Effects of virginiamycin and monensin on milk production efficiency and blood metabolites in Holstein cows.

By

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LISTE OF ABBREVIATIONS

ADF   : Acid detergent fiber

BCS   : Body condition score

BHBA : Beta-hydroxybutyrate

BW   : Body weight

CP   : Crude protein

CRC   : Capsule release control

DMI   : Dry matter intake

ECM   : Energy corrected milk

ME   : Metabolisable energy

MUN   : Milk urea nitrogen

NDF   : Neutral detergent fiber

NEFA : Non-esterified fatty acids

NFC   : Non fiber carbohydrate

NE_L : Net energy for lactation

NSC   : Non structural carbohydrate

OM   : Organic matter

RUP   : Ruminally undegradable protein

TCA   : Tricarboxilic acid

TMR   : Total Mixed Ration

VFA   : Volatile fatty acids

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DECLARATION

I declare that this dissertation hereby submitted in partial fulfillment for the requirements of the degree **Magister Scientiae Agriculturae (Production management)** at the University of Pretoria, has not been submitted by me or anyone else for any other degree at any other institution.

Claude Mukengela Muya

LISTE OF CONFERENCE PROCEDINGS

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ABSTRACT

Effects of virginiamycin and monensin on milk production efficiency and blood metabolites in Holstein cows.

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Degree : **MSc (Agric) Production Management**

Virginiamycin (V) and Ionophores, such as Poulcox (active ingredient monensin sodium), are antimicrobial feed additives approved for use in cattle to improve performance. The effect of virginiamycin on Gram positive bacteria is similar to that of monensin (M) although the modes of actions differ. Very little information is available on the potential synergistic effects of V and M, especially in dairy cattle diets. The objectives of this study were to investigate the effect of combinations of V and M on the performance of dairy cows. Forty high producing Holstein cows were blocked according to previous milk production and randomly allocated to one of the following lucerne based total mixed diets: 1) Control, no medication (C); 2) Control plus 20 ppm virginiamycin (V); 3) Control plus 15 ppm monensin (M); 4) Control plus 20 ppm virginiamycin and 15 ppm monensin (V+M). The experimental period was from 21 days prepartum until 60 days postpartum. Data were analysed according to a randomized block design, using the model GLM procedure (SAS, 2001). Dry matter intake varied from 23.6 kg/d to 25.4 kg/d and did not differ between treatments (P>0.10). Milk production was higher (P<0.10) for cows receiving V+M (41.2 kg/d) when compared to cows receiving only V (36.6 kg/d), but did not differ from other treatments (P>0.10). Milk fat % was lower for cows receiving M (3.42 %) and the control (3.62 %) when compared to treatment V+M (3.86 %) (P<0.10). Milk protein and MUN did not differ. Body weight

loss for the period from calving until day 60 postpartum, tended ($P<0.15$) to be less for cows receiving V+M (-8.1 kg) when compared to the control (-34.2 kg) and M (-31.9 kg) treatments. Both treatments M and V respectively, decreased blood BHBA and treatment M increased blood glucose ($P<0.10$) when compared to the control diet. Results suggest a complementary effect between the two additives monensin and virginiamycin when supplemented to early lactation cows.

CHAPTER 1

INTRODUCTION

Nutrition and management of the transition cow continue to be an active field of research attention, focusing on understanding the biology of transition cows and implementing management systems to optimise production and profitability (Overton, et al., 2003).

The transition from a pregnant, non-lactating cow to a non-pregnant, lactating cow is too often a disastrous experience for the cow (Goff, 2003). Despite the tremendous quantity of research conducted on nutrition and physiology of transition cows, the transition period remains a challenging and problematic management aspect on many dairy farms, and metabolic disorders continue to occur at economically important rates (Burhans et al., 2003). Numerous changes in the physiology, metabolic and endocrine status of the cow take place during the transition period. If nutritional management does not accommodate these changes, the transition cow is at risk of developing a wide range of health problems soon after calving (Goff and Horst, 1997).

The primary challenge faced by transition cows is a sudden and marked increase of nutrient requirements for milk production at a time when dry matter intake, and thus nutrient supply, lags far behind (Drackley, 1999). High-yielding dairy cows are typically in a state of negative energy balance for approximately the first 60-70 days postpartum, because the amount of energy required for maintenance of body tissue functions and milk production exceeds the amount of energy cows can consume. The degree of negative energy balance in the early postpartum period and the recovery rate from negative energy balance are critical for health status and productivity. Insufficient energy supply postpartum generally results in a higher risk for metabolic disorders, e.g., ketosis, fatty liver and displaced abomasum (Anderson, 1988). The metabolic adaptations that take place to meet energy demands can also contribute to the development of metabolic disorders. As the concentration of non esterified fatty acids (NEFA) in blood increases around calving, more NEFA is taken up by the liver. If NEFA uptake by the liver becomes excessive, then fatty liver may develop. Low intake of carbohydrate and a negative energy balance after calving lead to elevated levels of ketone bodies, which can result in ketosis. Ketosis

is generally accompanied by fatty liver and cows that develop ketosis and fatty liver have lower feed intake, lower glucogenic capacity, lower milk production and increased risk for developing other metabolic and infectious diseases (Curtis et al., 1985).

Subclinical ketosis causes significant economic losses through low milk production and an increased incidence of periparturient diseases. It has a reported worldwide prevalence of 8.9 to 34% for cows in the first 2 months of lactation, whereas the reported lactational incidence of clinical ketosis varies from 2% to 15% (Duffield, 2000). It has been estimated that an incident of ketosis costs the dairy producer $140/cow in treatment costs. In a recent review, Gabriella (2004) reported that given a ketosis incidence rate of 17% in US cattle, the annual loss in a 120 cows dairy herd, due to clinical ketosis could be $2520 and the cost for treating a case of subclinical ketosis is approximately $78. Losses due to lower milk production are difficult to quantify. The same report stipulates that estimates of milk production loss range from 300 to 450 kg for a lactation and theoretically, if a reduced incidence of subclinical ketosis and fatty liver contributes to an increased peak milk production of 1.0 kg, it would result in an additional $2880 of income. Additionally, ketosis increases the risk of developing other metabolic diseases such as displaced abomasum ($334/case), retained placenta ($319/case), mastitis ($200/case) and other metabolic problems. Clearly, feeding management strategies that reduce clinical and subclinical ketosis will directly benefit dairy farm profitability, enhance animal well being and improve cow longevity (Gabriella, 2004).

It is generally accepted that energy intake must not be compromised during the transition period. Meeting the energy demands of lactation is one of the basic physiological functions that must be maintained (Goff, 2001). In practice different strategies are followed to increase total energy intake of the fresh cow. These include improving dry matter intake, increasing non-fibre carbohydrate (NFC) intake and supplementation with feed additives such as glucogenic precursors, various sources of fat, rumen protected CLA, and rumen fermentation modifiers. The latter group includes buffering compounds, ionophore antibiotics, microbial feed additives and enzymes.

Because the transition period has the highest impact on reproduction, milk production and health, the greatest marginal return for an investment that improves dairy cow profitability will occur for changes

and interventions made during this time. Feed additives, such as ionophores, buffers and yeast cultures, therefore, have their highest impact during the transition phase.

Ionophore antibiotics such as Poulcox (active ingredient monensin sodium) and non ionophore antibiotics such as virginiamycin have shown a similar effect on gram positive ruminal bacteria, although the mode of action differs. Both in vivo and in vitro studies demonstrated that ionophore antibiotics and virginiamycin alter rumen fermentation towards increased molar proportion of propionate, decreased lactic acid production, decreased methane production and decreased protein degradation (Nagaraja et al., 1997). These alterations are positive in the sense that it can contribute towards stabilising feed intake, increase milk production and help prevent metabolic disorders such as ketosis, acidosis and fatty liver.

Although monensin has been used widely in the feedlot industry for many years, mainly for the prevention of acidosis and bloat, it is only recently that large-scale studies have been undertaken to determine its effects on milk production, health and reproduction in dairy cows. Apart from the beneficial effects regarding feed effeciency and prevention of bloat and acidosis, ionophores can play an important role in reducing the incidence of subclinical ketosis in dairy cows (Bagg, 1997). The reduction of ketone bodies and ketosis in early lactation after ionophore supplementation has been demonstrated in several trials (Sauer et al, 1989; Green et al., 1999; Melendez et al., 2004; Plaizier et al., 2005).

Field observations have demonstrated a potential synergistic effect between Poulcox and virginiamycin (K. Botha, personal communication, kbotha@afgri.co.za). There is however surprisingly little information published on the potential synergistic, additive or complementary effects of feed additives such as ionophore antibiotics, non-ionophore antibiotics and yeast cultures (Erasmus et al., 2005). Furthermore, no published literature could be found on the effect of virginiamycin on productivity and blood level of ketone bodies in lactating cows fed a total mixed ration (TMR).

The objective of this study was to evaluate the effects of Poulcox, virginiamycin or both on the productivity and incidence of subclinical ketosis (indirectly through blood metabolites) in Holstein cows during the transition and early lactation phase. The active ingredient in Poulcox is monensin sodium and most published research refers to monensin and not Poulcox.

CHAPTER 2

ENERGY METABOLISM AND THE RELEVANCE OF KETOSIS IN THE TRANSITION COW

2.1 ENERGY STATUS OF TRANSITION COW

High-yielding dairy cows are typically in a state of negative energy balance postpartum because the amount of energy required for maintenance and milk production exceed the amount of energy that cows can consume. The degree of negative energy balance in the early postpartum period and the recovery rate from negative energy balance are critical for health and productivity. Using the traditional 56 days dry period management, Rastani et al (2005) reported that immediately after calving, the cow is in negative energy balance, reaching the lowest level (–15 Mcal/d) at approximately week +2 and recovers the positive energy status later, after 10 weeks of lactation (Fig. 1).
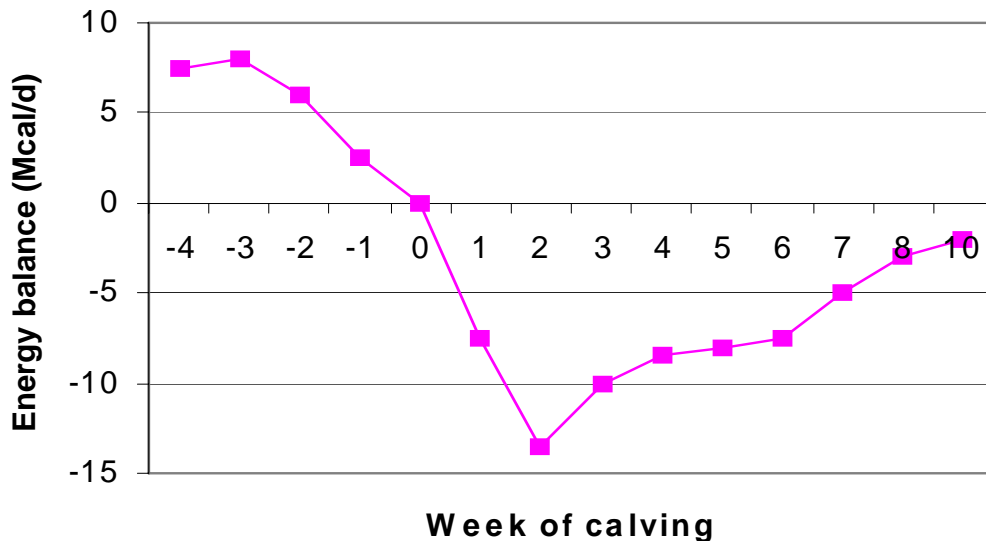

Fig. 1. Estimated prepartum energy balance of transition cows (Rastani et al., 2005).

