THE EFFECTS OF LASALOCID ON MILK PRODUCTION IN PASTURED SPRING-CALVING DAIRY COWS

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Agricultural Science at Massey University.

NHAMO GEORGE GOZHO

1995

Massey University Library

Thesis Copyright Form

Title of thesis: THE EFFECTS OF LASALOCID ON MILK PRODUCTION IN PASTURED SPRING-CALVING DAIRY COWS

(1) ✓(a) I give permission for my thesis to be made available to readers in Massey University Library under conditions determined by the Librarian.

(b) I do not wish my thesis to be made available to readers without my written consent for ..... months.

(2) ✓(a) I agree that my thesis, or a copy, may be sent to another institution under conditions determined by the Librarian.

(b) I do not wish my thesis, or a copy, to be sent to another institution without my written consent for ..... months.

(3) ✓(a) I agree that my thesis may be copied for Library use.

(b) I do not wish my thesis to be copied for Library use for ..... months.

Signed .....

Date 16/02/95

* * * * * * * * * * * * *

The copyright of this thesis belongs to the author. Readers must sign their name in the space below to show that they recognise this. They are asked to add their permanent address.

NAME and ADDRESS DATE

ABSTRACT

Ionophore supplements are widely used in the ration of fattening beef cattle, especially in the United States. Studies have indicated benefits in terms of a faster growth rate and live weight gain and a reduction in feed intake in beef cattle fed either monensin or lasalocid. In recent years interest has been growing on the possible use of ionophores in dairy cattle. This is because changes in rumen metabolism associated with ionophores could increase milk production in lactating ruminants and/or reduce health and reproductive problems.

Two experiments were conducted with dairy cows at grazing to evaluate the effects of Bovatec 20 (lasalocid) on milk production and performance in early and mid–lactation. In Experiment 1 thirty multiparous Friesian cows aged between three and nine years were assigned to two similar treatment groups of 15 cows balanced for age, previous lactation production, body weight and body condition prior to calving. Treatments consisted of no lasalocid (control) and 400 mg lasalocid per cow per day (drenched twice daily) and the experiment commenced 7 days postpartum. Milk yield and composition were measured at weekly intervals and the treatment continued for ten weeks. Gross energy content of milk was estimated from milk composition. Blood was sampled by tail venipuncture at weekly intervals during morning milking and serum harvested. Serum was analyzed for concentrations of non–esterified fatty acids, ß–hydroxybutyrate, glucose, magnesium and calcium. Reproductive parameters (calving to first oestrus, calving to conception intervals and the number of services per conception) were calculated from farm records collected during the experiment.

No differences in milk, milk fat, protein, or lactose yields were observed. Significant ($P<0.01$) lasalocid by period interaction was observed for milk fat yield. Gross energy content in milk did not differ between groups but period effects were significant ($P<0.10$) during weeks 3, 6, 7 and 9. Period by

lasalocid interaction for gross energy content of milk was also significant ($P<0.10$). Lasalocid treatment did not affect live weight changes of cows in early lactation. Lasalocid treated cows lost significantly ($P<0.05$) more condition than control cows. Plasma concentrations of ß–hydroxybutyrate, non–esterified fatty acids, glucose, magnesium and calcium were unaffected by lasalocid. Period by lasalocid effects for non esterified fatty acids and for magnesium were significant ($P<0.05$ and $P<0.10$, respectively). Reproductive parameters were unaffected by lasalocid supplementation.

In Experiment 2 forty–five multiparous Friesian cows in mid–lactation were divided into three groups using the criteria as in Experiment 1. The groups were randomly allocated to three treatments. Treatments consisted of a control group, a group treated with Bovatec 20 (lasalocid) as in Experiment 1, and a third group treated with Bloatenz (a bloat preventive formulation). Treatments lasted 10 weeks. Milk yield and composition, live weight and body condition scores were measured as in Experiment 1. Cows were also scored for intensity of bloat for two periods each of 7 days.

Treatment with either Bovatec 20 or Bloatenz did not affect milk, fat, protein or lactose yields of cows in mid–lactation. Period effects for fat yield were significant ($P<0.05$). Gross energy content in milk was unaffected by treatment. Live weight changes were unaffected by treatments but cows treated with Bovatec 20 and Bloatenz lost less condition compared to control cows. The pastures used failed to induce bloat and hence there were no data for this aspect of the study.

It was concluded that feeding lasalocid resulted in only small numerical increases in milk production in early lactation with no milk production responses in mid–lactation. Lasalocid had minor negative influence on body condition in early lactation and a significant positive influence in mid–lactation.

ACKNOWLEDGEMENTS

I would like to acknowledge the help of the following people in this study. My supervisors Dr G.F. Wilson and Professor C.W. Holmes who were a great help in both the planning and execution of the trial and in the preparation of the written text. Their technical expertise, critical advice, and patience have been invaluable in making this study an enjoyable and worthwhile learning experience, and for that I am grateful.

Mr G.S. Purchas, Ms M.F. Scott and Ms Y.H. Cottam for their assistance in the collection and processing of samples, and the staff at the Dairy Cattle Research Unit (DCRU) Massey, who assisted in running the trial.

Dr P.C.H. Morel and Professor D. Garrick for assistance and advice in statistical analysis, and Mrs B. Purchas for critically reviewing this manuscript every step of the way.

Thanks due to Dr Bob Elliott (Colborn–Dawes Ltd and Roche Ltd for their cooperation in undertaking the experiments and their sponsorship of the project.

I also thank my great Kiwi family that I have acquired over the two years of my stay, the Blackwell, the Dixon and the Purchas families and all the other students in the Faculty of Agriculture and Horticultural sciences.

I am also grateful to be the recipient of a Commonwealth scholarship from the New Zealand Vice–chancellors Committee.

Finally, I would like to thank my work colleague, Mr T. Mutsvangwa, for being a source of inspiration and encouragement all the way and my wife and daughter for making the sacrifice to allow me to come and study in New Zealand.

TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

TABLE OF FIGURES

TABLE OF TABLES

TABLE OF TABLES (CONTINUED)

TABLE OF TABLES (CONTINUED)

CHAPTER 1: GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

In ruminants nutrient inputs are subjected to fermentative digestion by micro–organisms and hydrolytic digestion by the animal's own enzyme systems. Fermentative digestion is advantageous for substances that cannot be digested by the animal's own enzymes. Fermentation of dietary protein in the rumen yields peptides, amino acids and ammonia which may be used in the synthesis of microbial protein. However, some of the ammonia formed will diffuse through the rumen wall into the bloodstream where it is converted to urea in the liver and is excreted in urine. This represents a loss to the animal. Dietary carbohydrates are fermented to the volatile fatty acids acetic, propionic, butyric and small quantities of other acids. Rumen fermentation is also associated with losses in dietary energy through the formation of methane. The main byproducts of rumen fermentation influence the efficiency of utilisation of nutrients for production of meat, milk and fibre. The efficiency of utilisation of dietary nutrients can be optimized by properly balancing fermentative and hydrolytic digestion to minimise losses associated with fermentative processes.

Fermentative digestion and outflow of nutrients from the rumen can sometimes be manipulated by protecting dietary components (especially protein) from rumen micro–organisms and by controlling the balance of microbial species and/or their activities. Intensive research efforts have been directed towards the use of chemical agents which will modulate ruminal fermentation. Ionophores have been used as chemical agents for modulating ruminal fermentation.

Ionophores such as monensin have been used widely in the beef fattening industry in the United States of America. Ionophores can improve feed efficiency when included in a variety of diets, when fed to various classes of cattle and when fed under a variety of environmental conditions. Ionophores can also improve dry matter digestibility, reduce methane losses, feed intake,

lactic acid production, and the likelihood of feedlot bloat or legume pasture based bloat.

Most studies reported in the literature have been done on beef cattle. More recently, interest has been growing on whether benefits of feeding ionophores to cattle can be applied to milk production. During early lactation, nutrient intake does not match nutrient requirements for milk production in high producing dairy cows. Therefore cows mobilise the energy in their body reserves to produce the high yields.

The relative quantities and proportions of nutrients supplied to the host animal by fermentation, digestion and absorption are important in determining the yield of milk and its components. Milk production is dependent on the production of glucose, volatile fatty acids (VFA), amino acids and long chain fatty acids (LCFA) supplied in the diet. Glucose is used to synthesize lactose in the mammary gland. The quantity of lactose determines milk yield. In the ruminant, small quantities of glucose are absorbed from the small intestines. The majority of glucose in the blood comes from gluconeogensis in the liver where propionic acid and non–essential amino acids are used as precursors for gluconeogensis.

The major lipogenic precursors are acetate and ß–hydroxybutyrate and these are used to synthesize short chain fatty acids. Fatty acids in milk with 18 or more carbon atoms are synthesized from blood plasma triglycerides which come either from the diet or from adipose tissue. Fatty acids with chain lengths between 10 and 18 carbon atoms are either obtained from the diet or are synthesized in the mammary gland.

Feeding ionophores can reduce the proportion of lipogenic volatile fatty acids and increase the proportion of glucogenic precursors. This would probably increase milk yield and reduce milk fat concentration. Other possible effects of ionophores reported in the literature, such as prevention of bloat and

improved mineral absorption, should be beneficial in pastured dairy cows.

The following chapter gives an overview of the effects of ionophores such as monensin and lasalocid on rumen fermentation, mineral absorption, as well as the subsequent effects of these changes on milk production, reproduction and the occurrence of metabolic disorders. Subsequent chapters describe experiments that were carried out to examine the effects of lasalocid on milk production, live weight and body condition changes as well as mineral absorption in spring calving pasture–fed dairy cows in early lactation. Effects of lasalocid on milk production and live weight and body condition changes in mid–laction were examined in the second experiment.

CHAPTER 2: REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Introduction

Ionophores are groups of natural or synthetic compounds capable of forming lipid soluble cation complexes (Painter et al., 1982). They are toxic to many bacteria, protozoa, fungi and higher organisms and hence they qualify as antibiotics. They usually have molecular weights of between 500 and 2000 (Russel and Strobel, 1989).

Lasalocid and monensin are classified as carboxylic ionophores because they contain a terminal carboxylic group. Ionophores such as monensin were used originally as coccidiostats in the poultry industry. Because they also affect ruminal fermentation, they are now used in the beef fattening industry particularly in the United States (Russel and Strobel, 1989) and, more recently, considered for possible use in stimulating milk production (Dye et al., 1988; Maas, 1990; Weiss and Amiet, 1990).

The primary mechanism of the carboxylic ionophores is to alter the flow of cations across cell membranes (Pressman, 1976). This affects microbial cell growth and survival in the rumen and hence leads to a change in rumen microflora with consequences on rumen fermentation. Feeding lasalocid and monensin to ruminants increases feed efficiency and/or rate of gain, mainly by promoting an energetically favourable shift in ruminal fermentation, with decreased acetate and increased propionate production.

In the following review, the use of ionophores in cattle production is considered. The effects of lasalocid on rumen fermentation, mineral metabolism, milk production and associated changes will be reviewed. Lasalocid and monensin belong to the same group; therefore experiments with both ionophores will be used to illustrate similarities and differences between the ionophores.

2.2 Use of ionophores in cattle production

a) Beef cattle

The literature abounds in reports that show the benefits of ionophores fed to beef cattle in terms of average daily gain (ADG). Goodrich et al (1984) summarised animal performance data from 228 trials with monensin involving 11,274 head of cattle. Using regression techniques to determine general (cross–trial) effects of monensin on animal performance, the data indicated that monensin–fed cattle grew 1.6% faster than cattle fed control diets. Feed consumption of monensin–fed cattle averaged 6.4% less than that of control cattle and feed required per kg gain was reduced 7.5%.

Animal responses to monensin treatment, however, have been variable. For example, mean responses $\pm$ standard deviations for daily gain (1.6 $\pm$ 8.5% increased gain), feed intake (6.4 $\pm$ 5.0% less feed) and feed/kg of gain (7.5 $\pm$ 6.5% less feed/kg of gain) indicate considerable variability in the response to monensin (Goodrich et al., 1984). In general ionophores increase feed efficiency through a reduction in voluntary food intake. With diets containing high levels of readily fermentable carbohydrates monensin depresses voluntary food intake without decreasing average daily gain; whereas with high roughage diets, ionophores do not depresses voluntary food intake but average daily gain is increased (Bergen and Bates, 1984). This may be related to the nutritive quality of the two feed types. Roughages generally have lower nutrient density than concentrates. Thus the maximum feed intake is likely to be governed by physical factors rather than animal requirements with roughage–based diets.

Live weight gain in beef cattle fed lasalocid have been variable. Valdes et al (1989) reported a 12.4% increase in total gain from the use of 122.3 mg/head/day of lasalocid with steers grazing African stargrass (*Cynodon plectostachyum*) pastures. Food intake was not determined in this study.

Other reports have also indicated increased gains and/or efficiency for grazing steers (Paterson et al., 1983; Spears and Harvey, 1984), for stocker calves fed whole plant grain sorghum silage (Gutierrez et al., 1982), and for steers fed alfalfa cubes ad libitum (Thonney et al., 1981).

Thonney et al. (1981) reported that at levels of 83, 175 and 122 mg lasalocid/day, there was a quadratic increase in gain with increasing dose. Recently, Worrel et al (1990) reported a 22% increase in average daily gain from 150 mg lasalocid/day in steers grazing ryegrass pastures and supplemented with cottonseed meal (CSM). The improved performance was recorded over the spring season only and was probably a result of an improved nitrogen metabolism which improved feed efficiency (Worrel et al., 1990).

In contrast, 200 mg lasalocid / day given in a grain based supplement did not improve performance of beef cows in the fall after calving (Hopman and Weber, 1986), nor did lasalocid added to protein supplemented corn–silage diets fed to growing animals (Horton and Brandt, 1981; Berger et al., 1981). Graded levels of lasalocid (0, 100, 200, or 300 mg/head/day) given in 1.82 kg grain based cubes did not improve average daily gain in mature beef cows in their last trimester of pregnancy (Jacques et al., 1987).

Daily gain in growing–finishing feedlot cattle increased by 8.6 and 7.9% in response to a daily rotation of lasalocid and monensin compared with continuous feeding of monensin or no ionophore, respectively (Morris et al., 1990). The mode of action of this benefit is unclear. Presumably a daily rotation of different ionophores would be too frequent to overcome any possible bacterial adaptation to ionophores (Morris et al., 1990). The daily rotation of these ionophores did not affect the site and extent of nutrient digestion; thus, it is likely that factors other than fermentative and digestive changes were responsible for the improvement in feedlot performance of cattle fed the ionophores.

Although many experiments have been carried out with beef cows, there is a paucity of data on the effects of ionophores on milk yield in beef cattle. It is often difficult to estimate milk production in beef cattle since they are dependent on a suckling stimulus for milk let down. Beef and dairy cows may also respond differently because they have been selected for different traits over many years.

Where milk yield has been measured in beef cows, there has either been no effect (Lemenager et al., 1978a; Hixon et al., 1982a; Hixon et al., 1982b; Grings and Males, 1988), or milk yield has been reduced (Rennick and Russel, 1982) by ionophore treatment. Hixon et al. (1982a) found that lasalocid fed to beef heifers increased milk production on a low energy diet but not on a high energy diet. This suggests that ionophore supplementation to diets marginally deficient in energy may improve milk production and calf performance with beef cows. Milk composition from beef cows was not affected by ionophore treatment (Lemenager et al., 1978a; Rennick and Russel, 1982; Gring and Males, 1988).

Differences in forage quality, sex, physiological status, supplementation practices and levels of inclusion of lasalocid are all confounding factors that make comparisons among experiments difficult.

b) Dairy Cattle

Information on the effects of ionophores on dairy cattle is limited. Relatively fewer studies have been done with growing heifers and lactating dairy cows than with beef cattle.

