CHAPTER II

BLACKGROUND INFORMATIONS

The pharmacology of monensin

Monensin, the carboxylic polyether ionophore is produced by *Streptomyces cinnanmonensis* (Haney and Hoehn, 1967). This substrate is used in the animals as a growth promoter, antiprotozoa, antiketogenic and antibloat agent. The mode of action of monensin is the direct growth inhibiting effect on some microorganisms such as bacteria and protozoa, which play the role as normal and abnormal microorganisms in the hosts. The new balance of microorganisms in the host after monensin treatment has been approved to be more profitable for the animal health and production.

In ruminants, the mode of action of monensin in rumen is the direct inhibition on acetate, butyrate, formate and H^+ producing gram positive bacteria resulting in the predominance of the succinate producing bacteria, which is the precursor of propionate source in the ruminal environment (Schelling, 1984). Monensin, like other ionophores can form the ionic channels on the biological membrane. This monensin cationic-H^+ antiporter channel is produced by the anionic form of the ionophore confined to the membrane interface. Sodium, potassium and lithium ions are cations, which exchange one by one with the hydrogen ion. The ionic channels formed on the microorganism membrane would affect bacterial energy derivation, the proton motive force (PMF) and inhibiting their growth rate (Bergan and Bates, 1984).

For the ruminal bacteria, sodium and potassium are the major cationics inside the cell having the concentration gradient of 70 folds for potassium ion and 2.5 fold

for sodium ion. Monensin has approximately ten times the affinity for the sodium ion as for the potassium ion. One might have expected a greater efflux of sodium ion than that of the potassium ion, but it is the potassium ion efflux that leads to the accumulation of hydrogen ion and decrease intracellular pH (Rusefi, 1987).

From its mode of action, monensin can affect ruminal fermentation pattern which can be consolidated into six categories: 1) The modification of the volatile fatty acid (VFA) proportion which is widely recognized of the great important. 2) The consequence modification of feed intake, which should be considered important. 3) The change in gas production from rumen during fermentation. 4) The modified digestibities. 5) The change in protein utilization and 6) The modified rumen filling and rate of passage (Schelling, 1984).

The studies of the pharmacokinetics of monensin indicate that orally administrated monensin is absorbed, extensively metabolized, excreted in the bile and eliminated in the feces. Bile from monensin treated cows contain very little monensin, but relatively large quantities of monensin metabolites have been found. Experiment steers have been shown no detectable plasma monensin when given monensin at a dose 30 mg/kg body weight orally while at the dose of 60 mg/kg body weight, intraruminally, the plasma monensin concentration would be below 0.02 ppm eventhough all animals died from the overdosing (Donoho, 1984). Using an assay with the sensitivity of less than 2.5 ng/ml, no monensin residues can be detected in the serial samples of milk from the cow treated by the intraruminal-slow releasing preparation of monensin at the dose of 320 mg/cow/day (Lowe et al., 1990).

The main and additional indications of monensin

Changing of the ruminal fermentation patterns in monensin treated animals shifted the effects of monensin into its systemic mode of actions. The greatest role of monensin is as an additive for feedlot cattle to improve feed efficiency when included in many diet rations. Monensin has also been shown to aid in the control of acidosis and bloat caused by high grain diet. In the pasture, monensin improves daily gain in beef cows (Schelling, 1984). Monensin can reduce the incidence and the severity of bloat in grazing dairy and beef cattle(Lowe et al., 1990).

In dairy cows, monensin has been used in replacement heifer to improve feed efficiency and increase growth rate. These effects will reduce the cost of growing heifers. Monensin can be used to lessen the postpartum ketonemia (Sauer et al., 1989). Due to the improvement in feed efficiency, the lower ratio of acetate to propionate and the protein sparing effect, monensin also has the positive effect on milk production in both dairy cow and goat. This effect is more apparent in pastured cows (Lowe et al., 1990; Lean et al., 1994; Hayes et al., 1996) than in feedlot cows (Baile et al., 1982; Sauer et al., 1989; Abe et al., 1994).

Monensin relating to mammary function

For the latter background information, it is important to review the role of monensin in the area that how the mammary gland can respond to monensin treatment in 4 areas: First, the interaction of monensin with normal ruminal fermentation patterns and with the dietary types. Second, the nutrients and hormones in the circulation after monensin treatment. Third, the stage of lactation and the response to

the monensin. Finally, the potential role of monensin supplementation contributing milk production.

