EFFECTS OF MONENSIN DELIVERED BY A SLOW RELEASE DEVICE ON ASPECTS OF PERFORMANCE IN DAIRY HEIFERS

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Abstract

Two series of trials (from August 1991 to February 1992 and from April to July 1992) were conducted to evaluate the effect of monensin on the growth, reproductive performance and *Eimeria* oocyst counts of dairy heifers in New Zealand. Two hundred heifers were divided according to their weight into two groups at the beginning of the trials. Heifers with similar weights were assigned randomly to either of two treatments: with or without monensin boluses that delivered 200 mg of monensin per day. Monensin had no effect on weight gains in both parts of the trial. A significant increase in height was observed during the second part of the trial. Monensin significantly decreased ($p<0.05$) the oocyst counts in both parts of the trial and plasma progesterone levels 100 days after the first administration of boluses. Although conception rates and age of heifers at calving were not affected by monensin, the weight of calves was significantly increased by the ionophore. These results indicate that monensin can influence reproductive performance of heifers without affecting their body weight. In addition, its properties as a coccidiostat were confirmed.

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The Effects of Monensin in Heifers

1.1 Introduction

After weaning, dairy herd owners seek to spend the minimum of time and effort in the management of future replacement heifers. Puberty is an important occurrence in heifers because it marks the beginning of their reproductive life. The earlier it occurs, the more offspring and milking days they may produce in their lifetime (Sorensen, 1979).

This means that if nutrition is adequate and parasite control measures are effective, heifers conceive at 15 months of age to calve at two years old (Macmillan, 1978). However, factors such as age, weight, nutrition, environment and congenital factors can affect puberty in heifers (Holmes *et al.*, 1987).

Age at puberty is an important factor in determining the efficiency of production in a herd. Heifers that are late maturing will not have the chance to be bred or will be bred late and either of these can decrease production efficiency (Short *et al.*, 1976). Regarding the effect of weight on puberty, Arije *et al.* (1971) concluded that heifers that grew faster preweaning tended to reach puberty at an earlier age and a heavier weight. Therefore, age at puberty is highly correlated with body weight.

Heifers calving early the first time continue calving early in subsequent years and wean heavier calves. Age at puberty can be altered by various genetic (Laster *et al.*, 1972), nutritional (Short *et al.*, 1971) or hormonal (González-Padilla *et al.*, 1975) factors although some approaches are not economical and other alternatives are required.

The manipulation of ruminal fermentation offers the possibility of integrating all these elements to attain the target of earlier puberty and heavier animals.

Fermentation efficiency and nutrient outflow from the rumen can be adjusted by manipulating the existing microflora and their metabolic activities. This can be achieved by several means such as:

- The use of chemical agents that modulate selected pathways of metabolism (volatile fatty acid production and ureolisis).
- Control of water removal from the rumen.
- Regulation of pH.

The type and extent of manipulation depend upon the nature of the diet and physiological functions performed. Animal productivity can be maximised by considering these two factors (Chalupa, 1977). During the past two decades, chemical manipulation of digestive end-products has focused on the utilization of ionophores. These compounds have become recognised as an important tool in ruminant nutrition (Rumsey, 1984).

1.2 Discovery of Ionophores

The discovery of ionophores started with earlier studies of bioenergetics. The topic investigated was how mitochondria converted metabolic energy into ATP and how this process was inhibited by certain guanidine derivatives such as synthalin and phenethylbiguanide (DBI). Pressman (1976) observed that the difference in potency of these inhibiting agents was due to their lipophilic properties and their ability to interact with mitochondrial membranes. He also noticed that for each carbon the alkyl chain was lengthened, the potency for the alkylguanidine to inhibit mitochondria increased by a factor of approximately 2.5.

Guanidine derivatives produced a slow inhibition of the respiration of mitochondria actively synthesizing ATP. This inhibition was reversed by compounds termed "uncoupling agents".

Ionophores are produced by organisms of the order *Actinomycetales*. The majority are produced by the genus *Streptomyces* (from where they were first isolated during the 1950s) and a few are by the genera *Actinomydura* and *Dactylosporangium* (Painter and Pressman, 1985).

These compounds were powerful uncouplers of mitochondrial oxidative phosphorylation. Pressman (1985) reported that when first studied in 1959, the cyclic depsipeptide valinomycin acted as an uncoupling agent at concentrations as low as 10^{-8} M. It was the most powerful uncoupling agent known at that time and was distinct from the other subclasses of uncoupling agents studied until then. Its existence was confirmed when another compound that exhibited the same behaviour as valinomycin - gramicidin - was discovered. Proton movement induced by valinomycin was established to be energy-dependent.

Valinomycin was observed to induce the mitochondria to take up cations in the reaction system in exchange for the protons that were expelled. Pressman *et al.* (1982) reported working with an electrode to sense the activity of H^+ and K^+ in a given medium. They demonstrated that the valinomycin-induced increase in H^+ activity sensed by the pH electrode was followed by a decrease in K^+ activity as this cation left the medium to enter the mitochondria. Ion selectivity was apparent when Na^+ proved a totally ineffective substitute for K^+.

Further studies established that there were two groups of substances that can alter the permeability of biological membranes by carrying ions across lipid barriers as lipid-soluble complexes: the valinomycin group, which transports cations as lipid-soluble, charged complexes, and the nigericin group, which contains a charged carboxyl group and transports cations as lipid-soluble, electrically neutral zwitterions. These substances were classified generically as *ionophores* (Greek: *ion bearer*) or ionophorous agents i.e., compounds that form lipid soluble, dynamically reversible cation complexes that act as vehicles for transporting ions across biological membranes (Pressman *et al.*, 1982).

Ionophore compounds can be divided into two classes depending on the mode of ion transport they promote:

- **Neutral Ionophores:** These lack ionizable functionality and catalyse electrophoretic transport across membranes. They form charged complexes with cations, i.e. Synthetic polyethers, Valinomycin, Nactins Macrotetralide.

- **Carboxylic Ionophores:** With an ionizable terminal carboxyl group. They form electrically neutral complexes that catalyse exchange diffusion transport of cations, i.e. Monensin, Salinomycin, Lasalocid (Painter and Pressman, 1985).

This distinction is fundamental to explain the profound differences in the biological behaviour of the ionophore subclasses. Valinomycin, that catalyses electrophoretic cation transport promotes the uptake of K^+. Under the same circumstances, nigericin that catalyses exchange diffusion transport promotes the release of K^+ down its concentration gradient in exchange for the uptake of H^+.

The beneficial effects of feeding ionophores to animals can be attributed to their ability to alter membrane permeability and perturb transmembrane ion gradients.

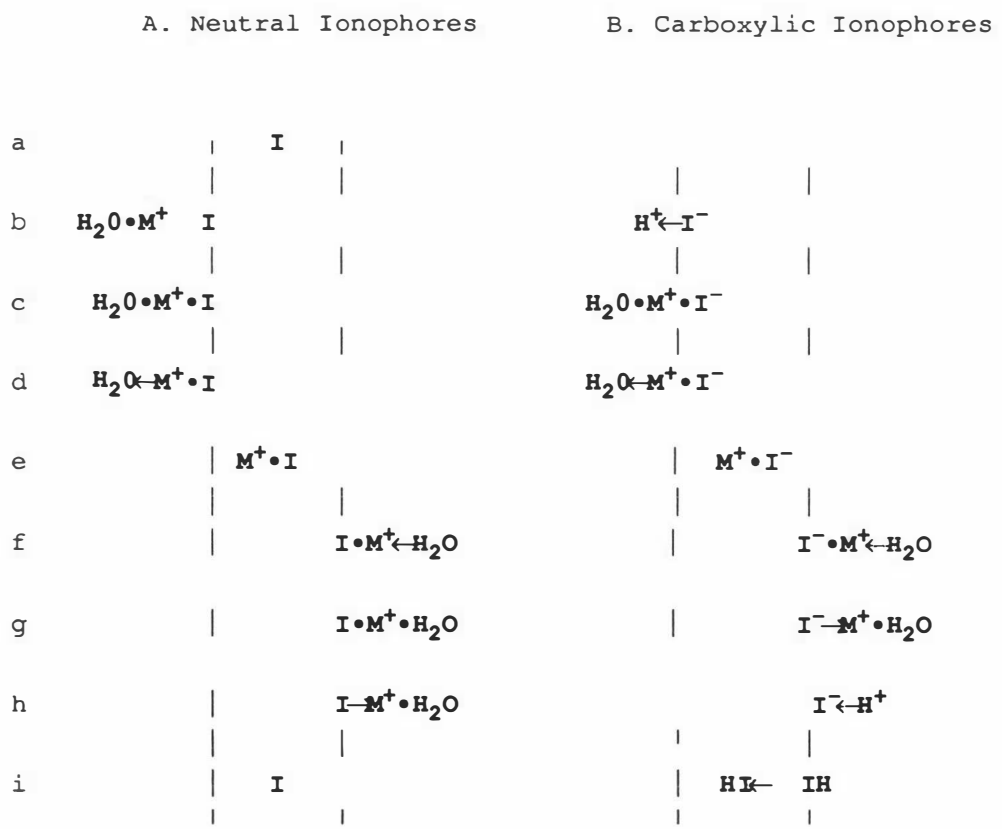
1.3 Mechanism of Ion Complexation of Ionophores

Both neutral and carboxylic ionophores form complexes by enveloping cations and displacing their solvation shell. Strategically placed oxygen atoms of the backbone serve as ligands. The limited flexibility of the molecular backbone defines a cavity of a preferred size for ions of specific ionic radius and therefore a high degree of ion selectivity is attained (Pressman *et al.*, 1982).

The difference in transport mode between neutral and carboxylic ionophores is shown

in figure 1. The *neutral* ionophore (figure 1A) within the membrane (a) diffuses to an interface (b) where it encounters a complexable ion. When the ion is in a suitable position to interact with the ionophore (c), its solvation water is stripped away and replaced by the liganding oxygen of the ionophore (d). The stable complex M^+I eventually diffuses from the interface to the interior of the membrane where processes f-h occur (which are the reverse of processes b-d). Finally the empty ionophore diffuses back into the membrane (i) restoring the original condition (Pressman, 1976).

Figure 1. Ionophore Transport Modes.



From: Pressman (1976).

Carboxylic ionophores, by contrast, only form cationic complexes in the deprotonated form. Figure 1B illustrates an ideal transport mode: a protonated ionophore within a membrane diffuses to one interface. Here, its proton is released and therefore the

ionophore is trapped at the polar interface owing to an increment of polarity. Afterwards, the ionophore anion encounters a cation (M^+) and engulfs it by displacing its water of solvation. The complex with its charges internally compensated, diffuses to the opposite interface and releases its cation where the anionic ionophore is able to combine with a proton. This decrease of polarity would permit the protonated ionophore to leave the interface and return to the membrane interior where it would available for another cycle (Pressman *et al.*, 1982).

Painter and Pressman (1982) indicated that the ion complexation-decomplexation reactions of carboxylic ionophores in water and methanol proceeded by *dissociative interchange*.

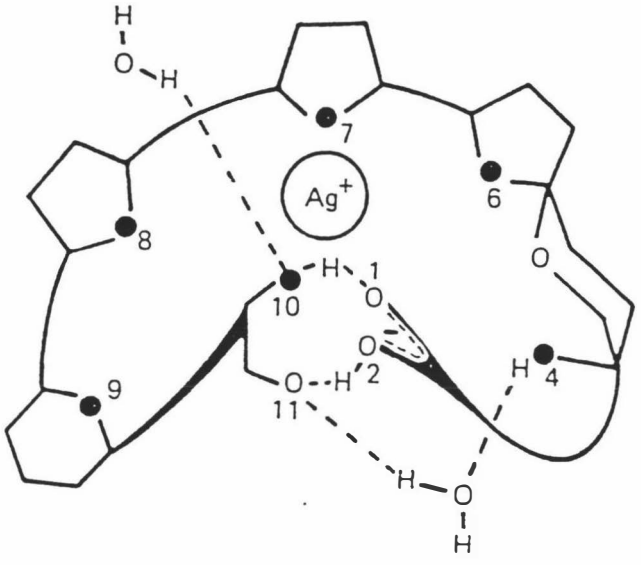
The ultimate stability of the inclusion complex depends on the ability of the ionophore to prevent the cation from reacting with the bulk solvent. In the reaction:

$$M^+ + I^- \longleftrightarrow M^+I^-$$

M^+ is a monovalent cation and I^- is an ionized carboxylic ionophore.

For monovalent ions the binding cavity is generally formed from the liganding atoms of a single ionophore in a 1:1 complex and for divalent cations the liganding cavity is formed by two ionophore molecules in a 1:2 complex. In both cases the complexes are electrically neutral zwitterions because of the ionized carboxyl group. The Ag^+ complex of monensin serves to illustrate the basic architectural conformation of a 1:1 ionophore ion complex shown in figure 2. The monensin anion is wrapped around the Ag^+ ion in the middle of an approximately spherical cavity co-ordinated by six oxygen atoms (Painter and Pressman, 1985).

Figure 2. Schematic Representation of the 1:1 Ag^+/Monensin Complex.



From: Painter and Pressman (1985).

1.3.1 Evaluation of the Transport Selectivity of the Carboxylic Ionophores

Although several biological test systems have been used to investigate carboxylic ionophore-catalyzed transport across biomembranes, isolated erythrocytes, rat liver or bovine heart mitochondria have been used most extensively owing to their ease of preparation and versatility.

In media enriched by metal salts, these compounds induce an exchange of internal for environmental cations. The cation exchange is accompanied by transient changes in the environmental pH. The direction and magnitude of this pH change depend on the relative positions of the internal and external cation in the ion selectivity sequence of the ionophore (Henderson *et al.*, 1969). In resting mitochondria, the effect of carboxylic ionophores on passive ion transport has been studied by

monitoring their ability to accelerate swelling in 100-150mM alkali salt solutions. The carboxylic ionophores cause mixed mitochondrial swelling in solutions of the K^+ salts of impermeant strong acids ($NCS^- > NO_3^- > Cl^-$) (Harris *et al.*, 1967).

The effect of ionophores on metabolically active mitochondria was more complex. The process of oxidative phosphorylation suggested that electron transport resulted in the build-up of an electrical potential difference (negative inside) across the inner membrane of respiring mitochondria (Harris *et al.*, 1967). In response to this membrane potential, the charge transferring neutral ionophores such as valinomycin could promote an influx of K^+ down the electrical potential gradient and up the K^+ concentration gradient. The dissipation of the membrane potential due to positive charge translocation by valinomycin accelerated the proton pump increasing the transmembrane pH gradient (Henderson *et al.*, 1969). Valinomycin increased the rate of mitochondrial respiration because the transport of substrate anions to the interior of the mitochondria depended on the magnitude of pH difference across the membrane. Valinomycin-induced proton expulsion has been found to facilitate substrate anion accumulation.

The carboxylic ionophores reversed the effects caused by the neutral ionophores on respiring mitochondria. They induced an outflow of accumulated cations from the mitochondria, proton uptake and shrinking (Painter and Pressman, 1985). In low concentrations, carboxylic ionophores have been observed to accelerate the respiration induced by valinomycin but as their concentration is increased they become inhibitors. The inhibition could be counteracted by increasing the K^+ or substrate anion concentration in the extramitochondrial media. The basic process underlying the activity of the carboxylic ionophore is again a K^+ for H^+ exchange. This exchange can lower or even reverse the transmembrane pH gradient established by the respiratory chain. If the fall in intramitochondrial pH is not very significant, dissipation of the pH difference leads to acceleration of respiration; if it is large, then respiration can be inhibited due to arrest of the substrate anion inflow.

Cation transport selectivities in mitochondria and erythrocytes as representatives of biological membranes are shown in table 1.

Table 1. Cation Transport Selectivities in Biological Test Systems

Ionophore	Selectivity sequence	Method
Monensin	$Na^+>K^+>Li^+>Cs^+$	Mitochondria and Erythrocytes.
Nigericin	$K^+>Rb^+>Na^+>Cs^+>Li^+$	Mitochondria and Erythrocytes.
Salinomycin	$K^+,Rb^+>Na^+>Li^+,Cs^+$	Mitochondria.
Narasin	$Na^+>K^+>Li^+$	Mitochondria.
Lasalocid	$Cs^+>Rb^+>K^+>Na^+>Li^+$	Mitochondria
Lysocellin	$Na^+>K^+$	Erythrocytes

From: Painter and Pressman, (1985).

Ionophores differ in their affinity and binding selectivity for cations. Monensin has a strong preference for Na^+ over K^+ and does not bind divalent ions to any extent (Pressman *et al.*, 1982). Salinomycin, by contrast, has a slightly greater affinity for K^+ than Na^+ but it has little affinity for divalent ions (Mitani *et al.*, 1975 cited by Spears, 1990). Lasalocid showed more affinity for transporting K^+ over Na^+.

1.3.2 Conformational Changes During Ion Capture and Membrane Transport in Ionophores.

The ion capture and release steps of the membrane transport mechanism involve a

rearrangement of the liganding heteroatoms on the ionophore backbone (Painter and Pressman, 1985). Two mechanisms have been proposed:

- In the highly polar environment in which the ionophore exists before ion capture, it is in an open, acyclic conformation that is radically different from the cyclic conformation of the ion inclusion complex. Conversion to the open conformation, which is energetically favoured in polar environments, facilitates the quick release of cations at the membrane interface (Painter *et al.*, 1982).

- The conformations of many carboxylic ionophores, whether free acids or ion inclusion complexes, are isomorphic in the solid state. The liganding cavity is preformed and ion capture and release involves minimal conformational change.

Conformational studies support the first hypothesis. Deber *et al.* (1976) postulated that there were specific regions in the backbone of carboxylic ionophores termed "hinges" that allow a significant amount of backbone flexibility. These hinge bonds not only play a role in the mechanism of ion capture but also modulate the effects that membrane microenvironments have on the precomplexation conformation of the ionophore.

1.4 Biosynthesis of Monensin

The antibiotic ionophore monensin is produced by *Streptomyces cinnamonensis* (ATCC 14513). It was first described by investigators from Eli Lilly and Co. (Haney *et al.*, 1968). Initial fermentation studies were presented by Stark (1968).

The microorganism that produces monensin was isolated from a sample of soil during a search for cultures that produce new compounds (Stark, 1969). The culture,

Streptomyces cinnamonensis (ATCC 15413) was classified as a strain of *Streptomyces cinnamonensis* Okami (NRRL B1588). The production of monensin by the culture was first observed by the detection of antibacterial activity. Five strains were chosen to find out which one produced more monensin in a given medium. Strain number 5 produced the most (5000 µg monensin per ml of fermentation broth). Other activities were recognised later when the antibiotic was tested broadly in other systems. Table 2 shows the requirements for monensin synthesis by fermentation. Other compounds closely related to monensin (factors B, C and D) were obtained from the fermentation process.

Table 2. Requirements for Monensin Synthesis by a Fermentation Process.

Requirement	Description
Most important mineral	Iron
Other minerals to be included	Manganese and Potassium
Aeration requirement	High
Carbohydrate as substrate	Glucose
Oil as substrate	Soybean oil (refined)
Optimal temperature	32°C

From: Stark (1969) and Stark *et al.* (1967)

Gorman *et al.* (1967) presented the results of a typical column chromatogram. The mixed sodium salts (30 g) in chloroform were chromatographed on 1 kg of silica gel in ethyl acetate. The volume of each fraction was 20 ml. Results of their experiment are shown in table 3.

Figure 3 illustrates the chemical structure of monensin where the radical R can be replaced by different chemical structures to form distinctive molecules of monensin.

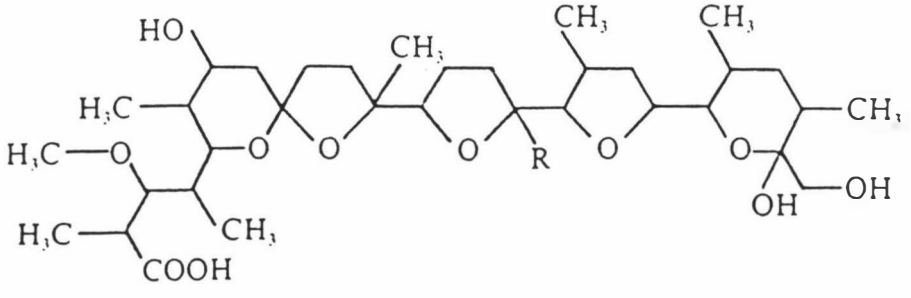
In factor B, the ethyl group on the ring C is replaced by a methyl group. In factor C, the methyl at the carboxyl end is replaced by an ethyl group.

Table 3. Results of a Column Chromatogram for Monensin and Closely Related Compounds Produced During Fermentation

Fraction Number	Weight (g)	Composition
0-95	0.0	-
96-98	0.1	Factor C
99-102	0.05	Mixed monensin and factors C and D
103-170	11.5	Monensin
171-295	14.5	Mixed monensin and factor B
296-450	3.0	Factor B

From: Stark, (1969)

Figure 3. Structure of Monensin



Factors B, C and D were more difficult to isolate. Factor D accounts for the smallest amount of all the compounds. Most of the fermentation broth samples contained only monensin with occasional traces of Monensin B (Stark, 1969).

The biosynthesis of monensin was studied by Day *et al.* (1973) using ^{14}C-labelled glucose, acetate, propionate, butyrate and methionine. Results indicate that the antibiotic ionophore is synthesized from five acetates, seven propionates and one butyrate molecules. The *o*-methyl group arises from methionine. Monensin methyl groups are derived from the "tails" of propionate, and the ethyl is contributed by the butyrate. The terminal hydroxymethyl group is incorporated from acetate.

Preliminary studies on biochemical characteristics of monensin and related compounds are summarized in table 4. Monensin has three reactive functional groups in the molecule that facilitate its chemical modification: a carboxyl group at the C-1 position and two hydroxy groups at the C-7 and C-26 positions. The two hydroxy groups are very important for the metal complex formation shown in figure 2.

Table 4. Properties of Monensin and Closely Related Compounds Produced During Fermentation.

Compound	Melting Point (°C) [a]	Molecular Weight [a]	Inhibitory Concentration(µ/mg) [b]
Monensin	267-269	692	1000
B	227-228	678	425
C	212-214	706 [b]	2000
D	251-252	706 [b]	1000

[a] : From: Gorman *et al.* (1967).

b: Compounds C and D are isomeric

c: Assayed against *Bacillus subtilis*. From: Haney *et al.* (1968).

Improvements in the mode of action of ionophores rely on their chemical modification. Sakakibara *et al.* (1988), Nakamura *et al.* (1991) and Suzuki *et al.* (1987) have investigated different methods such as lactonization to make the molecule of monensin more effective as an ion transporter.

1.5 Biological Applications of Ionophores

Ionophores affect bacterial transport as well as the light-driven cation transport of chloroplasts and bacterial chromatophores. For most cells, the stimulus is transmitted within the cell by a rise in cytosolic Ca^{2+} (that acts as a second messenger in activating cells). Examples of cellular activation *in vitro* mediated through elevated Ca^{2+} are outlined to demonstrate the importance of ionophores as ion carriers although they are not directly related to the effects of these compounds in the rumen microenvironment. These examples include:

- Contraction of skeletal, heart and smooth muscle
- Secretion by promoting exocytosis (discharge of intracellular secretory vesicles)
- Synaptic transmission of neural impulses
- Initiation of the prostaglandin cascade by stimulation of phospholipase that hydrolyses arachidonate from phospholipids
- Response of lymphocytes to antigens
- Initial events following the penetration of egg cells by sperm (Rasmussen *et al.*, 1977).

Monensin also stimulates the release of epinephrine from adrenals in intact animals as well as isolated chromaffin cells, stimulates the release of prostaglandins from the renal medulla and the release of serotonin from platelets. It inhibits the secretion of procollagen from cultured fibroblasts and enzymes from pancreatic acinar cells (Painter and Pressman, 1985).

1.5.1 Use of Carboxylic Ionophores as Food Additives

The use of carboxylic ionophores as agricultural feed additives is of particular economic importance. More than 70 ionophores have been identified so far and several of them have been studied to prove that they exhibit a similar degree of effectiveness as feed additives. Ionophores improve efficiency of production by intervening in three major areas of metabolism:

- Increasing efficiency of energy metabolism in the rumen
- Improving nitrogen metabolism
- Suppressing feedlot disorders such as lactic acidosis and bloat (Bergen *et al.* 1984).