The energy level of the diet is critical in dairy heifers particularly in the period before and around the onset of puberty. The age at the first oestrous period varies between 7 and 18 months (Holmes and Wilson, 1987). It is affected within a breed by the size of the heifer and therefore level of feeding from

birth. The use of ionophores in diets of growing dairy cattle can increase feed conversion efficiency and this result in heifers reaching puberty at a younger age. However, the feeding regimen for growing dairy heifers should be monitored because dietary energy supply leading to growth rates above 1000 g per day can permanently reduce the amount of parenchyma in the mammary gland which leads to reduced milk production (Sejrsen, 1978).

Baile et al. (1982) found that heifers given monensin conceived earlier than control animals. Earlier conception with monensin treatment appeared to be related to a faster growth rate and/or reaching a desirable breeding weight earlier than heifers in the control group. Some studies contend that the effect of ionophores on breeding may be mediated through both an increased body weight and attainment of the appropriate skeletal size (Moseley et al., 1977). In contrast to these results, Meinert et al. (1992) found no effect of monensin on growth rates of Holstein heifers, but age at breeding and calving were still reduced.

When ionophores are fed to ruminants, an increased proportion of the diet's rumen degradable protein (RDP) escapes rumen degradation and this may be digested in the small intestines. Therefore, in diets with a high proportion of RDP, feeding ionophores may reduce the potential waste of protein through deamination in the rumen. On the other hand feeding ionophores with diets containing a high proportion of undegradable dietary protein (UDP) may lead to impaired protein metabolism in the rumen (Steen et al., 1992).

Monensin has been reported to either increase milk production of pastured dairy cows (Lynch et al., 1990; Wilson et al., 1993) or to have no effect on milk yield in both autumn and spring calving herds (Maas, 1990). Milk fat content was unaffected by monensin in these grazing experiments. With concentrate based diets monensin included at 208.5 mg/head/day and 399 mg/head/day did not affect milk yield, milk protein and lactose concentrations (Sauer et al., 1989). However, monensin when added to the diet at the lower level (208.5

mg) lowered milk fat concentration.

There is a paucity of data with grazing dairy cows supplemented with lasalocid. Recent studies have been done in the United States with cows offered concentrate diets. Even in these studies, results have not been consistent and are difficult to summarise. Some of the lactation performance data of these studies are summarised in Table 2.1.

Three out of the four studies cited reported no significant changes in milk yield in lasalocid supplemented cows (Dye et al., 1988; Weiss and Amiet, 1990; Christensen et al., 1994). Milk yield was depressed in lasalocid supplemented cows in the study by Johnson et al. (1988). This depression in milk yield is an unlikely result for ionophores and could have been confounded with the effects of high levels of dietary fat in this experiment.

Milk fat per cent was significantly depressed in the studies reported by Dye et al. (1988) and Johnson et al. (1988). In one of these (Dye et al., 1988) milk fat per cent decreased linearly ($P<0.03$) as lasalocid intake increased. In other studies lasalocid did not significantly affect milk fat per cent (Weiss and Amiet, 1990; Christensen et al., 1994).

Milk protein content was unaffected in all four studies cited in Table 2.1. Milk protein per cent in all treatment groups in the study by Dye et al. (1988) were lower than expected for cows in early lactation and at the level of milk production reported. The inclusion of whole cottonseed in the diet may be a possible cause of this discrepancy.

All four studies showed a tendency towards a decrease in feed intake in lasalocid supplemented cows although such differences were significant in only three of the four studies (i.e., those by Johnson et al., 1988; Weiss and Amiet, 1990; and Christensen et al., 1994).

Variable milk production responses could be due to differences in diet, stage of lactation and duration of experiment. Although all diets used in these experiments could be described broadly as concentrate–based rations, their composition differed. Dye et al. (1988) included sorghum silage and a concentrate mix blended to supply 14.5% crude protein (CP) in the diet. Johnson et al. (1988) fed corn silage and a concentrate mix which supplied 16% CP in the diet. Weiss and Amiet (1990) offered alfalfa hay and silage, corn silage and a concentrate mix which provided between 17.7% CP and 19.5% CP.

The experiments also commenced at different stages of lactation (Dye et al., 1988: 35 days post partum; Johnson et al., 1988: 90 days post partum; Weiss and Amiet, 1990: 170 days post partum; and Christensen et al., 1994: 7 days post partum). Experiment duration varied from 4 weeks of data collection (Dye et al., 1988) to 98 days (Weiss and Amiet, 1990).

All these factors may thus have influenced the level of milk production and the magnitude of response obtained.

Table 2.1: Effects of feeding lasalocid on milk yield, composition and feed intake.

Variable Measured and Reference	Control	Lasalocid Treatment (mg/cow/d)			
		100–180	181–260	261–340	>340
Milk yield (kg/day)					
Johnson et al., 1988	21.1				20.3
Dye et al., 1988	25.0	25.2	25.1	24.7	
Weiss & Amiet,1990	20.7			21.6	
Christensen et al., 1994	33.5	36.0			36.1
Milk fat content (%)					
Johnson et al., 1988	4.61				3.78
Dye et al., 1988	3.20	3.09	3.10	3.06	
Weiss & Amiet,1990	3.80			3.60	
Christensen et al., 1994	3.74	3.53			3.87
Protein content (%)					
Johnson et al., 1988	3.25				3.21
Dye et al., 1988	2.74	2.76	2.72	2.69	
Weiss & Amiet,1990	3.30			3.20	
Christensen et al., 1994	2.96	2.80			3.00
Feed intake					
Johnson et al., 1988	16.3				14.3
Dye et al., 1988	19.3	19.3	18.2	17.9	
Weiss & Amiet,1990	20.6			19.6	
Christensen et al., 1994	22.0	21.5			21.2

2.3 Mode of action of ionophores

Ionophores may increase productivity of ruminants because of their ability to alter rumen microbial species and their growth which changes rumen fermentation patterns. Subsequently nutrient digestibility and utilization of the absorbed metabolites are altered.

2.3.1 Rumen microbes

a) Bacteria

Ionophores such as monensin and lasalocid are generally effective against gram positive bacteria. One known exception is *Streptococcus bovis 124* which was insensitive to monensin (Dennis et al., 1981). Among gram negative bacteria, only *Butyrivibrio fibrosolvens* and *Ruminococcus flavefaciens* are sensitive to lasalocid and monensin (Dennis et al., 1981). This is because they possess a cell wall structure similar to gram positive bacteria (Chen and Wolin, 1979). When exposed to ionophores, *Bacteriodes (Fibriobacter) succinoges* is initially inhibited but is eventually able to grow in the presence of lasalocid and monensin (Chen and Wolin, 1979).

Streptococcus bovis and lactobacillus species proliferate when starch is abundant. Lactate production by these species is often involved in the onset of rumen acidosis (Slyter, 1976). These species are sensitive to ionophores and are inhibited by monensin and lasalocid (Russel, 1987; Dennis et al., 1981). Therefore their inhibition is beneficial in grain–based diets.

Megasphaera elsdenii, the primary lactate using species in cattle and *Selenomonas ruminantium,* a less active lactate utiliser (Counotte et al., 1981), are resistant to monensin (Nagaraja and Taylor, 1987).

Generally the resistant bacteria fall into two categories (Dennis et al., 1981):
i) those that produce succinate as a major end product (*Bacteriodes, Selenomonas, succinimonas, succinivibrio*); and
ii) those that ferment lactate (*Anaerovibrio, Megasphaera, Selenomonas, Veillonella*).

b) Protozoa.

Ionophores have an inhibitory effect on some families of protozoa. Monensin appears to be more inhibitory than lasalocid (Dennis et al., 1986). Holotrichs (*Dasytricha, Isotricha and Charonina*) were unaffected by either lasalocid or monensin whereas some entodiniomorphs (*Entodinium, Diplodinium and Ophryoscolex*) were more sensitive than any other type (Dennis et al., 1986). Protozoal inhibition by ionophores has been reported to be transient and protozoal numbers returned to normal with prolonged treatment (Dennis et al., 1981).

The influence of ionophores on rumen protozoal population appears to be affected by the nature of the diet fed. Protozoal populations in the rumen were reduced by feeding monensin to cattle on a high grain diet but not on a high roughage diet (Richardson et al., 1976). The reduction in protozoa cells is greater in monensin–fed cattle on urea than on natural protein as a dietary nitrogen source (Poos et al., 1979). Such dietary influence on the effects of ionophores suggests different sensitivity of protozoal types to ionophores.

Some protozoa are cellulolytic but the major substrates appear to be sugars and starch which are rapidly assimilated and stored as poly–dextrans. These are mobilised as required and provide energy for growth and maintenance for the protozoa (Preston and Leng, 1987). By engulfing starch particles protozoa often 'buffer' the pH of the rumen and prevent it from falling to levels lower than required for microbial growth, particularly when grain–based diets are fed.

Protozoa are involved in recycling bacterial protein and defaunation often decreases rumen ammonia concentrations (Eadie and Gill, 1971). Defaunation increases feed efficiency in diets that are low in protein (Bird and Leng, 1978) because microbial protein is utilized by the ruminant rather than recycled in the rumen. Thus any rumen modifier that reduces protozoal cell biomass is likely to have a positive effect on protein supply to the host animal.

2.3.2 Mechanism of ionophore action on rumen microbes.

Lasalocid can bind a wide margin of cations, including mono– and divalent cations. The cations affected, in decreasing order of selectivity are:

$Ba^{2+}>>Cs^{+}>Rb^{+},K^{+}>Na^{+},Ca^{2+},Mg^{2+}>Li^{+}$ (Hanley and Slack, 1982).

Rumen microbes, like any other living organisms, require the maintenance of specific ionic gradients between internal and external cell fluids. Bacteria often contain higher concentrations of solutes than the external environment (Pressman, 1976).

The intracellular pH of most bacteria appears to be highly regulated within the narrow range of 7.6 to 7.8 (Bergen and Bates, 1984). Changes that affect this balance alter metabolic processes within the bacterial cell and thus changes the growth of the micro–organisms affected.

Within the rumen, sodium ions are the predominant extracellular cation (Durand and Kawashimi, 1980). Ruminal potassium ion concentration is four to five fold lower than that of sodium but it is the predominant intracellular cation of microorganisms (Russel and Strobel, 1989). It has been shown that when *Streptococcus bovis* is treated with monensin, there is a decrease in both intracellular potassium ion concentration and intracellular pH (Russel and Strobel, 1989). The low pH is a result of an influx of H^{+} into the cell as a result of the potassium gradient. The bacterial cell cannot maintain an alkaline intracellular environment and hence growth is inhibited.

Gram negative bacteria are generally ionophore–resistant due to biochemical mechanisms which can overcome the reduction in transmembrane ion gradients caused by ionophores (Bergen and Bates, 1984). Protozoa, fungi and gram positive bacteria which lack a protective outer membrane, and thus lack the ability to overcome the ion gradient reduction, are usually sensitive to ionophores (Russel and Strobel, 1989).

In general ionophores alter the flow across normal healthy cellular membranes. This action alters the internal–external ion gradient which is necessary for the normal metabolism of microbial cells. Some strains of microbes can overcome this reduction in ion gradient and are resistant to ionophores. The ionophore–sensitive strains which produce hydrogen, ammonia and lactic acid are reduced in number. The changes in fermentation patterns in the rumen are a result of altered rumen microbial species composition.

2.3.3 Ionophore-nutrient interactions.

The end products of rumen fermentation are: a) the volatile fatty acids, carbon dioxide and methane; which are all a result of carbohydrate fermentation; and b) ammonia, amino acids, and peptides, which are a result of protein degradation.

Feeding ionophores to ruminants results in changes in rumen fermentation patterns and the following changes occur:

a) changes in energy fermentation patterns that promote production of propionic acid at the expense of acetic and butyric acids,

b) a reduction in methane gas production,

c) changes in protein degradation in the rumen,

d) alteration in mineral metabolism, and

e) health related and other effects.

a) Energy metabolism

i) Volatile fatty acid production

Volatile fatty acids are end products of carbohydrate fermentation in the rumen that are used in intermediary metabolism in ruminants. The major volatile fatty acids that are produced in the rumen are acetic acid, butyric acid and propionic acid.

The molar proportions of the major volatile fatty acids are important because they determine the quantity of energy that is made available for use by the animal in the various metabolic processes. Fermentation of glucose to pyruvate by rumen microbes is associated with production of hydrogen. The largest quantities of hydrogen are used in the formation of propionate and butyrate and in the reduction of carbon dioxide to methane (Chalupa, 1977 and 1980) The differences in the production and utilization of metabolic hydrogen cause the efficiency of fermenting hexose to acetate, propionate and butyrate to be 62%, 109% and 78% respectively (Hungate, 1966; Chalupa, 1977 and 1980). Therefore metabolically useful energy recovered in the fermentation end–products can be increased by enhancing the production of propionate and to a lesser extent, butyrate at the expense of acetate; an effect facilitated by ionophores.

Cattle fed ionophores have lower acetate to propionate (A:P) ratios in rumen fluid than do cattle not fed ionophores. Decreases in A:P ratio have been observed in growing beef cattle (Bergen and Bates, 1984), lactating beef cows, (Lemenager et al., 1978b), as well as in dairy cows (Weiss and Amiet, 1990; Johnson et al., 1988; Dye et al., 1988).

Weiss and Amiet (1990) found that the acetate to propionate ratio of cows fed 340 mg lasalocid per day was significantly lower than that of control cows during the first 7 days of treatment. The effect was transitory and quickly

disappeared. The molar percentage of ruminal acetate was about 7% lower and propionate was 30% higher for lasalocid–fed cows than for control cows on day 7. This led to an A:P ratio of 2.7 for lasalocid cows and 3.6 for control cows.

Dye et al. (1988) also found that graded levels of lasalocid reduced the A:P ratio from 2.53 in the control cows to 2.28 with the highest lasalocid level. Total VFA concentration was depressed linearly with the addition of lasalocid. Johnson et al. (1988) found results similar to those by Dye et al. (1988) when they fed cows 333 mg of lasalocid per day. Other studies (Beede et al., 1986; Darden et al., 1985), reported no effect of ionophore supplementation on molar proportions of volatile fatty acids.

ii) Gas production

The main gases produced in the rumen during fermentation processes are carbon dioxide (40%), methane (30–40%) and hydrogen (5%), together with small and varying proportions of oxygen and nitrogen from ingested air (McDonald et al., 1988). The rate of gas production in the rumen is most rapid immediately after a meal and may exceed 30 l/h in cattle (McDonald et al., 1988). Methane alone can be produced at a rate of 12 l/h in cattle (Thornton and Owens,1981).

Carbon dioxide is produced partly as a by–product of fermentation and partly by the reaction of organic acids with the bicarbonate present in saliva. The basic reaction by which methane is formed is the reduction of carbon dioxide by hydrogen, some of which may be derived from formate.

The ruminant loses 7–12% of its food energy as methane (Russel and Strobel, 1989; McDonald et al., 1988). This is because the gases are mostly derived from food energy but they provide no energy to the animal and are lost through eructation. Ionophores can decrease this energy loss by 30% through

a reduction in methane production (Schelling, 1984).

Ionophores do not inhibit the growth of methanogenic bacterial species (Chen and Wolin, 1979), but they inhibit the growth of the micro–organisms that are involved in breaking up formate to carbon dioxide and hydrogen (Van Nevel and Demeyer, 1977). Carbon dioxide and hydrogen are the two important substrates needed to form methane by methanogenic bacteria. Thus when methane production from formate is inhibited, other reduction products, most notably propionic acid, increase at the expense of acetic acid (Russel and Strobel,1989).

iii) Digestibility.

