awassi sheep. Ind. J. Anim. Sci., 44, 252-256.

KAY, R. M., LITTLE, W. and KITCHENHAM, B. A. (1976). A comparison of the growth performance and blood composition of twin and singleton calves. Anim. Prod., 22, 19-25.

KEKWICK, R. A. (1959). The serum proteins of the foetus and young of some mammals. Adv. Protein Chem., 14, 244-247.

KILLEEN, I. D. (1967). The effect of body weight and level of nutrition before, during and after joining on ewe fertility. Aust. J. exp. Agric. Anim. Husb., 7, 126-136.

KNIGHT, R. A. and LEEK, R. G. (1973). Electrophoresis of serum proteins on cellulose acetate: Comparison of bottle-raised versus ewe-raised lambs from birth to 19 weeks of age. Am. J. Vet. Res., 34, 701-703.

KRUSE, V. (1970). Absorption of immunoglobulin from colostrum in newborn calves. Anim. Prod., 12, 627-638.

LARSON, R. E., WARD, A. C. S., FREDERIKSEN, W. B., ARDREY, W. B. and FRANK, F. W. (1974). Capability of lambs to absorb immunoproteins from freeze dried bovine colostrum. Am. J. Vet. Res., 35, 1061-1063.

LASCELLES, A. K. and McDOWELL, G. H. (1970). Secretion of IgA in the sheep following local antigen stimulation. Immunology, 19, 613-620.

LECCE, J. G. and MORGAN, D. O. (1962). Effect of dietary regimen on cessation of intestinal absorption of large molecules (closure) in the neonatal pig and lamb. J. Nutr., 78, 263-269.

LEE, C. S. and LASCELLES, A. K. (1970). Antibody producing cells in antigenically stimulated mammary glands and in the gastro-intestinal tract of sheep. Aust. J. exp. Biol. med. Sci., 48, 525-535.

LEES, J. L. (1971). Some aspects of reproductive efficiency in sheep. Vet. Rec., 88, 86-95.

LISOWSKI, J., JANUSZ, M., TYRAN, B., MORAWIECKI, A., GOŁAB, S. and BIAŁKOWSKA, H. (1975). Immunoglobulins of colostrum. III. Comparative studies of ovine serum and colostral IgG_1 and IgG_2. Immunochemistry, 12, 159-166.

LODGE, G. A. and HEANEY, D. P. (1973). Composition of weight changes in the pregnant ewe. Can. J. Anim. Sci., 53, 95-105.

LOGAN, E. F. (1974). Colostral immunity to colibacillosis in the neonatal calf. Br. vet. J., 130, 405-412.

LOGAN, E. F. and PENHALE, W. J. (1971). Studies on the immunity of the calf to colibacillosis. III. The local protective activity of colostrum within the gastro-intestinal tract. Vet. Rec., 89, 628-632.

LOGAN, E. F. and PENHALE, W. J. (1972). Studies on the immunity of the calf to colibacillosis. IV. The experimental reproduction of Enteric Colibacillosis. Vet. Rec., 91, 419-423.

LOGAN, E. F., STENHOUSE, A., PENHALE, W. J. and ARMISHAW, M. (1974). Studies on the immunity of the calf to colibacillosis. VI. The prophylactic use of a pooled serum IgM-rich fraction under field conditions. Vet. Rec., 94, 386-389.

LOUCA, A., MAVROGENIS, A. and LAWLOR, M. J. (1974). Effect of plane of nutrition in late pregnancy on lamb birth weight and milk yield in early lactation of Chios and Awassi sheep. Anim. Prod., 19, 341-349.

MacFARLANE, J. S. (1973). Serum immunoglobulin levels in lambs under a week old. Vet. Rec., 93, 237.

MacKENZIE, D. D. S. and LASCELLES, A. K. (1968). The transfer of [$^{131}_{I}$]-labelled immunoglobulins and serum albumin from blood into milk of lactating ewes. Aust. J. exp. Biol. med. Sci., 46, 285-294.

McBEATH, D. G., PENHALE, W. J. and LOGAN, E. F. (1971). An examination of the influence of husbandry on the

plasma immunoglobulin level of the newborn calf, using a rapid refractometer test for assessing immunoglobulin content. Vet. Rec., 88, 266-270.