Lasalocid and Salinomycin produce similar responses to monensin in improving beef cattle production. Narasin appears to be more potent than monensin, although it has undergone only moderate investigation (Potter *et al.*, 1979).

Schelling (1984) enumerated the biological effects of monensin. The items listed in table 5 embody potential modes of action that might influence animal performance. Modified ruminal microbial metabolism accounts for most of the listed observations although a direct influence of monensin on the gastrointestinal tissue could be involved with some observations related to rumen fill (Schelling, 1984).

1.6 Effects of Monensin in the Ruminal Microenvironment

1.6.1 Energy Transactions in Ruminants

The bulk of carbohydrates in ruminant feeds are polymers: cellulose, hemicellulose, starch, fructan and pectin. It has been estimated that fodder plants contain on a dry

basis 20-30 % of cellulose and 14-20% of hemicellulose.

Table 5. Biological Effects of Monensin in the Rumen.

- Greater ruminal propionate concentration
- Lower ruminal acetate concentration
- Lower ruminal butyrate concentration
- Lower ruminal lactate in stressed animals
- Higher ruminal pH in stressed animals
- Less ruminal methane production
- Decreased intake of grain diets
- Increased intake of forage diets
- Increased ruminal forage fill
- Decreased ruminal rate of passage
- Increased dry matter digestibility
- Increased protein digestibility
- Decreased ruminal deamination
- Decreased ruminal proteolysis
- Protein sparing effect
- Modified ruminal escape of protein
- Modified ruminal escape of starch
- Modified microbial population of the rumen
- Increased body glucose turnover
- Modified substrate gluconeogenesis
- Reduced 3-methylindole production

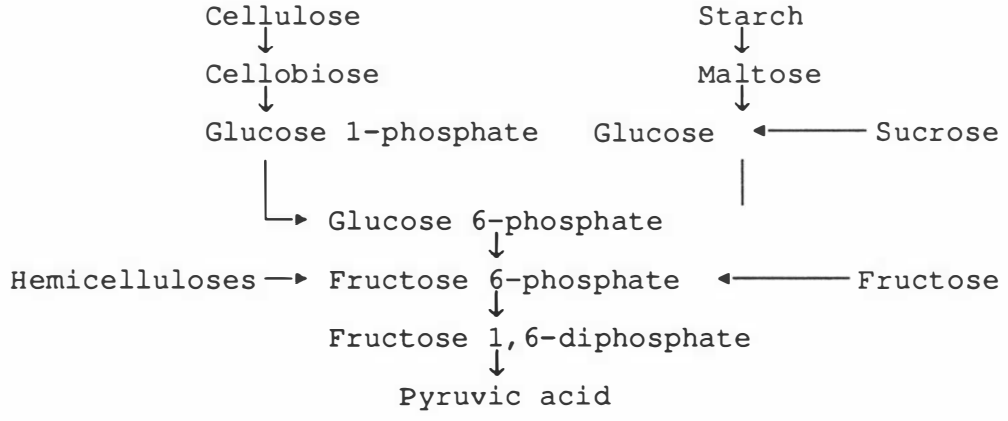
From: Schelling, (1984).

The major part of carbohydrate solubles (sugars and starch) and less solubles (cellulose and hemicellulose) are fermented in ruminants to volatile fatty acids when

the food passes through the rumen. This is due to the action of microbial enzymes. Carbohydrates undergo extracellular hydrolysis to be converted to short-chain oligosaccharides, mainly disaccharides, and simple sugars. The monosaccharide is then converted to pyruvate (figure 4). Ruminal microorganisms ferment glucose and pyruvate to produce metabolic hydrogen. Some of this metabolic hydrogen is used in the ruminal ecosystem for bacterial growth and biohydrogenation of unsaturated fatty acids. Nevertheless, the largest quantities are used in the formation of propionate and butyrate (from pyruvate) and in the reduction of carbon dioxide to methane (figure 5)(Czerkawski, 1974).

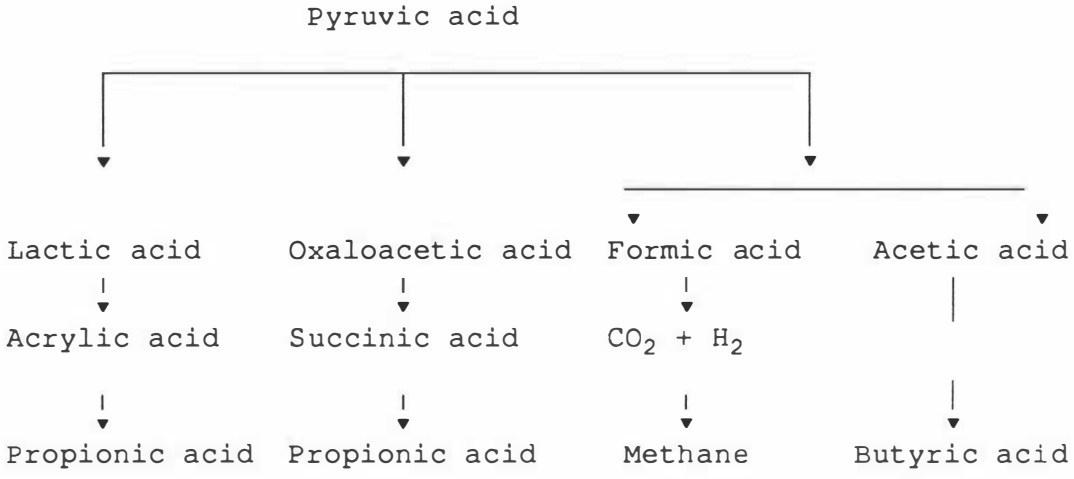
The mixture of volatile fatty acids in the rumen is composed mainly of acetic, propionic and butyric acids with small amounts of formic, isobutyric, valeric, isovaleric and caproic acids. The efficiencies of fermenting hexose to acetate, propionate and butyrate are 62, 109 and 78 % respectively. Therefore the energy recovered in the fermentation of these products can be increased by enhancing the production of propionate and inhibiting methanogenesis. It is thus possible to divert hydrogen normally deposited in methane into propionate and, to a lesser extent, the same diversion to propionate may reduce butyrate production.

Figure 4. Conversion of Carbohydrates to Pyruvate in the Rumen



From: Bondi, (1987)

Figure 5. Conversion of Pyruvate to Volatile Fatty Acids in the Rumen



From: Bondi, (1987).

Volatile fatty acids are absorbed through the ruminal walls into the circulation and are transported to the tissues. The metabolic processing of these compounds takes place in the animal tissues including catabolic processes that supply energy and biosynthetic processes such as the formation of fat from acetic and butyric acids or of glucose from propionic acid (Bondi, 1987).

Some gases such as methane, carbon dioxide and hydrogen are considered as by-products of ruminal fermentation. They are removed from the body by eructation.

1.6.2 Effect of Monensin on Ruminal End Products

Richardson *et al.* (1976) reported the effect of monensin on ruminal volatile fatty acid production. Their results are shown in table 6. The most consistent observation attributed to the use of ionophores is the increase in the molar proportion of propionic acid with a corresponding decline in the molar proportion of acetate and

butyrate (Bergen, 1984). Van Maanen (1978) carried out isotope dilution studies to conclude that propionate is produced at the expense of acetate. This ratio shift has been viewed as a favourable change for meat producing animals.

Table 6. Effect of Monensin on Ruminal Volatile Fatty Acid Production (mcM/ml)

Monensin ppm	Acetic	Propionic	Butyric	Isovaleric	Valeric
0	19.6	7.4	7.1	1.1	0.7
0.1	20.8	8.6	7.2	1.1	0.7
0.25	20.2	9.4	6.9	1.1	0.7
0.5	19.6	10.6	6.5	1.0	0.6
1.0	19.1	11.1	6.3	0.9	0.5
5.0	17.5	11.2	5.8	0.7	0.5
25.0	16.7	12.5	5.6	0.6	0.4

From: Richardson *et al.*, (1976)

Chalupa (1977) stated that propionate production by ruminal fermentation was more efficient than that of acetate. There is also evidence of propionate being utilized by the tissue more efficiently than acetate. Propionate metabolism produces a lower heat increment than acetate (Bergen, 1984) and it is more flexible as an energy source than acetate because it has the potential to be used for gluconeogenesis and direct oxidation by the citric acid cycle (Smith, 1971).

Henderson *et al.* (1981) studied the effect of monensin on pure and mixed cultures of ruminal bacteria to explain the origin of the extra propionate found in the rumen of animals receiving the ionophore. They suggested that the effects of monensin were

likely to result from the selective inhibition of the growth of rumen bacteria that were not important producers of propionate in the rumen. This favoured growth of organisms like *Anaerovibrio lipolytica, Selenomonas ruminantium, Magasphaera elsdenii and Bacteroides ruminicola* which all produce propionate.

Associated with this effect on propionate, a decline in methane production has been observed (Thornton *et al.,* 1981). This has been attributed to a reduction in the activity of formate lyase (Van Nevel and Demeyer, 1977). Studies carried out *in vitro* explained that the reduction in methane production also can be due to a sensitivity of methanogenic bacteria to monensin. This result cannot be extrapolated to *in vivo* conditions because many compounds and antibiotics can alter short-term metabolism *in vitro.*

Thornton *et al.* (1981) investigated the effect of monensin on *in vivo* methane production. They worked with three different types of diets that varied in roughage level, i.e., low, medium and high content and collected the expired gases for 24 hours from both treated and untreated groups. The total methane production is shown in table 7.

Table 7. Methane Production in Steers Fed Monensin at Three Levels of Roughage.

Roughage level	Treatment	Methane production	
		Kcal/hr	% of CO_2
Low	Monensin	61.2	6.81
	Control	72.5	7.58
Medium	Monensin	71.4	6.92
	Control	85.5	9.47
High	Monensin	73.6	7.55
	Control	96.4	9.84

From: Thornton *et al.,* (1981)

Monensin decreased ($p < 0.05$) methane production at all roughage levels (by 16% at the two lower roughage levels ($p < 0.05$) and by 24 % at the high roughage level) while intraruminal propionate concentrations were observed to increase. Monensin did not inhibit methanogenesis totally, which suggested that the additive might have influenced only one of the several methanogenic pathways. It has been postulated that monensin inhibits *Ruminococcus* and *Butirivibrio fibrisolvens* that are major acetate and H_2 producers. Decreases in those end products would contribute to a decrease in the acetate to propionate ratio and to a decrease in methane production due to a reduction in the availability of H_2 to the methanogenic bacteria (Dinius *et al.*, 1976).

Another theory suggested that methanogens were not directly inhibited by monensin. Depression in growth of *Ruminococcus* and *Butirivibrio fibrisolvens* (acetate and H_2 producers) could lead to a depression of their contribution to plant cell wall digestion. This could be important in determining the rate of *in vitro* dry matter digestion in the rumen. *In vivo* this situation was not observed because *Bacteroides succinogenes* could assume an enhanced role in plant cell wall digestion (Henderson *et al.*, 1981).

Gram-positive bacteria such as *R. albus, R. flavefaciens* and *B. fibrisolvens* are reported to be more sensitive to ionophore antibiotics than gram-negative bacteria. Gram-positive species do not produce propionate or succinate as fermentation products (Latham, 1977 cited by Chen *et al.*, 1979). The moderately sensitive *Bacteroides* and the insensitive *S. ruminantium* have gram-negative cell wall structures and are both important succinate producers. These species can easily be selected for resistant populations (Costerton *et al.*, 1974). Addition of ionophores to the rumen tended to decrease the production of the major fermentation products, acetate and butyrate, by inhibiting the growth of gram-positive bacteria. *S. ruminantium* is the major organism involved in the decarboxylation of succinate to propionate and CO_2 in the rumen and is resistant to monensin and lasalocid.

Factors that influence the internal and external cation concentration may be expected

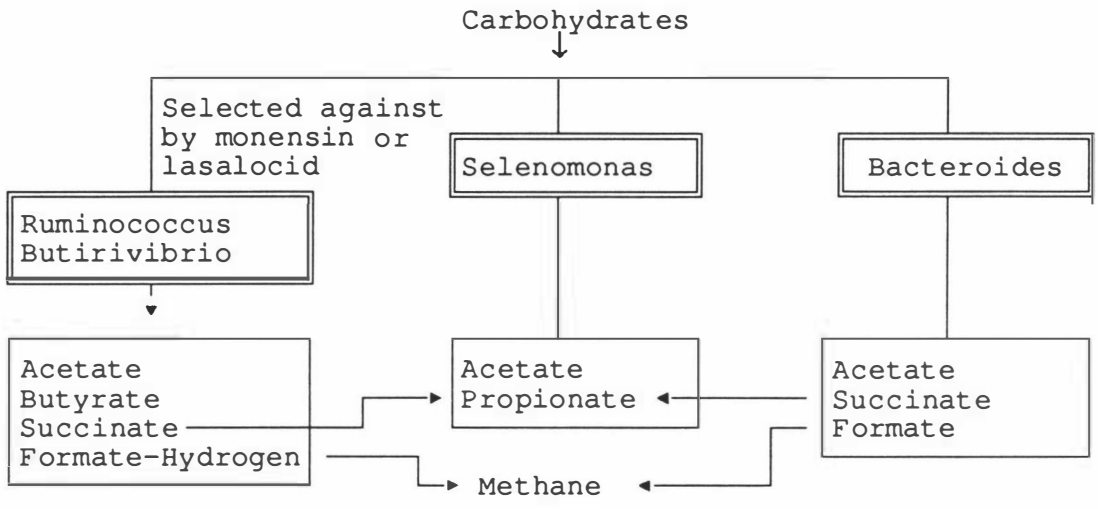
to influence the antimicrobial activity of ionophores in the rumen (Dawson *et al.*, 1987). Monensin and lasalocid alter microbial activities by dissipating the cation gradients that are normally established across bacterial cell membranes (Russell, 1987). *Streptococcus bovis* cells exposed to monensin decreased their intracellular K^+ concentration that was associated with the flow of protons and Na^+ and depended upon the relative concentration of ions both inside and outside the cells.
All strains of *B. ruminicola* tested were more sensitive to monensin and lasalocid in media containing low concentration of potassium and tended to be resistant to higher concentration of ionophores when potassium levels were high.

Strains of *B. ruminicola, B. succinogenes, E. ruminantium* and *R. albus* were unable to grow within 24 hours at the lowest (1.3mM) potassium concentration. They did grow in medium containing a greater potassium concentration.

Potassium ion concentrations decreased in cells exposed to ionophores as potassium ions flowed out of the cells in response to the concentration gradient. This flow of potassium out of the cell was accompanied by an influx of sodium ions and protons, a decrease in the transmembrane potential and limitations of energy production in the bacterial cells.

Increased external potassium concentrations decrease the magnitude of the potassium gradients and prevent the efflux of potassium from cells exposed to ionophores. Increased extracelluar potassium concentrations will increase the resistance of some ruminal bacteria to monensin and lasalocid. It was suggested that these compounds deplete intracellular potassium concentrations and therefore antimicrobial activities of some ionophores can be reversed by increasing the potassium concentration in the medium (Dawson *et al.*, 1987). A summary of the effects are presented in figure 6.

Figure 6. A Schematic Representation of the Hypothesized Mechanism by which Monensin and Lasalocid Alter Ruminal Fermentation by Altering the Microbial Community



From: Chen *et al.*, (1979)1.6.3

Effect of Ionophores on Ruminal Protozoa

Feeding lasalocid or monensin at a concentration of 6-12 µg/ml of ruminal content reduced total protozoal counts in cattle fed on either high grain or high roughage diets. Generic composition of protozoa was also influenced by these ionophores.

Holotrichs (Dasytricha and Charonina) were reported not to be affected by either lasalocid or monensin. Among entodiniomorphs, *Entodinium, Diplodinium* and *Ophryoscolex* were more sensitive than the other types. Protozoal inhibition was transient because prolonged antibiotic feeding resulted in the selection of a resistant population in the rumen of cattle. The protozoal changes associated with lasalocid and monensin were due to the direct action of the antibiotic on protozoa and were not a secondary response from alterations in ruminal bacteria or ruminal fermentation pattern.

1.7 Effect of Ionophores on Energy Metabolism

The effect of monensin on energy losses in faeces, urine and methane is shown in table 8. Thornton *et al.* (1981) found that urinary and faecal energy losses were not altered significantly by monensin at any roughage level. Monensin decreased ($p<0.05$) methane production at any roughage level.

Spears (1990) studied the influence of monensin and lasalocid on apparent digestible energy in cattle and sheep. In cattle, monensin and lasalocid increased apparent digestible energy by 2.0 percent (percentage digestibility) on average ($p<0.01$). Responses to monensin feeding in cattle have ranged from 0.9 percent decrease to a 9.2 (percent digestibility) increase. This tendency for improved digestible energy with monensin was not significant ($p<0.16$). Summary of the results are presented in table 9.

Table 8. Energy Subdivision when Fed Monensin and Three Roughage Levels.

Roughage level	Treatment	Energy loss		
		Faecal	Urine	Methane
Low	Monensin	20.4	3.8	7.3
	Control	21.3	3.7	8.0
Medium	Monensin	33.2	2.3	6.8
	Control	34.2	2.5	7.4
High	Monensin	36.6	3.3	7.0
	Control	38.9	2.9	8.2

Thornton *et al.*, (1981)

Table 9. Apparent Digestibility of Energy in Ruminants Fed Monensin or Lasalocid (% Digestibility).

Ionophore	Species	Control group	Treated group
Monensin	Cattle	70.3	72.4
	Sheep	68.2	67.5
Lasalocid	Cattle	75.7	77.7
	Sheep	70.7	70.7

From: Spears, (1990)

The response in energy digestibility to monensin depends upon the type of diet. There was not a consistent response with ionophores when the diet contained a high proportion of fibre.

The effect of ionophores on the extent and site of digestion and absorption has also been researched with inconsistent results. It was found that monensin initially produces a negative effect on digestibility which is followed by a slight positive effect. Simpson (1978) cited by Schelling, (1984) found that monensin decreased cellulose digestibility when no adaptation time was allowed.

Dinius *et al.* (1976) concluded that monensin had no effect on cellulose digestibility when animals had been adapted to monensin for 21 days. Beede *et al.* (1980) reported an increase in dry matter and gross energy digestibilities in cattle fed a grain-roughage diet and adapted to monensin.

The influence of ionophores on rumen fill and rate of passage was studied by Lemenager (1978). The inter-relation between rumen volume, fermentation rate and extent of digestion determines the turnover rate and its importance in the economy of feed utilization by the ruminant. Table 10 summarises the effect of monensin on rumen turnover rate and rumen fill.

Monensin decreased ruminal turnover rate and increased rumen fill at the percentages indicated in table 10 explaining observed reductions in feed intake when animals are treated with ionophores. Decreased ruminal turnover rate could partially account for the decreased feed intake observed with high roughage diets (Bolsen *et al.*, 1975).

Table 10. Effect of Monensin on Rumen Turnover Rate and Rumen Fill

Conditions	Phase	Rumen turnover rate %	% of change in Rumen fill
Cattle grazing pasture	solid	-7	9
Cattle grazing range grass	solid	-44	10
Cattle grazing range grass	liquid	-31	not measured
Cattle grazing range grass	liquid	-22	24

From: Schelling, (1984)

When monensin was fed, solids turnover and liquid turnover rates were decreased. Steers fed monensin had a 30.8% slower rumen liquid turnover rate ($p<0.10$) and 43.6% slower solid turnover rate ($p<0.001$) than control steers. This is reflected in daily forage intakes that were depressed by 15.6% ($p<0.02$). Decreased ruminal turnover matched the reduction in feed intake with monensin supplementation of high roughage rations (Bolsen *et al.*, 1975). Several theories might explain the relation between reduced forage intake and ruminal turnover rate when monensin is fed. First, ruminal turnover rate might have been decreased because intake was decreased (Balch *et al.*, (1965) cited by Lemenager, (1978)). However monensin depressed ruminal turnover rate independently of its depression of intake.

Lemenager (1978) reported that monensin decreased rumen turnover rate and increased rumen fill ($p<0.05$). Furthermore, there is a decreased rate of ruminal digestion. If digestion rate is depressed and omasal passage is limited by particle digestion, ruminal retention would be prolonged and rumen turnover would be slow.

Reduced rumen turnover would decrease feed intake, therefore bulk fill limits intake (Lemenager, 1978).

Table 11. Intake, Ruminal Turnover and Rumen Volume of Steers Fed Harvested Dry Winter Range Grass

Feature	0 mg monensin/steer/day	200 mg monensin/steer/day
Liquid turnover dilution (%/hour)	6.53	4.52
Intake (Kg)	4.60	3.88
Solid turnover dilution (%/hour)	2.73	1.54
Liquid rumen volume (litres)	236.2	174.2
Dry Matter in rumen (kg)	2.50	2.75

From: Lemenager, (1978)

Reffett-Stabel *et al.* (1989) found that steers fed salinomycin consumed less feed than control steers ($p<0.05$). Feed intake was lower ($p<0.05$) for steers given 100 mg salinomycin than for those fed 50 mg salinomycin/head daily. The amount of feed required per unit of gain was similar for control steers and treated ones ($p>0.10$). Free choice salt consumption was lower ($p<0.01$) for steers receiving either ionophore. The effect of salinomycin and lasalocid on feed intake and weight gain is shown in table 12.

Table 12 shows that steers fed salinomycin consumed less feed than control steers. Feed intake was lower for steers given 100 mg salinomycin than for those fed 50 mg salinomycin/head daily. In this experiment, feed/gain was 9.5 and 10.4% lower for the 100 mg salinomycin and lasalocid treatments respectively than for the control.

McClure *et al.* (1980) worked with cattle fed concentrate-based diets. Salinomycin

consistently improved feed conversion efficiency but responses in gain and feed intake were variable. Free choice salt consumption was lower for steers receiving the ionophore.

Table 12. Performance of Steers Fed Salinomycin or Lasalocid.

Item	Control	Salinomycin 50 mg	Salinomycin 100 mg	Lasalocid 250 mg
Animals	20	20	20	20
Initial wt	227	228	228	228
Final wt	309	307	305	308
ADG.[b] Kg.	0.73	0.70	0.69	0.72
DFI.[c] Kg.	5.25	5.00	4.49	4.64
Feed/gain	7.19	7.14	6.51	6.44
DSI.[d] g.	37	32	26	30

From: Reffet-Stabel *et al.* (1989)

[a]Weights are expressed in Kg.

[b]Average Daily Gain

[c]Daily Feed Intake

[d]Daily Salt Intake

Total gastrointestinal tract digestion of starch generally is not affected by ionophores. Ionophores reduce the percentage of starch digested in the rumen and increase the quantity of starch digested in the intestine (Muntifering *et al.*, 1981). This change in the site of digestion might represent more glucose being absorbed directly and therefore, more efficient use of energy. Higher amylase activities were found in the pancreas and faeces of cattle receiving monensin (Van Hellen *et al.* 1977 cited by Spears, 1990).

1.8 Effect of Ionophores on Nitrogen Metabolism and Mineral Absorption

Animals fed ionophores have higher apparent nitrogen digestibilities ($p<0.01$) according to studies carried out by Spears (1990). Table 13 summarises several experiments regarding this topic.

Table 13. Apparent Digestibility (%) of Nitrogen in Ruminants Fed Monensin or Lasalocid

Ionophore	Species	Control group	Treated group
Monensin	Cattle	62.2	65.7
	Sheep	64.8	67.5
Lasalocid	Cattle	70.8	74.6
	Sheep	66.9	70.2

From: Spears (1990)

A significant increase in apparent nitrogen digestibility was noted in cattle and sheep fed ionophores. This ranged from 2.6 to 3.8 percent with cattle showing a slightly greater response than sheep.

The nature of the diet and the protein level in the diet were important factors in digestibility. A tendency for higher nitrogen digestibility values were observed when cattle were fed at any roughage level with supplemental monensin, (Thornton *et al.* 1981 cited by Schelling, 1984). Dinius (1976) reported inconsistent responses in this respect.