Several reports indicate an influence of ionophores on carbohydrate digestibility although responses have been inconsistent. Different components of digestion have been examined in the various reports available in literature. Increases in dry matter and organic matter digestibilities have been observed with ionophore feeding (Rust et al., 1978; Horton et al., 1983; Beede et al., 1986). Other studies reported either no effect on dry matter digestibility (Morris et al., 1990), organic matter digestibility (Zinn, 1987; Dye et al., 1988; Morris et al., 1990) or a reduction in organic matter digestibilities (Muntifering et al., 1981). There appears to be no effect of ionophore on site and extent of carbohydrate digestion in the digestive tract (Darden et al., 1985; Zinn, 1987; Morris et al., 1990).

The observed contradictions in digestibility reported in the literature are probably due to differences in diet composition and feed processing methods. Most cited reports were from experiments with concentrate–based diets. Rust et al. (1978) used a high grain diet that was low in protein in their studies. Horton et al. (1983) fed 70% cracked corn and 30% corn silage. Zinn (1987) and Morris et al. (1990) fed a 90% concentrate diet.

Data on digestibility with grazing cattle is scarce, probably because of the difficulties involved in measuring intake and digestibility under such conditions. Where improved digestibility occurs when ionophores are fed, it is possible that the effect comes partly from an altered ruminal microbial population as well as a reduction in voluntary feed intake. A reduction in voluntary feed intake may lead to a decrease in rate of passage through the gastrointestinal tract and increases the exposure of digesta to digestive processes, making it possible for nutrient extraction to occur to a greater extent.

b) Nitrogen metabolism

Dietary proteins are hydrolysed to peptides and amino acids by rumen micro–organisms, but some amino acids are degraded further, to organic acids, ammonia and carbon dioxide. The ammonia produced, together with some peptides and amino acids, are used by rumen micro–organisms to synthesis microbial protein. Undegraded dietary protein (UDP) and microbial protein passes through to the small intestines where it is partially digested and absorbed.

Ionophores have been observed to have a protein "sparing effect" (Poos et al., 1978; Paterson et al., 1983; Goodrich et al., 1984; Beede et al., 1985). This effect could occur through ionophore feeding leading to: i) a reduction in ruminal proteolysis and/or deamination and the use of most of the rumen ammonia in microbial protein synthesis, ii) a greater proportion of preformed dietary protein escaping ruminal degradation, and iii) a larger portion of both microbial protein and UDP being digested and absorbed post–ruminally.

Deamination and proteolysis tend to be reduced in the rumen in the presence of ionophores (Bergen and Bates, 1984). Ruminal proteolysis appears to be inhibited by monensin to a lesser extent than deamination (Van Nevel and Demeyer, 1977; Whestone et al., 1981; Hino and Russel 1986). Thus ionophores may increase both quantity and quality of protein reaching the

lower gastrointestinal tract for digestion and absorption.

Protein by–pass was increased by 20% to 55% in five different experiments (Bergen and Bates, 1984). Isichei and Bergen (1980) reported an increase in protein escaping ruminal degradation when monensin was administered; but a comparable decline in microbial protein reaching the small intestine was observed as well. The total amount of protein reaching the lower gastrointestinal tract was increased with monensin in some experiments (Owens et al., 1978; Poos et al., 1979). Most studies with lasalocid have reported little or no effect on rumen ammonia levels (Darden et al., 1985; Dye et al., 1988; Steen et al., 1992).

Nitrogen digestibility is also improved by ionophores (Wedegaertner and Johnson, 1983; Paterson et al., 1983). Addition of lasalocid to protein supplements increased nitrogen digestibility by 6% in one trial and 13% in a second (Paterson et al., 1983). Beede et al. (1986) demonstrated improved nitrogen retention when monensin was fed to steers receiving a protein deficient diet.

It can be concluded from these observations that ionophores elicit their beneficial effects on protein metabolism possibly through a reduction in rumen degradation of dietary proteins which leads to increased digestion and absorption of protein of dietary and microbial origin in the lower gastrointestinal tract. Observations with lasalocid appear to suggest that this ionophore does not affect deamination to the same extent as monensin.

c) Mineral metabolism.

Mineral elements are dietary essentials for all animals and influence the efficiency of livestock production. In fact, approximately five percent of the body weight of an animal consists of minerals (McDowell et al., 1983).
Many factors affect mineral requirements including nature and level of

production, age, level and chemical form of elements in the feed ingredients, interrelationships with other nutrients, supplemental mineral intake, breed and animal adaptation. Mineral uptake can be enhanced by feeding ionophores. Minerals that are important in the metabolic function of the animal include magnesium and calcium. Low levels of minerals in forages can lead to low blood levels. In the case of magnesium this can cause hypomagnesaemic (grass) tetany whilst low levels of calcium can result in milk fever in recently calved cows.

The ability to mobilize skeletal magnesium in times of deficiencies, decreases in mature cattle. Thus mature cows fed on pastures are most susceptible to grass tetany during early spring or a particularly wet autumn (Holmes and Wilson, 1987). Feeding ionophores should reduce the occurrence of these disorders since several mineral balance trials have indicated that ionophores affect apparent absorption and retention of minerals.

Calcium absorption has not been affected by either lasalocid (Spears and Harvey, 1984; Reffett–Stabel et al., 1989; Valdes et al., 1988; Darden et al., 1985; Starnes et al., 1984) or by monensin (Starnes et al., 1984).

Phosphorus response to ionophores has been variable. Inorganic phosphorus metabolism was unaffected by lasalocid (Darden et al., 1985; Valdes et al., 1985; Reffett–Stabel et al., 1989) or monensin (Grings and Males, 1988). However, Starnes et al. (1984) reported an increased absorption and retention of phosphorus with both lasalocid and monensin.

Serum sodium levels are generally unaffected by ionophores (Spears and Harvey, 1984; Darden et al., 1985; Reffett–Stabel et al., 1989). The lack of response in serum sodium levels with ionophore feeding is because sodium levels are highly regulated and generally maintained within a narrow range (Morris, 1980).

The other minerals that have shown variable responses are: a) zinc, which has either been unaffected by lasalocid (Darden et al., 1985; Reffett–Stabel et al., 1989) or serum levels have been elevated by feeding ionophores (Starnes et al., 1984), and b) copper, which has either been unaffected (Darden et al., 1985; Valdes et al., 1988), or increased by feeding ionophores (Starnes et al., 1984; Reffett–Stabel et al., 1989).

The most consistent response of mineral metabolism in ruminants has been increased absorption and retention of magnesium (Greene et al., 1986; Spears and Harvey, 1984; Starnes et al., 1984; Wilson et al., 1993).
Wilson et al. (1993) found that monensin administered during the late pregnancy period significantly increased plasma magnesium concentrations and that this persisted into early lactation. However, when monensin was administered after calving, it did not affect plasma magnesium concentration. Some studies with lasalocid reported no effect of ionophores on absorption and retention of magnesium (Valdes et al., 1988; Reffett–Stabel et al., 1989).

The affinity of lasalocid for magnesium is lower than the affinity for calcium, sodium or potassium (Pressman, 1973). The ability of lasalocid to carry divalent ions across lipid bilayers is greater for magnesium than for other divalent cations (Greene et al., 1986). Ionophore feeding increased apparent absorption of magnesium in lambs and this was probably related to ionophore effects on the Na^+/K^+ ATPase of rumen epithelium and, in turn, the involvement of this enzyme in magnesium transport (Martens et al., 1978).

The lack of response in mineral absorption and retention where ionophores have been included in the diet was considered by Darden et al. (1985) to be because mature animals were used in the studies. However, it would be expected that even with lactating cows, some improved absorption and retention should have been noted in the studies cited and hence it is difficult to explain either the lack or the variability of the effects of lasalocid on mineral metabolism in these studies. Relative to the requirements for maintenance,

a cow in late pregnancy requires an extra 28% magnesium (Fontenot et al., 1989) and a lactating cow producing 20 litres of milk per day requires an extra 100% (Grace, 1983). Given such high demands for some minerals, it is difficult to explain the lack of response when mineral metabolism studies are undertaken.

d) Other effects of ionophores

i) Prevention of metabolic disorders

Ionophores can indirectly prevent metabolic disorders such as rumen lactic acidosis; mineral deficiency disorders such as milk fever and grass tetany and rumen upsets such as grain– and legume–bloat.

Excessive lactic acid production is involved in the etiology and pathology of lactic acidosis in beef cattle fed high grain diets (Bartley et al., 1975). Dennis et al. (1981) reported that monensin and lasalocid inhibited growth of the major lactic acid producing microorganisms within the rumen. While lasalocid was the more potent of the two, both ionophores inhibited growth of *Streptococcus bovis* which is the major lactate producer which proliferates under acidotic conditions.

Magnesium is involved in the metabolism of carbohydrates and lipids as a catalyst of a wide array of enzymes. Magnesium is required for cellular oxidation and exerts a potent influence on neuromuscular activity. About 70% of the total magnesium is found in the skeleton and the remainder is distributed in the soft tissue and fluids (McDonald et al., 1988). Magnesium deficiencies can lead to hypomagnesemic tetany in grazing ruminants (McDowell et al., 1983). Supplementation with lasalocid or monensin may help to prevent hypomagnesia (Martens et al., 1978).

Calcium is another important constituent of the skeleton system and teeth. About 99% of body calcium is found in the skeleton and teeth and the remainder is found in tissue fluids and cells where it is essential for the activity of various enzyme systems including those necessary for the transmission of nerve impulses and for the contractile properties of muscle (McDonald et al., 1988). Inadequate calcium causes weakened bones, slow growth and milk fever in severe cases (McDowell et al., 1983).

Cattle grazing ryegrass/red clover pastures can suffer from bloat. Legume bloat in cattle is caused by the formation of a stable, proteinaceous foam in the rumen, which traps gases produced by microbial fermentation and prevents eructation (Clarke and Reid, 1974). Rapid degradation of protein by micro–organisms in the rumen contributes towards foaming. The rate of gas production in cattle that bloat is higher than in those that are not prone to bloat (Majak et al., 1983). The accumulation of gas in the rumen causes an increase in pressure within the rumen. When intraruminal pressure exceeds 70 mmHg, the animal has difficulty breathing (Clarke and Reid, 1974). This situation is exacerbated by carbon dioxide diffusion from the rumen into the lungs. If not treated, the animal will die of asphyxia.

In New Zealand the incidence of bloat is high. It occurs in 60% of the dairy herds and in 40% of the herds, at least one cow dies due to bloat every year, accounting for 0.6 per cent of the cows culled (Holmes and Wilson, 1987). Ionophores reduce the incidence and intensity of bloat because they affect rumen micro–organisms (Bartley et al., 1983). Altering microbial species and numbers in the rumen reduces the rates of proteolysis and deamination in the rumen and thus reduces the incidence and severity of bloat.

ii) Improved reproductive performance

The use of ionophores in breeding beef cattle has been extensively studied in the cow. Monensin or lasalocid at levels of 150 to 200 mg per cow per day had no adverse effect on fertility (Sprott et al., 1988). First service conception or pregnancy rates were unaffected in either lactating cows (Turner et al., 1980; Walker et al., 1980a,b; Hixon et al., 1982a,b), non–lactating cows (Smith et al., 1980) or virgin heifers (Moseley et al., 1977; Baile et al., 1982).

Nutrition during the pre– and postpartum period affects weight gain and body condition and influences the postpartum interval to oestrus. It is aimed to have cows calving at condition score of 4–5 on the 9 point scale since lower condition scores can lead to fertility problems (Holmes and Wilson, 1987). Ionophore supplementation has been shown to reduce the interval from calving to first oestrus. The postpartum interval to oestrus may be decreased in cows in which weight or body condition are improved by ionophore feeding (Turner et al., 1977; Hixon et al., 1982a). Turner et al. (1977) reported that feeding monensin to cows in moderate body condition decreased postpartum interval to oestrus but that monensin had no effect on the same cows if they were in good condition. Therefore the impact of ionophores on the postpartum interval to oestrus may be influenced by body condition and weight gain at the time of ionophore treatment. It has been suggested that the effect of ionophores on the postpartum interval to oestrus becomes apparent when there is a response in terms of body condition and weight gains to ionophore feeding (Sprott et al., 1988). However, results of studies in which cow weight change was not influenced by ionophore feeding showed no impact on postpartum interval to oestrus (Turner et al., 1980; Walker et al., 1980b; Hixon et al., 1982b).

2.4 Scope of the study

Ionophores offer a way of manipulating rumen fermentation resulting in an improved net efficiency, possibly through increased digestibility and/or efficiency of utilisation of feed nutrients. It also reduces the occurrence of metabolic disorders such as grain and legume bloat and acidosis in high grain diets.

The improved milk yields that have been observed with diets containing ionophores are partially a result of a low acetate to propionate (A:P) ratio. A low A:P ratio is desirable because propionic acid is the major source of glucose production in the liver in ruminants. Propionic acid, after activation to its coenzyme A (coA) ester, is carboxylated to methylmalonyl coA and then converted to succinyl coA. This metabolite is an intermediate of the citric acid cycle and is converted to glucose by the phosphoenolpyruvate pathway. In lactating dairy cows glucose is needed for lactose synthesis.

Since high producing dairy cows are in a negative energy balance in early lactation (from calving to about 12 weeks post partum), an improvement in glucose synthesis from the diet reduces the negative energy balance in early lactation. Therefore cows fed ionophores would not need to draw on body reserves so quickly; a consequence of which may be fewer cases of clinical or subclinical ketosis.

Legume based pastures have a high proportion of readily degradable protein. Feeding ionophores may reduce the degradation of such protein in the rumen, leading to a greater supply of dietary protein to the animal. Therefore more amino acids would be available for milk protein synthesis, and/or for the supply of precursor to synthesize glucose.

The literature shows that there are benefits in feeding ionophores to both dairy and beef cattle. Under conditions found in New Zealand, the effect of

ionophores such as lasalocid on rumen metabolism and subsequently on milk production and the incidence of bloat in cows fed legume–based pasture is of interest. This is because the benefits from high protein legume–based pasture are compromised by an excessive protein breakdown in the rumen and the risk of bloat.

Nutrient requirements of lactating dairy cows are influenced by stage of lactation, physiological status, body weight and condition. Studies reported in the literature have been mainly of a short term nature. It is therefore important for long term studies to be undertaken so that seasonal and stage of lactation effects can also be assessed.

The objectives of the following study were;

a) To measure comparative milk production parameters to determine the effects of lasalocid on milk yield, fat, protein yields as well as concentrations in both early and mid–lactation.

b) To measure the effects of lasalocid on selected serum minerals and metabolites and on body weight and condition in early lactation.

c) To assess the effects of lasalocid on mastitis and reproduction performance.

d) To determine the efficacy of lasalocid for the control of bloat in pastured, lactating cows in mid–lactation.

CHAPTER 3: MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Experiment 1: Early lactation.

Thirty multiparous Friesian cows aged between three and nine years were selected from a dairy herd at the Dairy Cattle Research Unit (DCRU), Massey University. The animals were assigned to two similar groups of 15 cows balanced for age, previous lactation production (milk yield and fat yield), body weight and condition prior to calving. The groups were randomly assigned to either the control or lasalocid (Bovatec 20, Colborn Dawes, Australia Pty Ltd) groups. The experiment commenced in early August, 1993 and was terminated on 12 November, 1993.

The animals were run with about 100 other cows that were part of the herd at the DCRU. They were grazed on mixed perennial ryegrass/white clover pastures and they had access to clean water at all times. Cows were milked at 0530 and 1530 hours daily.