McBEATH, D. G. and LOGAN, E. F. (1974). Influence of neonatal management on serum immunoglobulin levels of suckled calves. Vet. Rec., 95, 466.

McCANCE, I. and ALEXANDER, G. (1959). The onset of lactation in Merino ewe and its modification by nutritional factors. Aust. J. agric. Res., 10, 699-719.

McCANCE, R. A. and WIDDOWSON, E. M. (1959). The effect of colostrum on the composition and volume of the plasma of newborn piglets. J. Physiol., 145, 547-550.

McCARTHY, E. F. and McDOUGALL, E. I. (1953). Absorption of immune globulin by the young lamb after ingestion of colostrum. Biochem. J., 55, 177-182.

McCLELLAND, T. H. and FORBES, T. J. (1973). The effect of level and pattern of energy intake during late pregnancy on the performance of housed Scottish Blackface ewes. Anim. Prod., 16, 165-171.

McDONALD, I. W. (1961). Physiological limitations on reproduction in the sheep. Aust. vet. J., 37, 99-104.

McDONALD, I. W. (1962). Ewe fertility and neonatal lamb mortality. N. Z. vet. J., 10, 45-52.

McDONALD, J. W. (1966). Variation in perinatal mortality of lambs with age and parity of ewes. Proc. Aust. Soc. Anim. Prod., 6, 60-62.

McEWAN, A. D., FISHER, E. W. and SELMAN, I. E. (1968). The effect of colostrum on the volume and composition of the plasma of calves. Res. vet. Sci., 9, 284-286.

McEWAN, A. D., FISHER, E. W. and SELMAN, I. E. (1970). Observations on the immune globulin levels of neonatal calves and their relationship to disease. J. Comp. Path., 80, 259-265.

McEWAN, A. D., FISHER, E. W., SELMAN, I. E. and PENHALE, W. J. (1970). A turbidity test for the estimation of immune globulin levels in neonatal calf serum. Clinica chim. Acta, 27, 155-163.

McFARLANE, D. (1961). Perinatal lamb losses. Aust. vet. J., 37, 105-109.

McFARLANE, D. (1965). Perinatal lamb losses. 1. An autopsy method for the investigation of perinatal losses. N. Z. vet. J., 13, 116-135.

McMENIMAN, N. P. and YOUNG, R. B. (1968). Lamb losses in late pregnancy and early life. Qd. agric. J., 94, 589-595.

MALIK, R. C. and ACHARYA, R. A. (1972). A note on factors affecting lamb survival in Indian sheep. Anim. Prod., 81, 29-32.

MANCINI, G., CARBONARA, A. O. and HEREMANS, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry, 2, 235-254.

MANSTON, R., RUSSELL, A. M., DEW, S. M. and PAYNE, J. M. (1975). The influence of dietary protein upon blood composition in dairy cows. Vet. Rec., 96, 497-502.

MARAIS, P. G. (1974). Lamb mortality of autumn and spring mated ewes. Agroanimalia, 6, 133-137.

MATHEWS, D. H. and OGDEN, P. R. (1957). Lamb mortality. A production loss sheepmen can ill afford. Fm. Home Sci., 18, 12-13 and 26-27.

MAXWELL, T. J. (1977). In "Perinatal losses in lambs". East of Scotland College of Agriculture, Edinburgh. 56-58.

MEAT AND LIVESTOCK COMMISSION (1972). Sheep improvement. Scientific study group report. MLC publication, Bucks., England.

MEAT AND LIVESTOCK COMMISSION (1973). Feeding the ewe. Sheep improvement Service, Tech. Rep. No. 2.

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (1971). Nutrient allowances and composition of feeding stuffs for ruminants. Agricultural Development and Advisory Service (A.D.A.S.), Advisory paper No. 2.

MOORE, R. W., DONALD, I. M. and MESSENGER, J. J. (1966). Fox predation as a cause of lamb mortality. Proc. Aust. Soc. Anim. Prod., 6, 157-160.

MOULE, G. R. (1954). Observation in mortality amongst lambs in Queensland. Aust. vet. J., 30, 153-171.