2.1.1 Energy requirement

During the transition from late gestation to early lactation, dairy cows experience a tremendous increase in the demand for energy and glucose resulting from the rapidly growing fetus and cow's tissues (e.g. gastrointestinal tract) and from the onset of milk synthesis (Drackley et al., 2001). The feeding program must therefore be based on more than just meeting the basic daily nutrient requirements. Meeting the energy demands involves adaptation of the rumen and involves enhancing total feed and therefore energy intake (Goff, 2001).

The bovine fetal-placental mass and its demand for energy, protein, and minerals increase dramatically as gestational age increases (Goff and Horst, 1997). Therefore, dairy cows increase their demand for energy supply during the transition period to satisfy the increased requirements of the uterus and fetus (Bell et al., 1995). In the latter study, the rates of growth and chemical composition were measured in multiparous Holstein cows that were serially slaughtered from 190 days to 270 days of pregnancy. They found that the estimated rates of accretion of energy in the gravite uterus of a mature Holstein cow increased from 2.4 MJ/day at 190 d of gestation to 3.4MJ/day at 270 d of gestation.

Results of this research (Table 1) clearly illustrate the increased nutrient requirement during the final 30 days of gestation.

Table 1 Rates of energy and protein deposition in uterus and fetus during pregnancy in Holsteins (Spain and Scheer, 2002).

	Energy (MJ/d)		Protein (g/d)	
Gestation (d)	Uterus	Fetus	Uterus	Fetus
210	2.64	2.10	76	54
230	2.90	2.51	90	73
250	3.17	2.94	103	91
270	3.43	3.37	117	110

Energy demand also increases to support the increase in mass of the gastrointestinal tract (Baird et al., 1980), mainly for supporting the growth of the rumen and small intestinal epithelia (Bertics et al., 1992). Johnson et al (1990) also suggest that the gut and liver have a high maintenance requirement for energy and protein, and their growth increases the cow's total nutrient requirements considerably.

Energy demand also increases to support the high demand for the rapid increase in milk production in early lactation (Sauer et al., 1989). The production of just 10 kg of colostrum on the day of calving requires that 11 Mcal of energy be supplied from the diet or be mobilised from body stores (Goff and Horst, 1997). High milk yield in early lactation requires the synthesis of large quantities of lactose, which requires large amounts of glucose. During the synthesis of lactose in the mammary gland, glucose is converted to galactose. Galactose then condenses with another glucose molecule to form lactose (Horton, 2004). The mammary gland consumes about 72 g of glucose for every kg of milk. Taking into account the glucose required for other metabolic pathways, a cow requires up to 6.5 kg of glucose per day (BASF, 1997). Total energy requirement can increase three-fold or more in a matter of three to four weeks at a time when dry matter (energy) intake fails to meet demand (McGuffey et al., 2001). Prediction of whole body glucose requirements and supply is shown in Fig. 2.
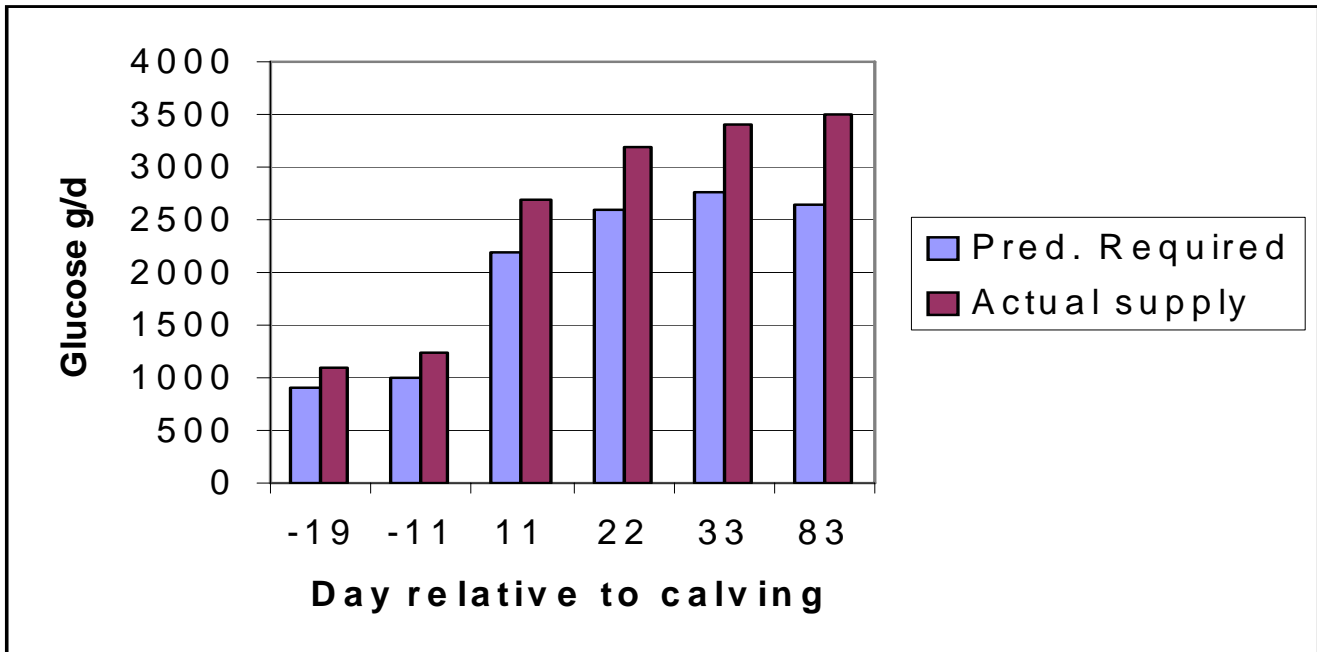
Fig.2 Predicted whole-body glucose requirement compared with actual supply of glucose by gut and liver during transition period and early lactation (Overton, 2004).

2.1.2 Energy intake

During late gestation and early lactation, the cow becomes anorexic. This condition severely limits consumption of energy in amounts necessary to meet demands for maintenance and milk production. As shown in Fig. 3, energy intake is limited by the low dry matter intake (DMI) caused by different physiological, metabolic and endocrine changes that take place.
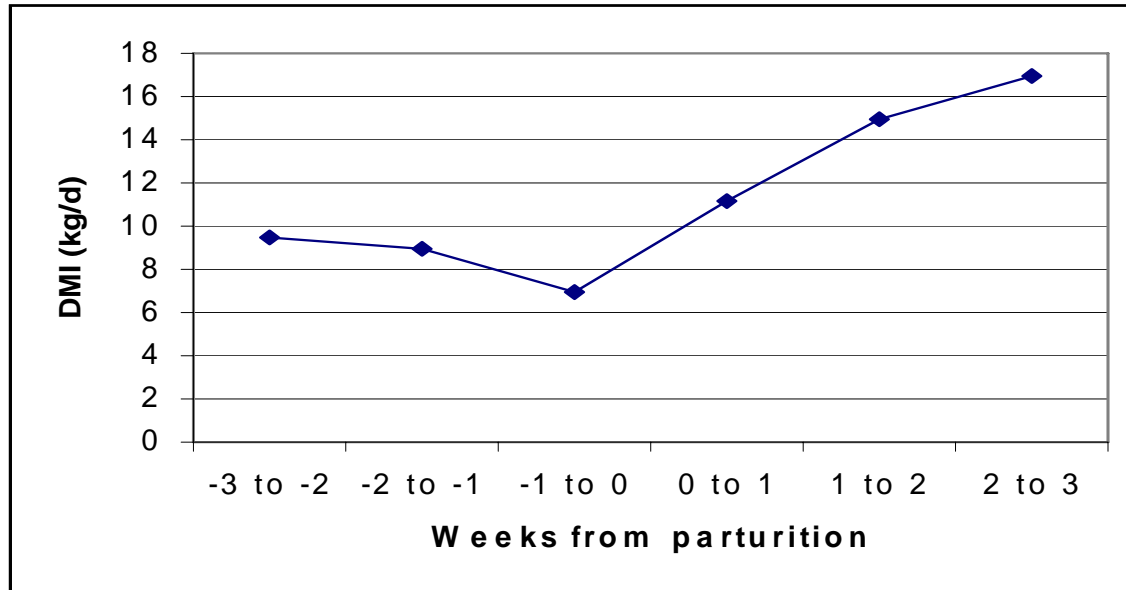
Fig. 3. Dry matter intake of transition cows pre- and postpartum (Spain and Scheer, 2002).

In the later stage of pregnancy the effective volume of the abdominal cavity is reduced as the fetus increases in size, and so is the space available for expansion of the rumen. As a result, intake will be decreased, especially if the diet consists predominantly of roughage (McDonald et al., 2002). Ruminal capacity decreases by as much as 20% during the last 60 days prior to calving (Table 2) then increases again (Stanley et al., 1993). A 20 to 40% gradual decline in DMI during the final 3 weeks of gestation may initiate a negative energy balance and compromise the ability of dairy cows to adapt to physiologic changes (Bell, 1995). These cows are changed from a roughage based diet to a transition diet, which is high in concentrates (Sauer et al., 1989). Even with this change in diet, intake of energy by transition cow is generally limited, due to metabolic and fill constraints, resulting in negative energy balance (Bertics et al., 1992).

Table 2 Periparturient change in ruminal water-holding capacity and fill (Stanley et al., 1993).

	Average days from calving						
	-61	**-48**	**-34**	**-20**	**-6**	**+8**	**+22**
Rumen capacity, L	127	119	108	106	102	142	133
Total fill capacity, %	46.5	51.9	57.3	55.5	53.1	51.0	58.9
DM fill capacity, %	6.7	6.2	6.6	6.0	6.2	6.4	7.4
Fluid fill capacity, %	39.9	45.7	50.7	49.5	47.0	44.6	51.5

As parturition approaches, plasma oestrogen rises sharply in response to secretion of fetal cortisol (Goff and Horst, 1997). High levels of oestrogen are thought to contribute to the decline in dry matter intake that occurs around parturition (Bell, 1995).