Schelling, (1984) stated that monensin reduced the dietary protein requirement. Chalupa (1980) reported that monensin significantly reduced the ruminal degradation of dietary protein and Hillaire *et al.* (1989) reported a decrease in the nitrogen

degradability of peanut meal (-38%). They concluded that there was a protective effect of monensin with respect to dietary proteins.

Schelling (1984) found that monensin decreased the rate of free amino acid degradation in rumen fluid. Dinius (1976) stated that rumen ammonia reductions were consistent with the depression of deamination and proteolysis. Monensin decreased bacterial nitrogen reaching the abomasum of steers adapted to monensin and therefore, more dietary protein reached the abomasum (Poos *et al.* (1979) cited by Schelling, (1984)). Adaptation of the rumen microflora to monensin can play an important role in microbial growth (Van Nevel *et al.*, 1977). Schelling (1984) concluded that monensin does exhibit a protein-sparing effect by making more effective use of amino acids.

Chalupa (1980) indicated that in the presence of monensin, ruminal ammonia nitrogen production is decreased. Therefore, more dietary protein escaped ruminal degradation and was available for digestion in the small intestine. The explanations of this outcome are either that a depression of available proteolytic and deaminative enzymes occurs owing to a depressing effect of monensin on total cell numbers or a direct effect on protease/deaminase activity. The extent of escape has been summarised by Bergen (1984) as shown in table 14. The escape from ruminal degradation of preformed dietary protein was increased from 22 to 55% ($p<0.05$) in the presence of monensin.

Ionophores also may affect the rate of amino acid absorption because this process requires a sodium co-transport mechanism. The energy required for transport is provided by the Na^+/K^+ - ATPase. Ionophores increase cellular sodium and increase activity of the sodium-potassium pump (Smith *et al.*, 1980 cited by Spears, 1990).

Table 14. Effect of Monensin on the Extent of Escape (%) from Ruminal Degradation of Various Protein Sources

Diet and Protein source	Extent of escape (%) Control	Extent of escape (%) Monensin
Feedlot plus brewers dried grain	100	137
Feedlot plus urea supplement	100	155
Rolled corn plus protein supplement to 16%	100	122
Corn silage plus soybean meal	100	152
Ground corn, corn silage plus soybean meal	100	136

From: Bergen, (1984)

Monensin has been proven to influence mineral absorption in ruminants. A significant increase in magnesium and phosphorus absorption was reported with the use of ionophores. Although this effect was observed in animals consuming high concentrate diets, steers fed tall fescue greenchop also showed the same trend (Spears, 1990). The increase in absorption occurred both pre-intestinally and in the small intestine.

Table 15. Effect of Lasalocid and Monensin on Apparent Absorption of Magnesium and Phosphorus in Cattle.

Item	Control	Monensin	Lasalocid
Magnesium	25.2	34.3	35.0
Calcium	32.3	40.3	37.5
Potassium	84.1	83.7	84.8
Sodium	64.2	77.2	72.7
Phosphorus	47.8	58.6	58.8

From: Starnes *et al.*, (1984)

According to the results summarised in table 15, monensin significantly increased apparent absorption of magnesium, phosphorus and sodium. No effect was demonstrated with potassium and differences in calcium absorption between treated and untreated groups were not statistically significant.

Monensin increased calcium absorption in studies carried out by Gado *et al.* 1986 (cited by Spears, 1990). The apparent absorption of potassium, sodium and trace elements was not consistent and depended upon several factors such as diet and the interaction with other minerals.

1.9 Effect of Monensin on Coccidia

Two theories have been proposed to explain the effect of monensin on *Eimeria* spp. Initially, experiments with *Eimeria tenella* showed that when treated with monensin, extracellular sporozoites became swollen, specially at the anterior end (Smith *et al.*,

1979). It was concluded that the site of action of monensin in coccidia was at the cell membrane and not at the mitochondrial membrane. Another theory suggests that increase of intracellular sodium in the parasite caused by monensin, stimulates the activity of the sodium pump in extruding these extra sodium ions from the parasite. Increased consumption of energy by the sporozoite to counteract the effects of monensin may reduce the organism's ability to penetrate epithelial cells and initiate infection (Stockdale, 1981).

Considering these reported observations on the effect of monensin on animal production efficiency, ruminal ecology and on cellular responses of ruminal anaerobes and microorganisms present in the ruminant tract, the aim of this study was to evaluate if these reported effects of monensin are valid for New Zealand dairy heifers.

The purposes of this study were to determine whether supplementing monensin to Friesian heifers would

- influence weight and height changes,
- affect coccidial counts,
- influence progesterone levels as an indication that heifers reached puberty earlier
- influence reproductive parameters such as conception rate, age at first calving and weight of calves.

Effect of Monensin on Weight Gain and Growth of Heifers

2.1 Introduction

The influence of feed additives on rate of gain is variable and depends on the amount of roughage in the diet. The effect of monensin on the efficiency of gains in cattle is controversial since some researchers have found positive weight gains (Baile *et al.*, 1982; Cochran *et al.*, 1990) while others have reported little or no weight change with its administration (Meinert, 1992; Watkins, 1987; Moseley, 1977; Raun *et al.*, 1976). Effects of monensin on weight gain depend not only upon the type of diet but on other variables such as stocking rate and the age of animal treated. Whatever factors affect the action of monensin, it has been consistently shown to decrease feed intake (Ilan, 1981; Potter *et al.*, 1986) and improve feed efficiency (Baile *et al.*, 1982).

2.1.1 Influence of the Type of Diet on the Effect of Monensin

Several authors have reported significant increases in live weight gain, carcass weight and carcass fat thickness on high-concentrate diets supplemented with monensin (Berger 1981, Mader *et al.*, 1985). By contrast, Zobell (1987) observed no benefit in average daily gain and feed efficiency when monensin was used on a barley-based high-concentrate finishing diet. He concluded that inclusion of monensin or lasalocid in the high concentrate finishing diet did not significantly affect the average daily gain or dry matter intake.

Perry *et al.* (1976) found that for pasture fed cattle, those given monensin treatments gained weight at nearly identical rates as those receiving no monensin. By contrast, Horn *et al.* (1981) reported an increment in daily gain of 25% in stocker cattle on

wheat pasture with monensin supplementation.

Beacom (1988) stated that cattle receiving high-forage diets supplemented with monensin gained weight faster than those fed a concentrate diet. Pomar *et al.* (1988) observed that monensin improved feed conversion efficiency whether the rations were rich in roughage or cereals. This effect of monensin was probably due to the increase in propionic acid production by rumen microbes. The fibre components of the ration were better digested when monensin was present, which suggests an increased activity of fibrolytic bacteria at high levels of roughage in the rations. Monensin did not seem to affect either dry matter (DM) intake or average daily gain (ADG) of growing calves regardless of the type or level of roughage.

2.1.2 Monensin and Feed Intake

Ilan (1981) demonstrated that the main site of activity for monensin is the rumen because the limiting effect of this ionophore on feed intake is not present until the rumen has developed. This hypothesis is supported by the observation of little change in the molar proportions of acetic, propionic and butyric acids induced by monensin in calves younger than 30 days (Ilan *et al.*, 1981).

Potter *et al.* (1986) evaluated the effect of monensin on the growth of cattle fed pasture plus a limited amount of concentrate. Pasture plus supplement supported daily gains of control cattle of 0.24 to 0.96 kg with an average of 0.56 kg. The addition of 200 mg monensin per day to the supplement increased daily gain by an average of 0.09 kg daily (16.3%) in their experiment. Moreover, monensin reduced feed intake by 3.1% and improved feed conversion efficiency by 15.3%.

2.1.3 Monensin and Feed Efficiency

Baile *et al.* (1982) reported that monensin improved feed use efficiency by decreasing the amount of feed per unit of growth. Effects upon growth of feeding 200 mg/head per day of monensin to Friesian heifers during 448 days were investigated. It was concluded that a weight gain advantage of 0.09 kg daily was achieved on dry matter intakes of 7.47 and 7.46 kg/day for the control and 200 mg treatment groups respectively. Thus, heifers fed 200mg of monensin were 12.6% more efficient in converting feed to gain than control heifers. A summary of these results is presented in table 16.

Table 16. Performance of Growing Friesian Heifers Fed Monensin for 448 Days

Item	0 mg/head/day	200 mg/head/day
Initial weight	197	195
Average weight gain (kg/day)	0.60	0.69
Average dry feed intake (kg/day)	7.47	7.46
Feed efficiency (intake/gain)	12.41	10.85

From: Baile *et al.*, (1982).

Reports vary about the appropriate dose of monensin for weight gain. Watkins (1987) stated that consumption of 200 mg per head daily by cows produced performance equal to or slightly better than that of cows not receiving monensin under *ad libitum* feeding conditions. Potter *et al.* (1976) concluded that monensin at two different dose rates: 14.5 ppm and 79 ppm, did not alter carcass composition nor live weight gain, carcass weight, dressing percentage, fat thickness and rib eye area. In another experiment, Potter *et al.* (1976) worked with 5 different dosages of monensin: 50,

100, 200, 300 and 400 mg/head/day. The best results in terms of live weight gain were obtained when a 200 mg/head/day dosage was used. This result agrees with the one presented by Watkins *et al.* (1987).

2.1.4 Monensin and Digestibility

The effect of monensin on nutrient digestibility depends upon the nature of the diet. In high fibre rations, the digestibility of organic matter can be slightly improved by monensin as a result of increasing the digestibility of the fibrous fraction of the feed. Improvement in fibre digestibility observed with monensin resulted from both a decrease in intake and an increase in the retention time of feed particles in the rumen. Each of these factors possibly influenced the other (Horton *et al.*, 1980). In contrast, Pomar (1988) stated that monensin did not affect DM digestibility but does reduce the digestibility of fibrous fractions (ADF and NDF) in calves receiving a diet with 20% pelleted roughage. For diets containing 40-60% pelleted roughage, monensin improved the digestibility of these fractions in calves (Pomar, 1988).

2.1.5 Monensin Ruminal Delivery Device

The use of a Monensin Ruminal Delivery Device tended to promote an increase in average daily gain when cattle were managed under the highest stocking rate (1.25 acres/steer) when compared with stocking rates (SR) of 1.75 and 1.50 acres/steer. The response was not consistent across years for these two lower SR groups. The degree with which monensin enhanced weight gain in the 1987 study was lower than that observed in 1986. This indicated that differences between years in quantity or quality of forage available influenced the response (Cochran *et al.*, 1990).

Table 17. Influence of Intraruminal Monensin Administration and Oestradiol 17ß Implants on the Average Daily Gain (lb/d) of Steers Grazing Early - Summer Bluestem Range at Three Stocking Rates (steer/acres)

Average Daily gain (lb/d)				
Stocking rate	Forage only	Monensin	Oestradiol 17ß	Monensin + Oestradiol 17ß
Low	2.18	2.30	2.64	2.62
Moderate	2.22	2.18	2.80	2.71
High	2.29	2.50	2.66	2.84

From: Cochran *et al.*, (1990)

Gain responses from the monensin ruminal delivery device (RDD) and oestradiol implant were not always additive. However, there was a treatment x SR interaction. At the low and moderate SR (1.75 and 1.50 acres/steer respectively) treatment groups that had received an oestradiol 17ß implant gained more weight than non-implanted steers. Steers receiving a monensin RDD, however, did not gain more than groups not receiving the monensin RDD.

In contrast, under the high SR (1.25 acres/steer) steers receiving a monensin RDD gained significantly more than the untreated controls. Although the combination of an implant and a monensin RDD resulted in the greatest daily gain at the high SR, both hormonal implant groups gained more than the control group or the group receiving only a monensin RDD.

Cochran *et al.* (1990) concluded that intraruminal administration of monensin via a slow-release device tended to promote increased rate of gain for beef steers grazing

early summer bluestem range when SR were high (1.25 acres/steer). They found that the response was not consistent across years at lower SR. Thompson (1990) could not demonstrate a synergistic effect with monensin - oestradiol 17ß administration when 100 steers were treated. Treatment with oestradiol or oestradiol + monensin improved growth rate but monensin alone did not have any effect on growth rate.

A study was conducted in the tropics to determine the effect of a RDD on the weight gain of beef steers grazing guinea grass (*Panicum maximum*) plus legume (*Neocotonia wightii*). There were significant effects ($p<0.05$) on daily weight gain, i.e., 1.10 kg vs 1.17 kg/animal for control and treated animals, respectively during the first 50 days of the trial (Oliveira *et al.*, 1987).

2.1.6 Monensin in Heifers

Overseas reports regarding the effect of monensin in heifers tend to agree that there is no effect of the ionophore on weight gains. Meinert (1992) determined the effect of feeding monensin on growth performance, average age at breeding and body composition of Friesian heifers. It was concluded that none of the growth measurements - body weight, height at withers, heart girth and length, and body condition scores - were affected by monensin feeding. Moseley (1977) concluded that gains in body weight and heart girth were not different between monensin and control heifers although monensin heifers grew significantly more in height and length than control heifers. Raun *et al.* (1976) have reported that body weight gains of feedlot steers were not increased by feeding monensin.

Feeding monensin has minimal, if any, effect on the carcass measurements or the proportions of fat, lean and bone in the edible portion of the carcass. However, the increased efficiency of energy retention in the carcass indicates that monensin allows the animal to use more of the feed energy for carcass gain (Potter, 1986).

Considering this observed effect of the ionophore on the efficiency of energy retention, the objective of this work is to investigate the effect of monensin in dairy replacements heifers under New Zealand conditions. If monensin has an influence on energy retention in the carcass, it is expected that heifers under treatment might gain weight and height faster than untreated ones. Since these measurements are determinants of the onset of puberty in heifers, it is expected that this would influence puberty in dairy heifers.

2.2 Materials and Methods

2.2.1 The Rumensin ABC

A delivery device was used throughout this study to provide continuous dosing of cattle with monensin for approximately 100 day periods. The device is a plastic capsule, approximately 16 cm long with a vented cap bearing collapsible wings at one end, a 22 mm orifice at the other end and weighing 300 g approximately. The capsule is represented in figure 7.

The Monensin Anti - Bloat Capsule (Rumensin ABC - ELANCO) was based on an original Commonwealth Scientific and Industrial Research Organization invention by Dr. Ralph Laby, (ELANCO Rumensin Anti Bloat Capsule booklet). It contains 32.0 g of monensin incorporated into a controlled release polymer formulation (hexaglycerol distearate matrix 45%) that ensures an effective dose of 200 mg of monensin per day over a 100-day period.

Once the capsule is inside the rumen, the core of matrix material at the orifice is exposed to rumen fluids. The copolymer matrix has an average molecular weight of 3000 to 3800 g/mole and is subject to degradation by means of hydrolysis and hydrolytic cleavage. The pressure exerted by the spring and plunger on the solid

Rumensin core is sufficient to keep it moving inside the capsule body so that the dissolving surface remains flush with the opening. It takes approximately 5 days from the time of dosing for the capsule to provide a steady release rate.

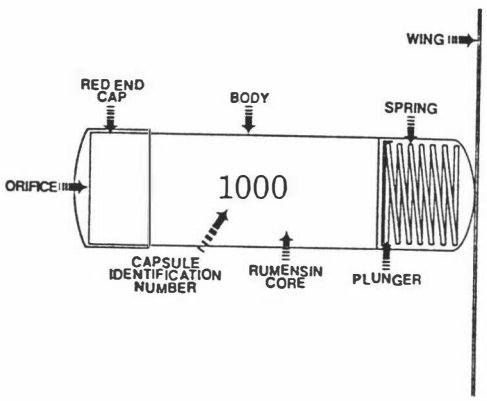
2.2.2 Administration of the Rumensin ABC

The capsule was administered orally to the experimental heifers which all weighed over 200 kg, using the Elanco Rumensin ABC administration tool. Excessive force was avoided to minimise the risk of injury for the animal.

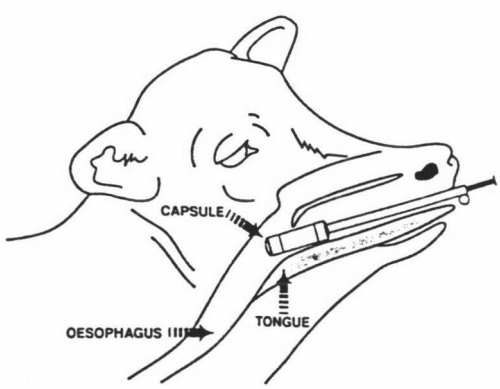
Each capsule had an identification number from 1 to 1,000. At the time of administration, this number was recorded with the animal's identification number. Although no cases of regurgitation were noticed, should it have happened, the animal that lost it could have been easily identified had the capsule been found.

Figure 7. Rumensin ABC and Administration.

a. Capsule



b. Administration



From: Rumensin ABC User's guide. ELANCO

The red plastic end cap was removed before administration. The wings were folded down along the capsule body and then it was placed in the head of the Rumensin capsule administration tool, orifice end first.

Figure 8 shows how to administer the device. The person who conducted the action stood to one side of the animal, restrained it with its neck stretched forward and held firmly against his side. The animal was grasped with one hand in the corner of the animal's mouth and the head of the administration tool containing the capsule introduced into the animal's mouth, avoiding the front teeth.

Once the tool was inside the animal's mouth, it was centred to avoid the molar teeth. The tool was pushed over the base of the tongue and as the animal swallowed, the tongue came forward allowing the tool to move easily over it. If resistance was encountered, the tool was withdrawn and the procedure repeated.

It was important to observe that the head of the administration tool passed the base of the tongue. It was determined that the correct position of the tool was generally indicated by the animal commencing to swallow. When this feature was noticed, the capsule was ejected from the tool by pressing the plunger. In this way, the capsule was deposited in the top of the oesophagus, not at the back of the throat. The tool was left in this position for several seconds before removing it from the animal's mouth to ensure that the capsule was swallowed and not regurgitated.

2.2.3 The Study Population

Two hundred heifers distributed over six farms located in the Manawatu area of New Zealand were used in the experiment. The cattle were grazed on pasture. The approximate location of the farms is shown in figure 9. Table 18 illustrates the distribution of the number of animals per farm.

Figure 8. Administration of the Rumensin ABC

Table 18. Distribution of Animals Over the 6 Farms that Participated in this Trial

Farm	Location	No. of Animals	Control	Treated
1	Foxton	18	9	9
2	Shannon	82	46	36
3	Rongotea	27	14	13
4	Foxton	39	14	25
5	Foxton	14	7	7
6	Rongotea	20	10	10

A total of 232 animals were weighed at the beginning of the trial to stratify them by weight. After this procedure was completed, a number was assigned to each of them and 200 animals were selected out of this total using a computerized random selection procedure which stratified them for weight and divided them into two equal groups: treated and untreated, regardless of farm since there were no significant differences in animal weights between farms.

The trial started in August 1991, when the heifers were an average of 11 months old and weighed over 200 kg. The first part of the experiment lasted 100 days. During that period, samples of faeces (for *Eimeria* diagnosis), blood (to evaluate progesterone levels) and measurements of weight and height were taken at day 0 (when the drug was administered), then approximately 45 and 100 days after the administration. A further weighing, height measurement and sample collection of faeces was carried out approximately 100 days after this period (i.e. about 200 days after initial treatment) to investigate possible carryover effects of monensin on changes in weight and height as well as on *Eimeria* oocyst counts.

The second part of the experiment started 100 days before the expected calving date (April 1, 1992). During this second trial period, measurements of height and weight and samples of faeces were taken at day 0 and 100 after the second administration of the drug, which occurred approximately 35 days after the first treatment.

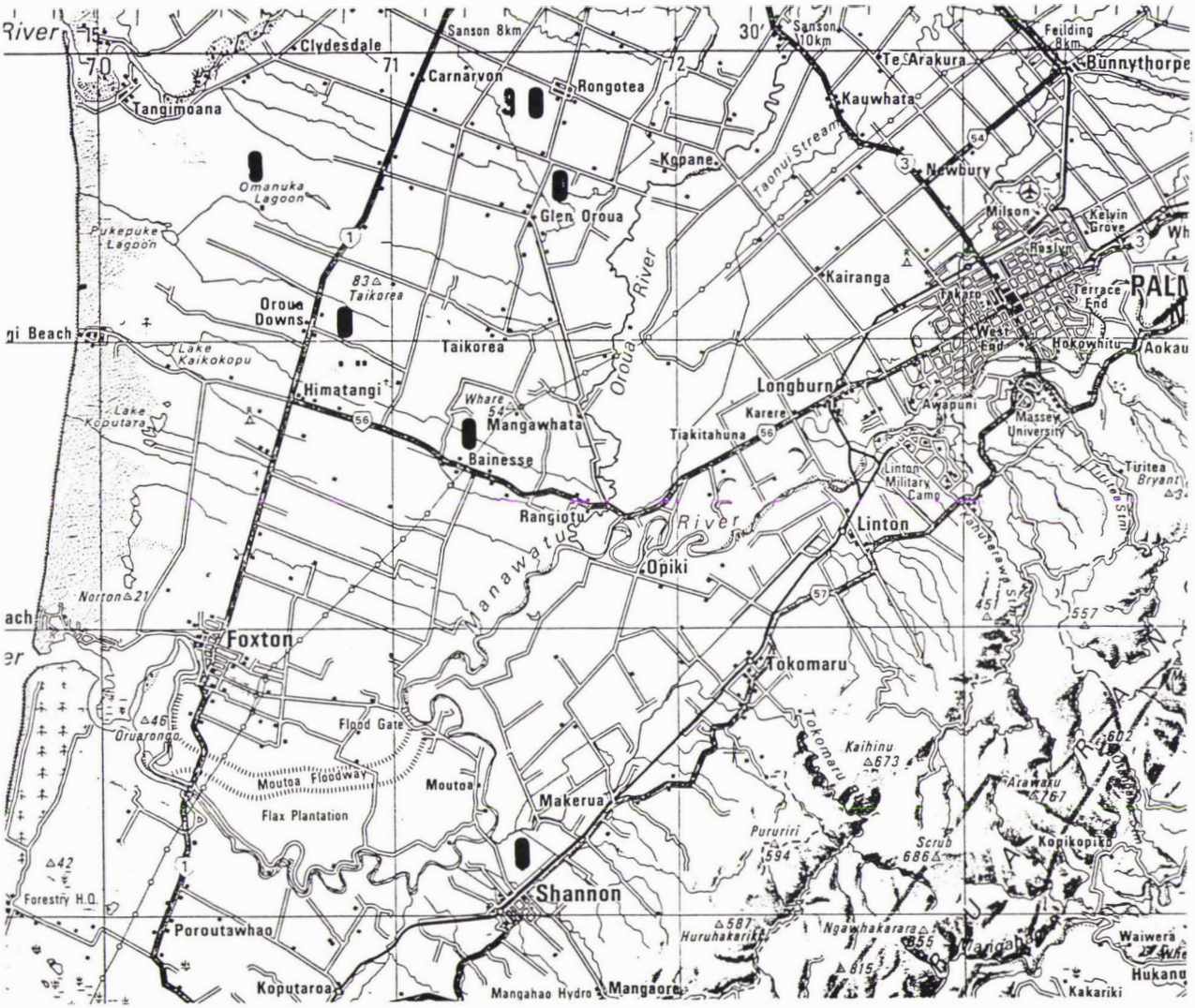
The total number of animals changed during the two parts of the trial due to sales and culling. Specification of these changes is given below.

Table 19. Changes in the Number of Animals per Farm at the Beginning of the Second Part of the Trial

Farm No.	Heifer No.	Cause	Total of heifers used after removals	Control	Treated
1	5 7	Died Culled (Empty)	16	8	8
2	9066, 9069, 9039, 9043, 9023, 9017, 9014.	Sold	75	42	33
3	-	-	27	14	13
4	236	Sold	38	14	24
5	6, 22 21, 13	Sold	10	6	4
6	-	-	20	10	10
Total			186	94	92

The total number of heifers at the beginning of the second part of the trial was 189. However, this number continued to change since farm No. 2 raised replacement heifers for other farms.

Figure 9. Approximate Location of the Six Different Farms Used in this Experiment.



2.2.4 Weighing Procedure

Two different sets of electronic scales were used during the experiments[1]. Both performed satisfactorily.