MULLANEY, P. D. (1969). Birth weight and survival of Merino, Corriedale and Polwarth lambs. Aust. J. exp. Agric. Anim. Husb., 9, 157-163.

MULLANEY, P. D. and LEAR, D. (1969). Duration of pregnancy in Merino ewes in relation to survival of lambs. Aust. vet. J., 45, 366-367.

NATIONAL RESEARCH COUNCIL (1968). Nutrient requirements of domestic animals. No. 5. Nutrient requirements of sheep. Publs. Natn. Res. Coun., Wash. No. 1693.

OBST, J. M. and EVANS, J. V. (1970). Genotype-environment interactions in lamb mortality with particular reference to birth coat and haemoglobin type. Proc. Aust. Soc. Anim. Prod., 8, 149-153.

O'HARA, P. J., WOLFF, J. E. and OLIVER, R. E. (1975). The effect of starvation on the pregnant ewe. Proc. N.Z. V. A. Sheep Society, 5th Seminar, Massey Univ.

ORCUTT, M. L. and HOWE, P. E. (1922). The relation between the accumulation of globulins and the appearance of agglutinins in the blood of newborn calves. J. exp. Med., 36, 291-308.

OUCHTERLONY, O. (1953). Antigen-antibody reaction in gels. IV. Types of reactions in co-ordinated systems of diffusion. Acta path. microbiol. scand., 32, 231-240.

OWEN, J. B. (1976). Sheep production, 1st ed., 154-160. London: Baillière Tindall.

PAHUD, J. J. and MACH, J. P. (1970). Identification of secretory IgA, free secretory piece and serum IgA in ovine and caprine species. Immunochemistry, 7, 679-686.

PAN, I. C., KAPLAN, A. M., MORTER, R. L. and FREEMAN, M. J. (1968). Spectrum of ovine immunoglobulins. Proc. Soc. exp. Biol. Med., 129, 867-870.

PARKER, B. N. J. and BLOWEY, R. W. (1976). Investigations into the relationship of selected blood components to nutrition and fertility of the dairy cow under commercial farm conditions. Vet. Rec., 98, 394-404.

PARRY, H. B. (1956). Prevention of toxaemia of late pregnancy in sheep. Nature, Lond., 177, 288-289.

PAYNE, J. M., DEW, S. M., MANSTON, R. and FAULKS, M. (1970). The use of a metabolic profile test in dairy herds. Vet. Rec., 87, 150-158.

PAYNE, J. M., ROWLANDS, G. J., MANSTON, R. and DEW, S. M. (1973). A statistical appraisal of the results of metabolic profile tests on 75 dairy herds. Br. vet. J., 129, 370-381.

PEARSON, L. D. and BRANDON, M. R. (1976). Effect of fetal thymectomy on IgG, IgM and IgA concentrations in sheep. Am. J. vet. Res., 37, 1139-1141.

PENHALE, W. J., LOGAN, E. F., SELMAN, I. E., FISHER, E. W. and McEWAN, A. D. (1973). Observations on the absorption of colostral immunoglobulins by the neonatal calf and their significance in colibacillosis. Ann. Rech. Vét., 4, 223-233.

PERRIN, D. R. (1958). The chemical composition of the colostrum and milk of the ewe. J. Dairy Res., 25, 70-74.

PIERCY, D. W. T. (1973). Natural resistance to infection in the newborn sheep: Competence of local and systemic defences in normally-suckled and colostrum deprived lambs. Res. vet. Sci., 14, 350-357.

PIERCY, D. W. T. (1974). Natural resistance to infection in the neonatal sheep: Comparison of the ability of colostrum deprived, normally-suckled and passively-immunized lambs to localize Erysipelothrix rhusiopathiae. Res. vet. Sci., 17, 210-214.

PLANT, J. W., BEH, K. J. and ACLAND, H. M. (1972). Laboratory findings from ovine abortion and perinatal mortality. Aust. vet. J., 48, 558-561.

PORTER, P. (1972). Immunoglobulins in bovine mammary secretions. Quantitative changes in early lactation and absorption by the neonatal calf. Immunology, 23, 225-238.