Absorptive capacity of the rumen also changes during the dry and transition periods. Beginning at dry-off, cows are most often fed high-forage, low-concentrate diets that are higher in neutral detergent fibre (NDF) and less energy dense than the lactation diet. The lower energy diet causes a decrease in length and surface area of rumen papillae. Long wide papillae projecting into the rumen fluid increase the surface area of the rumen wall and allow for more rapid transfer of the volatile fatty acids (VFA) produced during fermentation of the feedstuffs into the blood for transport to the liver and other tissues (Goff, 2003). The physiological decrease in length of rumen papillae corresponds to a 50% loss of absorptive capacity of VFA during the first 7 weeks of the dry period (Dirksen et al., 1997). The changes that take place in size of rumen papillae during the pre- and post partum phase are shown in Fig.4.
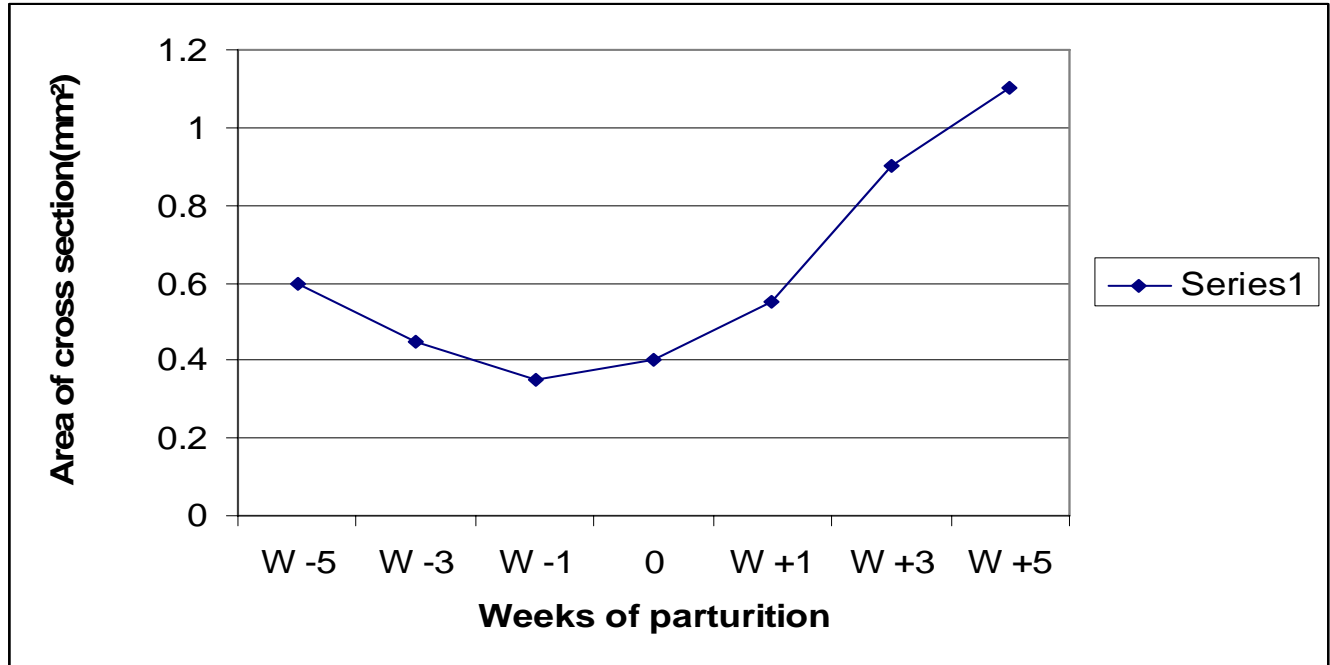
Fig. 4. Changes in the area of cross sections of rumen papillae of cows fed low-energy prepartum and high-energy postpartum (Spain and Scheer, 2002).

2.2 METABOLIC ADAPTATIONS AND KETOSIS

2.2.1 Lipid metabolism

To minimize the energy deficit during early lactation, animals have to mobilize energy from fat depots (Arieli et al., 2001). This mobilization of body fat occurs through release of NEFA into the bloodstream (Overton, 2002). Plasma NEFA concentration increases two-fold during the last 17 days of gestation, peaks around gestation and remains higher than pre-partum levels until about 2 weeks post-partum. The liver oxidizes NEFA to ketone bodies and CO_2 via the tricarboxilic acid cycle or esterifies them to triacylglycerols, which are exported from the liver as very low density lipoproteins (Spain and Scheer, 2002).

High producing cows are generally programmed to utilize body reserves in early lactation to support high milk production. However, there is a limit to the amount of fatty acids that can be processed and used for energy by the liver (and to some extent other body tissues). When this limit is reached, the fats

are no longer burned for energy but begin to accumulate as ketones (Goff, 2003). The three major ketone bodies are acetone, acetoacetate, and B-hydroxybutyrate (BHBA) (Duffield, 2003).

2.2.2 Ketosis and subclinical ketosis

2.2.2.1. Definition

Ketosis is a common disease in high producing dairy cows. It is caused by a negative energy balance, and typically occurs within 2 months after calving when energy demands exceed energy intake and results in increased concentrations of ketone bodies in tissues and milk of the cows (Enjalbert et al., 2001). This is in response to the homeorhetic drive to sustain high levels of milk production, at a time when dry matter intake is reduced (Baird, 1982). Clinical signs of the disease include loss of appetite, decreased milk production, loss of body weight, fatty liver, hypoglycemia, and increased NEFA and ketones in the blood (Baird, 1982). Even when clinical signs do not appear, ketosis can affect milk production and reproduction (Enjalbert et al, 2001). Subclinical ketosis which is characterised by the presence of an excess level of circulating ketones bodies in the absence of clinical signs of ketosis, is a common disease in lactating dairy cows with a lactational incidence rate above 40% in many herds. On a herd basis, subclinical ketosis is much more costly than clinical ketosis and has been associated with decreased milk yield; increased risk of clinical ketosis, metritis, and cystic ovarian disease; and impaired reproductive performance (Duffield et al., 1997).

2.2.2.2. Impact on milk production and composition

In general, there is consensus that a negative association between hyperketonomia and milk production exists; however, there are conflicting reports (Duffield, 2000). Higher concentrations of BHBA have been found in higher-producing cows (Herdt et al., 1981). Duffield (2000) suggested that it is possible that higher milk yields put cows at increased risk of developing subclinical ketosis due to increased milk production being associated with increased fat mobilisation and greater risk of hyperketonemia. Dohoo and Martin (1984) reported a loss of 1.0 to 1.4 kg of milk per day (4.4% - 6% of test day milk) associated with a positive milk ketone test. In contrast, Kauppinen (1983) reported a significant positive correlation between the BHBA and acetoacetate concentration in blood and milk. Detileux et al. (1994)

evaluated the relationship between milk production and clinical ketosis in over 60 000 Finnish Ayshires. They demonstrated a lactation curve depression associated with ketosis and a loss of 44.3 kg/d of milk in the first 100 days of lactation. In a review of studies, Erb (1987) concluded that higher milk yields in the previous lactation did not increase the risk of ketosis in the subsequent lactation. One Finnish study determined increased previous milk yield to be a risk factor for ketosis, but this association was not identified in two North American studies (Curtis et al., 1985; Dohoo and Martin, 1984).

Hals and Tveit (1994), reported that fat-corrected milk (FCM) yield increased with increasing level of plasma acetoacetate in two groups of half-sister related heifers , but the average plasma acetoacetate levels did not exceed 100 μmol /L until peak milk yield was attained. Milk fat percent was significantly higher in both subclinically ketotic and clinically ketotic cows compared with healthy cows (Solbu, 1983). The association between milk fat and hyperketonemia is, presumably, because of increased availability of BHBA and fatty acids for milk fat synthesis (Duffield, 2000). Milk protein percent has been reported to be lower in cows with subclinical ketosis (Mietttenen, 1994). This may be the result of a reduced energy supply, because milk protein percent is positively associated with net energy balance (Grieve et al., 1986).

2.2.2.3. Measurements of ketones bodies

Ketone bodies arise from the incomplete utilisation of fat as an energy source. Acetoacetic acid, acetone and BHBA are ketone bodies produced in the cow during this process (Bass, 2001). Ketone bodies are water soluble acetate derivative produced in the liver and synthetised from acetyl-CoA. The production of ketone bodies occurs at a relatively low rate under conditions of normal physiological status. Normal physiological responses to carbohydrate shortages cause the liver to increase the production of ketone bodies from the acetyl-CoA generated from fatty acid oxidation. During high rates of fatty acid oxidation, a large amount of acetyl-CoA is generated exceeding the capacity of the TCA cycle, and resulting in the synthesis of ketone bodies, or ketogenesis.

Ketone precursors may also exist in high levels in legume and grass silages containing high levels of butyric acid. This can increase the risk of ketosis by increasing the supply of ketone precursors to cattle (Bass, 2001). Ketone bodies are present in blood, urine, and milk and a range of thresholds and methods

for detection of subclinically elevated ketone body concentration have been reported in the literature. Beta-hydroxybutyrate is the predominant circulating blood ketone body, but there is a strong correlation between the whole blood concentrations of BHBA and acetoacetate (Kauppinen, 1983). Acetoacetate increase more compared with BHBA with increasing levels of total ketone bodies, despite a continuing rise in BHBA (Baird et al., 1968). This is most likely because of a relative increase in liver ketogenesis through increased fatty acid metabolism as well as a reduction or cessation in BHBA synthesis in the ruminal epithelium (Baird, 1981). Acetoacetate is unstable in both tissues and fluid samples as it readily decomposes to acetone and carbon dioxide (Bergman, 1971). In contrast, BHBA is relatively stable in whole blood, plasma, or serum, both in vivo and vitro (Custer et al., 1983). Beta-hydroxybutyrate measured using an enzymatic method in either serum or whole blood provides similar results; however, BHBA measured with the same test in plasma is generally systematically lower, possibly because of interference from anticoagulants (Custer et al., 1983). The presence of hemolysis in serum has been shown to interfere with BHBA analysis, causing elevated measurements (Duffield et al., 1998). All ketones bodies show some diurnal variation in relation to feed intake, but BHBA demonstrates the most marked diurnal variation of all the ketone bodies with peak levels of BHBA occurring approximately 4 hours post feeding (Tyopponen and Kauppinen, 1980).

Anderson (1984) determined that milk acetone was approximately 95% of blood acetone, milk acetoacetate was 45% of blood acetoacetate, and blood acetoacetate was 13% of blood BHBA. Beta-hydroxybutyrate levels in milk are only 10% to 15% of circulating blood levels, Probably because of the role of ketone body in fat metabolism. There is a high coefficient of correlation between circulating levels of acetone plus acetoacetate and BHBA (Anderson, 1984).

2.2.2.4. Diagnosis of ketosis

The preferred diagnostic test for subclinical ketosis is blood BHBA. This ketone body is more stable in blood than acetone or acetoacetate (Oetzel, 2003). Based on several studies, subclinical ketosis may start at serum BHBA concentration above 1000 μmol/L (10.4 mg /dL) and clinical ketosis at about 2600 μmol/L (27 mg /dL); however, at exactly what level individual cows will express clinical signs is

extremely variable. Studies using BHBA for assessing subclinical ketosis report a range of values from 1000 μmol/L (10.4 mg /dL) to 1400 μmol/L (15 mg /dL) (Duffield, 2000).

Despite its instability, blood acetoacetate levels have been used by some authors to identify animals with subclinical ketosis. Baird (1982) for example suggested a threshold of 500 μmol/L (5.0 mg /dL) blood acetoacetate for hyperketonemic cows to display clinical signs of ketosis.

Elevated ketone body concentration can also be detected using a milk test based on the reaction of acetone and acetoacetate with sodium nitroprusside. Marstorp et al (1983) developed an injection flow spectrophotometric system to measure milk acetone. The milk BHBA test however appears to be the best cowside test. A routine test for subclinical ketosis available through a central milk testing facility would be a convenient method for monitoring energy status of dairy herds (Geishauser et al., 1998).

The Fossomatic 4000 milk analyser used by several milk testing facilities has the capacity to measure milk citrate (Duffield, 2000). Citrate plays an integral role in the energy metabolism of the cell as a key component in the Krebs' cycle but the biologic role of citrate in milk is essentially unknown (Faulkner and Peaker, 1982). Citrate is formed from the joining of acetyl-CoA with oxaloacetate (Baird, 1982). Theoretically, low citrate levels would be present during ketosis because oxaloacetate is in short supply; however, citrate has not been found to be closely associated with subclinical ketosis and does not appears to be useful for monitoring subclinical ketosis (Duffield, 1997). Because milk fat and milk protein percentage are altered under conditions of subclinical ketosis, these variables have been investigated for their use in defining subclinical ketosis. Among all protein and fat variables, a protein-to-fat ratio of 0.75 or greater was the best test for diagnosing subclinical ketosis in a Canadian study; however, the protein-to-fat ratio was not a good test overall, having a sensitivity of 58% and a specificity of 69% (Duffield et al., 1997).