The group assigned to the lasalocid treatment was drenched twice daily during milking with a 20 ml preparation containing "Bovatec 20". Fifty ml of Bovatec were diluted with a 25% solution of polyethylene glycol (Carbowax 8000; Union Carbide Corporation, USA) to one litre. This quantity of "Bovatec 20" supplied 200 mg lasalocid/20 ml. Treatment with "Bovatec 20" started immediately after an initial blood sample had been collected. Control cows were not drenched.

Milk yield and composition were measured at weekly intervals commencing on the day of treatment. At each sampling, evening and morning milk yields were recorded and each sample was analyzed for milk fat, protein and lactose concentrations. Milk yield and milk composition data were used to calculate the gross energy content of milk for the treatment groups in both Experiments 1 and 2 using the following equation (Holmes et al., 1993):

$$GE = 38.5\ F + 24.5\ P + 15.7\ L$$

where GE = gross energy in MJ

F = fat yield in kg

P = protein yield in kg

and L = lactose yield in kg.

Cows were weighed and condition scored at four weekly intervals starting immediately prior to calving. Condition score for each cow was assessed by the nine point score system described by Holmes and Wilson (1987).

Blood was sampled by tail venipuncture at weekly intervals during morning milking. Serum was harvested by centrifugation at 3000 rpm and 4° C for 20 minutes and then transferred into duplicate vials and stored at –12° C.

Somatic cell count data and mating records (i.e., period from calving to first oestrus, calving to first mating, and calving to conception intervals and number of inseminations per cow) were used to assess the effects of treatments on cow performance.

3.2 Experiment 2: Mid-lactation: The bloat study.

Forty–five multiparous Friesian cows were used in the experiment. They were divided into three groups of 15 cows selected using the same criteria as in Experiment 1. The treatment groups were:

1) the control group used in Experiment 1,

2) lasalocid group, in which animals were treated as in Experiment 1,

3) and Bloatenz group in which cows were treated with a bloat preventive formulation called "Bloatenz" (Ecolab, Hamilton, New Zealand).

One part of "Bloatenz" was diluted in four parts of water, according to manufacturer's specifications. The diluted "Bloatenz" was administered to cows at a rate of 20 ml per cow at each milking time. The experiment started a few days before the end of Experiment 1 (8/11/93) and was terminated on 15/01/94.

Milk yields and composition, body weight and condition scores were measured as in Experiment 1. Cows were also scored for intensity of bloat for two periods each of 7 days. During these bloat challenges cows were grazed on 'bloat prone' pastures containing a high proportion of red clover following the morning milking. They were closely observed and scored for bloat at hourly intervals for the first four hours of grazing.

3.3 Chemical Analysis

Milk samples were analyzed for fat, protein and lactose content using an infra–red milk analyzer (Milkoscan, N Foss Electric, Denmark). Blood serum was analyzed for concentrations of the following metabolites: non–esterified fatty acids (NEFA) using the modified colorimetric method described by McCutcheon and Bauman (1986); ß–hydroxybutyrate (ßOHB) using the method of Williamson and Mellenby (1974); glucose using the autoanalyser method described by Trinder (1969). Levels of magnesium and calcium in blood were determined by the colorimetric methods described by Faulkner (1982) and Michaylova and Illkova (1971), respectively, and determined on a Cobas Fara II autoanalyser (Hoffman LaRoche Ltd, Switzerland). Intra– and inter–assay coefficients of variation were NEFA 2.4%, 8.8%; ß–hydroxybutyrate 1.1%, 6.2%; glucose 1.9%, 4.6%; magnesium 2.2%, 4.7%; and calcium 2.0%, 2.5%.

3.4 Statistical Analysis

Since the groups used in the experiments were balanced for date of calving, age, production, body weight and condition, the design was a completely randomised design. Performance data (milk yield, milk fat, protein and lactose yields and concentrations and gross energy content of milk) were analyzed using the Generalised Linear Model (GLM) for repeated measures procedures (SAS, 1985). In Experiment 1 previous lactation milk, fat, protein and lactose yields were used as covariates for the respective variables. The following model was fitted for such data:

$$y_{ijk} = x + t_{ij} + w_k + (t_{ij})*(w_k) + (x)*(w_k) + (t_{ij})*(x) + e_{ijk}$$

where y_{ijk} = dependent variable of cow j fed treatment i during week k,

x = covariate

t_{ij} = fixed effects of treatment i,

w_k = fixed effects of week k,

$(t_{ij})*(w_k)$ = first order interaction between treatment and week of lactation,

$(x)*(t_{ij})$ = first order interaction between covariate and treatment,

$(x)*(w_k)$ = first order interaction between covariate and week of lactation,

e_{ijk} = error term

The same model was used for performance data in Experiment 2, except that the milk, fat, protein and lactose yields immediately before the beginning of Experiment 2 were used as covariates.

Although the first order interactions between covariate and treatment and between covariate and week were included in the analysis, they were not important for providing information relating to the objectives of the study and hence are not reported in the results.

Data for blood metabolite concentrations (ßOHB, NEFA and glucose) and mineral concentrations (calcium and magnesium) were analyzed using the model outlined above and data from the initial blood sampling were used as covariates.

GLM procedures were used to analyze for the differences in $\log_{10}$ somatic cell counts, calving to first oestrus and calving to conception intervals as well as number of inseminations per cow for data collected in Experiment 1. Live weight changes and body condition score changes were estimated by subtracting the first measurements obtained at the beginning of each experiment from the measurements taken at the end of the experiment. GLM procedures were then used to analyze for the differences in the values obtained.

CHAPTER 4: RESULTS

LEVELS OF STATISTICAL SIGNIFICANCE

NS Not significant $P > 0.05$

\+ Approaching significance $0.10 > P > 0.05$

* $0.05 > P > 0.01$

** $0.01 > P > 0.001$

*** $0.001 > P$

4. RESULTS

4.1 Experiment 1.

4.1.1 Milk Production

Treatment with "Bovatec 20" (lasalocid) did not affect milk, fat, protein, or lactose yields (Table 4.1). Least square means for milk, fat, protein and lactose yields are shown in Tables 4.2, 4.3, 4.4 and 4.5, respectively.

The period (week) effect was not significant for any of the yield components measured. A significant ($P<0.01$) period (week) by lasalocid interaction was observed for milk fat yield. Weekly milk yield, fat, protein and lactose yields are shown in Figure 4.1. Lasalocid treated cows produced slightly more fat than control cows except in weeks 4 and 8 of the experiment when control cows produced slightly more milk fat. Lactose yields were higher in lasalocid treated cows throughout the experiment but only significantly different ($P<0.05$) in weeks 3, 6, 7 and 9 (Table 4.5).

Gross energy content in milk (Table 4.1) did not differ significantly between the two groups but analysis of variance on a weekly basis revealed that the difference between the groups approached significance ($P<0.10$) during weeks 3, 6, 7 and 9 (Figure 4.2). A period (week) by lasalocid interaction for gross energy content of milk also approached significance ($P<0.10$).

Significance of effects of lasalocid on percent fat, protein, and lactose are given in Table 4.6. Lasalocid treatment did not affect milk composition. Protein content was slightly depressed in the treated cows during weeks 1 to 3 of the experiment. Mean daily fat corrected milk yields (4% FCM) for the entire period of Experiment 1 were 26.6 and 28.0 kg /day for the control and lasalocid groups, respectively. The fat corrected milk yields were not

significantly different ($P<0.10$) in the two groups. Milk composition of lasalocid treated and control cows is given in Table 4.7.

Table 4.1: Significance of effects of lasalocid treatment, period of treatment and lasalocid by period interaction on milk yield, fat yield, protein yield, lactose yield and gross energy in milk (GE_{milk}) for Experiment 1.

Variable	Milk yield	Fat yield	Protein yield	Lactose yield	GE_{milk}
Covariate	**	***	***	***	***
Lasalocid (L)	NS	NS	NS	NS	NS
Period (week)	NS	NS	NS	NS	NS
Week x L	NS	**	NS	NS	+

GE_{milk} = Gross energy content in milk

Table 4.2: Covariate adjusted least square means of milk yield (kg\day) for the first ten weeks of lactation for control and lasalocid treated dairy cows (Experiment 1).

Week of lactation	Milk yield (kg\d)		
	Control	Lasalocid	SEM
1	23.22	24.40	1.54
2	24.33	25.40	1.15
3 *	25.45	28.19	1.28
4	26.85	26.95	1.37
5	26.47	27.27	0.97
6	25.02	26.69	1.30
7 *	24.98	27.36	1.12
8	24.82	25.88	0.82
9 *	23.74	25.95	0.96
10	24.75	25.16	0.97
Over all Means	24.96	26.33	2.76

Table 4.3: Covariate adjusted least square means of fat yield (kg\day) for the first ten weeks of lactation for control and lasalocid treated dairy cows (Experiment 1).

Week of lactation	Fat yield (kg\d)		
	Control	Lasalocid	SEM
1	1.12	1.12	0.084
2	1.09	1.08	0.062
3	1.11	1.25	0.088
4	1.19	1.15	0.075
5 *	1.09	1.20	0.062
6 *	1.01	1.15	0.061
7	1.07	1.25	0.071
8	1.10	1.08	0.063
9	1.01	1.11	0.059
10	1.14	1.16	0.057
Over all Means	1.09	1.15	0.137

SEM = standard error of means
*Weeks followed by a symbol have significantly different group means.

Table 4.4: Covariate adjusted least square means of milk protein yield (kg\day) for the first ten weeks of lactation for control and lasalocid treated dairy cows (expt. 1).

Week of Lactation	Milk protein yield (kg\d)		
	Control	Lasalocid	SEM
1	0.93	0.92	0.059
2	0.88	0.88	0.041
3	0.88	0.94	0.040
4	0.91	0.89	0.044
5	0.89	0.90	0.035
6	0.83	0.89	0.040
7 *	0.83	0.91	0.035
8 +	0.83	0.87	0.032
9	0.80	0.86	0.034
10	0.85	0.85	0.031
Over all Means	0.86	0.89	0.091

Table 4.5: Covariate adjusted least square means of lactose yield (kg\day) for the first ten weeks of lactation for control and lasalocid treated dairy cows (expt. 1).

Week of Lactation	Milk lactose yield (kg\d)		
	Control	Lasalocid	SEM
1	1.14	1.20	0.084
2	1.21	1.28	0.063
3 *	1.28	1.42	0.070
4	1.35	1.36	0.074
5	1.32	1.37	0.050
6 *	1.24	1.36	0.060
7 *	1.25	1.39	0.052
8	1.23	1.29	0.042
9 *	1.17	1.31	0.053
10	1.23	1.27	0.052
Over all Means	1.24	1.35	0.142

SEM = standard error of means
Means in the weeks followed by a symbol (* or +) are significantly different at the 5% or 10% level of significance, respectively.

Table 4.6: Significance of effects of lasalocid treatment, week of lactation and lasalocid by week of lactation interaction on per cent fat, per cent protein and per cent lactose for Experiment 1.

Variable	% Fat	% Protein	% Lactose
Covariate	***	***	***
Lasalocid (L)	NS	NS	NS
Period (Week)	NS	+	NS
Week x L	NS	*	NS

Table 4.7: Milk composition of control and lasalocid treated cows for the first ten weeks of lactation.

Week of Lactation	% Fat			% Protein			% Lactose		
	C	L	SEM	C	L	SEM	C	L	SEM
1	5.0	4.6	0.23	4.0	3.8*	0.10	4.9	5.0	0.05
2	4.5	4.3	0.15	3.6	3.5*	0.08	5.0	5.0	0.04
3	4.4	4.4	0.20	3.5	3.2**	0.05	5.0	5.0	0.04
4	4.5	4.3	0.17	3.4	3.3*	0.04	5.0	5.0	0.04
5	4.2	4.3	0.19	3.4	3.3	0.04	5.0	5.0	0.03
6	4.1	4.3	0.13	3.3	3.3	0.04	4.9	5.0	0.03
7	4.3	4.5	0.16	3.3	3.3	0.05	5.0	5.0	0.03
8	4.5	4.2	0.16	3.4	3.3	0.05	5.0	5.0	0.03
9	4.3	4.3	0.16	3.4	3.4	0.05	4.9	5.0	0.04
10	4.7	4.6	0.15	3.4	3.4	0.04	5.0	5.0	0.03
Overall Means	4.4	4.4	0.25	3.5	3.4	0.19	5.0	5.0	0.07

C = control L = Lasalocid SEM = standard error of means

Means in the same row in the same part of the table followed by a symbol are significantly different.