POUT, D. D. (1973). Intensive stocking of sheep in an arable system. Part II. Lamb growth studies. Expl. Husb., 25, 9-21.

PURSER, A. F. and YOUNG, G. B. (1959). Lamb survival in two hill flocks. Anim. Prod., 1, 85-91.

PURSER, A. F. and YOUNG, G. B. (1964). Mortality among twin and single lambs. Anim. Prod., 6, 321-330.

REID, R. L. (1958). The nutritional physiology of ewe in late pregnancy. J. Aust. Inst. agric. Sci., 24, 291-295.

REID, R. L. (1960). The determination of ketone bodies in blood. Analyst, Lond., 85, 265-271.

REID, J. F. S. (1972). Serum immunoglobulin concentration of newborn hill lambs. Vet. Rec., 90, 371-372.

REID, J. F. S. and CLIFFORD, D. J. (1974). Use of the refractometer in estimating immune globulin levels of neonatal lamb serum. Vet. Rec., 95, 22-23.

REYNEKE, J. and COLYN, P. C. (1966). Single versus twin lambs. Proc. S. Afr. Soc. Anim. Prod., 5, 122-126.

ROBINSON, J. J. (1973). Some aspects of ewe nutrition. Vet. Rec., 92, 602-606.

ROBINSON, J. J. (1974). Intensifying ewe productivity. Proc. Br. Soc. Anim. Prod., 3, 31-40.

ROBINSON, J. J. and FORBES, T. J. (1968). The effect of protein intake during gestation on ewe and lamb performance. Anim. Prod., 10, 297-309.

ROBINSON, J. J., FRASER, C. and BENNETT, C. (1971). An assessment of the energy requirements of the pregnant ewe using plasma free fatty acid concentrations. J. agric. Sci., Camb., 77, 141-145.

ROBINSON, J. J. and ØRSKOV, E. R. (1975). An integrated approach to improving the biological efficiency of sheep meat production. Wld. Rev. Anim. Prod., 11, 63-76.

ROBINSON, W. I., BROWN, W. and LUCAS, I. A. M. (1973). Effect of shelter, exposure and level of feeding over winter on the productivity of Welsh Mountain ewes. Anim. Prod., 17, 21-32.

ROWE, D. S. and FAHEY, J. L. (1965). A new class of human immunoglobulin. 1. A unique myeloma protein. J. exp. Med., 121, 171-184.

ROWLANDS, G. J., PAYNE, J. M., DEW, S. M. and MANSTON, R. (1973). A potential use of metabolic profiles in the selection of superior cattle. Vet. Rec., 93, 48-49.

RUSSEL, A. J. F. (1971). Relationships between energy intake and productivity in hill sheep. Proc. Nutr. Soc., 30, 197-204.

RUSSEL, A. J. F. Personal communication (1977).

RUSSEL, A. J. F., DONEY, J. M. and REID, R. L. (1967a). The use of biochemical parameters in controlling nutritional state in pregnant ewes and the effect of undernourishment during pregnancy on lamb birth weight. J. agric. Sci., Camb., 68, 351-358.

RUSSEL, A. J. F., DONEY, J. M. and REID, R. L. (1967b). Energy requirement of the pregnant ewe. J. agric. Sci., Camb., 68, 359-363.

RUSSEL, A. J. F., DONEY, J. M. and GUNN, R. G. (1969). Subjective assessment of body fat in live sheep. J. agric. Sci., Camb., 72, 451-454.

RUSSEL, A. J. F. and FOOT, J. Z. (1973). The effect of level of nutrition at two stages of pregnancy on the performance of primiparous ewes. Proc. Nutr. Soc., 32, 27A-28A.

RUTTER, W., LAIRD, T. R. and BROADBENT, P. J. (1971). The effect of clipping pregnant ewes at housing and of feeding different basal roughages. Anim. Prod., 13, 329-336.

SAUNDERS, R. W. (1977). In "Perinatal losses in lambs". East of Scotland College of Agriculture, Edinburgh. 5-9.

SCHEIDEGGAR, J. J. (1955). Une mico-méthode de l'immuno-électrophorèse. Int. Arch. Allergy, 7, 103-110.