The weighing platform was placed on an even surface and the load bars were inserted under it. Shims were placed under the feet of the load bars if instability was detected. The indicator holder was installed in a convenient position to protect the unit from possible damage caused by the animals. Caps were removed from the electronic unit and from the platform cable and endings were connected to the input terminal. It was checked that connections were dry and that nothing was affecting the weighing ability of the weighing platform since the machine automatically tared out what was on it. When the animal had all feet on the scale, the weighing button was pressed and within 2 seconds the machine computed its weight (figure 10). Then, the exit gate was opened and the animal was let off the scale. Weights were recorded on paper against the matching animal identification number.

2.2.5 Measurement of Height

The altitude stick used throughout the trial consisted of a horizontal cross arm that contained a fluid filled spirit level, as an indicator of the horizontal position. This part was inserted into a square measuring tube that displayed heights that ranged from 35 to 55.5 inches. These two components were placed into a tube that allowed the reading of height.

The animal was placed on a flat surface and the height stick was used when the animal stood still. When the horizontal indicator was in the appropriate position the animal's height was recorded against the corresponding identification number. Hip height measurement was registered and during the second part of the experiment the wither measurement was also recorded (figure 11).

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[1] A Micropower 2000^{R} system was used in the first part whilst a Tru-Test^{R} scale was used in the second part.

Figure 10. Scales Used for the Measurement of Weight

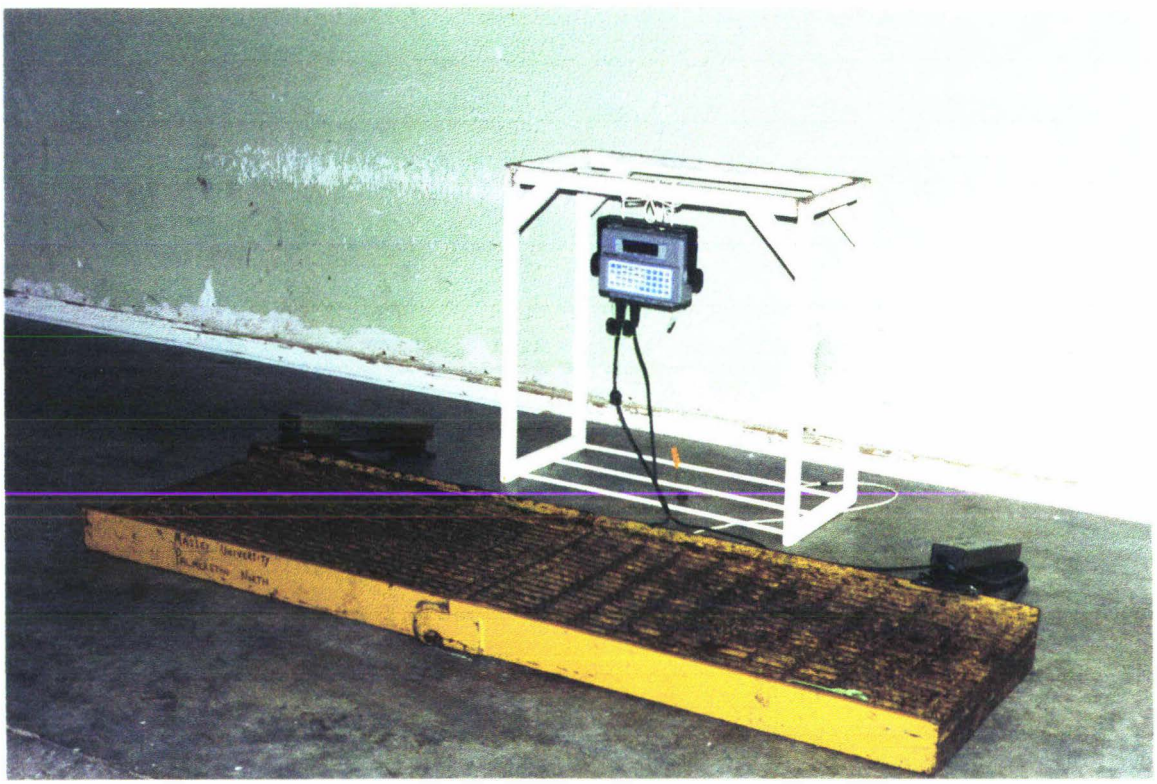
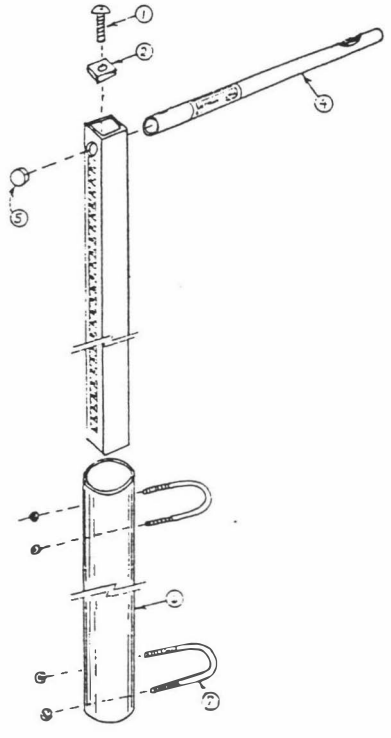
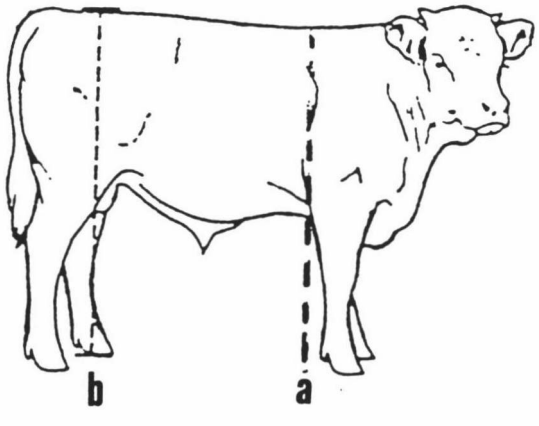


Figure 11. Measurement of Height

Altitude bar



Height taken to Withers (a) and Hips (b)



2.2.6 Statistical Procedure

The statistical analysis was carried out using SAS software[2]. A Multivariate Analysis of Variance (MANOVA) was chosen to examine the data. This test carries out the equivalent of an ANOVA where some variables have been measured for several samples. The discrete independent variable (treatment) was specified and the continuous outcome variables (gain of weight and height) were incorporated into the MODEL statement of the Proc GLM procedure. The statistical model used is shown in appendix 1.

2.3 Results

Results corresponding to the first and second part of the trial are presented in tables 20 and 21 respectively. Statistical analysis of the difference between the final measurement of weight and height - taken in the second part of the trial - and the first corresponding measurements in the first part of the trial (August 1991) were also computed. These results are presented in table 22.

Figures 12, 13, 14, 15 and 16 illustrate the comparison between treated and untreated heifers and their respective weight and height increases.

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[2] SAS/STAT™ User's Guide. SAS Institute Inc. Cary, USA 1988.

Table 20. Effect of Monensin on Mean (SE) of Weight and Height During the First 100 days of the Experiment (August 1991 to February 1992).

Variable[a]	Number of animals per group	Weight Gain 100 days p.a.***	Weight gain 200 days p.a.***	Height Increment 100 days p.a. ***	Height Increment 200 days p.a. ***
Farm 1 (n=16)	UT=8 T=8	88.55 (12.9) 94.06 (11.76)	142.27 (8.81) 154.81 (7.61)	2.44 (0.97) 3.06 (0.26)	4.11 (1.16) 4.56 (0.46)
Date*	02AUG91[b]	16NOV91	20FEB92	16NOV91	20FEB92
Farm 2 (n=75)	UT=42 T=33	91.49 (6.04) 105.69 (5.32)	189.61 (5.32) 190.96 (7.65)	2.55 (0.19) 2.21 (0.31)	4.52 (0.21) 4.86 (0.44)
Date*	05AUG91[b]	18NOV91	21FEB91	18NOV91	21FEB91
Farm 3 (n=27)	UT=14 T=13	114.64 (3.07) 114.77 (3.16)	157.57 (5.65) 146.15 (6.43)	2.50 (0.24) 2.53 (0.30)	1.16 (0.32) 1.34 (0.39)
Date*	06AUG91[b]	19NOV91	24FEB91	19NOV91	24FEB91
Farm 4 (n=38)	UT=14 T=24	104.3 (11.23) 105.7 (13.47)	151.77 (7.90) 150.60 (5.71)	1.69 (0.42) 1.44 (0.19)	3.17 (0.41) 2.70 (0.20)
Date*	08AUG91[b]	21NOV91	25FEB92	21NOV91	25FEB92
Farm 5 (n=10)	UT=6 T=4	93.50 (8.01) 77.50 (12.17)	45.33 (9.46) 136.00 (13.17)	3.56 (0.62) 2.75 (0.57)	5.65 (0.46) 5.75 (0.76)
Date*	09AUG91[b]	22NOV91	27FEB92	22NOV91	27FEB92
Farm 6 (n=20)	UT=10 T=10	102.64 (2.57) 98.00 (3.99)	129.90 (6.28) 138.77 (4.59)	1.86 (0.23) 2.02 (0.40)	3.41 (0.32) 3.69 (0.35)
Date*	10AUG91[b]	25NOV91	28FEB92	25NOV91	28FEB92
All Farms (n=186)	UT=94 T=92	97.21 (3.62) 103.94 (4.66)	167.80 (3.77) 160.72 (3.87)	2.40 (0.15) 2.11 (0.14)	3.61 (0.22) 2.30 (0.25)
Sig**		p=0.25	p=0.19	p=0.16	p=0.75

* Dates for farms when measurements occurred (ddmmyy)

** Probability that the difference in the change in weight or height during the first 100 days of the experiment was due to chance

*** Postadministration

UT: Untreated

T: Treated

[a]: Weight gain 100 days p.a., weight gain 200 days p.a., Height increment 100 days p.a. and Height increment 200 days p.a. were not significantly affected by farm ($p>0.05$)

[b]: First treatment starting date

Table 21. Effect of Monensin on Mean (SE) of Weight and Height During the Second Part of the Experiment (April - July 1992).

Variable[a]	Number of animals per farm	Weight gain 100 days p.a.	Height increment to withers	Height increment to Hips
Farm 1 (n=16)	UT=8 T=8	15.12 (4.94) 20.75 (6.71)	0.22 (0.28) 1.00 (0.20)	0.25 (0.20) 0.19 (0.19)
Date*	01APR92[b]	06JUL92	06JUL92	06JUL92
Farm 2 (n=75)	UT=42 T=33	2.05 (10.74) 14.91 (49.34)	0 (0.39) 0.02 (0.59)	0.29 (0.42) 0.14 (0.68)
Date*	02APR92[b]	07JUL92	07JUL92	07JUL92
Farm 3 (n=27)	UT=14 T=13	10.23 (6.00) 5.25 (5.99)	0.54 (0.25) 0.97 (0.16)	1.31 (0.46) 0.54 (0.24)
Date*	06APR92[b]	09JUL92	09JUL92	09JUL92
Farm 4 (n=38)	UT=14 T=24	55.08 (4.66) 52.74 (5.50)	0.35 (0.20) 0.87 (1.23)	0.62 (0.23) 0.69 (0.29)
Date*	07APR92[b]	10JUL92	10JUL92	10JUL92
Farm 5 (n=10)	UT=6 T=4	-1.16 (5.66) 8.25 (1.75)	1.50 (0.45) 1.62 (0.37)	1.96 (0.28) 1.50 (0.88)
Date*	08APR92[b]	13JUL92	13JUL92	13JUL92
Farm 6 (n=20)	UT=10 T=10	39.55 (10.18) 31.00 (3.17)	0.03 (0.33) 0.46 (0.61)	0.22 (0.21) 1.03 (0.30)
Date*	09APR92[b]	14JUL92	14JUL92	14JUL92
All Farms (n=186)	UT=94 T=92	19.97 (4.31) 27.29 (4.17)	0.34 (0.14) 0.78 (0.16)	0.69 (0.16) 0.53 (0.18)
Sig**		p=0.22	p=0.04	p=0.52

* Dates for farms when measurements occurred (ddmmyy)

** Probability that the difference in the change in weight and height during the second part of the experiment was due to chance

*** Postadministration

UT: Untreated

T: Treated

[a]: Weight gain 100 days p.a., height increment to withers and height increment to hips were not significantly affected by farm ($p>0.05$)

[b]: Second treatment starting date

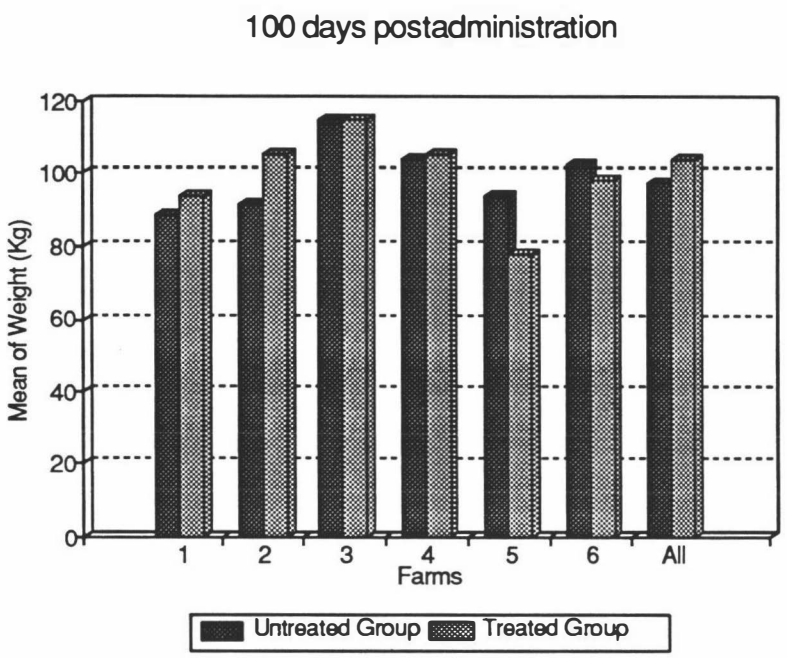
Table 22. Effect of Monensin on Mean (SE) of Weight Gain and Increment in Height Between Initial (August 1991) and Final Measurements (July 1992).

Variable[a]	Number of Animals per group	Gain of Weight	Increment in Height
Farm 1 (n=16)	UT=8	198.37 (9.15)	5.71 (0.45)
	T=8	216.56 (10.33)	6.21 (0.35)
Farm 2 (n=75)	UT=42	225.33 (12.69)	4.83 (0.45)
	T=33	232.29 (14.13)	5.23 (0.49)
Farm 3 (n=27)	UT=14	224.15 (8.60)	5.25 (0.23)
	T=13	209.91 (10.06)	5.00 (0.30)
Farm 4 (n=38)	UT=14	232.42 (9.51)	4.54 (0.42)
	T=28	235.48 (8.45)	4.25 (0.25)
Farm 5 (n=10)	UT=6	161.83 (5.64)	6.32 (0.90)
	T=4	147.75 (12.89)	6.00 (0.77)
Farm 6 (n=20)	UT=10	182.11 (7.76)	3.83 (0.32)
	T=10	183.57 (4.76)	4.53 (0.57)
All Farms (n=186)	UT=94	211.45 (5.23)	4.97 (0.20)
	T=92	217.14 (5.36)	4.94 (0.18)
Sig**		p=0.45	p=0.91

** Probability that the difference in the change in weight and height measurements in the first and second part of the trial was due to chance

UT: Untreated
T: Treated

Figure 12. Effect of Monensin on Heifers' Weight Gains by Group. Part 1 of the Trial



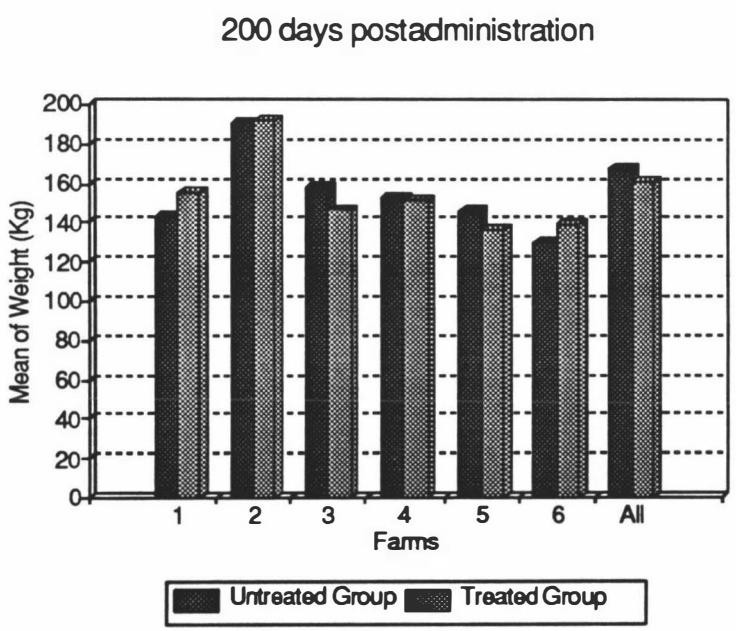


Figure 13. Effect of Monensin on Height Increment Part 1 of the Trial

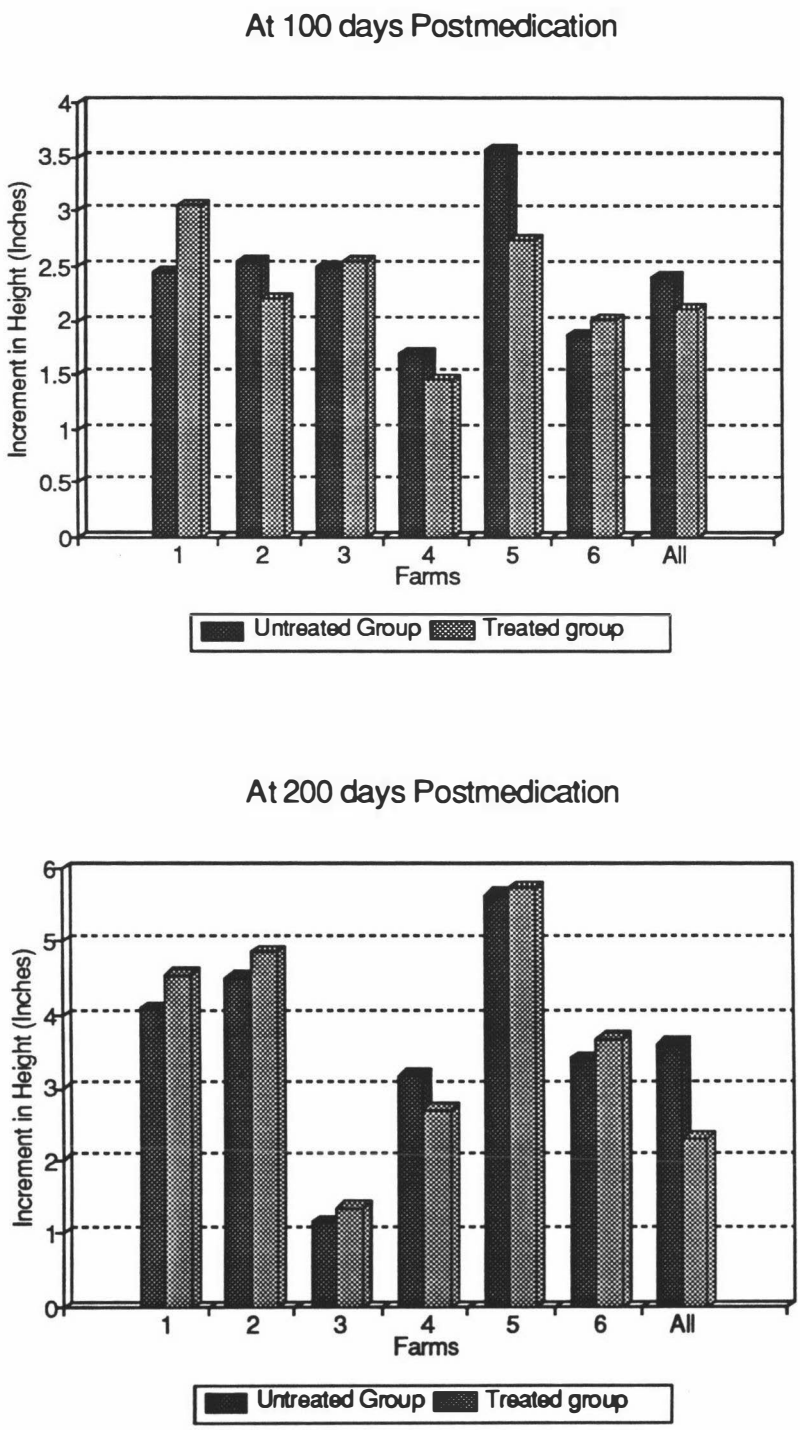


Figure 14. Effect of Monensin on Heifers' Weight Gain
Part 2 of the Trial

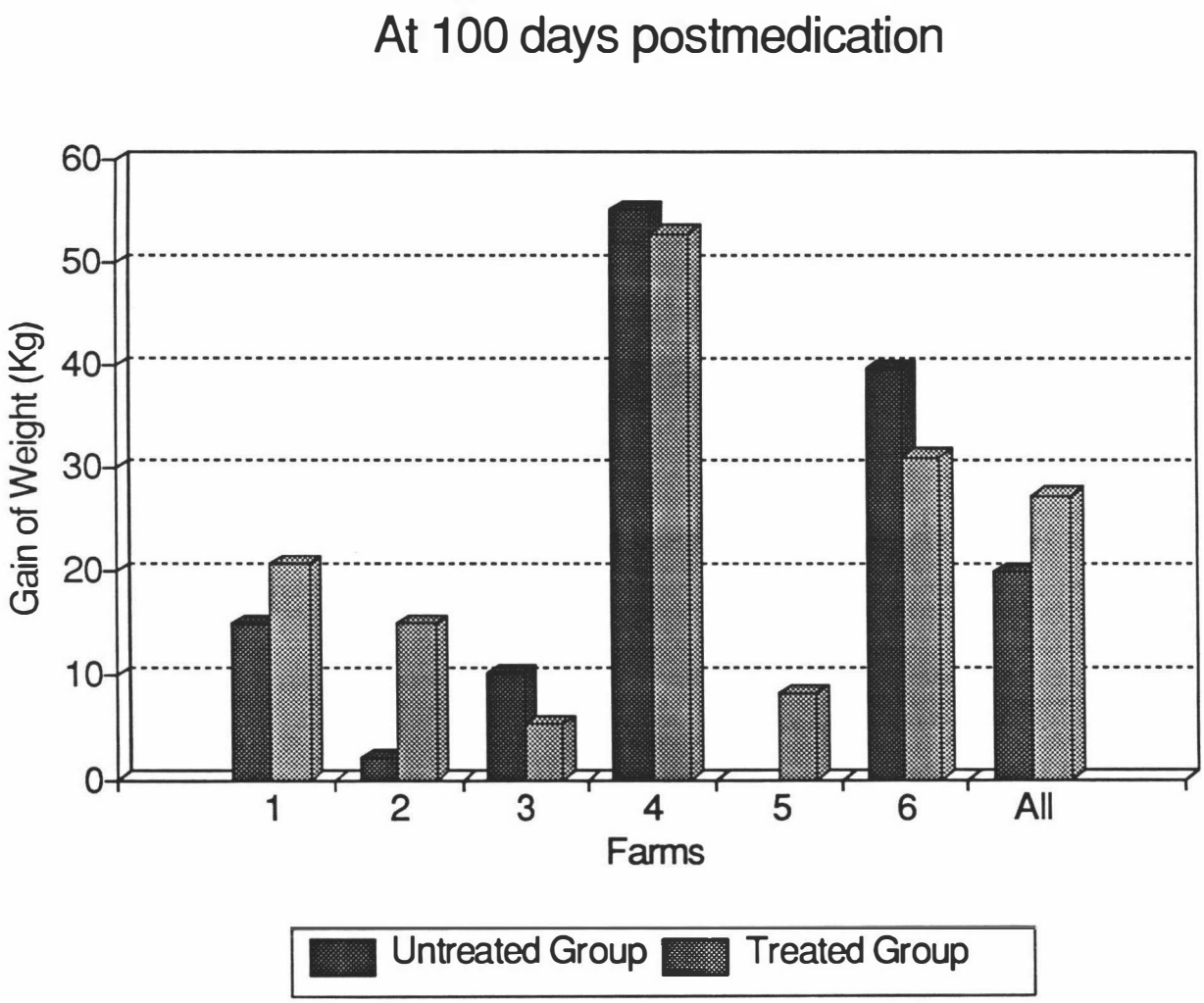
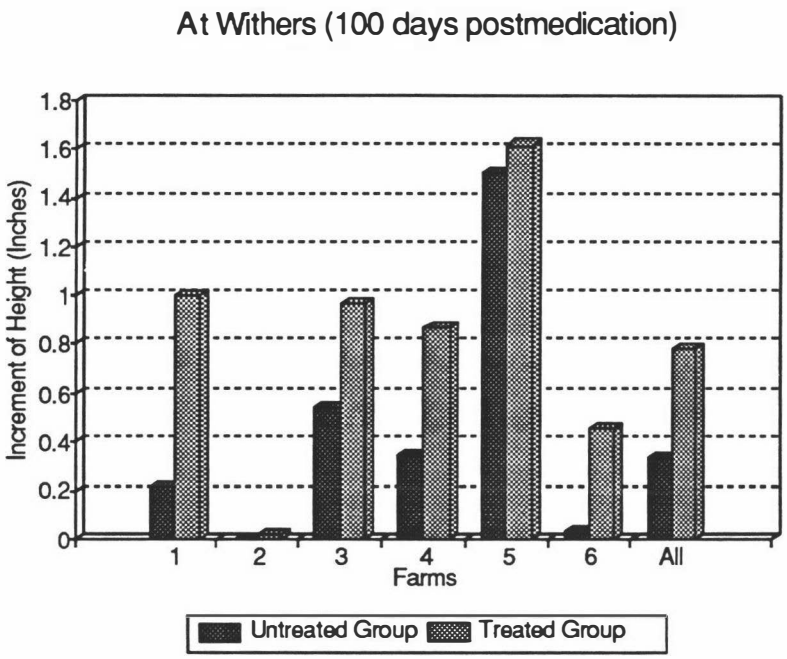