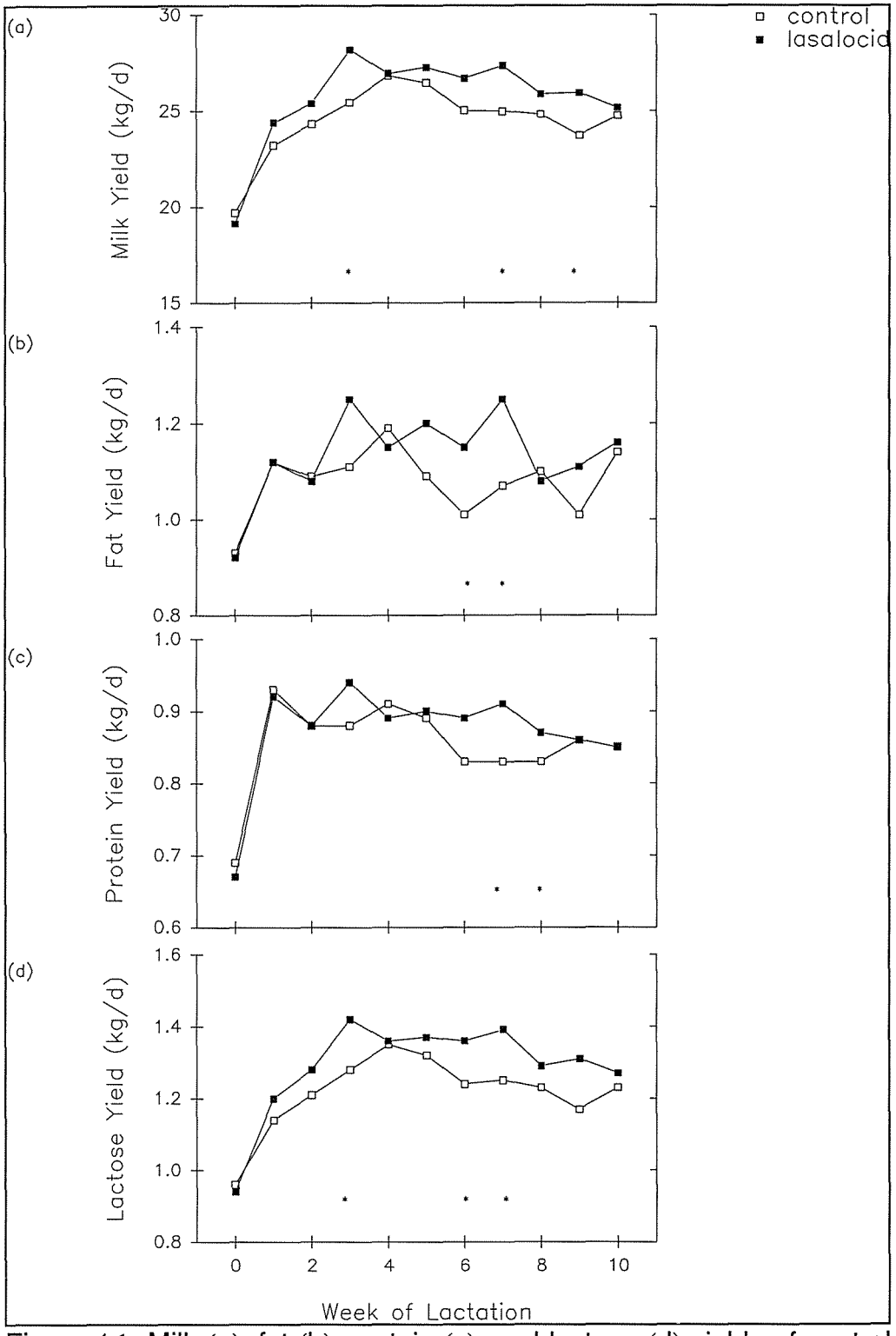
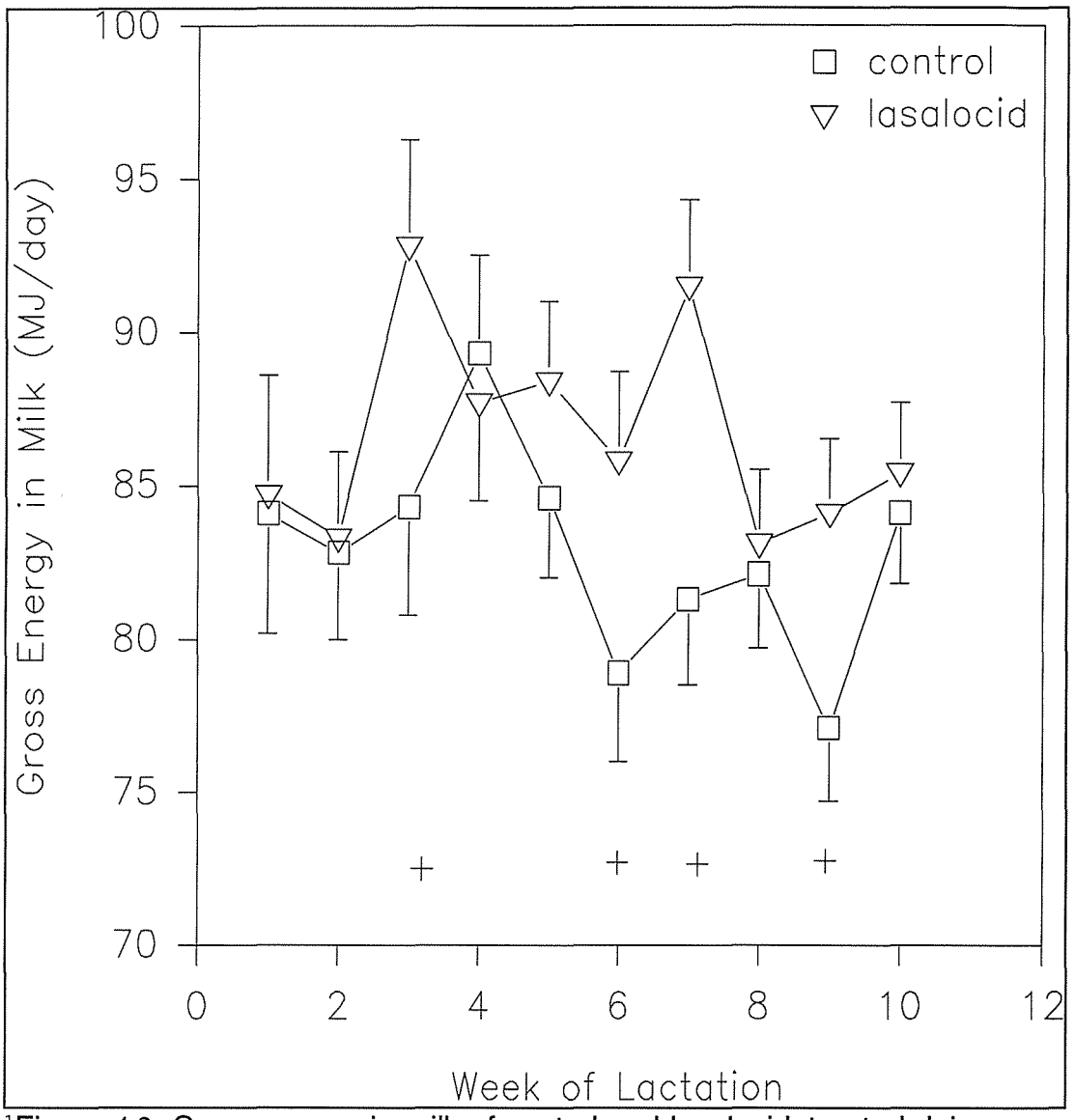


Figure 4.1: Milk (a), fat (b), protein (c), and lactose (d) yields of control and lasalocid–treated dairy cows in early lactation.

1



[1]Figure 4.2: Gross energy in milk of control and lasalocid–treated dairy cows in early lactation in Experiment 1.

[1]Differences between the groups in the weeks marked by a symbol (+) approached significance (P<0.10).

4.1.2 Live weight and condition score.

Cows in both groups lost weight during the period from calving to 4 weeks into lactation (Figure 4.3). The loss in live weight was confounded with the loss of the products of conception because initial live weights were measured before calving.

Lasalocid treatment did not affect the pattern of live weight change of cows in early lactation (Table 4.8). However, lasalocid treated cows lost significantly ($P<0.05$) more condition than control cows. Period (month) effects on live weight were significant ($P<0.05$). Changes in live weight and body condition score in lasalocid treated and control cows are shown in Figure 4.3.

Table 4.8: Significance of effects of lasalocid (L), stage of lactation (month) and lasalocid by month of lactation interaction on body weight and condition of cows during the first 3 months of lactation (Experiment 1).

Variable	Body Weight	Condition Score
Covariate	***	***
Lasalocid (L)	NS	*
Period (month)	*	NS
Month x L	NS	NS

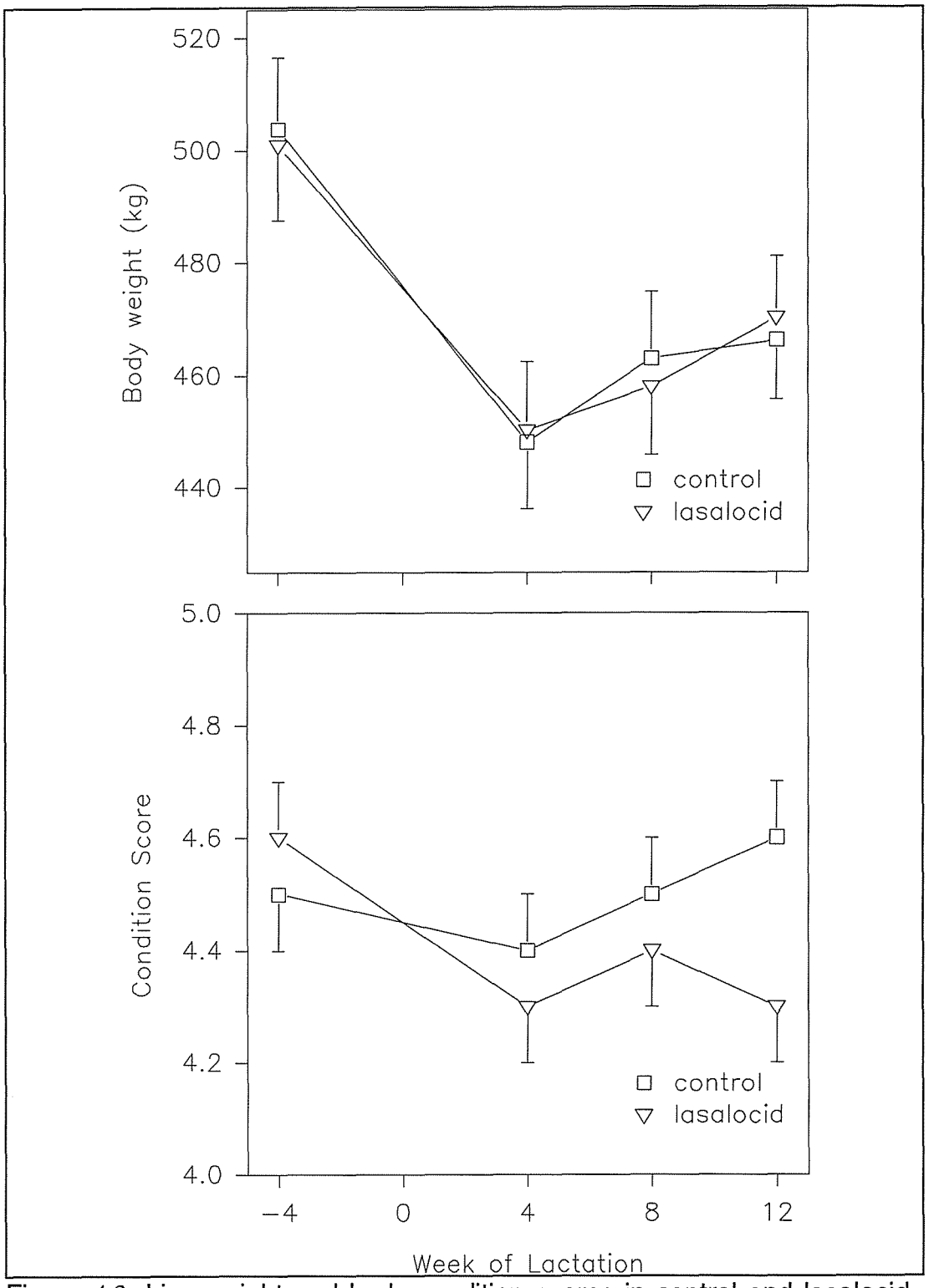


Figure 4.3: Live weight and body condition scores in control and lasalocid–treated dairy cows in early lactation in Experiment 1.

4.1.3 Blood metabolites

Lasalocid treatment did not affect plasma levels of glucose, ß–hydroxybutyrate (ßOHB), non–esterified fatty acids (NEFA), calcium, (Ca) and magnesium (Mg) (Table 4.9). Mean plasma concentrations of ßOHB and NEFA were slightly higher in the lasalocid than in control cows (Table 4.10). Lasalocid treated cows had significantly higher ($P<0.05$) plasma ßOHB concentrations than control cows during week 5 of the experiment. Period (week) by lasalocid interaction for NEFA was statistically significant ($P<0.05$).

Period (week) and period by lasalocid interaction for magnesium approached significance ($P<0.10$). Plasma levels of glucose, ßOHB and NEFA are shown in Figure 4.4 and those of calcium and magnesium are shown in Figure 4.5.

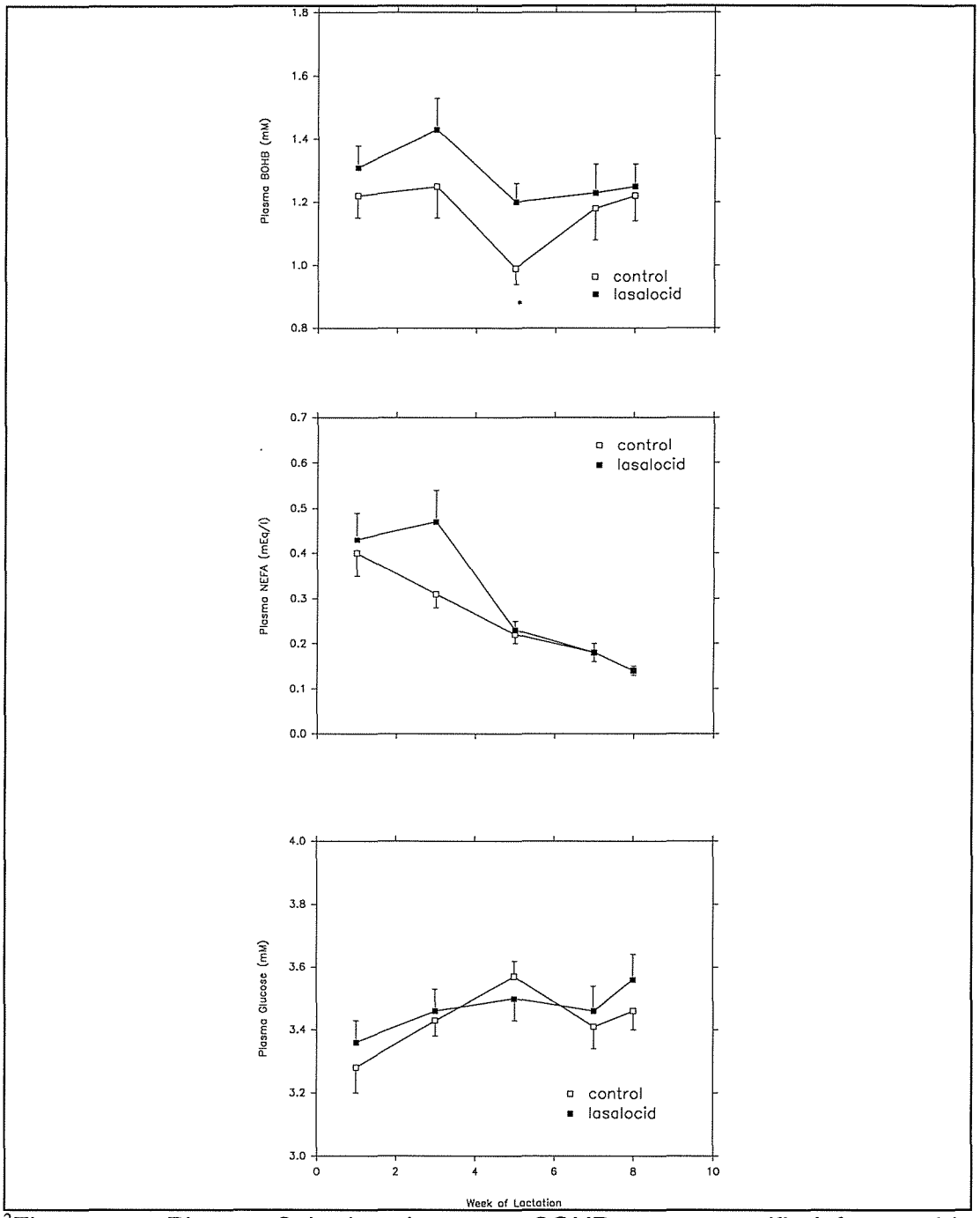
Table 4.9: Significance of effects of lasalocid (L), week of lactation and lasalocid by week of lactation interaction on plasma concentrations of glucose (GLU), ß–hydroxybutyrate, (ßOHB), non–esterified fatty acids, (NEFAs), magnesium, (Mg) and calcium, (Ca).

Variable	GLU	ßOHB	NEFA	Mg	Ca
Covariate	NS	+	NS	**	NS
Lasalocid	NS	NS	NS	NS	NS
Week	NS	NS	NS	+	NS
Week x L	NS	NS	*	+	NS

Table 4.10: Least square means for plasma levels of B–hydroxybutyrate (mM), non–esterified fatty acids (mEq\l), glucose (mM), calcium (mg\dl) and magnesium (mM) in early lactation.

Variable	Control	Lasalocid	SEM
ß–hydroxybutyrate (ßOHB) (mM)	1.160	1.277	0.179
Non–esterified fatty acids (mEq\l)	0.213	0.253	0.049
Glucose (mM)	3.474	3.493	0.135
Calcium (mM)	2.179	2.162	0.068
Magnesium (mM)	0.772	0.776	0.071

2



[2]Figure 4.4: Plasma ß–hydroxybutyrate (ßOHB), non–esterified fatty acids (NEFA) and glucose concentrations of control and lasalocid–treated dairy cows in early lactation.

[2]Differences in plasma ßOHB between the treatment groups for the weeks marked by a symbol (*) are significantly different ($P<0.05$).