SELMAN, I. E., McEWAN, A. D. and FISHER, E. W. (1971). Absorption of immune lactoglobulin by newborn dairy calves. Res. vet. Sci., 12, 205-210.

SETH, O. N., PANDEY, M. D. and ROY, A. (1972). A note on live-weight growth and mortality in lambs born as singles, twins or triplets. Ind. J. Anim. Sci., 42, 695-698.

SHAW, W. B. (1971). Escherichia coli in newborn lambs. Br. vet. J., 127, 214-219.

SHELTON, M. (1964). Relation of birth weight to death losses and to certain productive characters of fall-born lambs. J. Anim. Sci., 23, 355-359.

SHEVAH, Y. (1974). A note on the use of Dexamethasone for inducement of parturition of Finn cross Dorset ewes. Anim. Prod., 18, 89-92.

SHEVAH, Y., BLACK, W. J. M. and LAND, R. B. (1975). Differences in feed intake and the performance of Finn cross Dorset ewes during late pregnancy. Anim. Prod., 20, 391-400.

SILVERSTEIN, A. M., UHR, J. W., KRANER, K. L. and LUKES, R. J. (1963). Fetal response to antigenic stimulus. III. Gamma-globulin production in normal and stimulated fetal lambs. J. Immunology, 91, 384-395.

SINGH, O. N. and SINGH, L. B. (1970). Lamb mortality in Rambouillet sheep in relation to birth type and birth weight. Ind. J. Anim. Sci., 40, 430-433.

SINGH, V. K., TIWARI, S. B., SINGH, L. B. and HONMODE, J. (1973). Efficiency of milk production and its conversion into lamb weight in Malpura, Chorkla and cross-bred ewes. Ind. vet. J., 50, 1199-1204.

SLEE, J. (1976). Cold stress and perinatal mortality in lambs. Vet. A., 16, 66-69.

SLEE, J. (1977). In "Perinatal losses in lambs". East of Scotland College of Agriculture, Edinburgh. 30-34.

SMEATON, T. C. (1969). Studies of gamma-globulin metabolism in the foetal and neonatal lamb. Ph.D. Thesis. Aust. Natl. Univ., Canberra.

SMITH, T. and LITTLE, R. B. (1922). The significance of colostrum to the newborn calf. J. exp. Med., 36, 181-198.

SMITH, I. D. (1962). Reproductive wastage in a Merino flock in Central Western Queensland. Aust. vet. J., 38, 500-507.

SMITH, I. D. (1964). Ovine neonatal mortality in Western Queensland. Proc. Aust. Soc. Anim. Prod., 5, 100-106.

SMITH, I. D. (1972). Ovine perinatal mortality associated with Bacillus cereus. Res. vet. Sci., 13, 499-501.

SMITH, W. D. (1975). Studies on the immune response of the ovine respiratory tract to Parainfluenza 3 virus. Ph.D. Thesis. University of Edinburgh.

SMITH, W. D., DAWSON, A. McL., WELLS, P. W. and BURRELLS, C. (1975). Immunoglobulin concentrations in ovine body fluids. Res. vet. Sci., 19, 189-194.

SNEDECOR, G. W. and COCHRAN, W. G. (1971). Statistical methods, 6th ed. (4th printing), 543-546. Iowa: The Iowa State University Press.

STAMP, J. T. (1967). Perinatal loss in lambs with particular reference to diagnosis. Vet. Rec., 81, 530-534.

SULLIVAN, A. L., PRENDERGAST, R. A., ANTUNES, L. J., SILVERSTEIN, A. M. and TOMASI, T. B. (1969). Characterization of the serum and secretory immune systems of the cow and sheep. J. Immun., 103, 334-344.

SVEHAG, SVEN-ERIC (1964). The formation and properties of poliovirus neutralizing antibody. J. exp. Med., 119, 517-535.

THOMAS, J. F. M. (1945). Losses affecting lambs. In "Sheep". 88-91. London: Faber and Faber Ltd.

THOMAS, W. J. K. (1971). Lowland sheep: An economic analysis of lamb production, 1970. Economic report No. 8: University of Exeter, England.