2.3 FEED ADDITIVES TO IMPROVE ENERGY METABOLISM: MONENSIN AND VIRGINIAMYCIN

Monensin and virginiamycin, respectively an ionophore antibiotic and a non-ionophore antibiotic have been used for many years as feed additives in ruminant diets (Coe et al, 1999, McGuffey et al, 2001). Virginiamycin's effects on ruminal microorganisms and rumen fermentation patterns appear to be

similar to those of ionophore antibiotics (Nagaraja et al, 1997). Monensin supplemented during the transition period improves energy balance in early lactation, and this improved energy balance reduces the risk of energy associated diseases such as ketosis, abomasal displacement and retained placenta (Duffield, 2001). Monensin has been studied more intensively than virginiamycin and more information about monensin effects is available compared to virginiamycin. In addition, the slow release bolus Monensin Control Release Capsule (CRC) has been studied more extensively than feed delivered monensin for its impact on health (Duffield, 2001).

2.3.1 Monensin

The use of monensin as feed additive is one of the strategies that can be implemented for prevention of a negative energy balance and ketosis. Monensin is a carboxylic polyether ionophore produced by naturally occurring strains of *Streptomyces cinnamonensis* (Haney and Hoehn, 1967). Monensin has been reported to have a variety of beneficial effects in ruminants. Claims are for increased milk production, improved feed efficiency, control of subclinical and clinical ketosis and control of bloat (McGuffey et al., 2001). Feed cost is still the most important factor affecting profit margins in dairy herds. Therefore, any improvement in the conversion of feed to milk has a direct impact on the profit margin of the dairy farm (Britt et al., 2004). In the USA, Rumensin® (active ingredient, monensin sodium), supplementation of dairy cows was recently approved, based on the claim of improved milk production efficiency (Shaver, 2005). Applied at the recommended dosage levels in fowls, monensin is practically not absorbed by the gastrointestinal tract and is not deposited in muscles and internal organs (Biovet JSC, 2000). Scientific data indicate that meat and milk produced from animals fed monensin is safe for human consumption. Likewise, monensin is biodegradable in manure and soil, and is not toxic for crop and plants (Ipharraguerre and Clark, 2005).

2.3.1.1 Action of monensin on rumen bacteria

Monensin exerts its many effects by shifting the microbial population in the rumen, selectively inhibiting gram positive bacteria, because of differences in bacterial cell wall structure (Duffield et al., 2002). Monensin binds to the bacterial cell membrane and first causes an efflux of potassium from the cell and influx of hydrogen into the cell (Russel, 1996). The increase of hydrogen is exported out of the

cell either by active transport involving adenosine triphosphate or passively via sodium entry into cell in exchange for hydrogen. In order to maintain inner cell equilibrium, the bacteria cell expends energy and this results in death or reduced growths of the bacterium (Bergen and Bates, 1984).

Rumen bacteria fall into two categories, gram (+) and gram (-). Gram (+) bacteria produce acetate, butyrate, lactate and ammonia and gram (-) bacteria produce propionate and succinate (Bagg, 1997). Gram (-) bacteria have a complex outer membrane with layers of protein, lipopolysaccharide and lipoproteins which make them impermeable to large molecules such as ionophores and are therefore usually resistant to ionophore action. Gram (+) bacteria, lacking the complex outer membrane, are usually sensitive to ionophores.

2.3.1.2 Effect of monensin on rumen fermentation

When supplemented to ruminants, monensin modifies ruminal metabolism, directing it predominantly to the formation of the energetically richer propionic acid at the expense of the other VFA's. Propionate enters the tricarboxilic acid cycle and replenishes oxaloacetate, the main substrate for gluconeogenesis and energy generation (Richardson et al., 1976). The maximal calculated contribution of propionate to net glucose release by liver ranged from approximately 50 to 60% during the transition period; the contribution from lactate ranged from 15 to 20%; and the glycerol contribution ranged from 2 to 4% (Reynolds et al., 2003). Consequently, more glucose becomes available for lactose synthesis in the mammary gland, and milk volume increases (Van der Werf et al., 1998). In addition, propionic acid has a positive effect on mucosal development (Dirkens et al., 1997). The increase in rumen propionate is accompanied by a reduction in the amount of methane produced in the rumen, and inhibition of methane production is suggested to be responsible for one-third of the improved energy utilization of monensin-fed animals (Wedegaertner and Johnson, 1993).

2.3.1.3. Improved energy metabolism

The gluconeogenic and antiketogenic potential of monensin has been investigated by many researchers. In a trial involving 3 groups of 12 Holstein cows, monensin included at 30 grams per ton of total diet, decreased the incidence of subclinical ketosis and significantly reduced blood BHBA in the first 3 weeks postpartum (Sauer et al., 1989). In their study the incidence of subclinical ketosis defined as total blood

ketone > 900 µmol/L was decreased and blood BHBA levels were reduced by 40 % for the high monensin group.

Erasmus et al. (1993) have also investigated the antiketogenic effects of monensin. Cows receiving diets supplemented with either 10 or 20 ppm of monensin, from precalving to early lactation, showed significant reductions in blood acetone and acetoacetate but no significant effects on BHBA. Treatment with monensin at 300 or 450 mg/animal/day, commencing 2 to 4 weeks precalving, reduced serum BHBA and NEFA in lactating dairy cows during the first 28 days pospartum but not at a daily dose of 150 mg/ animal (Thomas et al., 1993). Serum glucose levels were not affected by monensin supplemetation.

Granzin and Dryden (1999) reported that milk yields of cows supplemented with monensin at 150 mg/day (23.0 kg/d) and 300 mg/day (23.7 kg/d) were significantly higher than those of unsupplemented cows (21.1 kg/d). Monensin fed at either 150 or 300 mg/day significantly increased the ratio of plasma glucose: BHBA, but had no effect on concentrations of blood acetoacetate, serum NEFA, plasma glucose or BHBA when compared to the control.

2.3.1.4 Effects of Monensin on dry matter intake, body condition score and body weight

Studies have shown a large range of effects of ionophores on dry matter intake (DMI), body condition score (BCS) and body weight (BW), with either increase, decrease or no effects (Table 3).

Table 3 Effects of monensin supplementation on dry matter intake, body condition score and body weight compared to control.

Reference	Monensin	BW change	BCS change	DMI(kg/d)
Phipps, et al, 1997	300 mg/d	1	0.1	NS
Phipps, et al, 1997	300 mg/d	-2	0.1	NS
Sauer, et al, 1989	200 mg/d	14	ND	-0.6
Sauer, et al, 1989	400 mg/d	8	ND	-1.2
Erasmus et al, 1993	300 mg/d	-8	ND	0.3
Broderick et al, 2004	10.2 ppm	I	ND	N
Erasmus et al, 2005	10 ppm	N	NS	NS

N: Did not differ; I: Higher; NS: Change not significant; ND: Not determined

From these experimental data, it is difficult to draw a meaningful conclusion regarding the effect of ionophores on above-mentioned parameters.

2.3.1.5 Monensin and health

The reported health benefits of administering ionophores to dairy cattle include bloat prevention and a reduction in the incidence of subclinical ketosis and associated clinical diseases (Duffield et al., 2002). Monensin administered in CRC format has been helpful in preventing pasture bloat in dairy cows (Lowe et al., 1991) reducing the incidence and duration of subclinical ketosis (Duffield et al., 1998) and reducing the risk of abomasal displacement and multiple illness as well as culling (Duffield et al., 1999). In feedlot steers, monensin has an impact on reducing rumen acidosis and the effects are thought to be mediated through monensin's effect on reducing lactic acid-fermentating bacteria and enhancing lactic acid utilisers (Duffield 2001).

2.3.1.6 Effects of monensin on milk production and composition

The advantage of supplementing with monensin to dairy cattle include increased milk volumes as a result of increased blood glucose (Van der Werf et al., 1998).

Phipps et al (2000) reported that efficiency of milk production was increase by 5 %, with supplementing monensin at dose of 150,300 and 450 mg/d. In the same study, Milk fat and milk protein content declined at 300 mg/d compared to the control. In the study of Becket et al. (1998), for cows receiving a slow-release intraruminal bolus containing 32 g of sodium monensin. Monensin significantly increased milk production by 0.75 L/d per cow and tended to increase milk fat and protein yields but had no significant effect on milk fat or milk protein percentages. Nevertheless, monensin supplementation did not affect milk production in the study of Odongo et al. (2007).

2.3.1.7 Estimated economics of preventing subclinical ketosis using monensin

No published South African data are available on the economics of monensin supplementation. Duffield (2000) reported that monensin has been shown to reduce the incidence of subclinical ketosis by 50% in a study involving 25 herds, and that the cost of subclinical ketosis has an approximate range of CAN $50 to CAN $100 per cow per lactation. In another Canadian field study of 95 herds, the return over costs for Rumensin was 69 cents (Canadian dollar) per day including milk improvement, rumen health, and BCS impact (Hutjens, 2006).

2.3.2 Virginiamycin

Virginiamycin is a fermentation product of *Streptomyces virginiae* (Rogers et al., 1995). It is composed of two majors factors, M and S, that function synergistically (Boon and Dwart, 1974). The mode of action is the blocking of protein synthesis (Cocito, 1979). Virginiamycin M blocks the elongation of polypeptide chains by binding to peptidyl transferase, and virginiamycin S, which has a similar action, enhances and fixes the bound components. Singly the two components of virginianycin are bacteriostatic but together they are bactericidal. Virginiamycin is an antimicrobial feed additive approved for use in cattle to improve performance (Ives et al., 2002). The poultry and swine industries have used virginiamycin for many years as a performance enhancer. Virginiamycin acts by altering ruminal microbial populations. The gram (+) bacteria antimicrobial activity and subsequent alterations in ruminal fermentation products are similar to those of monensin, namely an increase in propionate at the expense of acetate and methane, although the mode of action differs (Nagaraja et al., 1997). Antibiotics such as virginiamycin have been shown to improve the growth and feed efficiency of broilers

(Woodward et al., 1988; Miles et al., 1984), to decrease flock variability (Miles et al., 1984), and improve the intestinal digestion and absorption of carbohydrates and fats (Eyssen and De Somer, 1963). There are limited data on the effect of virginiamycin supplementation to dairy cattle and the effect of virginiamycin on blood ketone bodies, has to date not been described.

CHAPTER 3

MATERIALS AND METHODS

3.1 COWS, DIETS AND EXPERIMENTAL DESIGN

Forty multiparous high producing Holstein cows were used in a randomized complete block design experiment. Approximately 3 weeks prepartum, cows were assigned to one of 10 blocks of four cows, based upon parity, previous milk production, BW and BCS. The four experimental treatments were 1) Control diet, no medication (C); 2) Control plus 20 ppm virginiamycin (V); 3) Control plus 15 ppm monensin sodium (M) and 4) Control plus 20 ppm virginiamycin plus 15 ppm monensin sodium (V+M). The commercial products used to supply monensin and virginiamycin were Poulcox and Stafac respectively, both supplied by Phibro Animal Health, P.O. Box 40492, Moreleta Park, 044, South Africa. Monensin and virginiamycin supplements were added to the TMR's in one of four vitamin mineral premixes (Table 4).