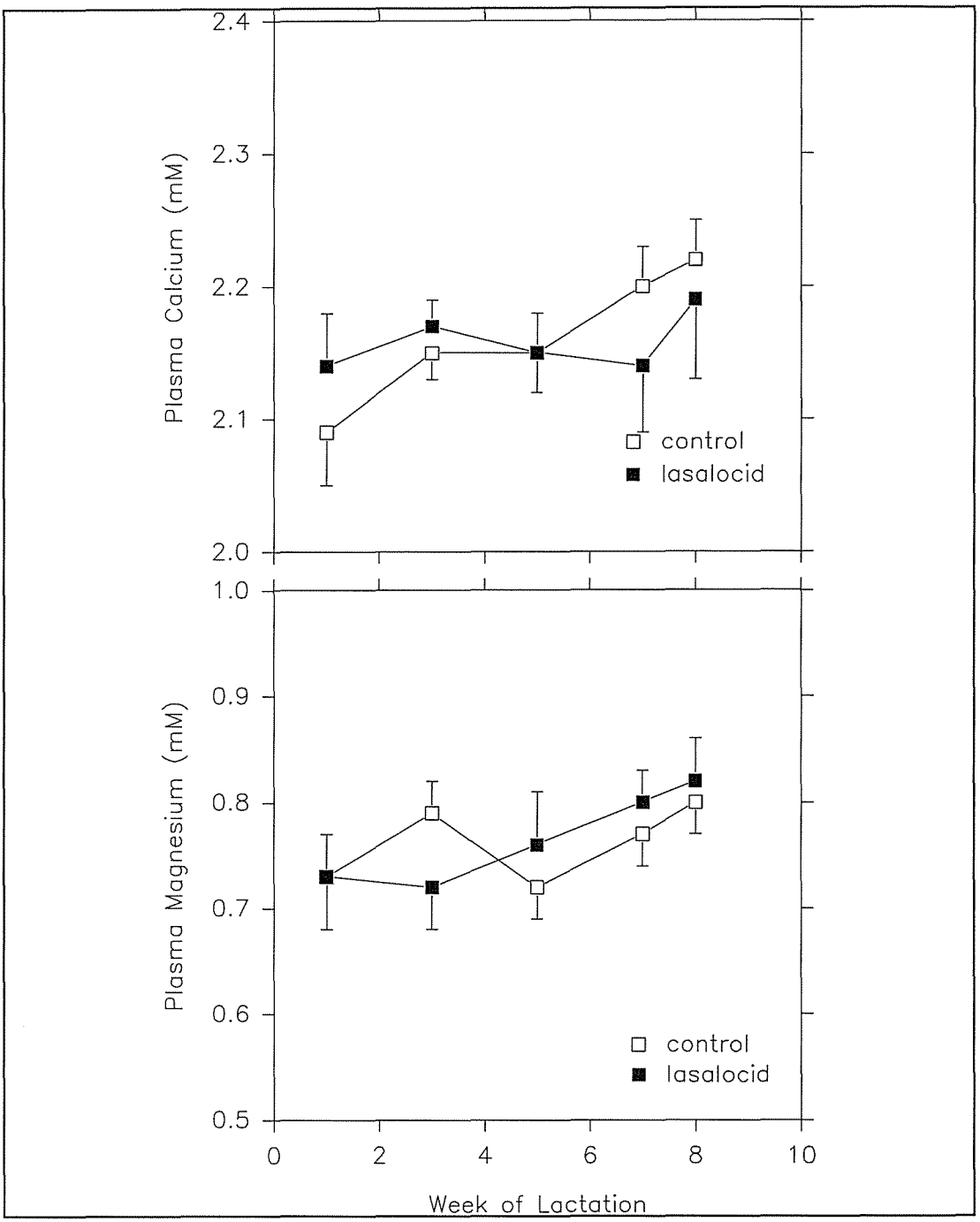


Figure 4.5: Plasma calcium and magnesium in control and lasalocid–treated dairy cows in early lactation.

4.1.4 Somatic cell counts and reproductive performance

Log_{10} somatic cell counts (Log_{10} SCC) and reproductive parameters (calving to first oestrus, calving to conception intervals, and number of services per conception) are given in Table 4.11. There was no indication of an effect of lasalocid on any of these parameters.

Table 4.11: Effects of lasalocid treatment on somatic cell counts (SCC) resumption of ovarian activity and conception in pasture–fed dairy cows in early lactation.

	Treatment Means		
Variable	Control	Lasalocid	SEM
Log_{10} SCC	4.6	4.5	0.5
Reproduction Parameters			
Calving to 1st heat interval (days)	39.2	42.1	6.6
Calving to conception (days)	97.5	92.1	6.8
No. of inseminations \ cow	1.4	1.7	0.2

4.2 Experiment 2

4.2 1 Milk production, Live weight and body condition

Treatment with "Bovatec 20" or "Bloatenz" did not affect milk, fat, protein or lactose yields in cows in mid–lactation. Period (week) effects on fat yield were significant ($P<0.05$) (Table 4.12), because milk yield decreased as expected at this stage of lactation. Treatment with either "Bovatec 20" or "Bloatenz" did not affect live weight, but effects of these treatments on body condition approached significance ($P<0.10$) (Table 4.12). Cows treated with either "Bovatec 20" or "Bloatenz" did not lose as much body condition as cows in the control group. The gross energy content of milk was not affected by either "Bovatec 20" or "Bloatenz".

Least square means for milk, fat, protein and lactose yields, as well as milk composition and gross energy content of milk are given in Table 4.13. Weekly milk yield, fat, protein and lactose yields are presented in Figure 4.6. Initial and final live weights and body condition scores for the three treatment groups are given in Table 4.14.

Table 4.12: Significance of effects of lasalocid or Bloatenz treatment, stage of lactation and treatment by stage of lactation interaction on milk, fat, protein and lactose yield (kg\d), body weight and body condition score (CS) and gross energy content of milk (GE_{milk}) in pasture–fed dairy cows in mid–lactation for Experiment 2.

Variable	Milk	Fat	Protein	Lactose	Weight	CS	GE_{milk}
Covariate	***	***	***	***	***	***	***
Treatment	NS	NS	NS	NS	NS	+	NS
Period	NS	*	NS	NS	NS	+	NS
Period*Trt	NS	NS	NS	NS	NS	NS	NS

Table 4.13: Least square means for milk, fat, protein and lactose yields (kg\d) and milk composition and gross energy content of milk for control, lasalocid and Bloatenz treated dairy cows in mid–lactation for Experiment 2.

	Treatment Group			
Variable	Control	Lasalocid	Bloatenz	SEM
Milk yield	20.94	21.23	21.06	1.800
Fat yield	0.922	0.959	0.966	0.110
Protein yield	0.713	0.724	0.725	0.061
Lactose yield	1.030	1.042	1.034	0.286
Composition				
Fat %	4.43	4.55	4.58	0.47
Protein %	3.41	3.42	3.45	0.13
Lactose %	4.93	4.91	4.91	0.06
$\mathbf{GE_{milk}}$(MJ/day)	749.75	819.60	811.71	73.20

$\mathbf{GE_{milk}}$ refers to the gross energy content of the milk.

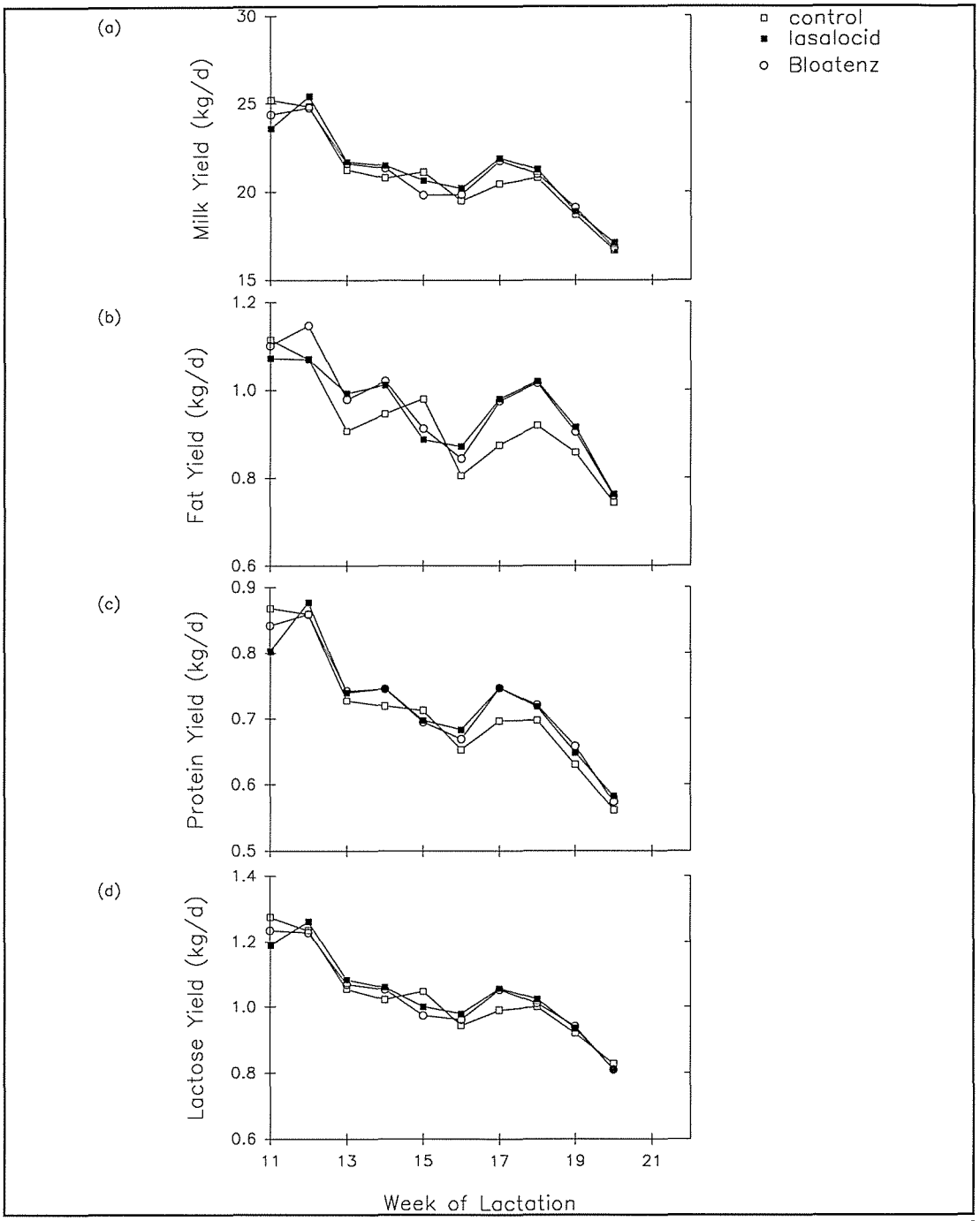


Figure 4.6: Milk yield (a), fat yield (b), protein yield (c) and lactose yield (d) of control, lasalocid and Bloatenz–treated dairy cows in mid–lactation in Experiment 2.

Table 4.14: Initial and final body weight and condition score means (± SE) for control, lasalocid and Bloatenz treated cows in mid–lactation for Experiment 2.

	Treatment Means ± SE		
Variable	Control	Lasalocid	Bloatenz
Initial body weight (kg)	469 ± 11.11	466 ± 10.35	458 ± 10.35
Final body weight (kg)	493 ± 9.81	492 ± 9.13	480 ± 9.13
Changes in live weight	24.79±3.81	26.13±3.68	22.60±3.68
Initial body condition	4.5 ± 0.13	4.7 ± 0.12	4.4 ± 0.12
Final body condition	4.3 ± 0.10	4.6 ± 0.09	4.4 ± 0.09
[a]Changes in condition score	–0.19 ± 0.08	–0.11 ± 0.07	0.07 ± 0.07

[a]Differences between control and Bloatenz cows were significant ($P<0.05$) and differences between control and lasalocid cows approached significance ($P<0.10$).

CHAPTER 5: DISCUSSION

5. DISCUSSION

The purpose of this study was to determine the effect of "Bovatec 20" (lasalocid) on performance and health of dairy cows in early and mid–lactation. The effect of lasalocid on the incidence of bloat in pastured dairy cows in mid–lactation was also of interest.

The aim of Experiment 1 was to evaluate the effects of lasalocid on milk production, live weight changes and reproductive performance in dairy cows in early lactation. Lasalocid treatment increased milk production by an average of 5.5%, or approximately 1.4 kg/cow /day, but this difference did not reach statistical significance. Composition of the milk did not change with regard to fat. Hence fat corrected milk followed a trend similar to that of milk yield.

Lasalocid has resulted in small improvements in milk yield in some studies (Christensen et al., 1994; Weiss and Amiet, 1990;) but depressions in milk yield at high levels of lasalocid inclusion in others (Dye et al., 1988; Johnson et al., 1988) (see Table 2.1, page 13). Similar variable results on milk production have also been reported for monensin–fed dairy cows (Sauer et al., 1989; Maas, 1990).

Treatment with ionophores is associated with a decreased acetate : propionate (A:P) ratio in the rumen. Such a shift results in improved live weight gains in beef cattle (Goodrich et al., 1984). In the dairy cow a decreased A:P ratio should increase glucogenic precursors and subsequently milk production but may result in a depression in milk fat concentration. Johnson et al.(1988) and Dye et al. (1988) reported depressions in milk fat concentration at 550 mg and 333 mg lasalocid per day, respectively. Sauer et al. (1989) also reported fat depression when monensin was fed. In the present study, both milk yield and fat yield increased by 5.5% in lasalocid treated cows (Tables 4.2 and 4.3 respectively) and hence milk fat concentration remained unchanged (Table

4.7). The lasalocid level used in this experiment was 400 mg lasalocid per day, which was higher than the level offered by Dye et al., 1988 but lower than the highest level offered by Johnson et al. (1988).

There were no significant effects of lasalocid on either milk protein or milk lactose concentrations. Milk fat, protein and lactose yields however, were increased by 5.2%, 3.5% and 8.9%, respectively. These results are in general agreement with those of Hopman and Weber (1986) who found no difference in milk protein concentration in lasalocid–fed beef cows, and those of Brown and Hogue (1985) who fed monensin to lactating goats. It is generally accepted that it is very difficult to alter protein production from the mammary gland because of inherent genetic limitations to protein production and/or a possible negative feedback system for actual amount of protein produced (Larson, 1969; Hopman and Weber, 1986).

Concentrations of VFA in rumen fluid were not measured in the present study. However, Maas (1990) reported a decrease in acetate to propionate ratio in monensin treated sheep grazing ryegrass/white clover pastures similar to those used in the present study. The ratio of lipogenic (acetic and butyric) to glycogenic (propionic, isobutyric, valeric and iso–valeric) volatile fatty acids were 3.27 for the control sheep and 2.74 for the treated sheep. Similar changes in the rumen of the cows used in the present experiment would account for the slightly higher milk production in the treated group. However, it is probable that there was only a slight shift towards glucogenic VFA production judging from the low milk production response and the lack of any change in milk composition.

Plasma concentrations of glucose were unaffected by lasalocid treatment. In the ruminant, blood glucose is mainly produced via synthesis from propionate and non–essential amino acids in the liver by gluconeogensis (Bergman et al., 1966). Glucose is required for the synthesis of lactose in the mammary gland of lactating dairy cows. When the supply of glucose precursors is inadequate,

the cow mobilises triglycerides from adipose tissue and synthesizes glucose from the glycerol produced (Manston and Allen, 1981). In this way glycaemia is kept within a specific range.

During maximum energy demand states such as early lactation, lipogenesis and esterification in adipose tissue decreases (McNamara and Hillers, 1986). Glucose and acetate utilisation by the adipose tissue is at a minimum since these substrates are diverted into the process of milk production. Plasma glucose concentration is affected by factors such as age and physiological status (Rowlands, 1980) and thus its use as an indicator of either energy balance or dietary intake energy is limited, particularly in early lactation (Lee et al., 1978; Erfle et al., 1974; Jenny et al., 1974). The fact that blood glucose concentration is of limited value in diagnosing dietary sufficiency of energy may explain why there were no appreciable differences in plasma glucose levels over time in either control or lasalocid treated cows in early lactation.