Figure 15. Effect of Monensin on Height Part 2 of the Trial



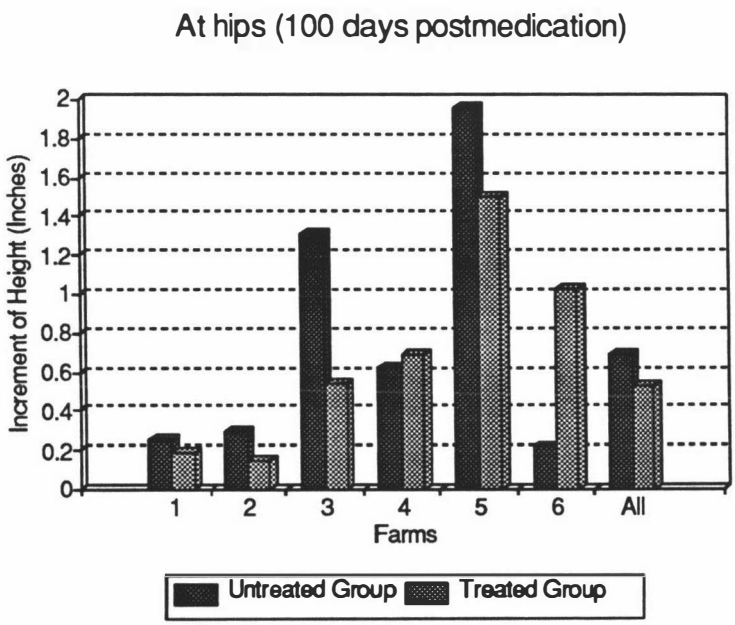
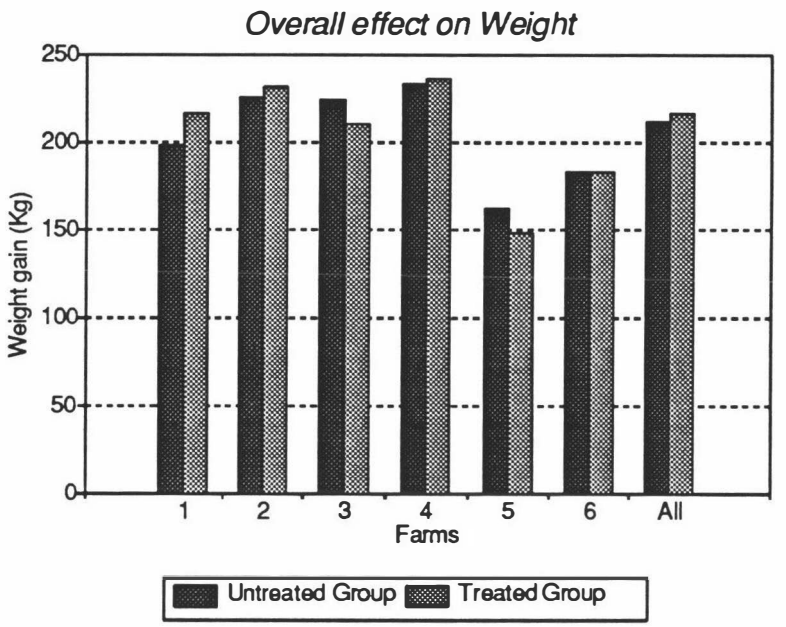
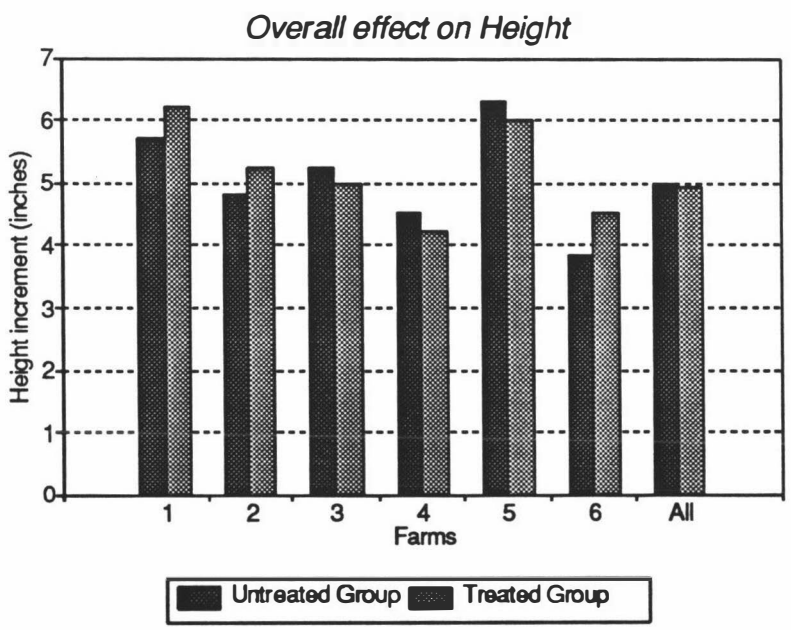


Figure 16. Overall Effect of Monensin on Weight and Height. Parts 1 and 2 of the Trial





2.4 Discussion

There was no significant difference in weight gain between the treated and control groups in either part of the trial. Gain of weight was also similar when the first and last measurements of the total trial were considered ($p>0.1$). These results agree with Meinert *et al.* (1992), Perry *et al.* (1976) and Brown *et al.* (1974). Level of production and quality of food available could have played an important role on the effect of monensin in these heifers. Groups of animals under low energy regimes and low productivity show incosistent responses to feed additives. Improvements in forage quality and inclusion of supplements potentially could enhance the opportunity for monensin to elicit a better performance response.

Although administration of monensin did not significantly increase weight in these heifers, the treated group had a tendency to have higher weights ($p=0.25$ and $p=0.22$ for the first and second parts of the trial, respectively). Treated groups were on average 6.9% heavier than untreated groups in the first part of the trial, 7.3% heavier in the second part and 2.7% heavier when considering the first and last measurements of the trial. This trend towards heavier heifers persisted over time. This observation agrees with Meinert *et al.* (1992) who found that although weight of heifers was not significantly affected ($p>0.05$) by monensin feeding, monensin-fed heifers were heavier in their experiment.

Observation of this weight gain trend with monensin supplementation may reflect differences in the quantity or quality of forage available during the trial which have been associated with differing responses to monensin. The supply of feed varies during the year because of changes in the growth rate of pasture. The pasture growth rate on average for this district starts to increase in August and it is maximum in November when it reaches a quantity of 70 kg DM/ hectare grown daily (Holmes *et al.*, 1987). From April to July, the average of pasture growth is 25 kg DM per hectare daily.

Although feed intake was not measured, cattle included in the treatment group of this trial could have required less dry matter per kilogram of gain than control ones. Perry *et al.* (1976) found intake to be reduced by 10%. If this was the case, monensin supplementation could be useful in those months when pasture growth decreases, so the animal could make more efficient use of the feed present on the farm.

There was no significant effect of monensin from the first treatment of the trial on height changes which agrees with Meinert *et al.* (1992). In their experiment, feeding monensin to heifers had no effect on growth rates. After the second treatment, the administration of monensin significantly ($p=0.04$) improved the height of the withers as shown in table 21. Treatment differences in height to the hips between the first measurement in August and the last one in July 1992 were not significant. Height to the withers was not taken in August 1991 so comparisons with the corresponding measurement in July 1992 could not be made.

Reports indicate that monensin may increase growth rate by altering the concentration of circulating hormones and metabolites, thus altering cellular metabolism (Raun *et al.*, 1976). They reported that plasma growth hormone (GH) tended to be higher in monensin - fed cows. By contrast, Plouzek *et al.* (1982) reported that feeding monensin to yearling steers resulted in a trend toward lower plasma GH. It appears, then, that monensin may have stimulated the increase in height of the heifers but this observation was not consistent throughout the experiment.

Statistical analysis of the heifers' weight and height in the period between the first and the second part of the trial (February to April 1992) showed that there was no significant change in these parameters in the treated group during that period when compared to the controls ($p>0.1$). A valid explanation of these results is related to the fast degradability of monensin. Donoho, (1984) demonstrated that 12 hours after removal, the ionophore had been degraded almost completely and therefore no effect in ruminal fermentation after its withdrawal must be expected.

Results concerning the correlation between height and weight were difficult to interpret. If monensin had a positive effect on height, the same outcome may not necessarily be observed in weight gain. These two parameters may conceivably vary independently. While the fluctuation of height was minimal as time passed, weight measurements changed drastically over time according to feeding. Thus, responses to the use of feed additives in cattle on pasture are not always predictable. Rate of weight gain may be restricted by the amount and type of feed available.

2.5 Conclusion

The results of this trial demonstrate that monensin can be safely fed to dairy heifers under practical field conditions. Consumption of 200 mg per head daily produced body weight change equal to that of heifers not receiving monensin. These results agree with previous studies that reported little or no body weight change in heifers supplemented with 200 mg monensin/head/day.

Based on the results of this experiment and those conducted by Moseley *et al.* (1977), Turner *et al.* (1980) and Lemenager *et al.* (1978), it appears that the benefit of monensin in cattle is variable. Weight gains of heifers were not affected significantly but less feed might have been required. A trend toward increased heifer weight was associated with monensin supplementation.

Height changes due to monensin administration were not statistically significant during the first part of the trial. Height at the withers was significantly improved after the second treatment. Height at the hips, by contrast, was not significantly affected by monensin. This inconsistency may be clarified by designing another experiment that looks specifically at the effect of monensin on height and weight changes, but ensures that feeding levels are kept constant.

Effects of Monensin on *Eimeria spp.* (Apicomplexa: Eimeriidae) of Heifers

3.1. Introduction

Bovine coccidiosis is a significant disease of cattle throughout the world. Greiner *et al.* (1984) reported that in countries like the United States, mortality due to coccidiosis has reached 20% of the young cattle treated annually for clinical acute coccidiosis by veterinarians. In addition, if losses resulting from subclinical or mild or untreated cases of clinical coccidiosis are added to this, the financial impact on the cattle industry is considerable.

In New Zealand, the disease is considered to be most important as a cause of diarrhoea in calves one to three months old. However, reports from the North Island laboratories show that it also can cause serious clinical disease in 1-2 year old cattle (Anonymous, 1990). These animals and older ones also may serve as symptomless carriers.

The carboxylic ionophore monensin has proven to be an effective preventive agent against coccidiosis in calves at a dose of 1 mg/kg (Parker *et al.,* 1986). However, its effect in the form of a slow release intraruminal device on oocyst discharge of heifers has not been evaluated under New Zealand conditions.

The purpose of this part of the study was to examine the effect of monensin on coccidia. In addition, the proportions of different species of *Eimeria* found during the experiment were examined to see if there were seasonal changes in them.

3.1.1 Life Cycle of *Eimeria* species

The first coccidium, and evidently the first protozoan, that was seen in the world was *Eimeria stiedai,* which Leeuwenhoek observed in 1674 (Levine and Ivens, 1970). The parasite was not named until 1865 and did not receive its present name until 1907.

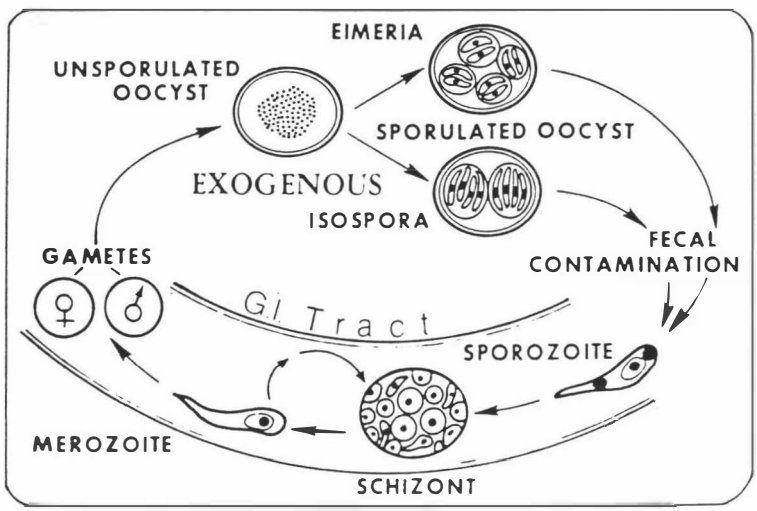
Although the taxonomic position of coccidia has been a subject of controversy, Levine (1973) clarified the situation when he stated that coccidia have the following characteristics: "Macrogamete and microgametocyte develop independently; syzygy absent, microgametocyte typically produces many microgametes; zygote not motile; sporozoites typically enclosed in a sporocyst; endodyogeny absent; monoxenous or heteroxenous".

Members of the genus *Eimeria*, with few exceptions, are considered monoxenous because the life cycle is completed in only one host, and stenoxenous because each species usually parasitises a single species of host. Unsporulated oocysts are shed in the faeces. Oocysts sporulate outside the body (exogenously) under aerobic conditions. With appropriate temperature and in the presence of moisture and oxygen the oocysts sporulate to an infective state. The sporulated oocyst is tetrasporic and the sporocyst is dizoic; i.e., the oocyst contains four sporocysts, each of which contains two sporozoites. Figure 17 shows a classical coccidian life cycle.

After the oocyst is ingested, the sporozoites (motile infective stage) excyst, enter intestinal epithelial cells, and become rounded; each becomes a trophozoite, which in turn grows and becomes a schizont (Todd *et al.,* 1977). Through asexual multiplication, many nuclei form in each schizont, and a merozoite develops from each nucleus. The number of merozoites in a schizont depends on the species of coccidia. The schizont ruptures and the merozoites enter new host cells to form a second generation of schizonts. Two, three or more asexual generations of schizogony may result. After the last schizogony, merozoites enter new host cells and initiate gametogony, the sexual phase of the cycle. Gametogony may occur as early

as 15 days postinfection. Most become macrogametes, each of which contains a nucleus that does not divide (female). Others become microgametocytes in which nuclear division takes place (male).

Figure 17. Eimeria Life Cycle



From: Fayer, (1980).

Biflagellate sperm-like microgametes that develop within the microgametocytes eventually break out and fertilize the macrogametes (Todd *et al.*, 1977). The fertilized macrogamete forms an oocyst wall around itself and, as an oocyst, leaves the host cell, enters the intestinal lumen, and is shed in the faeces (Fayer, 1980).

3.1.2 Coccidia of Cattle

Fifteen species of intestinal coccidia have been recognised in cattle: *E. bovis* Fiebiger, 1912; *E. zuernii* Martin, 1909; *E. canadensis* Bruce, 1921; *E. ellipsoidalis* Becker and Frye, 1929; *E. auburnensis* Christensen and Potter, 1939; *E.*

bukidnonensis Tubangui, 1939; *E. alabamensis* Christensen 1941; *E. cylindrica* Wilson, 1931; *E. illinoisensis* Levine and Ivens, 1967; *E. kosti* Elibihari and Hussein, 1974; *E. thianethi* Gwéléssiany, 1935; *E. pellita* Supperer, 1952; *E. brasiliensis* Torres and Ramos, 1939; *E. wyomingensis* Huizinga and Winger, 1942; and *E. subspherica* Christensen, 1941 (Levine, 1982).

The following species of coccidia have been reported in New Zealand: *E. bovis, E. zuernii, E. canadensis, E. ellipsoidalis, E. auburnensis, E. alabamensis, E. cylindrica, E. brasiliensis, E. wyomingensis* and *E. subspherica* (McKenna, 1972).

The most pathogenic species are *E. bovis* and *E. zuernii.* They are the most common cause of clinical coccidiosis in cattle. *E. auburnensis* is moderately pathogenic.

3.1.3 Epidemiology of *Eimeria* Infection

Confinement of animals in small damp areas, either in tropical or temperate environments, facilitates disease from coccidial infection because large numbers of oocysts excreted by infected animals become accessible to potential hosts (Fayer, 1980).

Parker *et al.* (1984) stated that the causes of bovine coccidiosis, particularly by *E. zuernii* are poorly identified because the ingestion of large number of sporulated oocysts does not always result in disease. Their work in dry environments demonstrated that disease may result in the absence of abundant oocysts or even when environmental conditions are not favourable for oocyst sporulation. They concluded that various factors combine to produce coccidiosis and stress of calves at weaning is one of them. Precipitation of disease by weaning stress rather than a heavy new oocyst challenge was then suggested.

The number of oocysts produced by an infected animal is affected by factors such as

immunity or resistance developed by the host, nutrition of the host, stress factors, competition with other species of coccidia or other infectious agents, genetic differences in strain of parasites, and the use of anticoccidial drugs. Another factor to be considered is the variation in the inherent reproductive potential (number of oocysts produced from each inoculated oocyst) of the different species of *Eimeria*. This factor may contribute greatly to the observed differences in prevalence of species (Fayer, 1980).

The oocyst is the only exogenous stage in the coccidian life cycle and is found in every species. Therefore, factors affecting the number of oocysts produced, their sporulation and their physical and biological dispersal as well as factors affecting the oocyst survival time such as moisture, temperature and chemicals will determine the epidemiology of coccidian infections.

3.1.4 Factors Affecting the Number of Oocysts Produced by an Infected Animal.

3.1.4.1 Immunity and Resistance

Immunity to *Eimeria* results in the decreased production of oocysts after ingestion of infective oocysts. Immunity is specific to each coccidian species and immunity to one species does not confer immunity to other species in the same host. Schizonts are thought to provide the antigenic stimulation that results in immunity. Apart from acquired resistance, natural resistance is thought to increase with age of the host (Fayer, 1980).

3.1.4.2 The Crowding Factor

As more oocysts are inoculated into a host, the number of oocysts produced per oocyst inoculated decreases. This phenomenon is called the "crowding" factor (Brackett and Bliznick, 1952). One possible explanation for it is the very rapid development of an immune response. Another possibility is that as the number of first and second generation schizonts increases, the area of healthy tissue left for development of subsequent stages decreases. Simultaneously, tissue damage from infection with one species may decrease the oocyst output of another species (Fayer, 1980).

3.1.4.3 Nutrition

The nutritional state of the host affects the development of coccidia. Vitamins A and K provide protection for the host without being nutritional requirements of the parasite. Vitamins such as thiamine, riboflavin and biotin are required by the parasite and known chemical antagonists of such nutrients are used as coccidiostats in poultry (Ryley, 1973).

3.1.4.4 Stress

Continual or intermittent shedding of small numbers of oocysts by livestock is relatively common. Marsh in 1938 (cited by Fayer, 1980) hypothesized that coccidia are normally present in the intestine of cattle but do little harm until host resistance is decreased by external factors. Stress factors such as shipping, exposure to heat, cold or other climatic extremes, change in diet, specific types of feed and crowding cause outbreaks of coccidiosis. Determination of the influence of these stress factors

on coccidiosis is extremely difficult (Fayer, 1980).

3.1.4.5 Coccidiostats

Most coccidiostats protect against clinical illness while reducing but not eliminating oocyst output. In theory, the continued low-level shedding of oocysts is desirable because animals can then be exposed to small numbers of oocysts and can develop immunity without clinical disease (Fayer, 1980).

3.1.5 Conditions Affecting Sporulation and Survival of Oocysts

Oocysts are not infectious until they sporulate. *Eimeria* oocysts sporulate exogenously and are subject to the influence of environmental conditions. Three factors are known to affect sporulation: temperature, moisture and availability of oxygen. Cold or freezing temperatures retard sporulation and temperatures above 35°C seem to inhibit or reduce sporulation of several species of *Eimeria.* Pellérdy (1974) states that the sporulation time is 2-3 days for most of the species of *Eimeria* but it can be as long as 14 days for *E. brasiliensis*.

Marquardt (1960) found that sporulation of *E. zuernii* was a two-stage process: the first or presegmentation stage was shortened by exposure to high temperatures, whereas the second or segmentation stage was inhibited by high temperatures. In the same experiment, sporulation of *E. zuernii* was greatly reduced at relative humidities of less than 75% and at oxygen tensions of less than 10%. Under controlled conditions of temperature and moisture, only 33% of *E. zuernii* oocysts survived 4 h of exposure to sunlight and none survived 8 h of exposure (Marquardt *et al.,* 1960).

Sporulated oocysts can remain viable and infective for relatively long periods.

Eimeria ellipsoidalis and *Eimeria bovis* oocysts used for experimental infection of cattle were routinely stored in suspensions of 2.5% potassium dichromate at 5°C and were infectious for approximately one year (Fayer, 1980). Low temperatures apparently were not detrimental because oocysts were still highly infective after the lowest winter temperatures (7°C). The oocysts remained infective longer in part-shade than in direct sunlight. Moisture was the limiting factor for length of survival.

Oocysts are extremely resistant to the effects of harsh chemicals. The most effective chemicals are so caustic or toxic that they are generally not suitable for use in livestock-rearing facilities. Small-molecular-weight compounds such as ammonia are effective oocysticides under special conditions but are ineffective if direct contact with oocysts is impaired, as when oocysts are mixed with litter (Fayer, 1980).

Farquhar *et al.* (1979) proposed the ensiling of waste-blended diets made from bovine excreta and basal feedstuffs as an economical and convenient means of conserving available nutrients and avoiding direct transmission and continual reinoculation of coccidia. They concluded that the fermentation process that occurs in a properly ensiled, manure-blended diet inhibits sporulation of bovine coccidial oocysts. The degree of inhibition depends on the ensiling temperature, with 25°C to 35°C having the greatest detrimental effect over a 3 week storage period.

In addition, exposure of unsporulated and sporulated oocysts to high levels of gamma radiation, X-rays, ultrasonic waves, and low-accelerating-voltage electrons only reduces viability or destroys relatively small numbers of oocysts. Such energy sources may be potentially useful for attenuation of oocysts for immunization. However, their use for on-site destruction of oocysts does not appear practical (Fayer, 1980).

3.1.6 Physical and Biological Dispersal of Oocysts

Because oocysts are relatively small (most are between 10 and 50 µm) and resistant to environmental factors except heat and drying, they may be easily dispersed by

wind and water. They may remain in faeces on the soiled clothes, boots or hands of animal handlers or veterinarians and be transported from one location to another. They may be transported by contaminated tools or machinery. Animals themselves may transport oocysts on their bodies and infections may result when cattle lick themselves or others in the same yard.