Cows received the experimental treatments starting at assignment approximately 3 weeks prepartum. During the prepartum period cows received 8 kg/day (as fed) of the assigned treatment as well as ad lib access to a good quality *Eragrostis curvula* hay. Additionally, anionic salts (100g $MgSO_4$ + 100g NH_4Cl_2) and limestone (200g) were top dressed and mixed into the diet in daily basis. After calving, cows were moved to a semi-intensive housing unit equipped with Calan head gates (American Calan Inc., Northwood, NH, USA) for monitoring of individual feed intake and were fed only their assigned experimental diet as a total mixed ration in equal allocations at 08h00 and 16h00 for a period of 56 days. A group of 10 cows had access to a dirt exercise lot of 500 m², and fresh water was continuously available. Water was added at 30 % of the daily postcalving feed allocation to improve intake and prevent separation of feed ingredients. Cows were exposed to continuous lighting and were milked at 06h00 and 17h00 daily in a 10-point DeLaval herringbone parlour equipped with an Alpro Herd Management System (DeLaval. (Pty) Ltd, Heilbron, 9650, South Africa).

All prepartum and postpartum animal care was consistent with the guide for the Care and Use of Animals in Agriculture Research and Teaching (1999) and animal use was approved by the Animal Ethics Committee of the South African Agricultural Research Council, Livestock Business Division, Irene, South Africa.

Table 4 Ingredient and chemical composition of the basal TMR fed both prepartum and postpartum (DM basis)

Ingredients (%)	
Lucerne hay	38.0
Ground maize	34.0
Cottonseed meal	3.0
Whole cottonseed (linted)	6.0
Sunflower meal	5.5
Soybeans roasted	4.0
Cane molasses	6.0
Brewers grains	2.0
Megalac[a]	0.79
Sodium chloride	0.40
Vitamin / Mineral premix[b,c]	0.30
Chemical composition	
OM, %DM	92.3
Fat, %DM	5.5
CP, %DM	16.6
Soluble CP, %CP	27.8
RUP, %CP	41.2
NDF, %DM	29.1
ADF, %DM	21.9
NSC, %DM[d]	41.1
ME, MJ /kg DM[e]	11.2
NE_L, MJ /kg DM[e]	7.51
Ca, %DM	0.81
P, %DM	0.39
K, %DM	1.52
Mg, %DM	0.29

[a]Arm & Hammer (Church & Dwight, Inc., Princetown, NJ)

[b]Contains per kg of premix: 7,000k Iu of Vitamin A; 1,500k Iu of Vitamin D_3; 1300 mg of Vitamin B_1; 4000 mg of Vit B_{12}; 15,000 mg of Vit E; 130,000 mg of niacin; 1000 mg of Co; 3000 mg of I; 375 mg of Se; 100,000 mg of Mn; 20,000 mg of Cu; 100,000 mg of Zn; 350,000 mg of S; 60,000 mg of Fe

[c]There were four mineral/vitamin premixes, with the treatments being created by addition of virginiamycin or monensin, or both, at a level to provide 20 ppm of virginiamycin or 15 ppm of monensin in the DM of the TMR

[d]Non-structural carbohydrates (NFC) = 100 – (CP + Fat + Ash + NDF)

[e]Calculated as metabolizable energy (ME) using the database of Van der Merwe and Smith (1991) and converted to NE_L as: ME X 0.67 (NRC, 1989)

3.2 SAMPLE COLLECTION AND ANALYSIS

3.2.1 Feed sampling and analysis

Samples of TMR and orts, within cow, were collected weekly, frozen and composited by treatment (TMR) or cow (orts). The DM contents of the TMR and orts were determined by oven drying at 60° C for 48 h. Dried TMR were ground and analysed for organic matter (OM) by ashing in a muffle furnace at 600° C for 2 h, crude protein (CP) according to AOAC, (2000) procedure 968.06 and ether extract according to AOAC (2000) procedure 920.39. Buffer CP was determined according to Krishnamoorthy et al. (1982). Calcium (Ca), potassium (K) and magnesium (Mg) were determined according to Giron, (1973) using a Perkin Elmer Atomic Spectrophotometer. Phosphorus (P) was assayed according to AOAC (2000) procedure 965.17. The NDF was determined according to Robertson and Van Soest (1991) and ADF according to Goering and Van Soest (1988). Non fibre carbohydrate (NFC) was calculated from other assayed nutrients (Hall, 1998). Dried orts were analysed for CP and NDF.

3.2.2 Milk yield and composition

Milk production from each cow was recorded on a daily basis. Composite milk samples were prepared from consecutive morning and afternoon milking once every 7 days, and were analyzed for fat, CP, lactose and milk urea nitrogen (MUN). Analyses were done at Lacto Lab (Pty) Ltd, Irene by means of a System 4000 Infrared Analyser (Foss Electric, Hillerod, Denmark).

3.2.3 Body weight and body condition score

Cows were weighed and body condition scored using the five-point BCS scale (i.e., 1 very thin; 5, very fat) at assignment and at subsequent 2 week intervals (Wildman et al., 1982).

3.2.4 Blood metabolites

Samples were collected by jugular venipuncture from each cow 7 days prepartum and thereafter on days 7, 14 and 28 postpartum. All Samples were taken at the same time of the day; approximately 1 hour before the 08h00 feeding.

3.2.4.1 Beta-hydroxybutyrate and Acetone

Blood was collected using a 10 ml labeled glass tube, (green stopper lithium / heparin vacutainers, without vacuum), and cooled immediately to 2-4° C. Two ml of blood were then transferred to two clean test tubes (in duplicate). Cold 30% perchloric acid was added in a 1:1 ratio to the blood samples, for the precipitation of protein. After thorough mixing, the precipitated protein was removed by centrifuging in a refrigerated centrifuge at 2000 rpm, for 20 min. The clear supernatant was transferred to clean glass tubes and recapped with clean screw caps, as quickly as possible to prevent evaporation of acetone, and stored at –20° C, until analyses for acetone and BHBA. The determination of BHBA was carried out by means of an enzymatic analysis (Williamson et al., 1962). Acetone was determined after the completion of the enzymatic analysis of aceto-acetic acid, by adding a color reagent. The color reagent consisted of a well mixed solution of 1 ml 2-hydroxybenzaldehyde (GR) in 80 ml 4 N potassium hydroxyde. Five ml colour reagent was added to 3 ml of the samples and standards, after which an incubation period of 3

hours at 40° C followed. The absorbance was then read at 450 nm using a Beckman DU 650 spectrophotometer.

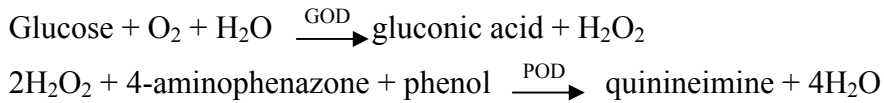### 3.2.4.2 Non-esterified fatty acids

Blood samples were collected in lithium / heparin vacutainer tubes, kept on ice and thereafter centrifuged in a refrigerated centrifuge at 2500 rpm for 20 min. The plasma was stored in Eppendorf tubes at –20° C until analyses could be done; 250 µl plasma was added to 1 ml phosphate buffer (pH=6.4), and then 6 ml of CHM (chloroform heptane methanol solution) was added to each sample (in triplicate). Standards and blanks were prepared in the same manner. The tubes were vigorously shaken for 60 sec. on a multiple vortexer and then centrifuged for 20 min. at 2000 rpm. The buffer was removed by suction, 3ml of the organic phase was transferred into clean test tubes. Cobalt reagent (2.5 ml) was then added, mixed thoroughly, spun down, and analysed with a color reagent added, mixed thoroughly and centrifuged for 20 minutes at 2000 rpm.. Two ml of the organic phase was then transferred into clean test tubes and 2.5 ml color reagent was added. The stock color reagent consisted of a 4% (m/v) 1-nitroso-2-naphtol solution in 96% ethyl alcohol.

Just before use, the stock reagent was diluted by a factor of 12.5 with ethyl alcohol. Triplicate samples were read on a Beckman DU 650 spectrophotometer at 450 nm. The extraction procedure of Falholt et al. (1973), with a modification of the colorimetric technique of Novak (1965), further developed and refined by De Villiers et al. (1977) was used.

3.2.4.3 Glucose

Blood samples were collected into 5 ml vacutainer tubes containing potasium oxalate and sodium fluoride 12.5 mg, centrifuged at 2500 rpm for 10 min. The plasma was stored in Eppendorf tubes at –20° C until analyses. Glucose was determined using the ACE Glucose method. The ACE™ Glucose Reagent is intended for the quantitative determination of glucose in serum using the ACE tm Clinical Chemistry system. In this method glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to form a red-violet quinoneime dye as indicator. The absorbance of the reaction is bichromatically measured at 505 nm / 692 nm.

Principle of the procedure:

Glucose + O_2 + H_2O $\xrightarrow{\text{GOD}}$ gluconic acid + H_2O_2

$2H_2O_2$ + 4-aminophenazone + phenol $\xrightarrow{\text{POD}}$ quinineimine + $4H_2O$

3.3 STATISTICAL ANALYSIS

Data were analysed according to a randomized block design with the statistical program using the GLM model (Statistical Analysis Systems, 2001) for the average effects over time. An analysis of variance was used to determine the significance of difference between different treatments and blocks. The control diet (C) was contrasted with the addition of monensin (M) and virginiamycin (V) or both (V+M). Parameters were analysed as average and repeated measures. Last square means and standard deviations (SD) were calculated and significance of difference between means was determined using Fisher's test (Samuels, 1989). Significance was declared at $P<0.10$ and tendency to differences were accepted if $P<0.15$.

The linear model used is described by the following equation:

$$Y_{ij} = \mu + T_i + B_j + e_{ij}$$

Where Y_{ij} = variable studied during the period

μ = overall mean of the population

T_i = effect of the ith treatment

B_j = effect of the jth block

e_{ij} = error associated with each Y_{ij}

CHAPTER 4

RESULTS AND DISCUSSION

4.1. EXPERIMENTAL DIETS

The control diet was formulated to fulfill the minimum nutrient requirement of an early lactating 680 kg Holstein cow producing 40 kg of milk with 4% fat and 3.5% protein (NRC, 2001). Chemical analyzes of orts (data not shown) indicated that little selective feeding occurred and the chemical composition of consumed diets differed little from the mean chemical composition of the formulated diets (as shown in Table 4).

4.2. FEED INTAKE, MILK PRODUCTION, MILK COMPOSITION AND FEED EFFICIENCY

Results on DMI, milk production, milk composition and feed efficiency are presented in Table 5. Mean postcalving DMI varied from 23.6 kg/d to 25.4 kg/d and did not differ between treatments (P>0.10), however it tended to be lower for cows supplemented with M when compared to cows supplemented with both additives (P=0.14). Although the effect of ionophores on DMI have been variable, with some reporting a decrease in DMI (Sauer et al., 1989, Green et al., 1999), no effect on DMI have been reported in most studies. In a recent review by Ipharraguerre and Clark (2003), in 8 of 12 studies with lactating cows, no significant effects of monensin on DMI were observed. The level of ionophore supplementation, however, also affected DMI. Symanoski et al., (1999) supplemented Holstein cows with either 0,8,16 or 24 ppm monensin and found DMI to be decreased in the 16 and 24 ppm groups. Similarly, in a series of nine trials monensin was supplemented to 966 Holstein cows at level of 0, 7 and 22 ppm (McClary et al., 2005). Dry matter were decreased on both the 15 and 22 ppm monensin treatments. A similar trend was observed in our study.

Table 5. Mean dry matter intake (DMI), milk production and composition and feed efficiency as affected by monensin and virginiamycin supplementation.