THOMSON, W. and FRASER, A. M. (1939). Feeding concentrates to the in-lamb ewe. The effects in lambing results. Scot. J. Agric., 22, 71-73.

THOMSON, A. M. and THOMSON, W. (1949). Lambing in relation to the diet of the pregnant ewe. Br. J. Nutr., 2, 290-305.

THOMSON, W. and THOMSON, A. M. (1953). Effect of diet on milk yield of the ewe and growth of her lambs. Br. J. Nutr., 7, 263-274.

THOMSON, W. A. and AITKEN, F. C. (1959). Diet in relation to reproduction and viability of the young. Part II. Sheep. World survey of reproduction and

review of feeding experiments.
Commonwealth Agric. Bur., England.

TOPLEY, W. W. C. and WILSON, G. S. (1975). Principle of bacteriology, virology and immunity. 6th ed., 1358-1384. London: Edward Arnold.

TREACHER, T. T. (1970). Effects of nutrition in late pregnancy on subsequent milk production in ewes. Anim. Prod., 12, 23-26.

TRIBE, D. E. and SEEBECK, R. M. (1962). Effect of live-weight and live-weight change on the lambing performance of ewes. J. agric. Sci., Camb., 59, 105-110.

TUMBLESON, M. E., LITTLETON, C. A., TICER, J. W., KOMER, E. G. and BLOOMFIELD, R. A. (1968). Serum biochemical parameters during neonatal development of lambs.
Am. J. Vet. Clin. Path., 2, 97-103.

TURNER, H. N. (1969). Genetic improvement of reproduction rate in sheep.
Anim. Breed. Abstr., 37, 545-563.

UNDERWOOD, E. J. and SHIER, F. L. (1941). Studies in sheep husbandry in Western Australia. I. The effect of nutritional "flushing" upon fertility.
J. Dep. Agric. West. Aust., 18, 13-21.

UNDERWOOD, E. J. and SHIER, F. L. (1942). Studies in sheep husbandry in Western Australia.

II. The influence of feeding during late pregnancy on birth weight, growth rate and quality of the lamb. J. Dep. Agric. West. Aust., 19, 37-47.

UNDERWOOD, E. J., SHIER, F. L. and CARISS, H. G. (1943). Influence of a high level of prenatal feeding on lamb production. J. Dep. Agric. West. Aust., 20, 288-297.

UPCOTT, D. H., HERBERT, N. C. and ROBINS, M. (1971). Erythrocyte and leucocyte parameters in newborn lambs. Res. vet. Sci., 12, 474-477.

VAERMAN, J. P. (1971). Comparison between several mammalian IgA_S, including the Bovine. J. Dairy Sci., 54, 1317-1318.

VAN HORN, J. L., BURKITT, W. H., WILLSON, F. S., FLOWER, A. E., DRUMMOND, J. and PAYNE, G. F. (1951). Production of range ewes increased by adequate feeding. J. Anim. Sci., 10, 1065.

VARELA-DIAZ, V. M. and SOULSBY, E. J. L. (1972). Immunoglobulin synthesis in sheep: IgG_2 deficiency in neonatal lambs. Res. vet. Sci., 13, 99-100.

VENKATACHALAM, G., NELSON, R. H., THORP, F., LUECKE, R. W. and GRAY, M. L. (1949). Causes and certain factors affecting lamb mortality. J. Anim. Sci., 8, 392-397.

VETTER, R. L., NORTON, H. W. and GARRIGUS, U. S. (1960). A study of pre-weaning death losses in lambs. J. Anim. Sci., 19, 616-619.

VIOR, C., TOMA, A., GRIGORE, C., CONSTANTINESCU, V., SECAŞIU, N. and CONTORA, N. (1973). Investigations on immunoglobulins in calves. Arch. Vet. (Rom.), 10, 13-22.

WALLACE, L. R. (1948a). The growth of lambs before and after birth in relation to level of nutrition. Part I. J. agric. Sci., Camb., 38, 93-153.

WALLACE, L. R. (1948b). The growth of lambs before and after birth in relation to level of nutrition. Part II. J. agric. Sci., Camb., 38, 243-302.

WALLACE, L. R. (1948c). The growth of lambs before and after birth in relation to level of nutrition. Part III. J. agric. Sci., Camb., 38, 367-401.