Non esterified fatty acid concentrations are elevated in ruminants in a negative energy balance and they have been used as indicators of fat mobilisation or to show when there is an energy deficit (Manston and Allen, 1981). Non esterified fatty acid concentrations were not significantly different ($P>0.05$) between the control and lasalocid groups in Experiment 1. Mean plasma NEFA concentrations were highest in the third week of lactation in lasalocid treated cows (Figure 4.4), whereas concentrations in the control group showed a gradual decline from parturition to the 8th week of lactation. The peak concentrations of NEFA in lasalocid treated cows corresponded with peak milk production in lasalocid treated cows (Figure 4.1), indicating that these cows were under a greater negative energy balance than their counterparts. Thus this result in the present study is difficult to interpret, since it was postulated that lasalocid treated cows would have more glucose precursors from the diet, and hence they would not need to draw on their body reserves as much as control cows to produce an equal quantity of milk.

Concentrations of ß–hydroxybutyrate increase when an animal is under increasing energy stress or deficit (Manston and Allen, 1981).
ß–hydroxybutyrate concentrations were not changed by lasalocid treatment (Figure 4.4). However, there was a tendency for plasma concentrations to increase from calving to the third week of lactation and then gradually decline. Data from animals with multiple catheters indicated no significant effect of ionophores on net portal flux of ß–hydroxybutyrate (Harmon and Avery, 1987; Harmon et al., 1988; Harmon et al., 1989).

It is generally accepted that dairy cows lose live weight in early lactation for up to 10 weeks into lactation (Garnsworthy, 1988). Unfortunately body weight and condition scores measurements were missed for the first month of lactation and hence body weights measured at 4 weeks into lactation were considered as the initial measured weights. Lasalocid treated cows did lose more body condition (Figure 4.3), although this was statistically nonsignificant. This loss in body condition therefore suggests that cows treated with lasalocid mobilised more body tissue than control cows in early lactation. Jacques et al. (1987) reported that lasalocid did not affect condition score change in beef cows in winter.

Voluntary food intake was not measured in the present study; therefore, it was not possible to measure gross energy efficiencies of cows in the present experiments. The gross energy content of milk was used as a measure of energy output. Since the composition of milk was unaffected by lasalocid, gross energy content of milk followed a similar trend to milk yield (Figure 4.2), and differences between groups approached significance in weeks 3, 6, 7 and 9. This may have been a random effect brought about by the daily variation in milk fat, protein and lactose yields or an indication that lasalocid marginally improved the efficiency of milk production.

Plasma calcium concentrations were unaffected by lasalocid treatment. This agrees with most workers who have reported no change in calcium absorption in lasalocid treated cattle (Spears and Harvey, 1984; Starnes et al., 1984; Darden et al., 1985; Valdes et al., 1988; Reffett–Stabel et al., 1989).

Magnesium concentrations were also unaffected by lasalocid treatment. Mean plasma levels of magnesium were the same at the beginning of the experiment but mean levels in the control group initially increased then declined, whereas the levels for the lasalocid treated group showed a gradual and steady increase. Greene et al. (1988) reported an increased absorption of magnesium from a concentrate/cottonseed hull diet fed to steers given monensin. Wilson et al. (1993) also reported higher plasma magnesium concentrations in grazing cows treated with monensin before calving. Plasma magnesium alone may be a poor indicator of magnesium absorption and status because magnesium absorbed in excess of need is excreted through urine (Ammerman et al., 1972). Urinary magnesium has been reported to be a better indicator of magnesium status than plasma magnesium when plasma concentrations are within the normal range (Halse, 1970).

The 'normal range' for both calcium and magnesium quoted by Manston and Allen (1981) are; calcium 2.18 – 2.58 mmol/l and magnesium 0.85 – 1.25 mmol/l. The lack of an effect of lasalocid on plasma mineral concentration in the present experiment could imply that animals were neither hypocalcaemic nor hypomagnesaemic. However, initial plasma calcium levels were marginal and plasma magnesium concentrations were lower than the range quoted above.

There are many interrelationships among dietary factors affecting blood concentrations of minerals. Grazing of early spring pasture often results in high concentrations of rumen ammonia which interfere with absorption and/or utilisation of magnesium (Hays and Swenson, 1970; O'Dell, 1960). High potassium in young plants can create a magnesium–potassium imbalance reducing absorption and utilisation of magnesium (Hays and Swenson, 1970).

In the present experiment, the opposing trends in magnesium and calcium concentrations are of interest (Figure 4.5) but no explanation for this can be offered.

There were no obvious differences in the reproductive parameters in Experiment 1. Several researchers including Lemenager et al. (1978a), Walker et al. (1980a) and Hixon et al. (1982a) have reported no differences in first service conception rate with the use of monensin. However, Turner et al. (1977), Lemenager et al., (1978), and Hixon et al. (1982b) found that in monensin–fed cows there was a significant reduction in number of days to first postpartum oestrus and this was associated with a higher live weight or antiketogenic effects (see Review section 2.3.3 d (iii)). In the current experiment, there were no apparent effects on glucose concentration, weight gain (or condition) or antiketogenic effects (NEFA and ßOHB) so the lack of an effect on reproduction is not surprising.

The aims of Experiment 2 were to investigate the effects of lasalocid on milk production, live weight and body condition changes as well as effects on the incidence and intensity of bloat in cows in mid–lactation. It has been observed that with continual exposure to ionophores, rumen microorganisms become adapted and can grow in the presence of ionophores (Chen and Wolin, 1979; Dennis et al., 1981) and hence, the responses to ionophores may diminish with time. In order to avoid this, a new group of cows that had not been exposed to lasalocid prior to this experiment were used.

There was no bloat observed in either the control, or the lasalocid or Bloatenz "treated cows". Thus this aspect of the experiment will not be discussed since there were no data. Milk and milk component yields of cows in Experiment 2 were not affected by either lasalocid or Bloatenz treatment. Milk yield had started to decline when Experiment 2 commenced. The trend in milk yield changes was similar in all the three groups. This agrees with observations of Sauer et al. (1989) who found that the effects of monensin on milk production

and composition disappeared by week 12 of lactation.

Mean body weight gains over the 10 week period were 24.8, 26.1 and 22.6 kg for the control, lasalocid and Bloatenz groups, respectively and were not significantly different. Mean condition score changes in the treatments over the same period were –0.19, –0.11 and 0.07 units for the control, lasalocid and Bloatenz cows, respectively, and these changes were significantly different among the groups (P=0.056). The improved rate of gain of body condition could imply an increased rate of body fat deposition in lasalocid and Bloatenz–treated cows.

To summarize, previous research has suggested that supplementing with ionophores may increase nutritional and production performance in ruminants. In the present study however, lasalocid did not significantly affect milk yield and milk composition. Body weight and gross energy in milk were unaffected by lasalocid both in early and late lactation. Lasalocid–treated cows lost more body condition in early lactation but lasalocid treatment improved body condition in mid–lactation. Plasma levels of glucose, NEFA, ß–hydroxybutyrate, calcium and magnesium in early lactation were all unaffected by lasalocid feeding.

In conclusion, it may be stated that feeding lasalocid resulted in positive, but very small increases in production (significant in some weeks) in early lactation and possibly a minor negative influence on body condition. Therefore the use of lasalocid to improve performance in dairy cattle may not be viable unless other effects of lasalocid such as its effects on bloat are considered. Since this aspect was not adequately covered in the present study, further study is needed in this area. It also appears from the literature that emphasis has been focused on the effects of ionophores on protein and energy utilisation with only limited research on mineral metabolism. More research into the uptake and utilisation of minerals is needed since ionophores are directly involved in cation utilisation by bacterial and mammalian cells.

REFERENCES

REFERRENCES

Ammerman, C.B., Chicco, C.F., Loggins, P.E. & Arlington, L.R. 1972. Availability of different inorganic salts of magnesium to sheep. *Jounal of Animal Science* **34**: 122–126.

Baile, C.A., McLaughlin, C.L. & Chalupa, W.V. 1980. Effects of monensin fed to replacement dairy heifers during the growing and gestation period upon growth, reproduction and subsequent lactation. *Journal of Dairy Science* **65**: 1941–1944.

Bartley, E.E., R.M. Meyer & Fina, L.R. 1975. Feedlot and grainbloat. In: Digestion and Metabolism in the Ruminants. McDonald, I.W. and Warner, A.C.I (eds), pp 551–562, The University of New England Publishing Unit. Armidale, N.S.W., Australia.

Bartley, E.E.,T.G. Nagaraja, E.S. Pressman, A.D. Dayton, M.P. Kartz & Fina, L.R. 1983. Effects of lasalocid or monensin on legume or grain (feedlot) bloat. *Journal of Animal Science* **56**: 1400–1406.

Beede, D.K.,G.T. Schelling, G.E. Mitchell, Jr., R.E. Tucker, W.W. Gill, S.E. Koenig, & Lindsey, T.O. 1986. Nitrogen utilisation and digestibility by growing steers and goats that contain monensin and low crude protein. *Journal of Animal Science* **62**: 857–863.

Beede, D.K., Schelling, G.T., Mitchell Jr, G.E. & Tucker, R.E. 1985. Utilization by growing goats of diets that contain monensin and low or excess crude protein: Comparative slaughter experiment. *Journal of Animal Science* **61**: 1230–1242.

Bergen, W.G. & Bates, D.B. 1984. Ionophores: Their effect on production efficiency and mode of action. *Journal of Animal Science* **58**: 1465–1483.

Berger, L.L., Ricke, S.C. & Fahey Jr, G.C. 1981. Comparison of two forms and two levels of lasalocid with monensin on feedlot cattle performance. *Journal of Animal Science* **53**: 1440–1445.

Bergman, E.N., Roe, W.E. & Kon, K. 1966. Quantitative aspects of propionate metabolism and gluconeogensis in sheep. *American Journal of Physiology* **211**: 793–799.

Bird, S.H. & Leng, R.A. 1978. The effects of defaunation of the rumen on the growth of cattle on low–protein high–energy diets. *British Journal of Nutrition* **40**: 163–167.

Brown, D.L. & Hogue, D.E. 1985. Effect of feeding monensin sodium to lactating goats: Milk composition and ruminal volatile fatty acid acids. *Journal of Dairy Science* **68**: 1141–1147.

Chalupa, W. 1977. Manipulating rumen fermentation. *Journal of Animal Science* **45**: 585–599.

Chalupa, W. 1980. Chemical control of rumen microbial metabolism. In: Digestive Physiology and Metabolism in Ruminants: Proceedings of the 5th International Symposium on Ruminant Physiology. Ruckebush, Y and Thivend, P., (eds), pp325–347, MTP Press Ltd.

Chen, M. & Wollin, M.J. 1979. Effect of monensin and lasalocid–sodium on the growth of methanogenic and rumen saccharolytic bacteria. *Applied and Environmental Microbiology* **38**: 72–77.

Christensen, D.E., Wiedmeier, R.D., Shenion, H.T., Bowman, B.R. & Olson, K.C. 1994. Effects of graded levels of dietary lasalocid on performance of Holstein cows during early lactation. *Proceedings, Western Section, American Society of Animal Science* **45**: 328–330.

Clarke, R.T.J. & Reid, C.S.W. 1974. Foamy bloat of cattle: A review. *Journal of Dairy Science* **57**: 753–785.

Counotte, G.H.M., Prins, R.A., Janssen, R.H.A.M. & deBie, M.J.A. 1981. Role of *Megasphaera elsdenii* in the fermentation of DL–[2–^{13}C]lactate in the rumen of dairy cattle. *Applied and Environmental Micribiology* **42**: 649–655.

Darden, D.E., Merchen, N.R., Berger, L.L. & Fahey Jr, G.C. 1985. Effects of avoparcin, lasalocid and monensin on sites of nutrient digestion in beef steers. *Nutrition Reports International* **31**: 979–989.

Dennis, S.M., Nagaraja, T.G. & Bartley, E.E. 1981. Effect of lasalocid or monensin on lactate producing or using bacteria. *Journal of Animal Science* **52**: 418–426.

Dennis, S.M., Nagaraja, T.G. & Dayton, A.D. 1986. Effect of lasalocid, monensin and thipeptin on rumen protozoa. *Research in Veterinary Science* **41**: 251–256.

Durand, M. & R. Kawashimi, 1980. Influence of minerals in rumen microbial digestion. In: Digestive Physiology and metabolism in Runinants: Proceedings of the 5th International Symposium on Ruminant Physiology. Ruckebusch, Y and Thivend, P., (eds), pp375–408, MTP Press.

Dye, B.E., Amos, H.E. & Froetschel, M.A. 1988. Influence of lasalocid on rumen metabolites, milk production, milk composition and digestibility in lactating cows. *Nutrition Reports International* **38**: 101–115.

Eadie, J.M. & Gill, J.C. 1971. The effect of the absence of rumen ciliate protozoa on growing lambs fed on a roughage–concentrate diet. *British Journal of Nutrition* **26**: 155–161.

Erfle, J.D.,Fisher, L.J. & Sauer, F.O. 1974. Interrelationships between blood metabolites and an evaluation of their use as criteria of energy status of cows in early lactation. *Canadian Journal of Animal Science* **54**: 293–303.

Faulkner, W.R. 1982. In: Selected Methods for the Small Clinical Laboratory, AACC, Washington DC, USA pp277–280.

Fontenot, J.P., Allen, V.G., Bunce, G.E. and Goff, J.P. 1989. Factors influencing magnesium absorption and metabolism in ruminants. *Journal of Animal Science* **67**: 3445–3455.

Garnsworthy, P.C. 1988. The effect of energy reserves at calving on performance of dairy cows. In: Nutrition and Lactation in the Dairy Cow. Garnsworthy, P.C., (ed), pp157–170, Butterworths, London.

Goodrich, R.D., Garrett, J.E., Gast, D.R., Kirick, M.A., Larson, D.A. & Meiske, J.C. 1984. Influence of monensin on the performance of cattle. *Journal of Animal Science* **58**: 1484–1498.

Grace, N.D. 1983. Magnesium. In: The mineral requirements of grazing ruminants. New Zealand Society of Animal Production, Occasional Publication No.9. pp92–99, Grace, N.D, (ed).

Greene, L.W., May, B.J.,Schelling, G.T. & Buyers, F.M. 1988. Site and extent of apparent magnesium and calcium absorption in steers fed monensin. *Journal of Animal Science* **66**: 2987–2991.

Greene, L.W., Schelling, G.T., & Byers, F.M. 1986. Effects of dietary monensin and potassium on apparent absorption of magnesium and other macro elements in sheep. *Journal of Animal Science* **63**: 1960–1967.

Grings, E.E. & Males, J.R. 1988. Performance, blood and ruminal characteristics of cows receiving monensin and a magnesium supplement. *Journal of Animal Science* **66**: 566–573.

Gutierrez, G.G., L.M. Schake & Byers, F.M. 1982. Whole plant grain sorghum silage processing and lasalocid effects on stocker calf performance and rumen fermentation. *Journal of Animal Science* **54**: 863–868.

Halse, K. 1970. Individual variation in blood magnesium and susceptibility to hypomagnesemia in cows. *Acta Veterinaria Scandinavica* **11**: 394–414.

Hanley, H.G. & Slack, J.D. 1982. Pharmacology of lasalocid. In: Polyether Antibiotics Volume 1. Westley, J.W. (ed), Marcel Dekker Inc. New York, NY.

Harmon, D.L. & Avery, T.B. 1987. Effect of dietary monensin and sodium propionate on net nutrient flux in steers fed a high concentrate diet. *Journal of Animal Science* **65**: 1610–1615.