	Treatment[1]				
	C	**M**	**V**	**V+M**	**SEM**
DMI (kg/d)	24.6	23.6	24.3	25.4	0.83
Milk (kg/d)	38.9[ab]	37.4[ab]	36.6[b]	41.2[a]	1.87
Fat (%)	3.62[bc]	3.42[c]	3.75[ab]	3.86[a]	0.13
Fat (kg)	1.41[ab]	1.28[b]	1.37[b]	1.59[a]	0.09
Protein (%)	3.13	3.02	3.14	3.14	0.03
Protein (kg)	1.21[ab]	1.13[b]	1.14[b]	1.29[a]	0.03
Lactose (%)	4.68	4.71	4.61	4.61	0.03
MUN (mg/dl)	12.5	12.2	12.1	13.0	0.43
ECM (kg/d)	39.7[ab]	36.9[b]	37.9[b]	43.3[a]	2.00
Feed efficiency	1.61	1.57	1.56	1.70	0.08

[1]C, control; V, Virginiamycin; M, Monensin; V+M, Virginiamycin + Monensin.
[abc]Means in the same row with different superscripts differ (P< 0.10)
ECM= ((0.327x kg milk)+(12.95x kg fat)+(7.2x kg protein)) (Orth, 1992); Feed efficiency = ECM/DMI

Very little information is available on the supplementation of virginiamycin to dairy cattle. Clayton et al., (1999) reported no difference in DMI between the control and experimental diet when virginiamycin was supplemented to grazing dairy cows receiving concentrate pellets at a level of 30 ppm. Rogers et al., (1995) reported no effect on DMI when virginiamycin was supplemented to feedlot steers over a dose range of 7.5 to 27.6 ppm. Similar results have been reported by Wagner et al. (2000) with growing heifers and by Ives et al. (2002) with steers fed corn-based finishing diets. This is in agreement with our results where virginiamycin supplemented at 20 ppm, did not affect mean DMI (P>0.10). Mean postcalving intake of dry matter was not affected by the combination treatment (V+M), but tended to be higher (P<0.15) when compared to M group (table 5). An illustration of DMI over time is shown in Fig. 5, and after 60 days, the general trend was still for the DMI to increase.
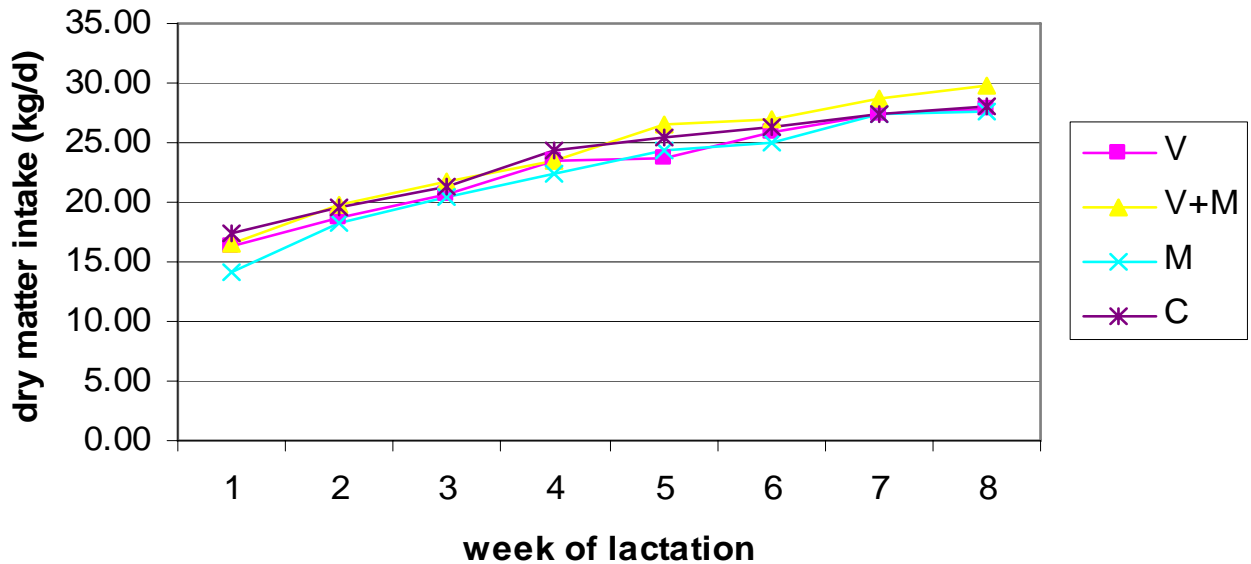
Fig.5. Change in DMI (kg/d) over time for cows receiving no additives (C), monensin (M), virginiamycin (V), or both (V+M).

Dry matter intakes were also compared within weeks and results are presented in Table 6.

During week 1, monensin supplementation suppressed DMI when compared to both C (P=0.03) and V+M (P=0.07), but tended to be lower (P<0.15) compared to V group. Dry matter intake in the V+M treatment group was higher (P<0.10) at week 8 compared to M group, when it tended to be higher (P<0.15) compared to V and C group.

Table 6. Dry matter intake at different stages of lactation as affected by monensin and virginiamycin supplementation.

	Treatment[1]				
DMI (kg/d)	C	M	V	V+M	SEM
Week +1	17.40^a	14.06^b	16.32^{ab}	16.55^a	1.01
Week +2	19.67	18.16	18.62	19.69	1.30
Week +3	21.27	20.45	20.76	21.80	1.43
Week +4	24.36	22.46	23.40	23.44	1.16
Week +5	25.37^{ab}	24.25^{ab}	23.76^b	26.45^a	1.09
Week +6	26.24	25.06	25.79	27.00	0.93
Week +7	27.43	27.44	27.47	28.64	0.89
Week +8	27.95^{ab}	27.55^b	27.85^{ab}	29.69^a	0.81

[1]C, control; V, Virginiamycin; M, Monensin; V+M, Virginiamycin + Monensin.
[abc]Means in the same row with different superscripts differ (P< 0.10)

Mean milk production did not differ between C, M, and V treatments, but differed (P<0.10) between cows receiving V (36.6 kg) and V+M (41.2 kg) treatments respectively (Table 5). Feeding the same dose of monensin has either increased milk yield (Phipps et al., 2000; Heuer et al., 2001) or not (Van der werf et al., 1998). The lack of difference in mean milk yield has also been observed by Erasmus et al (2005) when studying the influence of yeast culture and monensin or both on ruminal fermentation and performance of multiparous dairy cows. Duffield et al., (1999) observed that the magnitude of the increase in milk production is partially dictated by the BCS of the cows at the moment of treatment administration. They have found a significant interaction of treatment by BCS, and the results showed that monensin administration had no significant effects on milk yield for cows with BCS<3.25. In this study, all the cows had a BCS<3.25 (Table 8) and this probably contributed to the lack of response. Van Amburgh has also concluded that the effects of ionophore on growth and lactation are not consistent and appears to be diet related. In the study of Ipharraguerre and Clark (2003), it was calculated that ionophore supplementation increased milk production by 1.5 kg/d (9.4%) when feeding high forage diets (>50%) and 0.7 kg /d (1.5%) when feeding low forage diets (<50%). The basal diet in our study contained 38% forage. This can also have contributed toward the lack of difference on milk production. In a study where 30 ppm virginiamycin were supplemented to grazing lactating cows, virginiamycin did

not affect milk yield (Clayton et al., 1999). Thus, the level in the current study, 20 ppm, might not have been sufficient to increase production. More studies with virginiamycin on lactating dairy are needed. In the V+M treatment group, mean milk production was higher (P<0.10) when compared to cows receiving only V, but did not differ from the other treatments (Table 5). Milk production by stage of lactation as affected by monensin and virginiamycin supplementation is presented in table 7. During week 1, milk production in V+M group tended to be higher (P<0.15) when compared to M group, and remained significantely high from week 7 to week 8 compared to V group. As shown in Fig. 6, after 60 days, the general trend was still for the milk yield to increase.

Table 7. Milk production by Stage of lactation as affected by monensin and virginiamycin supplementation or both.

Milk production (kg/d)	Treatment[1]				
	C	M	V	V+M	SEM
Week +1	25.78	23.20	24.47	27.31	1.74
Week +2	34.62	31.03	32.33	35.64	1.91
Week +3	35.70	35.10	36.26	39.19	2.18
Week +4	39.80	37.71	37.73	41.56	2.16
Week +5	41.44	39.52	42.74	39.67	2.12
Week +6	42.54	40.80	40.14	40.08	2.08
Week +7	42.86[ab]	41.76[ab]	39.15[b]	44.70[a]	2.08
Week +8	42.42[ab]	41.83[ab]	37.99[b]	44.98[a]	2.21

[1]C, control; V, virginiamycin; M, monensin; V+M, virginiamycin + monensin.
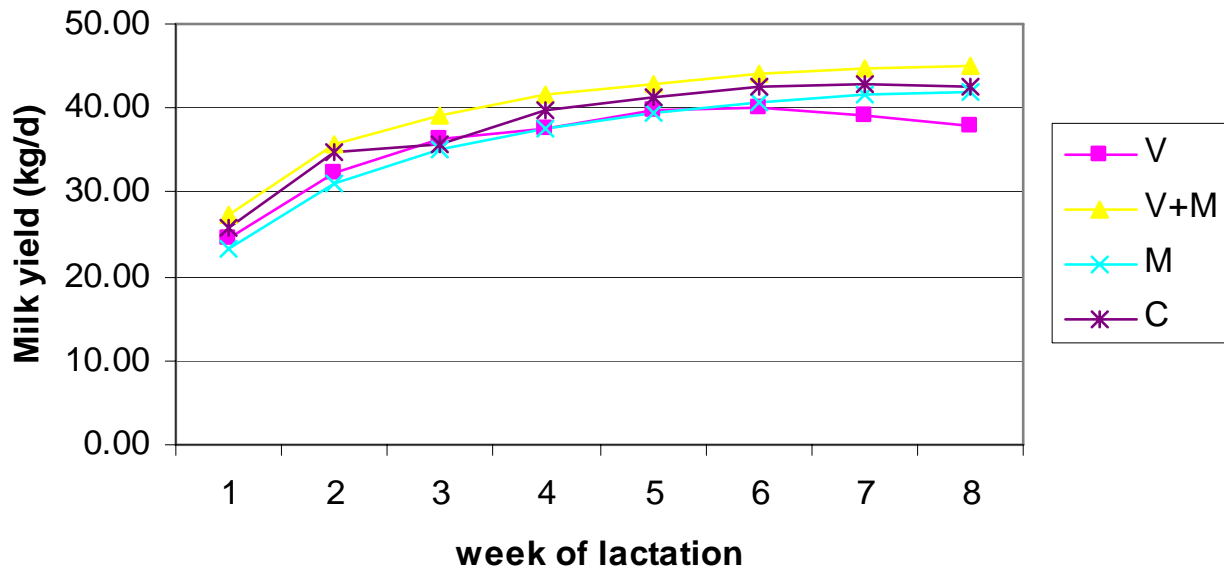[abc] Means in the same row with different superscripts differ significantly at P< 0.1

Fig.6. Change in milk production (kg/d) over time for cows receiving no additives (C), monensin (M), Virginiamycin (V), or both (V+M).