WATSON, R. H. (1972). Observed levels of mortality in relation to lambing and early stages of growth of sheep in Australia. Wld. Rev. Anim. Prod., 8, 104-113.

WATSON, R. H. and ELDER, E. M. (1961). Neonatal mortality in lambs: Further observations in a Corriedale flock with a history of high lamb mortality. Aust. vet. J., 37, 283-290.

WATSON, D. L. and LASCELLES, A. K. (1971). IgA in the body fluids of sheep and cattle. Res. vet. Sci., 12, 503-507.

WATSON, D. L., BRANDON, M. R. and LASCELLES, A. K. (1972). Concentrations of immunoglobulin in mammary secretion of ruminants during involution with particular reference to selective transfer of IgG_1. Aust. J. exp. Biol. med. Sci., 50, 535-539.

WATSON, D. L. and LASCELLES, A. K. (1973a). Mechanism of transfer of immunoglobulins into mammary secretion of ewes. Aust. J. exp. Biol. med. Sci., 51, 247-254.

WATSON, D. L. and LASCELLES, A. K. (1973b). Comparisons of immunoglobulin secretion in the salivary and mammary glands of sheep. Aust. J. exp. Biol. med. Sci., 51, 255-258.

WATSON, W. A. (1971). Toxoplasma abortion in sheep. Vet. A., 12, 52-56.

WATSON, W. A. and BEVERLEY, J. K. A. (1971). Ovine abortion due to experimental Toxoplasmosis. Vet. Rec., 88, 42-45.

WATT, J. A. (1965). Abortion and neonatal losses in sheep. Vet. Rec., 77, 637-642.

WATT, J. A. (1977). In "Perinatal losses in lambs. East of Scotland College of Agriculture, Edinburgh. 41-42.

WEIR, D. M. (1973). Handbook of experimental immunology. 2nd ed., 10.1. Oxford: Blackwell Scientific Publications.

WELLS, P. W. and EYRE, P. (1972). Preliminary characterization of bovine homocytotropic antibody. Immunochemistry, 9, 88-91.

WELLS, P. W., DAWSON, A. McL., SMITH, W. D. and SMITH, B. S. W. (1975). Transfer of IgG from plasma to nasal secretions in newborn lambs. Vet. Rec., 97, 455.

WERNET, P., BREU, H., KNOP, J. and ROWLEY, D. (1971). Antibacterial action of specific IgA and transport of IgM, IgA and IgG from serum into the small intestine. J. infect. Dis., 124, 223-226.

WHITELAW, A. (1976). Survey of perinatal losses associated with intensive hill sheep farming. Vet. A., 16, 60-65.

WIENER, G. (1967). A comparison of body size, fleece weight and maternal performance of five breeds of sheep kept in one environment. Anim. Prod., 9, 177-195.

WIENER, G. (1969). Lamb mortality in commercial sheep flocks with reference to genetic factors. Anim. Prod., 11, 282.

WIENER, G., DEEBLE, F. K., BROADBENT, J. S. and TALBOT, M. (1973). Breed variation in lambing performance

and lamb mortality in commercial sheep flocks. Anim. Prod., 17, 229-243.

WILLIAMS, M. R., MAXWELL, D. A. G. and SPOONER, R. L. (1975). Quantitative studies on bovine immunoglobulins. Normal plasma levels of IgG_2, IgG_1, IgM and IgA. Res. vet. Sci., 18, 314-321.

WILSON, M. R., DUNCAN, J. R., HEISTAND, F. and BROWN, P. (1972). The influence of preparturient intramammary vaccination on immunoglobulin levels in bovine mammary secretions. Immunology, 23, 313-320.

WORLD HEALTH ORGANIZATION (1964). Nomenclature for human immunoglobulins. Bull. Wld. Hlth. Org., 30, 447-450.

ZIVIN, J. A. and SNARR, J. F. (1973). An automated colorimetric method for the measurement of 3-hydroxybutyrate concentration. Analyt. Biochem., 52, 456-461.

Abbreviations were made according to the World List of Scientific Periodicals (1965), 4th ed., London: Butterworths.