Harmon, D.L., Gross, K.L. & Avery, T.B. 1989. Influence of ionophore addition to a high–concentrate diet on net nutrient flux in steers. *Nutrition Reports International* **40**: 1127–1135.

Harmon, D.L., Avery, T.B., Huntington, G.B. & Reynolds, P.J. 1988. Influence of ionophore addition to roughage and high–concentrate diets on portal blood flow and net nutrient flux in cattle. *Canadian Journal of Animal Science* **68**: 419–429.

Hays, V.W. & Swenson, M.J. 1970. Minerals. In: Duke's Physiology of Domestic Animals, (8th edition). Swenson, M.J, (ed), pp663–690, Comstock Publishing Association, London.

Hino, T. & Russel, J.B. 1986. Relative contributions of ruminal bacteria and protozoa to the degradation of protein in vitro. *Journal of Animal Science* **64**: 261–270.

Hixon, D.L., Fahey Jr, G.C., Kesler, D.J. & Neumann, A.L. 1982a. Effects of creep feeding and monensin on reproductive performance and lactation of beef heifers. *Journal of Animal Science* **55**: 467–474.

Hixon, D.L., Fahey Jr, G.C., Kesler, D.J. & Neumann, A.L. 1982b. Effects of energy level and monensin on reproductive performance and lactation of beef cows. *Theriogenelogy* **17**: 515–525.

Holmes, C.W. & Wilson, G.F. 1987. Milk Production From Pastures Butterworths, Wellington, New Zealand. 319 p.

Holmes, C.W., Wilson, G.F.,Kuperus, W., Buvaneshwa & Wickham, B. 1993. Live weight, feed intake and feed conversion efficiency of lactating dairy cows. *Proceedings of the New Zealand Society of Animal Production* **53**: 95–99.

Hopman,B. & Weber, D.W. 1986. Effect of lasalocid on fall calving beef cows. *Journal of Animal Science* **63**: 1722–1727.

Horton, G.M.L., Farmer, M.J. Bassendowski, K.A. & Steacy, G.M. 1983. Rumen fermentation and digestibility in steers as influenced by level of intake and monensin. *Canadian Journal of Animal Science* **63**: 855–860.

Horton, G.M.J. & Brandt, W.E. 1981. Response of backgrounding calves fed a high silage diet and different protein sources to zeranol implants and lasalocid. *Journal of Animal Science* **53 (suppl. 1)**: 406.

Hungate, R.E. 1966. The Rumen and its Microbes. Academic Press, London. 533p.

Isichei,.C.O. & Bergen, W.G. 1980. The effect of monensin on the composition of abomasal nitrogen flow in steers fed grain silage ration. *Journal of Animal Science* **51 (suppl.1)**: 371.

Jacques, K.A., Cochram, R.C., Corah, L.R., Avery, T.B., Zoellner, K.O. & Higginbotham, J.F. 1987. Influence of lasalocid level on forage intake, digestibility, ruminal fermentation, liquid flow and performance of beef cattle grazing winter range. *Journal of Animal Science* **65**: 777–785.

Jenny, B.F.,Polan, C.E. & Thye, F.W. 1974. Effects of high grain feeding and stage of lactation on serum insulin, glucose and milk fat percentage in lactating cows. *Journal of Nutrition* **104**: 379–385.

Johnson, J.C.Jr.,P.R. Utley, B.G. Mullinix, Jr & Merrill, A. 1988. Effects of adding fat and lasalocid to diets of dairy cows. *Journal of Dairy Science* **71**: 2151–2165.

Larson, B.L. 1969. Biosynthesis of milk. *Journal of Dairy Science* **52**: 737–747.

Lee, A.J., Twardock, A.R., Burbar, R.H., Hall, J.E. & Davis, C.L. 1978. Blood metabolic profiles: Their use and relation to nutritional status of dairy cows. *Journal of Dairy Science* **61**: 1652–1670.

Lemenager, R.P., Owens, F.N., Lusbey, K.S. & Totusek, R. 1978a. Monensin, forage intake and lactation of range beef cows. *Journal of Animal Science* **47**: 247–254.

Lemenager, R.P., Owens, F.N. Shockey, B.J. Lusbey, K.S. & Totusek, R. 1978b. Monensin effects on rumen turnover, 24 hour VFA patterns, nitrogen components, and cellulose disappearance. *Journal of Animal Science* **47**: 255–261.

Lynch, G.A., Hunt, M.E. & McCutcheon, S.N. 1990. A note on the effect of monensin sodium administered by intraruminal controlled release devices on productivity of dairy cows at pasture. *Animal Production* **51**: 418–421.

Maas, J.A. 1990. The effect of monensin sodium on lactational performance of autumn calved dairy cows. *M.Agr.Sc. Thesis. Massey University, Palmerston North, New Zealand.* 80p.

Majak, W.,R.E. Howarth, K.J. Cheng & Hall, J.W. 1983. Rumen conditions that predispose cattle to pasture bloat. *Journal of Dairy Science* **66**: 1683–1688.

Manston, R. & Allen, W.M. 1981. Modern diagnostic methods in practice: The use of blood chemistry in monitoring the health of farm livestock. *British Veterinary Journal* **137**: 241–247.

Martens, H., J.Harmeyer & Michael, H. 1978. Magnesium transport by isolated rumen epithelium of sheep. *Research in Veterinary Science* **24**: 161–168.

McCutcheon, S.N. & Bauman, D.E. 1986. Effect of chronic growth hormone treatment on responses to epinephrine and thyrotropin releasing hormone in lactating cows. *Journal of Dairy Science* **69**: 44–51.

McDonald, P., Edwards, R.A. & Greenhalgh, J.F.D. 1988. Animal Nutrition, 4th edition. Chapter 8. Longman Scientific and Technical, London.

McDowell, L.R., Conrad, J.H., Ellis, G.L. & Loosli, J.K. 1983. Minerals for Grazing Ruminants in Tropical Regions. U.S. Agency for International Development Bulletin. University of Florida, Gainesville, pp 5–20.

McNamara, J.P.& Hillers, J.K. 1986. Regulation of bovine adipose tissue metabolism during lactation. 1. Lipid synthesis in response to increased milk production and decreased energy intake. *Journal of Dairy Science* **69**: 3032–3041.

Meinert, R.A., Yang, C.M.J., Heinreichs, A.J., & Varga, G.A. 1992. Effect of monensin on growth, reproductive performance, and estimated body composition in Holstein heifers. *Journal of Dairy Science* **75**: 257–261.

Michaylova, V. & Illkova, P. 1971. Photometric determination of micro amounts of calcium with Arsenazo III. *Analytica. Chimica Acta* **53**: 194–198.

Morris, F.E., Branine, M.E., Galyeau, M.L., Hubbert, M.E., Freeman, A.S. & Lofgreen, G.P. 1990. Effect of rotating monensin plus tylosin and lasalocid on performance, ruminal fermentation and site and extent of digestion in feedlot cattle. *Journal of Animal Science* **68**: 3069–3078.

Morris, J.G. 1980. Assessment of sodium requirements of grazing beef cattle. A review. *Journal of Animal Science* **50**: 145–152.

Moseley, W.M., McCartor, M.M. & Randel, R.D. 1977. Effects of monensin on growth and reproductive performance of beef heifers. *Journal of Animal Science* **45**: 961–968.

Muntifering, R.B., Theurer, B. & Noon, T.H. 1981. Effects of monensin on site and extent of whole corn digestion and bacterial protein synthesis in beef steers. *Journal of Animal Science* **53**: 1565–1573.

Nagaraja, T.G. & Taylor, M.B. 1987. Susceptibility and resistance of ruminal bacteria to antimicrobial feed additives. *Applied and Environmental Microbiology* **53**: 1620–1625.

O'Dell, B.L. 1960. Magnesium requirements and its relation to other dietary constituents. *Federation Proceedings* **19**: 648–654.

Owens, F.N., Shockey, B.J., Fent, R.W. & Rust, S.R. 1978. Monensin and abomasal protein passage of steers. *Journal Animal Science* **47(suppl. 1)**: 114.

Painter, G.R., Pollock, R & Pressman, B.C. 1982. Conformational dynamics of the carboxylic ionophore lasalocid A underlying cation complexation–decomplexation and membrane transport. *Biochemistry* **21**: 5613–5620.

Paterson, J. A., Anderson, B.M., Bowman, Morrison, R.L.& Williams, J.E. 1983. Effect of protein source and lasalocid on nitrogen digestibility and growth by ruminants. *Journal of Animal Science* **57**: 1537–1544.

Poos, M.I., Hanson, T.L. & Klopfenstein,T.J. 1979. Monensin effects on diet digestibility, ruminal protein by–pass and microbial protein synthesis. *Journal of Animal Science* **48**: 1516–1524.

Pressman, B.C. 1973. Properties of ionophores with broad range cation selectivity. *Federation Proceedings* **32**: 1698– 1703.

Pressman, B.C. 1976. Biological application of ionophores. *Annual Review of Biochemistry* **45**: 501–530.

Preston, T.R. & Leng, R.A. 1987. Matching Ruminant Production Systems with Available Resources in the Tropics and Sub–tropics. Chapter 3. Penambul Books, Armidale, NSW.

Reffet-Stabel, J., Spears, J.W. Harvey, R.W. & Lucas, D.M. 1989. Salinomycin and lasalocid effects on growth rate, mineral metabolism and ruminal fermentation in steers. *Journal of Animal Science* **67**: 2735–2742.

Rennick, K.K. & Russel, J.R. 1982. The effects of monensin supplementation of baled corn stover in lactating beef cows. *Journal of Animal Science* **51 (suppl 1)**: 391.

Richardson, L.E., Raun, A.P., Potter, E.L., Cooley, C.O. & Rathmacher, R.P. 1976. Effect of monensin on rumen fermentation *in vitro* and *in vivo*. *Journal of Animal Science* **43**: 657–664.

Rowlands, G.J. 1980. Concentrations of glucose, albumin, inorganic phosphate and sodium in the blood of beef calves at 2½ months of age and their relationships with subsequent weight gain. *Journal of Agricultural Science (Cambridge)* **94**: 95–104.

Russel, J.B. 1987. A proposed model of monensin action in inhibiting rumen bacterial growth: effects on ion flux and protonmotive force. *Journal of Animal Science* **64**: 1519–1525.

Russel, J.B. & H.J. Strobel, 1989. Effect of ionophores on ruminal fermentation. A minireview. *Applied and Environmental Microbiology* **55**: 1–6.

Rust, S.R., Owens, F.N., Thornton, J.H. & R.W. Fent. 1978. Monensin and digestibility of feedlot rations. *Journal of Animal Science* **47 (suppl.1)** : 437.

S.A.S. 1985. Statistical analysis systems. *User's guide.* SAS Institute Inc. North Carolina, USA. 956pp.

Sauer, F.D.,Kramer, J.K.G. & Cantwell, W.J. 1989. Antiketogenic effects of monensin in early lactation. *Journal of Dairy Science* **72**: 436–442.

Schelling, G.T. 1984. Monensin mode of action in the rumen. *Journal of Animal Science* **58**: 1518–1527.

Sejrsen, K. 1978. Mammary development and milk yield in relation to growth rate in dairy and dual purpose heifers. *Acta Agriculturae Scandinavica* **28**: 41–45.

Slyter, L.L. 1976. Influence of acidosis on rumen function. *Journal of Animal Science* **43**: 910–929.

Smith, M.F., Shipp, L.D., Sangster, W.N., Wiltbank, J.N. & Carroll, L.H. 1980. Effect of monensin, energy level and low body condition on pregnancy rate in non lactating beef cows. *Theriogenology* **14**: 91–104.

Spears, J.W. & Harvey, R.W. 1984. Performance, ruminal and serum characteristics of steers fed lasalocid on pasture. *Journal of Animal Science* **58**: 460–464.

Sprott, L.R., Goehring, T.B., Beverley, J.R. & Corah, L.R. 1988. Effects of ionophores on cow herd production: A review. *Journal of Animal Science* **66**: 1340–1345.

Starnes, S.R., Spears, J.W., Froetschel, M.A. & Croom Jr., W.J. 1984. Influence of monensin and lasalocid on mineral metabolism and ruminal urease activity in steers. *Journal of Nutrition* **114**: 518–525.

Steen, T.M., Quigley III, J.D., Heitmann, R.N., & Gresham, J.D. 1992. Effects of lasalocid and undegradable protein on growth and body composition of Holstein heifers. J. Dairy Sci. **75**: 2517–2523.

Thonney, M.L., Heide, E.K., Duhaime, D.J., Hand, R.J. & Perosio, D.J. 1981. Growth, feed efficiency and metabolite concentrations of cattle fed high forage diets with lasalocid or monensin supplements. *Journal of Animal Science* **52**: 427–433.

Thornton, J.H. & Owens, F.N. 1981. Monensin supplementation and *in vitro* methane production by steers. *Journal of Animal Science* **52**: 628–634.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Annals of Clinical Biochemistry* **6**: 24–27.

Turner, H.A., Young, D.C.,Raleigh, R.J. & ZoBell, D. 1980. Effect of various levels of monensin on efficiency and production of beef cows. *Journal of Animal Science* **50**: 385–390.

Turner, H.A., Raleigh, R.J. & Young, D.C. 1977. Effect of monensin on feed efficiency for maintaining gestating mature cows wintered on meadow hay. *Journal of Animal Science* **44**: 338–342.

Valdes, J.L., McDowell, L.R., & Wilkinson, N. 1988. Lasalocid for grazing steers administered in a free–choice mineral mix. *Nutrition Reports International* **38**: 1–8.

Van Nevel, C.J. & Demeyer, D.I. 1977. Effect of monensin on rumen metabolism *in vitro*. *Applied Environmental Microbiology* **34**: 251–257.

Walker, P.M., Weichanthal, B.A & Cmarik, G.F. 1980a. Conception rates in synchronized beef cows with and without monensin feeding. *Journal of Animal Science* **51**: 526–531.

Walker, P.M., Weichanthal, B.A & Cmarik, G.F. 1980b. Efficacy of monensin for beef cows. *Journal of Animal Science* **51**: 532–538.

Wedegaertner, T.C. & Johnson, D.E. 1983. Monensin effects on digestibility, methanogenesis and heat increment of a cracked corn–silage diet fed to steers. *Journal of Animal Science* **57**: 168–177.

Weiss, W.P. & Amiet, B.A. 1990. Effect of lasalocid on performance of lactating dairy cows. *Journal of Dairy Science* **73**: 153–162.

Whetstone, H.D., C.L. Davis & Bryant, M.P. 1981. Effect of monensin on breakdown of protein by ruminal microoganisms *in vitro*. *Journal of Animal Science* **53**: 803–809.

Williamson, D.N. & Mellanby, J. 1974. D–(–)–3–Hydroxybutyrate. In: Methods of enzymatic analysis Volume 4. Bergmeyer, H.E. (ed), pp 1836–1839. Academic Press, London.

Wilson, G.F., Lynch, G.A. & van Der Wel, C. 1993. Effects of rumensin anti–bloat capsules on plasma magnesium concentration and aspects of health and performance of pastured dairy cows. *Proceedings of the New Zealand Society of Animal Production* **53**: 247–250.

Worrel, M.A., Undersander, D.J., Thompson, C.E. & Bridges Jr, W.C. 1990. Effects of time of season and cottonseed meal and lasalocid supplementation on steers grazing rye pastures. *Journal of Animal Science* **68**: 1151–1157.

Zinn, R.A. 1987. Influence of lasalocid and monensin plus tylosin on comparative feeding value of steam–flaked versus dry–rolled corn in diets for feedlot cattle. *Journal of Animal Science* **65**: 256–266.