The increased milk production of cows supplemented with both additives together, when compared to the V group, is more difficult to explain. Virginiamycin's effects on ruminal micro-organisms and fermentation patterns appears to be similar to these of ionophores in terms of altered fermentation towards increased molar proportion of propionate, decreased lactic acid production, decreased methane production and decreased protein degradation (Nagaraja et al., 1997). It possible that the combined positive effects of the two additives on stabilizing feed intake and rumen fermentation and increasing feed efficiency, together with a potential post-ruminal effect of virginiamycin, could have contributed to the increased production. The primary mode of action for virginiamycin in non-ruminant is increased nutrient availability due to selective inhibitions of several species of enteric bacteria inhabiting the digestive track. Studies with growing pigs showed that virginiamycin inhibits decarboxylation of AA and urea and thereby spares essential AA by reducing formation of ammonia and amines (Dierick et al., 1986). It has been reported by Henderickx et al., (1981) that virginiamycin contributes to improved growth in monogastrics through altering metabolic activities of intestinal microflora, thereby increasing quantities of ME and MP available to the host and also may influence permeability of intestinal mucosa to enhance nutrient absorption from the gut. These effects were demonstrated in a recent study where virginiamycin decreased intestinal tract weigh through thinning of intestinal wall, increased the number

of villi per unit area and improved performance of broilers (Miles et al., 2006). If virginiamycin causes effects in the gastro intestinal tract of ruminants, similar to those reported from research with monogastrics, then a combination between virginiamycin and monensin might ehance properties of both additives. This hypothesis is supported by results from this study where ECM production from cows supplemented with V+M was increased when compared to cows supplemented with only V (P=0.07) or M (P=0.02). In agreement with Rogers et al., (1995) additional research on the post ruminal effects of virgininiamycin should help improve our understanding of the mode of action of virginiamycin in ruminants.

In this study, mean milk fat % in M and V treatments did not differ from the control (Table 5). In a recent study (Duffield et al., 2003), monensin supplemented at a dose ranging from 9 to 14 ppm, significantly reduced milk fat percentage in herds receiving diets low in NSC (≤ 40 %), but not in those receiving diets high in NSC (≥ 40 %). These results suggest that there are significant interactions between monensin and certain dietary factors on milk fat suppression in Holstein dairy herds. The level of NSC used in the current study $\geq 40.2\%$ may explain the lack of effect of monensin on milk fat %.

When supplemented at a level of 30 ppm to grazing Holstein cows (Clayton et al., 1999) virginiamycin did not affect milk fat. In the current study, milk fat % was lower in cows supplemented with monensin, when compared to the V (P=0.02) and V+M (P=0.004) treatments (Table 5).

Milk protein percentage did not differ between treatments (P>0.10). The lack of effects of monensin on milk protein percentage is consistent with results from the studies of Heuer et al (2001) and Erasmus et al. (2005). Milk protein yield was greater for cows supplemented with V+M when compared to those receiving only M (P=0.06) and V (P=0.08). There was no difference in milk protein yield between the M, V, and C treatments. In agreement with the study of Mutsvangwa et al. (2002), monensin supplementation did not affect milk protein yield. No effects of the two antibiotics and their combination were observed on milk lactose and MUN.

Energy corrected milk (ECM) was greater for cows supplemented with V+M when compared to those receiving only M (P=0.03) and V (P=0.07). Feed efficiency values were similar for all the treatments and were in the range to be expected for early lactation mature high producing cows (Hutjens, 2005).

Although we could not find data with effects of virginiamycin on feed efficiency in dairy cattle, feed efficiency was improved in response to virginiamycin supplemented at the dose of 19.3 ppm in feedlot diets (Rogers et al., 1995).

4.3. BODY WEIGHT AND BODY CONDITION SCORE

Results on body weight and body condition score are presented on Table 8. All cows lost body weigh during the postcalving period. The decrease observed in BW and BCS during lactation indicates that body tissues were a source of energy and nutrients for cows on all treatments.
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Table 8. Mean body weight, body weight change, body condition score and body condition score change as affected by monensin and virginiamycin supplementation.

	Treatment [1]				
	C	**M**	**V**	**V+M**	**SEM**
Mean postcalving BW (kg)	641[ab]	614[a]	629[ab]	659[b]	20.73
BW change (kg)	-34.2	-31.9	-20.2	-8.1	12.23
Mean postcalving BCS	1.48	1.57	1.62	1.54	0.09
BCS change	-0.20[ab]	-0.30[b]	-0.05[a]	-0.25[ab]	0.10

[1]C, control; V, Virginiamycin; M, Monensin; V+M, Virginiamycin + Monensin.
[abc]Means in the same row with different superscripts differ (P< 0.10).

Mean BW of cows supplemented with both additives (V+M) were higher (P=0.09) when compared to cows supplemented with M (Table 8). This also reflected in the change in BW with cows supplemented with V+M experiencing the smallest numeric change in BW and tended to differ (P<0.15) from the control cows. Means BCS did not differ between treatments (P> 0.10). The change in BCS was lower in V group (P<0.10) when compared to M. The BCS values were lower than expected, but it should be noted that the individual who did the scoring throughout the trial in general, does score lower than other herd managers. Change in BW and BCS were in agreement with other studies where early lactation multiparous cows were fed TMR's ad libitum (Dann et al., 2005).

Compared to the C diet, M supplementation did not affect BW or BW change. This is in agreement with other studies where cows were administred monensin (Wang et al., 2001; Erasmus et al., 2005) or virginiamycin (Clayton et al., 1999). In our study, M supplementation did not affect the change in BCS and an explanation might be that the level of supplementation (15 ppm) was too low to induce a response. Lower BCS losses, however, were reported by Wagner et al. (1999) in cows receiving diets supplemented with 16 and 22 ppm monensin when compared to control cows and cows supplemented with monensin at 8 ppm. No studies could be found where the effects of V on BCS were reported and more studies are needed, Godfrey et al. (1993), however reported an improved gain in sheep supplemented with Virginiamycin.

4.4. BLOOD METABOLITES

Results of the effects of different treatments on blood metabolites are shown in table 9. Blood concentration of NEFA, BHBA, acetoacetate and acetone have been used as indicator of negative energy balance and severity of ketosis (Duffield, 2000). Clinical signs of disease include decreased milk production, loss of BW, fatty liver and increased NEFA and ketones in blood (Baird, 1982).

Prepartum glucose concentration did not differ (P>0.10) between treatments and averaged 64.8 mg/dl. Similar prepartum glucose values were reported in the studies of Stephenson et al. (1997) and Plaizier et al., (2005). Monensin supplementation to prepartum dairy cows increased blood glucose concentration postpartum (Duffield et al., 1998; Green et al., 1999) but not prepartum (Stephenson et al., 1997; Green et al., 1999) which is in agreement with our study (treatment M). The lack of a prepartum glucose response with monensin supplementation might be explained by stimulation of insulin release. This results in a lowered plasma glucose concentration due to increased partitioning of glucose to higher energy demanding organs such as the uterus and mammary gland (Arieli et al., 2001). No data could be found on the effect of virginiamycin supplementation on prepartum blood metabolites of dairy cows. Treatment did not affect prepartum plasma BHBA concentration and values are in the same order as those reported by Stephenson et al. (1997) and Green et al. (1999). Our results are in agreement with Plaizier et al. (2005), who found no effect of prepartum monensin supplementation on plasma BHBA concentration and Green et al. (1999), who observed that monensin had only a limited effect on BHBA precalving. Others, however, reported that monensin supplemented cows had significantly lower BHBA values precalving (Stephenson et al., 1997; Duffield et al., 2003). Prepartum plasma NEFA values did

not differ (P>0.10) and averaged 0.54 mmol/L, which is in the same order as pre-calving values reported by Duffield et al. (2003). Lower NEFA values indicate less fat mobolization and improved energy status; lower blood NEFA values have been reported after prepartum monensin supplementation (Stephenson et al., 1997; Duffield et al., 2003). Our results, however, are in agreement with Arieli et al. (2001) and Plaizier et al. (2005). The beneficial effects of monensin are more pronounced close to parturition and the time of sampling in relation to calving, and therefore, should be taken into account when interpreting results.

Table 9. Serum glucose, plasma NEFA, plasma acetone and plasma BHBA as affected by monensin and virginiamycin supplementation.

	Treatment[1]				
	C	M	V	V+M	SEM
Glucose (mg/dL)					
Glucose week -1	63.0	63.72	67.14	64.8	0.13
Mean postcalving glucose	65.0[b]	75.4[a]	65.2[b]	65.2[b]	0.10
Glucose week +1	62.46[b]	73.98[a]	61.92[b]	60.66[b]	0.19
Glucose week +2	66.78	75.24	65.88	65.52	0.23
Glucose week +4	61.02[c]	77.76[a]	68.94[b]	69.12[b]	0.17
Acetone (mg/dL)					
Acetone week -1	0.23[b]	0.46[a]	0.25[b]	0.28[b]	0.05
Mean postcalving acetone	0.25[b]	0.26[ab]	0.30[a]	0.28[ab]	0.03
Acetone week +1	0.28	0.29	0.32	0.29	0.06
Acetone week +2	0.25	0.24	0.27	0.27	0.04
Acetone week +4	0.277[a]	0.231[b]	0.230[b]	0.243[b]	0.01
NEFA (mmole/L)					
NEFA week -1	0.43	0.85	0.52	0.72	0.15
Mean postcalving NEFA	0.63	0.48	0.56	0.57	0.09
NEFA week +1	0.64	0.46	0.73	0.66	0.14
NEFA week +2	0.64	0.58	0.57	0.60	0.12
NEFA week +4	0.47	0.47	0.46	0.42	0.09
BHBA (μmole/L)					
BHBA week -1	975	1069	996	1148	131.98
Mean post calving BHBA	1586[a]	1144[b]	1254[b]	1319[ab]	126.59
BHBA week +1	1683[a]	1178[b]	1112[b]	1432[ab]	179.26
BHBA week +2	1667[a]	1113[b]	1341[ab]	1311[ab]	160.47
BHBA week +4	1408	1141	1309	1271	137.56

[1]C, control; V, virginiamycin; M, monensin; V+M, virginiamycin + monensin.
[abc]Means in the same row with different superscripts differ (P<0.10).

Mean postpartum serum glucose was increased (P < 0.01) by M supplementation when compared to the other treatments and glucose levels during week 4 were increased by all the treatments, when compared to the control (P<0.10). This was expected since monensin is known to increase propionic acid in the rumen which would result in more glucose production via the citric acid cycle (Lean et al., 1992). The lack of a response in mean glucose concentration by the V + M treatment group cannot be explained. In their review, Ipharraguerre et al. (2003) reported a significant increase in blood glucose after monensin supplementation in only 4 of 13 studies. The highest increases in blood glucose of 10.6% and 8.6% were from the studies by Van der Werf et al. (1998) and Green et al. (1999), respectively.
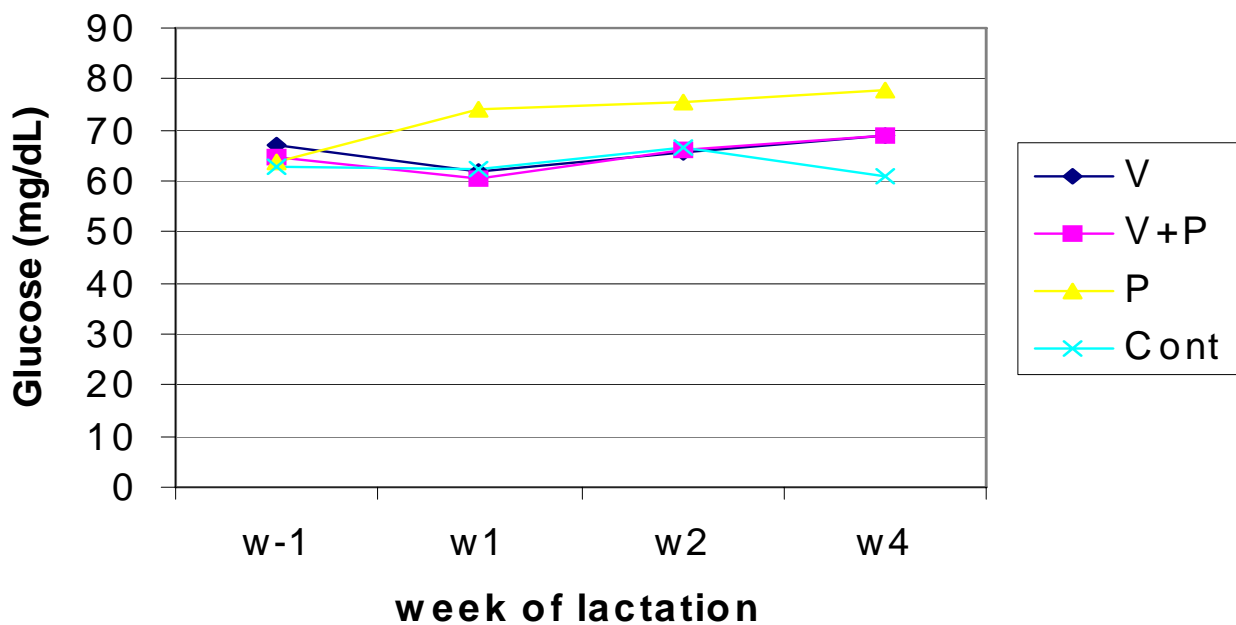

Fig. 7. Change in blood glucose concentration over time for cows receiving no additives (C), monensin (M), Virginiamycin (V), or both (V+M).