In addition, invertebrates also may transport oocysts. Under experimental conditions earthworms and worm casts were found to contain infective oocysts (Markus, 1974).

According to Fayer (1980), the role of wildlife is also important in the epidemiology of coccidial infections because some wildlife species are closely related to domestic animals and share species or strains of parasites with them. Disease problems arise when the territories of the wild and domestic animals overlap or when vectors or transport hosts have access to both groups of animals.

Eimeria infection can influence the development of other diseases. Moderate coccidial infection enhances the damage caused by bovine parvovirus (BPV) (Durham, 1985).

3.1.7 Pathogenesis of Coccidial Infections

Severe clinical coccidiosis usually causes diarrhoea, loss of blood, dehydration and loss of appetite. Reactions to toxins from the parasite have also been observed (Isler *et al.*, 1987). The general health of the animal usually decreases and the animal becomes anorexic and subsequently loses condition.

The physiological and pathological changes occurring in infected animals may significantly affect their ability to recover from disease. Changes in weight of animals after infections with *Eimeria* are accompanied by changes in blood components such as serum globulin, albumin, glucose and phosphorus as well as

haemoglobin content and packed cell volume. These changes have been reported to occur only when severe blood loss due to severe intestinal bleeding was observed. Stockdale (1977) stated that after the infection of the epithelium of the intestinal mucosa, *Eimeria zuernii* in cattle invade the host villous cells and attack the endothelial cells of the lacteals. After the first schizogonous generation, the merozoites invade epithelial cells in the crypts of the large intestine. Gametocytes, in turn, are found in the epithelial cells of the intestinal crypts to cause villous atrophy in the intestine.

Speer *et al.* (1985) stated that immunity involves both humoral and cellular responses. Merozoites and sporozoites of *E. bovis* have numerous cytoplasmic antigens but few surface antigens that elicit an IgG response in the host. Globule leucocytes play an important role in the immune reaction of the host to coccidia by cellular means. Examination of intestinal tissues from experimentally infected worm-free lambs, showed that the population of globule leucocytes was, on average, nine times higher in the mucosa overlying Peyer's patches (12.8 globule leucocytes per mm) than in the adjacent mucosa (1.6 globule leucocytes per mm). This indicates that antigenic stimulation in Peyer's patches by coccidial antigens may directly induce proliferation or differentiation of globule leucocytes. There was also evidence of increased migration of globule leucocytes into the villous epithelium in response to coccidial infection. In any event, calves that recover from infection are either immune to reinfection or, if reinfection occurs, little or no signs of disease are observed (Gregory *et al.*, 1981).

3.1.8 Main Features of the Disease Caused by *Eimeria zuernii* and *Eimeria bovis*

Levine *et al.* (1970) described *Eimeria zuernii* and *Eimeria bovis* as the two most pathogenic species of coccidia in cattle while some other species may contribute to disease. The disease processes resulting from infections with *E. bovis* and *E. zuernii*

have been described.

Calves fatally affected by *Eimeria* appeared to die in three different circumstances:

- Those that died early in infection (days 18-20 postinfection when the maximum production of oocysts occurred) had diarrhoea and were dehydrated on clinical and postmortem examination (Stockdale, 1976).

- Calves dying later in infection (days 21-25 postinfection) had diarrhoea that progressed to dysentery and were both dehydrated and anaemic at ante- and postmortem examination.

- Calves that survived beyond 25 days after the infection either rapidly improved in condition or progressively weakened until they could no longer stand, requiring euthanasia (Stockdale, 1977).

Gross lesions found after infection consisted of a diphtheritic enteritis located in caecum, proximal part of the spiral colon and the distal two metres of the ileum. Most of the damage to the intestinal mucosa is apparently caused by sexual stages (reported in *E. zuernii*). The section of the intestine affected differs according to the species involved. In *E. zuernii* infection, the caecum was always more severely affected while in *E. bovis* infection, the colon was the target organ.

Oocyst production of *E. bovis* usually peaks at 19-22 days postinfection. This coincides with the massive damage due to the formation of the sexual stages and oocysts at 19 days postinfection.

The effect of coccidial infection on weight gain of calves was studied by Stockdale (1981). Infected and control calves gained weight at the same rate for 20 days after the infection but after that, infected calves lost weight abruptly. This is explained by the destruction of epithelial cells by second generation schizogony and gametogony. This leads to a reduction of reabsorption of water, Na^+ and Cl^- from the intestinal

contents. Moreover, as the epithelium is lost, capillaries of the large intestinal lamina propria are exposed and rupture occurs with loss of erythrocytes and plasma.

Theoretically, each infective oocyst of *E. bovis* ingested may yield as many as 24 million second generation merozoites and thus 1,000 oocysts would infest and destroy 24 billion intestinal cells (Stockdale, 1981).

Another manifestation of coccidiosis in cattle is what has been described as "nervous coccidiosis". This entity has not been reported in New Zealand, but it affects up to 30% of the calves with coccidiosis confined in feedlot conditions (Isler *et al.*, 1987). The nervous signs vary in severity and frequency from minor muscular incoordination, twitching and loss of balance to intermittent or continuous seizures. Apparent blindness and hyperexcitability have been noted occasionally (Radostits *et al.*, 1980).

3.1.9 Control of Coccidiosis in Cattle with Monensin

Since any level of infection in the intestine elicits some degree of resistance (Fitzgerald *et al.*, 1984), the apparent effectiveness of a given compound against coccidiosis in calves depends upon whether the calves have been previously exposed to coccidial organisms. Therefore, before testing a coccidiostat, ideally, calves should not have been previously exposed to coccidia (Stromberg *et al.*, 1986).

Fitzgerald *et al.* (1984) carried out an experiment to test the effectiveness of different doses of monensin against experimental *Eimeria* spp. infection in calves. Fourteen days after inoculation, different degrees of coccidiosis were observed according to the level of ionophore given and accompanied by a marked decline in weight gain that was correlated with the occurrence of clinical coccidiosis. Non-medicated calves however, showed severe signs of coccidiosis while medicated ones were less severely affected.

Stockdale, (1978) found that monensin was effective at preventing and suppressing most of the clinical signs in bovine coccidiosis due to *E. zuernii and E. bovis* after experimental infection.

Fitzgerald *et al.* (1984) and (Stockdale, 1981) concluded that the ionophore antibiotics effectively control coccidial infections in ruminants when they are administered at a dose rate of 1 mg/kg in treatments that last from three days before infection until 30 days after infection.

In the tropics, monensin reduced the severe diarrhoea and dysentery associated with *E. zuernii* under natural conditions (Parker *et al.*, 1988). Here, the intra-ruminal controlled release capsules were a convenient and effective method of medicating weaner calves continuously for one to two months.

3.2 Experimental Procedure

The experimental procedure follows the same time pattern as described under Materials and Methods in Chapter II (effect of monensin on weight and height). During the first part of the trial, 4 samples of faeces for oocyst counts were collected from each animal directly from the rectum. Each sample weighed 10 g approximately. The first sample was collected when the capsule was administered, the second one at 45 days and the third one, 100 days after the first. A fourth sample was taken after a further 100 days to see if there were any carryover effects of monensin against *Eimeria*. Similarly, at the beginning of the second treatment regime, a sample was taken, with another 100 days later.

Samples for identification of *Eimeria* species were taken on three occasions: August 1991, November 1991 and February 1992.

3.2.1 Examination of Samples for Coccidia

3.2.1.1 Oocyst Counting

Faecal samples were stored at 4°C until examined. Oocysts were counted by a modified McMaster technique using 2 g of faeces suspended in 28 ml saturated NaCl solution (specific gravity 1.2). The total number of oocysts counted, multiplied by 50, represented the number of oocysts contained in one gram of faeces (OPG).

3.2.1.2 Separation of Oocysts for Sporulation.

- Five gram of faeces was taken from each sample and placed in a common container. When all subsamples from each farm were collected, the pooled material was thoroughly mixed using a glass stirrer to provide a representative sample from each farm. This procedure was done for each farm.

- Five gram of faeces from this homogenate was mixed with 50 ml tap water and filtered through a 500 µm aperture sieve. The filtrate was then passed through a 105 µm sieve. The residue in each sieve was washed with a jet of tap water.

- Approximately 50 ml filtrate was centrifuged at 800 X G for 6 minutes.

- Two-thirds of the supernatant was discarded. The sediment was resuspended in tap water and recentrifuged.

- The resultant sediment was resuspended in sodium chloride solution (specific gravity 1.2) and allowed to stand for 10 minutes for coarse material to sink

with little chance of trapping the oocysts. The suspension was then centrifuged at 400 X G for 6 minutes.

- The tube was removed gently from the centrifuge and allowed to stand for a further 10 minutes to compensate for any disturbance to the oocyst band at the top.

- Approximately 5 mls was sucked from the top by using a "J" pipette attached to a suction pump and collected in a 50 ml centrifuge tube.

- The oocysts were twice washed free of sodium chloride solution by suspension and centrifugation in distilled water.

- The washed sediment was transferred to a 15 ml graduated conical centrifuge tube and centrifuged at 150 X G for 6 minutes.

- The supernatant (approx: 12 mls) was discarded and the sediment resuspended in 2.5 per cent (W/V) potassium dichromate solution. The total volume was not > 5 ml. The suspension was then placed in a 35 mm petri dish.

- The petri dish, then, was placed in a 27°C room for 21 days to allow sporulation (McKenna, 1972). A few drops of potassium dichromate solution were added on the 7^{th} and 14^{th} day to protect the oocysts from desiccation.

- On day 21, the oocyst suspension contained in the petri dishes was stirred thoroughly to free the oocysts that usually adhere to the bottom.

- The suspension was washed into a 15 ml tube. Sucrose solution (specific gravity 1.2) was added carefully to form a convex meniscus and a coverslip was placed over it.

- After 10 minutes the coverslip was removed, placed on a microscope slide

and the sporulated oocysts examined at 400x magnification. One hundred oocysts from the pooled sample were identified to species level.

3.2.1.3 Identification of Species

Species were identified according to descriptions given by Levine and Ivens (1970), Levine (1985) and Levine and Ivens (1986) for sporulated oocysts. Sporulated oocysts were examined and identified from consideration of the following features: presence or absence of micropylar cap and its characteristics, the oocyst size and shape, presence or absence of a micropyle, number of polar granules, size and shape of sporocysts, characteristics of the residuum (if present), presence or absence of a Stieda body, number and size of refractile globules in each sporozoite. The following tables were taken as a guide: (Adapted from Levine and Ivens, (1970) and Levine and Ivens, (1981)).

To determine oocyst and sporocyst dimensions for comparative purposes, 100 oocysts were measured using an Olympus BH2 microscope with apochromatic objectives and a digital micrometer.

Table 23. Morphological Characteristics of Sporulated Oocysts of *E. alabamensis, E. auburnensis* and *E. bovis.*

	alabamensis	***auburnensis***	***bovis***
Size (µm)	18.9 x 13.4	38.4 x 23.1	27.7 x 20.3
Shape	Pyriform. Also subellipsoidal or subcylindrical	Elongate - ovoid. Flattened.	Stoutly ovoid. Blunted across the narrow end.
Micropyle	-	+	+
Micropyle cap	-	-	-
Oocyst wall	Thin, delicate homogeneous, transparent.	Thick: 1-1.5µ A membrane lines the oocyst wall.	Smooth and slightly thinner at the micropylar end
Sporocyst residuum	-	+ Rounded masses or individual granules	+
Stieda body	+ tiny	+	-
Number of large refractile globules	3-4	1 large at the large end. 1-2 small.	1

Table 24. Morphological Characteristics of Sporulated Oocysts of *E. brasiliensis*, *E. bukidnonensis* and *E. canadensis*.

	brasiliensis	***bukidnonensis***	***canadensis***
Size (µm)	36-38 x 26-27	33-54 X 24-35	32.5 x 23.4
Shape	Ellipsoidal	Pyriform. Yellowish brown to dark brown	Ellipsoidal. Slightly ovoid.
Micropyle	+	+ Conspicuous	+ Inconspicuous gap covered with a thin, dark refraction line.
Micropyle cap	+	-	-
Oocyst wall	Composed of 1 single layer, plaques.	Thick: 2-4 µ Yellowish-brown radially striated.	Transparent. 1 µ thick and thinner at each end.
Sporocyst residuum	+ Scattered	-	+
Stieda body	+ Small-dark	+	+ Small
Number of large refractile globules	1	1	2-3

Table 25. Morphological Characteristics of Sporulated Oocysts of *E. cylindrica*, *E. ellipsoidalis* and *E. pellita*.

	cylindrica	***ellipsoidalis***	***pellita***
Size (μm)	23 x 14	17 x 13	36-41 x 26-30
Shape	Cylindrical (sides parallel)	Ellipsoidal (vary from spherical to almost cylindrical	Ovoid with a flattened end).
Micropyle	-	-	+
Micropyle cap	-	-	-
Oocyst wall	Thin, smooth, homogeneous, a single layer.	Thin, smooth, a single layer.	Thick, dark brown, velvet appearance
Sporocyst residuum	+	+ Rounded masses or individual granules	-
Stieda body	-	-	-
Number of large refractile globules	1 or more and a central vesicle.	1 in the large end. 1 near the middle	2

Table 26. Morphological Characteristics of Sporulated Oocysts of *E. subspherica, E. wyomingensis* and *E. zuernii.*

	subspherica	***wyomingensis***	***zuernii***
Size (µm)	11 x 10.4	40 x 28	17.8 x 15.60
Shape	Subspherical.	Ovoid.	Spherical to bluntly ellipsoidal.
Micropyle	-	+	-
Micropyle cap	-	-	-
Oocyst wall	Thin, smooth, homogeneous, fragile appearance.	Yellow. 1 layer. 2-3.5 µ thick	Thin, homogeneous, transparent.
Sporocyst residuum	-	-	-
Stieda body	+ small	+ tiny-flat	+ tiny
large refractile globules	1	1 large	1 in the large end.

3.2.2 Data Analysis

Data relating to oocyst counts were analyzed in the SAS^{TM} statistical package using a nonparametric analysis of variance for independent samples by randomization (The Kruskal-Wallis Method). A common analysis of variance, which is a parametric test, assumes certain properties such as normality and homogeneity of treatment variances that in this case could not be met (Schefler, 1979).

The analysis involved the ranking of all oocyst counts corresponding to each set of samples, beginning with the smallest and ending with the largest count. If ties occurred, each member of the tie was assigned the same rank, which was the mean of the ranks making up the tie. The analysis was completed using a general linear model procedure.

3.3 Results

3.3.1 Oocyst Counts

The mean oocyst counts with corresponding Standard Errors (SE) are shown in table 27. Results referring to the second part of the trial are presented in table 28. Figure 18 shows the mean number of oocysts in treated and untreated groups by farm at 0 and 45 days postadministration during the first part of the experiment. Figure 19 shows the mean of oocyst counts for treated and untreated groups at 100 and 200 days postadministration in the first part of the trial. Figure 20 shows results for the second part of the trial.

According to the scoring system for level of oocysts per gram of faeces described by Parker *et al.* (1986), heifers in this trial were classified as belonging to score 1, i.e.,

from 20 to 98 oocysts per gram of faeces. At 100 days, a strong trend existed towards a positive effect (p=0.08) of treatment with rumensin on reducing the oocyst counts. Analysis of data obtained from the first part of the trial and taking animals from all farms as a whole, shows that a significant difference between treated and non-treated heifers occurred in the change in oocyst counts at 45 days and 100 days, but not at 200 days.

3.3.2 Classification of Species

Oocysts of 7 species of *Eimeria* belonging to the categories described by McKenna (1972) were present in the faecal samples during this trial (table 29 and figure 21). Although these species were present throughout the experiment, four of them were the most prevalent ones in all farms. Table 29 shows that in August 1991, the percentage of oocysts of *E. canadensis* was 33.3% of all the oocysts found; 39.2 and 38.7 were the percentages of oocysts found in November 1991 and February 1992 respectively. The average percentage of these three observations is 37%. Therefore, *E. canadensis* was the most predominant species in the farms included in this trial.

Similar calculations showed that overall, *E. bovis* was the second most prevalent species (16.6%) followed by *E. zuernii* (15.7%). *E. auburnensis* and *E. brasiliensis* which were found with a mean percentage prevalences of 12.2 and 11.1 respectively of all the oocysts found. *E. alabamensis* and *E. ellipsoidalis* were found least often during the trial and less than 6% of the total oocysts found belonged to these species. *E. cylindrica, E. wyomingensis, E. subspherica, E. bukidnonensis* and *E. pellita* were not found in any of the farms included in this study. The morphological characteristics of the sporulated oocysts found in this study are shown in figures 22 and 23.

Table 27. Mean (SE) of Oocyst Counts (OPG) in the First Part of the Experiment.

Days after dosing	Number of Animals per group	0	45	100	200
Farm 1 (n=16)	UT=8	266.5 (221)	388.5 (238)	272 (228)	2739 (6928.5)
	T=8	294 (363.5)	19 (25.5)	569 (1328.5)	425 (370)
Farm 2 (n=75)	UT=42	354.5 (501.5)	219 (385)	342 (1270.5)	223.5 (363.5)
	T=33	268 (295)	116.5 (264.5)	153 (200.5)	507.5 (1513)
Farm 3 (n=27)	UT=14	550 (786)	188.5 (326.5)	250 (323.5)	96.5 (115)
	T=13	227 (308.5)	7.5 (18.5)	11.5 (22)	65.5 (123)
Farm 4 (n=38)	UT=14	281 (177.5)	119 (149.5)	138 (153)	131 (123.5)
	T=24	448 (369)	19 (28.5)	34.5 (50.5)	318 (646)
Farm 5 (n=10)	UT=6	1400 (1036.5)	68.3 (388)	1033 (593)	358 (571)
	T=4	790 (862.5)	0 (0)	3687.5 (7176)	225 (155.5)
Farm 6 (n=20)	UT=10	245.5 (249.5)	70 (98)	213.5 (222.5)	222.5 (186)
	T=10	322 (627)	0 (0)	16.5 (35.5)	161 (116.5)
All Farms (n=186)	UT=94	415 (579)	226.5 (347.5)	322 (936)	424 (2111.5)
	T=92	349.5 (418)	48 (161.5)	280 (1576.5)	326.5 (917)
Sig*			p=0.03	p=0.08	p=0.34

* Significance of the effect of treatment on the change in the number of oocysts at sampling.

UT: Untreated

T: Treated

Table 28. Mean (SE) of Oocyst Counts in the Second Part of the Experiment.

Days after dosing	Number of Animals per group	0	100
Farm 1 (n=16)	UT=8	693.5 (505)	275 (159)
	T=8	412.5 (230.5)	18.5 (18.5)
Farm 2 (n=75)	UT=42	207 (41.5)	164.5 (29.5)
	T=33	490.5 (125.5)	181.5 (48)
Farm 3 (n=27)	UT=14	353.5 (124)	276.5 (128.5)
	T=13	119 (34)	58 (34)
Farm 4 (n=38)	UT=14	196 (36.5)	212.5 (79)
	T=24	154 (21.5)	32.5 (11)
Farm 5 (n=10)	UT=6	350 (51.5)	541.5 (314.5)
	T=4	262.5 (74.5)	0 (0)
Farm 6 (n=20)	UT=10	241 (69)	220 (60)
	T=10	133 (40)	135.5 (135.5)
All Farms (n=186)	UT=94	286.5 (54.5)	247 (45.5)
	T=92	271.5 (45.5)	70 (19)
Sig*			p=0.003

* Significance of the effect of treatment on the change in the number of oocysts at sampling

UT: Untreated

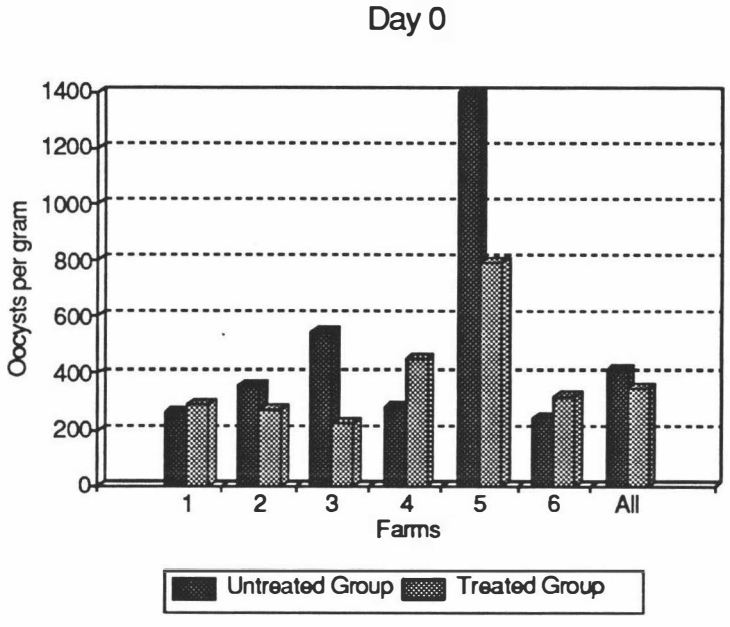
T: Treated

Table 29. Predominance of Coccidial Species in Heifer Faecal Samples for All Farms.