Both treatments M (P = 0.02) and V (P = 0.07) reduced mean postpartum plasma BHBA when compared to the control treatment. This might have resulted from several actions of monensin and virginiamycin. These include a decrease in ruminal production of butyrate which reduced dietary entry of BHBA precursors; decrease in acetate: propionate ratio which subsequently increased hepatic oxidation of glucose in the TCA cycle; decreased ruminal degradation of dietary protein which increased amino acid availability for gluconeogenisis or reduced demand for free fatty acids mobilization as a

result of increased glucose availability (Stephenson et al., 1997). The concentration of BHBA is consistently decreased by administration of monensin to dairy cows (Duffield, 2000); Ipharraguerre et al. (2003) reported an average decrease of 23% in a summary of 14 studies. In our study the average decrease in BHBA was 22% for the three treatments when compared to the control. Clayton et al. (1999) reported no effect of virginiamycin on blood BHBA, however, those cows were beyond the stage of lactation when ketosis is problematic.

High producing cows meet their energy requirements by mobilizing adipose tissue, thereby releasing NEFA into the blood. Non-esterified fatty acids may be oxidized, re-esterified or metabolized to ketone bodies. During times of severe negative energy balance ketone body formation and re-esterification of NEFA occurs at an increasing rate. Ketogenesis by the liver leads to elevated blood concentrations of BHBA, acetoacetate and acetone. Excessive ketogenesis often leads to an increased incidence of ketosis, mainly because of reduced feed intake. Blood concentrations of NEFA, BHBA and acetoacetate have been used as indicators of negative energy balance and severity of ketosis (Duffield et al., 1998; Green et al., 1999; McGuffey et al., 2001).

Subclinical ketosis causes economic losses through decreased milk production and an increased incidence of periparturient diseases. Results from various studies indicated a worldwide prevalence of 8.9 to 34% for sub-clinical ketosis in cows during early lactation and 2 to 15% for clinical ketosis (Duffield, 2000). Studies using BHBA for assessing sub-clinical ketosis range from 1000 μmol /L to 1400 μmol /L for defining a sub-clinical threshold and 2400 μmol /L for defining a clinical ketosis threshold (Duffield, 2000). Oetzel (2003) recommends the cut-point for sub-clinical ketosis to be 1400 μmol /L. Furthermore, Duffield (1997) reported a serum concentration of > 1400 μmol /L for BHBA during the first two weeks postpartum to cause a threefold greater risk for cows to develop either displaced abomasum or clinical ketosis. In our study both treatments V and M as well as the combination treatment reduced plasma BHBA below the threshold of 1400 μmol/L compared to the mean BHBA of 1586 μmol /L for treatment C (Fig. 8). It could be concluded, therefore, that both virginiamycin and monensin has potential to decrease the incidence of clinical and subclinical ketosis. This is in agreement with other studies where monensin reduced the incidence of subclinical ketosis (Sauer et al., 1989; Duffield et al., 1998; Green et al., 1999).
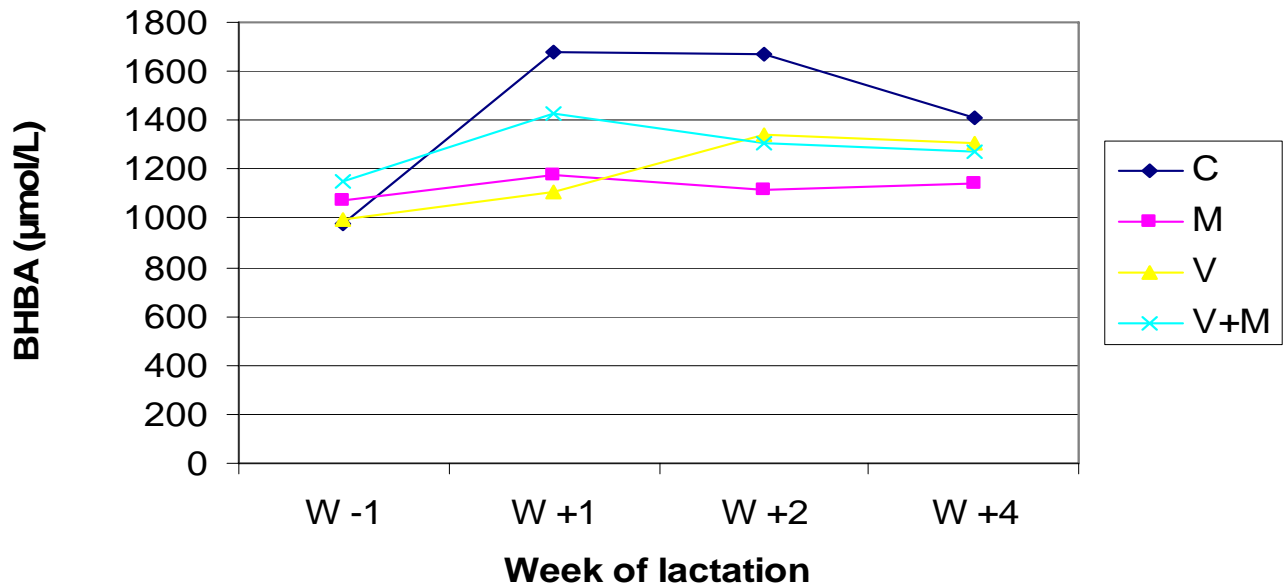
Fig. 8. Change in blood BHBA concentration (µmol/L) over time for cows receiving no additives (C), monensin (M), Virginiamycin (V), and their combination (V+M).

Mean concentration of plasma NEFA ranged from 0.48 mmol /L for cows supplemented with monensin to 0.63 mmol /L for control cows and did not differ between treatments (P > 0.10). Signs of clinical ketosis include loss of BW, fatty liver, decreased milk production and increased NEFA in blood, but cows do not exhibit all of these signs during subclinical ketosis (McGuffey et al., 2001). Prepartum administration of monensin have reduced prepartum NEFA values indicating improved energy status and reduced fat mobilization (Stephenson et al., 1997; Duffield et al., 2003). Despite changes in BHBA that improved energy status the results from the latter two studies indicated that monensin did not reduce NEFA postpartum. This is in agreement with results from the current study and from the review by Ipharraguerre et al. (2003).

The NEFA test is an indicator of negative energy balance (with subsequent risk for fatty liver, ketosis, retained placenta, displaced abomasums) primarily for prepartum transition cows. Negative energy balance is expected in early lactating cows, making the NEFA test more difficult to interpret and is therefore not typically evaluated after calving. The best application of NEFA testing is a secondary test in a herd with a high incidence of subclinical ketosis; the test helps determine whether the postpartum ketosis is due to pre-calving negative energy balance and fatty liver (Oetzel, 2003) In our study we did

analyze for NEFA postpartum since there is data available on monensin supplementation but none on the effect of postpartum supplementation of virginiamycin on NEFA concentration in blood. There is no published threshold value for plasma NEFA for postpartum cows, however, Nafikov et al. (2006), proposed that when postpartum cows have NEFA levels not exceeding 0.4 mM, the likelihood of developing fatty liver by these cows would be minimal. Although the mean NEFA value in our study for all treatments was slightly higher at 0.56 mM, the average for week 4 postpartum was only 0.45 mM.

CHAPTER 5

CONCLUSION

A major challenge to the dairy cattle nutritionist today is to fully utilise the genetic potential of cows by feeding high concentrate diets, without compromising efficient rumen function and fermentation. Feed additives and rumen modifiers such as yeast cultures, probiotics, ionophores, buffers and enzymes play an increasingly important role in helping to prevent metabolic disturbances such as acidosis and ketosis which most often occur during the transition period from 3 weeks prepartum to 3 weeks postpartum.

Ionophores have been used for many years in beef cattle diets and lately also in dairy cattle diets. Many of the benefits of ionophores are due to improved energy status through increased propionic acid production and a reduction in methane production. This results in less body fat mobilisation, lower incidence of ketosis and improved milk production efficiency. Virginiamycin (V), a non-ionophore antibiotic has shown growth promoting effects in ruminants through improved feed efficiency and decreased liver abscesses and acidosis in feedlot cattle. Although virginiamycin and monensin (M) affect gram-positive bacteria in a similar manner, very little is known on the effect of virginiamycin in dairy cattle, especially when fed in combination with monensin.

The forty experimental cows were fed either a control diet, control plus 15 ppm of monensin, and control plus 20 ppm of virginiamycin or a combination of monensin and virginiamycin. Although monensin suppressed DMI during week 1 postpartum there were no differences in average DMI (P>0.10). Energy corrected milk production, however, was increased by supplementing V+M compared to supplementation with only M or V. The change in body weight for cows supplemented with V+M tended to be lower when compared to cows receiving the control diet. Furthermore, both treatments M and V respectively, decreased blood BHBA and treatment M increased blood glucose compared to the control diet, thereby potentially decreasing the incidence of subclinical ketosis.

Results from this study suggest a complementary effect between the two additives monensin and virginiamycin. It is possible that combined positive effects of the two feed additives on stabilising feed intake and rumen fermentation and increasing feed efficiency, together with the potential post-ruminal

effect of virginiamycin could have contributed to increased production. It has been reported that virginiamycin contributes to improved growth in monogastrics through altering metabolic activities of intestinal microflora thereby increasing quantities of metabolisable energy (ME) and metabolisable protein (MP) available to the host and may also influence permeability of intestinal mucosa to enhance nutrient absorption in the gut. If virginiamycin causes effects in the gastro-intestinal tract of ruminants similar to those reported in research with monogastrics, then a combination of the two additives might enhance properties of both additives. This hypothesis is supported by the results from this study where ECM production from cows supplemented with V+M (43.3 kg/d) was increased compared to cows supplemented with only V (37.9 kg/d, P=0.07) or M (36.9 kg/d, P=0.02). Additional research on the post ruminal effects of virginiamycin should help improve our understanding of the potential complementary effects between virginiamycin and monensin.

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