Species	August 1991		November 1991		February 1992	
	%	OPG*	%	OPG*	%	OPG*
E. canadensis	33.3	93	39.2	165	38.7	1224
E. bovis	30	84	8.6	36	11.4	361
E. zuernii	13.4	37	5.4	23	28.2	892
E. auburnensis	-	-	32	135	4.7	149
E. brasiliensis	10	28	11.4	48	12	380
E. alabamensis	10	28	3.1	13	3.0	95
E. ellipsoidalis	3.3	9	-	-	-	-

* Overall mean of Oocysts per gram of faeces

Figure 18. Effect of Monensin on Oocyst Counts Part 1 of the Trial, Days 0 and 45



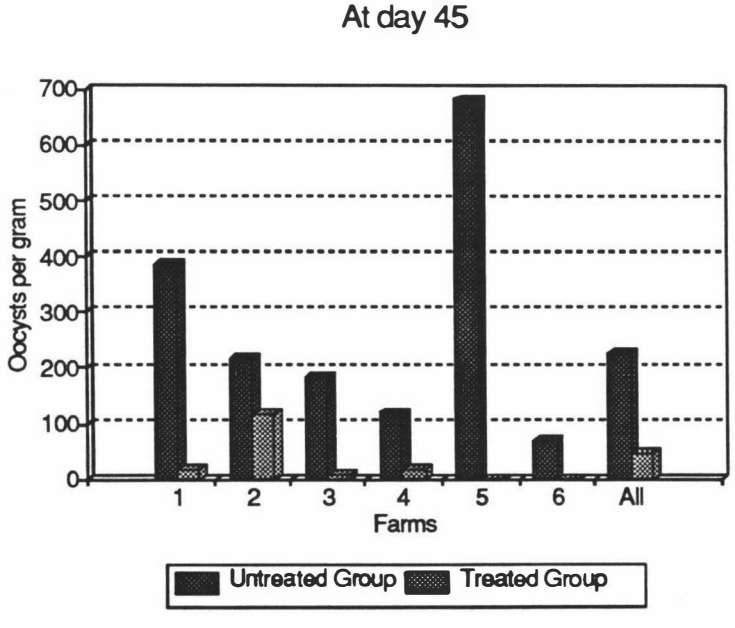
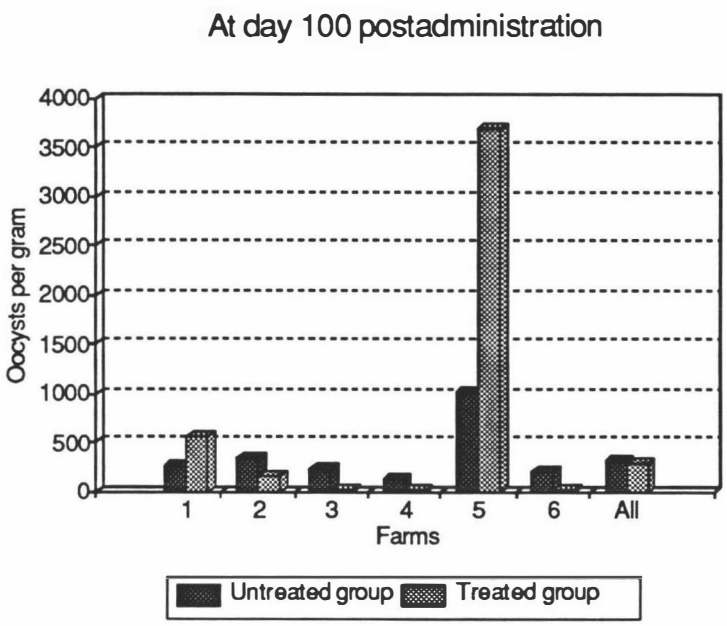


Figure 19. Effect of Monensin on Oocyst Counts Part 1 of the Trial, Days 100 and 200



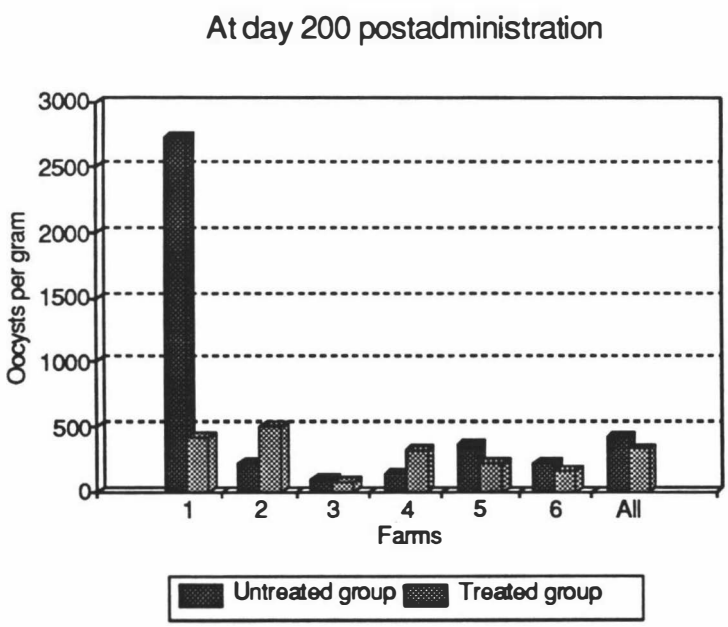
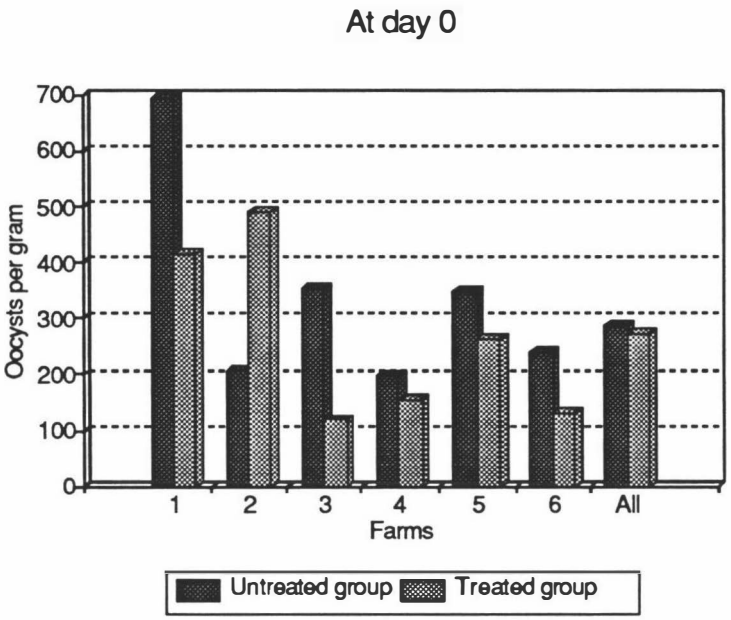


Figure 20. Effect of Monensin on Oocyst Counts Part 2 of the Trial



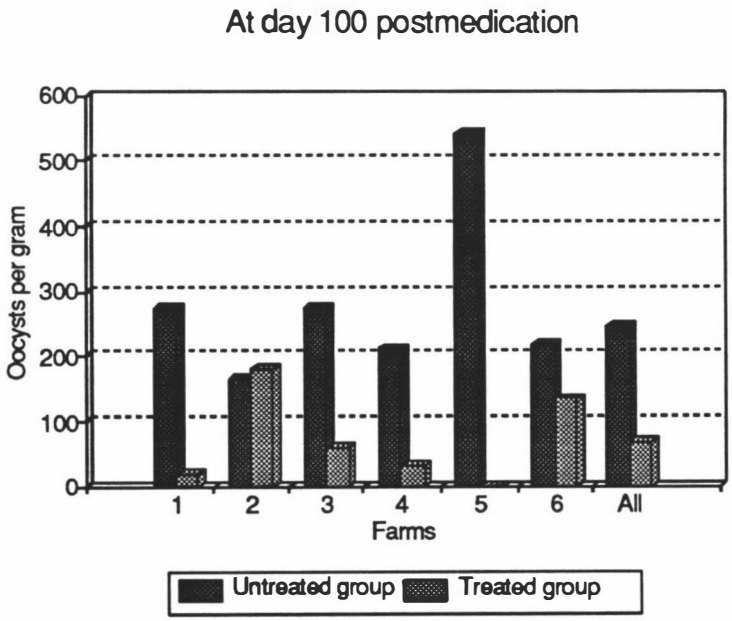
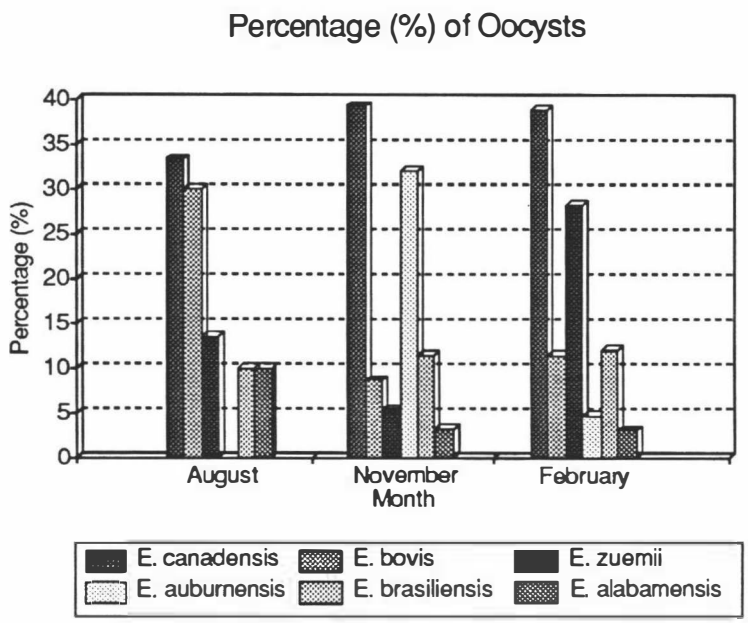


Figure 21. Predominance of Coccidial Species in Faecal Samples



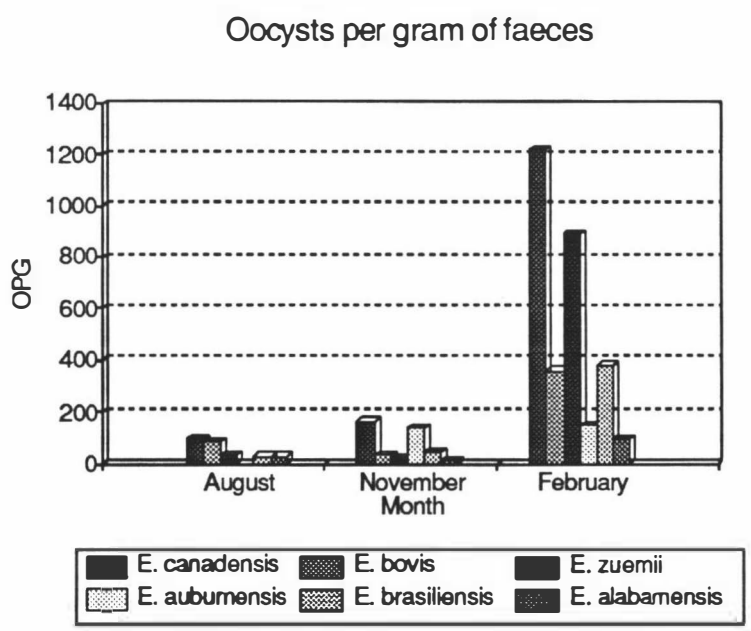


Figure 22. Photomicrograph of Sporulated Bovine *Eimeria* Oocysts (40X).

Eimeria canadensis

Eimeria zuernii

——— = 7 µm

Figure 23. Photomicrograph of Sporulated Bovine *Eimeria* Oocysts (40X)

Eimeria auburnensis

Eimeria bovis

——— = 7 µm

3.4 Discussion

This study has confirmed what other reports have shown, that is; when administered at 200 mg/head/day, monensin effectively ($p<0.05$) reduces the level of coccidia in heifers, even if they have low counts.

The effect was not statistically significant at 200 days. This is to be expected because the ionophore would have undergone a complete degradation by that time. Donoho, (1984) investigated the degradability of monensin and found that cattle killed at 12 hours after the last dose of monensin contained essentially no residue (less than 0.025 ppm) in muscle, kidney, fat and heart. Liver contained less than 0.59 ppm. These findings were determined to be due to degradation of the ionophore. Since monensin has such a fast degradability, its effect after withdrawal is nil.

In the second part of the experiment, a significant reduction in oocyst counts was observed at 100 days postadministration ($p=0.003$) which confirms the effect of monensin found in the first part of the trial.

The present study agrees with Watkins *et al.* (1986) and Fitzgerald *et al.* (1973) who reported that a dose of less than 1 $mgKg^{-1}$ (0.4 and 0.8 mg/kg body weight) has proven to be effective in reducing faecal scores of coccidia. Fitzgerald *et al.* (1973) recommended a dose rate of 1 $mgKg^{-1}$ of monensin to suppress most of the clinical signs of coccidiosis in experimental calves. The dose rate of 1 $mgKg^{-1}$ was attained at the beginning of the trial when heifers weighed, on average, 200 kg. This dose rate decreased as heifer weight increased. Watkins *et al.* (1986) reported significant reductions in oocyst counts at a dose rate of 0.4 mg/kg body weight. Since the bolus released 200 mg monensin per day, heifers that weighed up to 500 kilos were included in this range of action.

The histograms (figures 18, 19 and 20) show that mean oocyst counts in the treated group of each farm during both parts of the trial were lower than the mean of the

untreated group of the farm. This observed trend was confirmed by the statistical analysis showing significance when all of the animals were considered, regardless of farm.

The mean number of oocysts corresponding to *E. bovis* and *E. zuernii* decreased during November and increased again in February. By contrast, the number of oocysts of *E. auburnensis* increased during the same period to become the second most frequently found species eliminated by heifers during the Summer.

Heifers had low counts of oocysts possibly due to their immunological status and consequently the number eliminated may be clinically unimportant, but this information is relevant to establish whether cyclic variation in levels of these pathogenic species of *Eimeria* are passed to the environment.

Summer conditions appear to have a negative effect on the presence of *E. bovis* and *E. zuernii*. According to the results obtained, oocyst discharge of these two pathogenic species is lower in November. Since levels were higher in February, that represents a higher challenge for susceptible animals at that time of the year if they survive in the environment.

E. canadensis was the most prevalent species throughout this study. *E. canadensis* has not been reported as an important cause of severe illness (Pellérdy *et al.*, 1974). Heifers included in this experiment are not important source of infection due to both their low level of oocyst counts and the high proportion of non-pathogenic species in faeces.

3.5 Conclusion

Monensin was effective as a coccidiostatic agent when used in 194 animals in two different trials. Because of its fast biodegradability and its low level of accumulation

in organic tissues, monensin cannot act as a coccidiostat after its withdrawal of treatment. Other experiments need to be carried out to test monensin in the form of slow ruminal delivery device to prevent clinical coccidiosis under New Zealand conditions.

Since it has been concluded in a previous chapter that the effect of monensin on the increment of height was statistically significant during one treatment period, the question is if the role of monensin as a growth promotant is independent of its effect on coccidia. No statistically significant effect of monensin on weight gain was demonstrated. Since the effect of monensin as a coccidiostat is independent to its effect as a growth promoter, any efficiency in converting Metabolizable Energy (ME) into live weight due to monensin administration it is not attributable to the effect of the ionophore against coccidia.

An experiment designed to evaluate the effect of monensin on species of coccidia is required to determine which species are more affected by the ionophore. By doing this, it would be possible to define the degree of susceptibility of pathogenic species such as *E. bovis* or *E. zuernii*. Any effect of the ionophore of these species will be beneficial for the animal and the population at risk.

Effect of Monensin on Reproductive Performance of Heifers

4.1 Introduction

Manipulation of ruminal fermentation with monensin increases ruminal propionic acid, depresses ruminal proteolysis and deamination of dietary proteins as has been described in chapter I. Such shifts in rumen fermentation may influence the reproductive efficiency of ruminants.

Progesterone (P_4) plays a key role in the establishment of the reproductive cycle of the heifer because of the large degree of association observed between this hormone and luteinizing hormone (LH). The mature pattern of LH secretion is a gradual event resulted from the increase of levels of P_4. Changes in levels of LH as puberty approaches are important for the maturation of the hypothalamo-pituitary-ovarian axis (González-Padilla *et al.*, 1975). Besides hormonal levels, age at puberty in heifers also depends upon other factors such as weight and height of the heifer. Short *et al.* (1971) demonstrated that age at puberty was highly correlated with body weight. Heavier heifers (259 kg) reached puberty earlier (407 days old) than heifers that weighed 238 kg which reached puberty at 433 days old.

Monensin supplementation has been demonstrated to improve the average height of heifers included in this trial. Earlier puberty is expected and a change in the hormonal levels (specifically progesterone) due to the ionophore, is possible. Fluctuations in hormonal levels may have an influence on conception rates and subsequent in-calf rates. Improved energy utilization in pregnant heifers, in turn, could have an effect on their calves.

Various workers have characterized the changes following the use of monensin on

progesterone levels (Kirkwood *et al.,* 1990; Mears *et al.,* 1988) in heifers approaching ovulation. Changes in the weight of calves and conception rates due to monensin have also been reported (Clanton *et al.*, 1981; Lemenager *et al.*, 1978). However, no reports describe interrelationships of these three features in heifers under New Zealand conditions. This was the objective of this study.

4.1.1 Physiology of the Oestrus Cycle

Puberty is the time at which the ovaries become functional and reproduction becomes possible. Reproductive function, normally initiated around 1 year of age in heifers, can be affected by numerous environmental and genetic factors. Thus, age at puberty may be extremely variable ranging from 4 months to over 2 years. Onset of puberty in heifers results from a synchronized and interdependent cascade of maturational changes in the central nervous system, hypothalamus, pituitary and ovaries.

Hypothalamic inactivity is responsible for the prepubertal state rather than an inability of the pituitary to respond to GnRH or ovaries to gonadotropin secretions. Maturation of the GnRH - releasing system may involve the central nervous system since changes have been noticed in sensitivity to neurotransmitters in rats approaching puberty (Knickerbocker *et al.,* 1986).

The prepubertal GnRH-releasing system is extremely responsive to negative feedback (oestrogen inhibits the pulsatile mode of LH secretion). This threshold to negative feedback is elevated gradually as puberty approaches resulting in more frequent LH pulse patterns and ovarian activity (Schillo *et al.,* 1982).

Changes in levels of progesterone play an important role in puberty. Endogenous episodes of LH occur at a frequency of one to four episodes per 24 hours during the prepubertal period in heifers. Prior to 20 days before puberty, plasma progesterone (P_4) concentrations seldom rise above 0.5 ng/ml so that frequency of LH episodic

release is insufficient to support ovarian activity during this period (González-Padilla *et al.*, 1975).

Transition between prepuberty and puberty occurs 2 to 4 weeks before the first ovulation. Transition is characterized first by an increased frequency of LH episodes and then by the onset of ovarian activity, as determined by plasma P_4 concentrations. (González-Padilla *et al.*, 1975).

It is common to find that heifers in the transitional period before puberty exhibit one or two transient elevations in plasma P_4. Although these rises are of a lower magnitude (1 to 2 ng/ml plasma) than concentrations following corpus luteum formation during the first oestrus cycle. The first preovulatory surge of LH occurs only after the prepubertal P_4 elevation declines to baseline levels. Onset of ovarian steroidogenic activity may act later to establish regulation of hypothalamic GnRH and pituitary gonadotropin release patterns in subsequent cycles. This information regarding levels of P_4 in plasma is important for this trial. Progesterone levels were measured to determine the influence of monensin on hormonal changes, in order to assess a possible influence on puberty.

Results obtained by González-Padilla *et al.* (1975) regarding the serum concentration of hormones in heifers are summarized in table 30. Progesterone levels were low prior to 20 days before puberty (day -20). Between days - 20 and 0, heifers showed two periods when progesterone was elevated for 2 - 5 days. The first period occurred between days - 18 to -11 and always preceded the priming LH peak. The second occurred between the priming and the puberal LH peaks. This was assumed to be of ovarian origin since the priming LH peak may have induced luteinization of the follicles or formation of a small corpus luteum while the first elevation of progesterone was at least partially produced by the adrenals.

Since heifers included in this study were 11 months old when the trial began, observation of low levels of progesterone (less than 0.5 ng/ml) was expected then. Changes in those levels would occur according to time and ionophore

supplementation. Plasma progesterone concentrations comparable to cyclic levels have been reported in 15-18 month old brahman X Shorthorn heifers before their first oestrus (Donaldson *et al.,* 1970).

Table 30 also illustrates the interaction between progesterone and other hormones. The periods of lower concentration of GnRH were coincident with the periods when progesterone was elevated, while the highest values of GnRH concentration occurred when oestradiol levels were higher. Therefore, the ratio, progesterone : oestradiol 17ß, may be involved in the regulation of GnRH secretion.

In addition, the length of the oestrus cycle in Friesian heifers has been reported to be, on average, 18-24 days (Wishart *et al.,* 1972). Factors affecting the duration of oestrus are the breed of the animal, season of the year, presence of a bull and frequency of observation.

Table 30: Serum Concentration of Hormones in Prepuberal and Puberal Heifers

Days from first preovulatory LH peak	GnRH (pg/ml) x ± SE	FSH (ng/ml) x ± SE	LH (ng/ml) x ± SE	Progesterone (ng/ml) x ± SE	17ß E_2 (pg/ml) x ± SE
-64 to 39	26 ± 1.8	43.1 ± 0.5	1.11 ± 0.12	1.68 ± 0.24	18.3 ± 1.9
-38 to -20	23 ± 2.3	37.7 ± 0.9	1.22 ± 0.13	1.39 ± 0.23	8.0 ± 2.5
-19 to -12	22 ± 2.1	46.1 ± 1.5	1.59 ± 0.21	2.76 ± 0.39	7.1 ± 0.6
-11 to -6	18 ± 2.2	43.3 ± 1.9	2.57 ± 0.92	3.39 ± 0.57	8.7 ± 1.2
-5 to 0	18 ± 1.9	54.4 ± 6.7	0.72 ± 0.07	8.76 ± 1.40	7.1 ± 0.8
1 to 4	24 ± 2.7	42.0 ± 1.3	0.35 ± 0.03	4.58 ± 1.31	8.9 ± 1.1
5 to 16	19 ± 1.7	45.1 ± 1.2	0.34 ± 0.05	18.25 ± 3.22	9.9 ± 1.1

From: González-Padilla *et al.*, (1975)

4.1.2 Effect of Monensin on Puberty of Heifers

McCartor *et al.* (1979) reported that monensin decreased the time to puberty in beef heifers. These effects of monensin potentially could decrease their age at first calving. Meinert *et al.* (1992) conducted research utilizing dairy heifers and concluded that monensin had significant effects on the age at first breeding and at calving. Monensin was responsible for a reduction of 25 days in the age at first breeding and 48 days in age at calving when it was supplemented via a grain mixture.

However, Moseley *et al.* (1982) stated that the effect of monensin on puberty of heifers depends on the initial weight of heifers when monensin is supplemented. Monensin affected age and weight at puberty only in heavy heifers (weaning weight 226.9 k) as shown in table 31. Puberty in lighter heifers was not affected by monensin supplementation and this difference between the two groups was not due to increased average daily gain.

Moseley *et al.* (1977) had previously reported that feeding monensin might have had a positive influence in the onset of puberty on beef heifers. However, puberty was delayed ($p<0.05$) when treated heifers were exposed to a low programme of nutrition that restricted their growth. These results indicated that puberty was mediated by both a critical body weight and an appropriate size. All heifers gained at nearly identical rates from day 60 to 158. Total weight gains for the test period were not different between monensin treated and control heifers.

Meinert *et al.* (1992) concluded that monensin significantly reduced the age at first breeding by 25 days and age at calving by 48 days. This decrease in age at puberty caused by monensin supplementation was reported to be independent of weight gains.

4.1.3 Effect of Monensin on In-Calf Rate and Weight of Calves

Most reports regarding the effect of monensin on conception rate and weight of calves show that inclusion of the ionophore in the diet does not significantly affect these reproductive parameters.

Table 31. Average Reproductive Traits in Heavy and Light Heifers Fed 200 mg Monensin per Day

Trait	Light		Heavy	
	Roughage	Roughage plus monensin	Roughage	Roughage plus monensin
Age at Puberty (days)	377.6	372.7	383.1	368.8
Weight at Puberty (kg)	278.1	287.7	296.6	322.1
% Pregnant	86.4	78.3	82.6	95.8

From: Moseley *et al.*, (1982)

Walker *et al.* (1980) concluded that no difference in conception rate was noted between a monensin treated group and a control group of synchronized cows, nor were differences in calf weight at birth attributable to the use of monensin. Results from their experiment are presented in table 32. No statistically significant differences in conception rate due to treatment were observed in their trial.

The mean calf birth weight showed a minimal difference between the control and treatment groups (0.5 kg on average) but this effect was not statistically significant.

Table 32. Cow Reproductive Performance and Monensin Administration.

	mg monensin/head/day		
Item	Control	200	300
Total Conception (%)	33.3	56.3	30.8
Birth Weight (kg)	28.7	29.0	29.2

From: Walker *et al.*, (1980)

Therefore, Walker *et al.* (1980) concluded that the monensin treatments and the changes in the pattern of ruminal fermentation associated with them had no apparent effect on the first-service conception rate and calf birth weights in their experiment.

Table 33 shows results obtained by Baile *et al.* (1982) who carried out an experiment on heifers to evaluate the effect of monensin on reproductive performance. Calf birth weights were not affected by treatment. This result agrees with those presented by Turner *et al.* (1977) and Walker *et al.* (1980) who also reported that differences between birth weight due to treatment were minimal or not observed. Apparently monensin administration had a non-significant influence on conception rates in the experiment carried out by Baile *et al.* (1982). The percent conception - measured by palpation at an appropriate time after breeding - indicated that all heifers but 5% of the control group were pregnant.

Lemenager *et al.* (1978) concluded that conception rates of beef cows during winter supplementation, were not significantly different between treated and untreated groups.

Clanton *et al.* (1981) carried out three different experiments to evaluate the effect of monensin on beef calf weights at birth. Their results are presented in table 34.

Table 33. Effect of Monensin on Reproductive Performance of Heifers and Calves at Birth

	mg monensin/head/day		
Item	Control	200	600
Total Conception (%)	95.0	100	100
Birth Weight (kg)	36.7	33.3	35.4

From: Baile *et al.*, (1982)

Table 34. Effect of Monensin (200 mg/head/day) on Calf Weight (kg).

Experiment	No. animals	Age	Weight of Calves (Kg)	
			Control group	Treated Group
I	100	More than 3 years old	38	40
II	64	2-3 years old	36	38
III	32	Yearling heifers	26	30

From: Clanton *et al.*, (1981)

In experiment one, there were no significant differences in calf weight between cows given 200 mg monensin per head and controls. The birth and weaning weights of calves from the cows given monensin were slightly higher than those of calves from

cows not given monensin, but the differences reported were not significant.

In experiment III, the birth weights of calves from heifers fed monensin were significantly higher than those of calves from heifers not fed monensin. Some of this advantage persisted to weaning but by that time the differences were not significant. The benefit of monensin in beef cow diets is variable and depends upon both the quality of forage used and the feeding regime followed.

Turner *et al.* (1977) and Watkins *et al.* (1987) did not find a significant effect of monensin administration combined with hay supplementation on calf birth weight. Differences between birth weights due to treatment were minimal: calves from monensin supplemented cows averaged 37 kg and control cows' calves 36 kg.

No significant effects in pregnancy rates between treated and control groups were found. Control groups showed a conception rate of 58.8% while treated groups had a conception rate of 52.5%. Similar conception rates were observed at different levels of monensin treatment. However, monensin improved feed efficiency for maintaining pregnant cows wintered on a relatively low quality roughage (Watkins *et al.*, 1987).

The primary objective of this part of the study was to determine whether monensin supplementation would result in an increase in mean levels of progesterone in heifers. This, in turn, would determine if monensin influenced the onset of puberty as reflected in the increased average of P_4 levels due to a higher number of heifers with an elevated amount of P_4.

4.2 Materials and Methods

Two hundred heifers distributed over six farms located in the Manawatu area of New Zealand were used in this experiment (table 18). The cattle were grazed on pasture and were approximately 11 months old at the beginning of the experiment. Animals

were stratified according to weight and were then randomly divided into two equal groups; (treated and untreated), regardless of farm as described in chapter II.

4.2.1 Blood Sampling for Progesterone Assay

Blood was collected into evacuated tubes containing EDTA and plasma was separated by centrifugation within the 4 hours after collection. Samples were labelled and stored at -20^{o}C for 2 or 3 months until analysis. Levels of progesterone were determined by means of a radioimmuno-assay performed in the Veterinary Physiology and Anatomy Department laboratory at Massey University.

Equipment utilized included a shaker for extraction and tritium as a marker. The progesterone assay responds by competitive binding between tritium labelled hormone and the level of the hormone present in a sample of blood.

The procedure has been described by Scott, (1989) as follows:

- Plasma progesterone was extracted from the samples and standard sera using a toluene:hexane solution. After shaking horizontally for ten minutes and then centrifuging for another 5 minutes at 1000 rpm, samples were stood to freeze in an upright position. The resultant solvent was decanted off into tubes and dried under air flow.

- After drying, the tubes were washed with ethanol and then vortexed. From each of these tubes, 100 µl were placed into labelled plastic tubes in duplicate. Simultaneously, 100 µl of standards and zeros were also prepared in duplicate. Standards, standard sera and samples were then redried.

- Once dried, 600 µl of a tracer cocktail - containing tritium - was added to all tubes. Duplicates of total count tubes containing 600 µl of the tracer cocktail

plus 600 µl of phosphate buffer solution in EDTA and gelatine buffer (PBSEG) and duplicate blank tubes containing 100 µl of tracer working solution plus 500 µl PBSEG were prepared as well. All tubes were incubated overnight at 4°C.

- The next morning, 600 µl charcoal in PBSEG buffer was added to precipitate out the antiserum - tracer complex. Tubes were centrifuged and the supernatant decanted into scintillation vials. Finally, 5 ml. scintillation fluid was added and vials were placed on a Beckman LS 7500 Microprocessor Liquid Scintillation System beta counter (Beckman Instruments Inc., Fullerton, CA.). Counts per minute results were analyzed by LKB Wallac, RIACALC programme to give ng/ml values.

4.2.2 Collection of Data for Conception Rate, Weight of Calves at Birth and Age at Calving

Pregnancy was determined by rectal palpation on bred heifers when they were between 4-5 months pregnant. Five of six farmers agreed to participate in this part of the trial. Farm No. 2 was not included because it was not dedicated to raising replacement heifers for the farm itself but to sell them. By calving time most of the heifers had been distributed around the North Island. Therefore, collection of further data was not possible. Each remaining farmer was given a weighing band[3] and a recording form to write down the birth weight of calves and their date of birth.

Farmers were instructed in the method of obtaining the weight estimates for calves. The method described was to put the weighing band round the girth of the calf when it was born and had stood up, as near to the forelegs as possible, to pull firmly and to record that measurement on the information sheet. They were reminded of the

[3]Weighband. Dalton Supplies Ltd., Nettlebed, Henley-on-Thames, Oxon.

importance of keeping as similar conditions as possible while taking the weight estimate from girth measurement. To avoid spurious variations in the measurement of this data, a fixed technique of measuring the weight as described above was agreed before calving season started.

The recording form did not contain information regarding heifers that were included in the control group and which ones were in the treatment group.

Birth dates of heifers were taken from records available in each farm. The length of the period between birth and calving dates of each heifer was obtained with the help of the statistical programme SAS^{TM}. Variables were submitted to a MANOVA (Multiple Analysis of Variance) under the General Linear Model procedure included in the SAS^{TM} package.

4.3 Results and Discussion

The effect of monensin feeding on progesterone levels is shown in table 35. Results corresponding to the effect of monensin on birth weight, in calf-rate and age of the heifer at birth are presented in table 36.

Plasma progesterone was significantly affected ($p < 0.05$) by monensin in heifers at 100 days after the first administration of boluses.

This study was not designed to directly test the hypothesis that age at puberty could be reduced by shifting ruminal fermentation since the management of heifers did not allow regular monitoring of ovarian cycling by ultrasound examination or palpation. The purpose of this study was to determine if monensin could affect levels of plasma progesterone in heifers which would provide evidence that the ionophore is acting in this way under New Zealand field conditions.
The results obtained in this experiment show that monensin caused an increase of

mean progesterone levels in the plasma of heifers that had been treated with monensin boluses at 100 days after administration. This indicates that supplementing with monensin either speeded the onset of puberty in those heifers or enhanced progesterone secretion.

The changes in progesterone levels found in this study cannot be attributed to any other factor known to affect age at puberty since all heifers were subject to the same type of management regardless of treatment group.

Levels of fermentable carbohydrates in a diet can influence hormonal patterns in cattle, (McCartor *et al.*, 1979). Beal *et al.* (1978) indicated that the dietary energy content can affect certain endocrine variables controlling reproduction. In their experiment, heifers fed low energy rations tended to have lower concentrations of serum progesterone and higher concentrations of LH.

It could reasonably be postulated that variations in the availability of energy for animals due to the administration of monensin (e.g., shifting the ruminal fermentation pattern towards more propionate) could be expected to have the same effect on the onset of puberty as changes in the qualitative components of the diet.

The exact mechanism by which monensin increased the levels of progesterone in plasma cannot be determined from this experiment. The administration of monensin to the same heifers significantly increased their growth in stature during one experimental period while weight gains were not affected. Studies cited by Moseley *et al.* (1977) show that growth rate has an influence on age at puberty, since free fatty acid levels in plasma can interfere with the regulation of growth hormone in cattle (Hertelendy *et al.*, 1973). An explanation for the observed findings, is that animals treated with monensin have increased levels of propionate and higher levels of growth hormone than untreated ones. Therefore, their progesterone levels might be increased. This study agrees with the one presented by Moseley *et al.* (1982) in the sense that levels of hormones related to reproduction can be altered by monensin and this change is not due to increased average daily gains or increased body weight.

Table 35. Mean (SE) Levels of Progesterone (ng/ml) in Untreated and Treated Heifers at Three Different Samplings

Variable	Number of Animals per group	Day 0	Day 45	Day 100
Farm 1 (n=16)	UT=8	3.02 (1.12)	0.94 (0.52)	10.62 (1.23)
	T=8	0.49 (0.11)	2.51 (1.48)	8.74 (1.19)
Farm 2 (n=75)	UT=42	0.57 (0.15)	2.43 (0.41)	4.51 (0.38)
	T=33	0.77 (0.23)	3.38 (0.67)	4.93 (0.58)
Farm 3 (n=27)	UT=14	0 (0)	2.29 (0.38)	3.14 (0.56)
	T=13	1.48 (0.56)	3.28 (0.76)	8.53 (1.16)
Farm 4 (n=38)	UT=14	1.63 (0.52)	3.96 (0.59)	6.24 (0.57)
	T=24	1.62 (0.48)	3.13 (0.61)	7.78 (0.53)
Farm 5 (n=10)	UT=6	0.06 (0.02)	0.17 (0.12)	5.83 (1.38)
	T=4	0.37 (0.26)	1.36 (0.93)	2.93 (1.07)
Farm 6 (n=20)	UT=10	2.07 (0.54)	1.59 (0.48)	3.33 (0.89)
	T=10	1.15 (0.32)	0.93 (0.40)	4.19 (0.60)
All Farms (n=186)	UT=94	1.19 (0.17)	2.37 (0.25)	5.47 (0.34)
	T=92	1.23 (0.19)	2.83 (0.34)	6.59 (0.37)
Sig*			p=0.37	p=0.047

* Probability that observed differences between progesterone levels in treatment and control groups are due to chance.

UT: Untreated
T: Treated

Table 36. Mean (SE) of the Effect of Monensin on Conception Rate, Days between Birth and Calving and Weight of Calf in Dairy Heifers

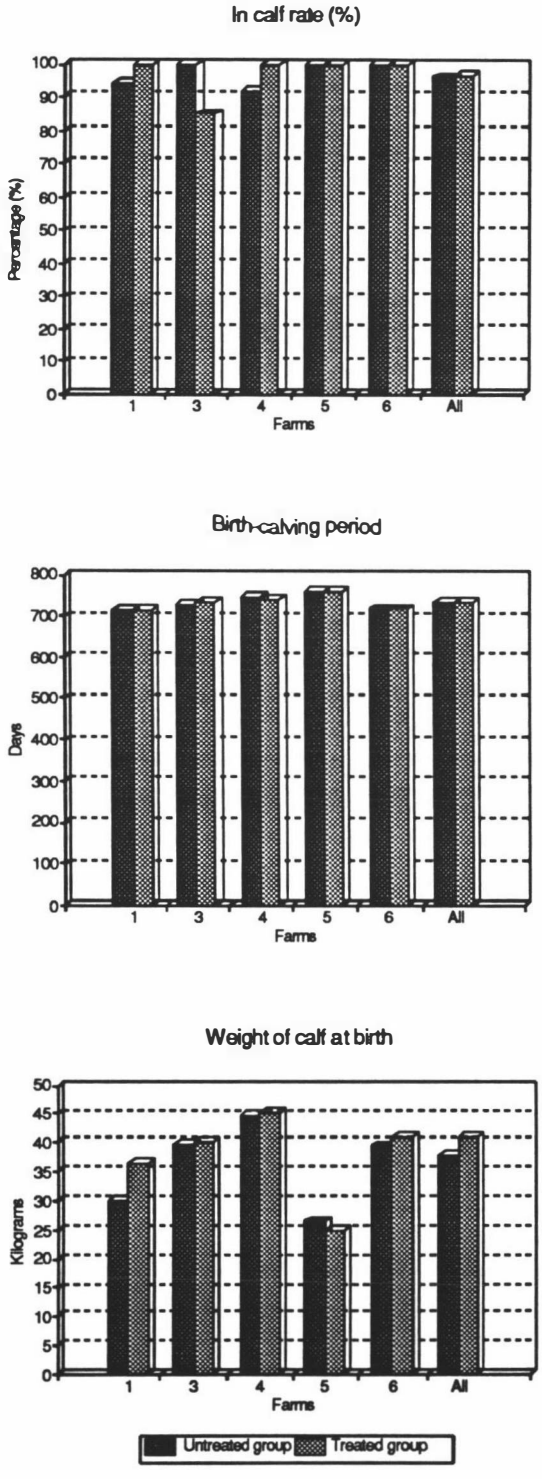
Variable	Number of Animals per group	In Calf Rate (%)	Birth-calving period	Weight of Calf
Farm 1 (n=16)	UT=8	94	716.0 (6.12)	30.12 (4.48)
	T=8	100	715.1 (6.23)	36.25 (0.77)
Farm 3 (n=27)	UT=14	100	729.1 (5.14)	39.64 (0.88)
	T=13	85	733.7 (5.06)	40.36 (1.61)
Farm 4 (n=38)	UT=14	92	748.2 (9.03)	44.8 (0.76)
	T=24	100	742.2 (0.54)	45.4 (0.45)
Farm 5 (n=10)	UT=6	100	761.2 (8.54)	26.5 (1.23)
	T=4	100	757.7 (15.4)	25.0 (2.48)
Farm 6 (n=20)	UT=10	100	718.0 (7.55)	39.5 (1.01)
	T=10	100	717.3 (5.16)	41.1 (0.83)
All Farms (n=111)	UT=52	96	733.2 (3.90)	37.7 (1.22)
	T=59	96.5	734.5 (0.86)	41.0 (0.86)
Sig*		p=0.92	p=0.80	p=0.02

* p values reflecting that observed that observed differences between treatment and control groups are due to chance.

UT: Untreated

T: Treated

Figure 24. Effect of Monensin on Some Reproductive Parameters



Monensin administration did not affect in-calf rates in these heifers. Considering the group of 111 animals, the difference is practically non-existent (96% Conception rate in untreated heifers included in this study compared to 96.5% in the control heifers). This result agrees with the findings of Walker *et al.* (1980), Lemenager *et al.* (1978), Turner *et al.* (1977) and Watkins *et al.* (1987).

Monensin significantly affected levels of progesterone in heifers 100 days postadministration and therefore it was expected that an effect on the age of the heifer at first calving may have occurred. Untreated heifers had their calves when they were on average 733.2 (SE 3.9) days old while treated ones calved when they were 734.5 (SE 3.4) days old. This difference was not statistically significant.

Age of heifers at calving in this trial was affected by management techniques designed for seasonal supply dairy farms to ensure that lactating cows have maximal forage available for milk production. In most dairy breeds, a cow should be mated about 280 days before the date on which it is expected to calve in the following season. In seasonal supply farms, calving is due to start around the 1 of August. Thus, mating should begin around the 22 October in the previous year (Holmes and Wilson, 1987).

Desired mating management in New Zealand aims to concentrate the calving period that allows the herd's feed requirements to be matched closely to the growth rates of pasture. Concentration of the calving season also reduces the number of late calving cows and increases the average number of days of lactation for the herd. It also concentrates the work of rearing calves into a short period of time. For these reasons, in seasonal supply farms the cows should calve as early as possible in spring, provided that they can be fed well from the day that they calve.

Concentration of the calving pattern could have played an important role in the results obtained in this trial because no matter how early heifers reach puberty, they are managed to follow a pre-established plan for calving. Although the results reported here show that there was not a significant effect of monensin on the age of

heifers at calving, it does not mean strictly that the ionophore does not influence the onset of puberty. Treated heifers could have reached puberty earlier but breeding at that time did not conform to the calving pattern desired by the farmer (Holmes and Wilson, 1987).

Results obtained in this trial agree with Meinert *et al.* (1992) in the sense that a decrease in age at puberty might have occurred independent of weight. However, they reported a statistically significant reduction in age at calving of 48 days that was not observed in the present study. Dissimilar dietary regimes can account for the differences in the results. Whilst heifers in this experiments were fed on grass, heifers in the Meinert *et al.* (1992) experiment were fed on a grain mixture.

The weight of calves born to the treated heifers was statistically significantly increased by monensin. Calves born from untreated heifers had an average weight of 37.7 kg while calves born from treated heifers averaged 41.0 kg ($p=0.02$). This result agrees with that reported by Clanton *et al.* (1981) who further observed that differences in weight were not significant by the time of weaning. In their experiment, heifers treated with 200 mg monensin per day delivered calves that weighed 30 kg while calves from control heifers weighed 26 kg at birth.

The birth weight of a calf is important because potentially it helps determine the length of the period from birth until weaning and even mating. It also affects the potential growth rate of calves. Calf birth weight is related to mature body weight. Monensin administration to the heifers in this trial resulted in calves being significantly ($p=0.02$) heavier than untreated ones. However, there were no differences in weight change of the heifers themselves as has been concluded and reported in chapter II.

4.4 Conclusion

Statistically significant changes in progesterone levels were observed when monensin was administered to growing heifers in the form of slow - release devices. This finding is consistent with puberty being reached earlier in those animals receiving the ionophore although definite conclusions in this respect could not be drawn from the information collected in this study.

Monensin supplementation caused a statistically significant increase in the birth weight of calves. Further studies might include the effect of monensin on puberty, aiming to establish the average number of days that medicated heifers spend to reach puberty compared to a control group. Differences in weight of calves at birth due to monensin administration have to be investigated to determine if this advantage persists in later life.

General Discussion and Conclusions

Although monensin has been reported to improve the daily weight gains of cattle grazing pastures, results from this experiment showed that monensin had no significant effect on the rate of body weight gain in the dairy heifers included in this trial. However, the ionophore may improve the efficiency of production. This indicates that although the treated heifers did not gain extra weight, less feed may have been required in the treated group to achieve the same weight gain, but this was not able to be measured in this trial.

During the second part of the trial, a significant increase in height due to monensin was observed. It could be postulated that the ionophore has an effect on height in pregnant heifers. However, the fact that only one measurement (height to the withers) in one of the two parts of the trial was affected and not the other (height to the hips), decreases the consistency of the results. The effect of monensin on growth hormone (GH) is still controversial and results obtained from this experiment could not help clarify this.

Monensin was confirmed as a coccidiostat in subclinically infected animals. Weight gain was not significantly affected by monensin administration in this study. It was not possible to conclude if reported effects of the ionophore on cattle weight gains are independent of the action of the ionophore on coccidia for this particular experiment.

Reports indicate that feeding monensin to cattle has wide ranging influences related to their productivity. The variety of modes of action of the drug on the ruminant organism have been reported to influence different systems and organs. It was therefore expected that monensin administration would have significantly influenced weight gains in these dairy heifers. There is not evidence from this experiment to explain why it did not happen. It can be suggested that inclusion of monensin in their diet should have been accompanied by a change in the substrate the heifers were

eating. In this case, the substrate kept being the same and the properties of monensin could not be revealed. Although changes in weight were not significant, treated heifers showed a variation in weight greater than in North American circumstances. The failure to demonstrate a significant response may have been due to the size of the experiment and also to the diverse genotype of New Zealand Friesian heifers resulting in a large weight variance.

Mean progesterone levels in the plasma of treated heifers were significantly higher than in control ones. Since no effect of the ionophore on body weight was observed, it could be validly postulated that levels of hormones related to reproduction can be altered in response to monensin treatment independently of manipulation of body weight. Parameters like conception rate and age of heifers at calving were apparently not affected by the monensin administration. However, definite conclusions cannot be drawn since these parameters were subject to constraints imposed by the management decisions of the farmers who participated in this trial. Any possible influences may have been masked by management strategies such as breeding the heifers only at the desired breeding season.

Weight of calves, by contrast, was significantly affected by monensin. This agrees with Clanton *et al.* (1981). This result is independent to the effect of monensin on the heifers' body weight. Further studies should follow to establish if this effect of monensin on calves weights persisted over time.

Although the literature reports that monensin causes the amount of energy available for the heifers to increase, lack of consistency in the results of many studies make the ionophore controversial in the field of ruminant nutrition. Different results under different conditions reveal that the action of monensin depends upon a series of factors, where the quality of feed appears to play an important role. The results obtained in this trial demonstrate that under New Zealand conditions, supplementing with monensin could benefit heifers in certain specific ways. However, more research has to be carried out to confirm or refute this result. The design of a further experiment should include groups of animals under different nutritional regimes and

different energy levels in their diets. Study of the metabolic effects (i.e. change in the population of microorganisms, influence in digestibility) of monensin in dairy heifers in New Zealand will give a concrete answer to the effect of the ionophore in animal performance.

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Appendix I

Statistical Models

Analyses for the variables height, weight, rank of oocyst counts, levels of progesterone, weight of calves and age of heifer at birth was by least-squares analysis of variance, which included treatment as the main fixed effect. The general model used per farm was:

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

Where,

Y_{ij} = Value of the response variable for the i^{th} treatment and the j^{th} observation.

μ = Overall mean

T_i = Effect of the i^{th} treatment

ϵ_{ij} = Random error for the i^{th} treatment and j^{th} observation

Therefore in this study, any given measurement (Y) in heifers under a particular treatment (i) differed from the population mean (μ) by an amount that was due to the combined effects of the treatment T_i to which the heifer was subjected, and the natural variation, or random error (ϵ) of the *j*th heifer under the treatment i. If the treatment had no effect, then T would be zero and the only source of difference from the true mean would be random variation. Similarly, the model used for the total number of animals was:

$$Y_{ijk} = \mu_i + T_{ij} + \epsilon_{ijk}$$

Where,

Y_{ijk}: Value of the response variable for the i^{th} farm, j^{th} treatment and k^{th} observation

μ_i : Overall mean for the i^{th} farm

T_{ij}: Effect of the j^{th} treatment on the i^{th} farm

ϵ_{ijk}: Random error for the j^{th} treatment and the $_{th}$ observation on the i^{th} farm.

Appendix 2
Commands for the SAS™ programme

VI.2.1 Analysis of Weight and Height

```
data one;
    infile 'H:\data\all1.dat' lrecl=145;
    input farm heifer height weight device fecha date8. wt2 ht2 wtfeb hthip htwit;*
    if device> 0 then treat=1; else treat=0;
    gain1=wt2-weight;
    gainfeb=wtfeb-weight;
    dht1=ht2-height;
    dht2=hthip-height;
run;
title '* * * * * * All Farms - I * * * * * * *';
proc format;
    value trt 0='No drug'
              1='Treated';
run;
proc sort; by treat; run;
run;
proc glm;
    class treat;
    model height weight ci = treat;
    means treat/lsd;
    format treat trt.;
run;
```

* Meaning of Variables:

farm: Number of the farm where measurements were taken

heifer: Number of the heifer

height: Initial height (August 1991)

weight: Initial weight (August 1991)

device: Number of the bolus administered

fecha: Date of Birth of the heifer

wt2: Weight taken after 100 days (November 1991)

ht2: Height measured after 100 days (November 1991)

wtfeb: Weight 200 days postadministration (February 1992)

hthip: Height at hips 200 days postadministration

htwit: Height at withers 200 days postadministration

A similar model was used for data obtained in the second part of the trial.

VI.2.2 Analysis of Oocyst Counts

```
data one;
    infile 'H:\data\all1.dat' lrecl=145;
    input farm heifer device fecha date8. oi ci oii cii oiii ciii oiv civ;*
    if device> 0 then treat=1; else treat=0;
d2a1=cii-ci;
d3a1=ciii-ci;
d4a1=civ-ci;
run;
title 'All farms - Oocyst counts';
proc format;
    value trt 0='Control'
              1='treated';
run;
proc sort; by treat; run;
proc rank;
```

```
    var d2a1 d3a1 d4a1;
    ranks rd2a1 rd3a1 rd4a1;
run;
proc glm;
    class treat;
    model rd2a1 rd3a1 rd4a1 = treat;
    means treat/lsd;
    format treat trt.;
run;
```

* oi: Number of oocysts per gram of faeces in first sample (August 1991)
ci: Number of oocyst counted by the McMaster method (August 1991)
oii: Number of oocysts per gram of faeces 45 days postadministration
cii: Number of oocysts counted by McMaster 45 days postadministration
oiii: Number of oocysts per gram of faeces 100 days postadministration
ciii: Number of oocysts counted by McMaster 100 days postadministration
oiv: Number of oocysts per gram of faeces 200 days postadministration
civ: Number of oocysts counted by McMaster 200 days postadministration

VI.2.3 Analysis of Progesterone Levels

```
data one;
    infile 'H:\data\prall.dat' lrecl=145;
    input farm heifer device birth date9. prog1 prog2 prog3;
    if device> 0 then treat=1; else treat=0;
    d2to1=prog2-prog1;
    d3to1=prog3-prog1;
run;
title '0 O o *  All Farms - Progesterone levels * o O 0';
proc format;
```

```
    value trt 0='No drug'
              1='Treated';
run;
proc sort; by treat; run;
run;
proc glm;
    class treat;
    model d2to1 d3to1 prog1 = treat;
    means treat/lsd;
    format treat trt.;
run;
```