Normal ruminal fermentation patterns, dietary types and their interactions with monensin

The role of rumen on dietary utilization and the synthesis of essential nutrients are well known. Ruminal digestion is a complex process that involves dynamics interaction among diet, microbial population and host. Unlike the enzymatic digestion in small intestine, microbial fermentation is the primary digestion process in rumen. The end productions of fermentation that have nutritional value for the host are the VFAs and the microbial cells. The VFAs are mostly absorbed from the rumen, while the microbial cells, which contain half of the protein available to their host, are digested in the abomasum and absorbed in the small intestine and provide the majority of the host's supply of amino acids.

The microbial nitrogen supplies sufficient essential amino acids for their host. The main substrates for microbial nitrogen synthesis are simple carbon compounds and dietary or endogenous non-protein nitrogen (NPN). High degradable protein diet is partly deaminated to form ammonia. Excess ammonia is absorbed, converted to urea and subsequently excreted in the urine or recycled. The other protein source is undegradable dietary protein which flows along with microbial protein to the abomasum and the small intestine. The nutritional value of the latter protein source depends on its spectrum of essential amino acids and the intestinal digestibility (Nolan, 1993) (Fig 1).
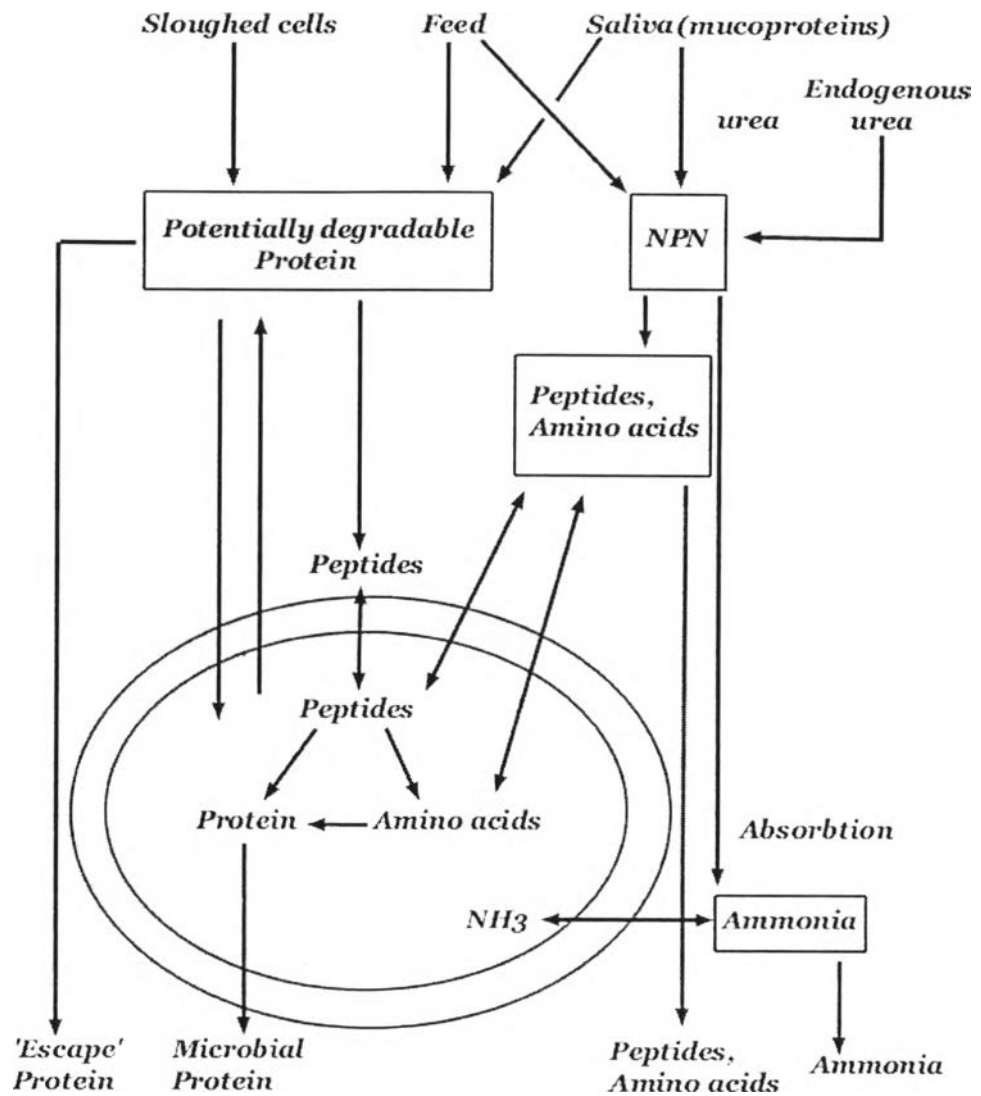
Figure 1. A model of nitrogen transactions in the rumen. The ovals delineate the microbial (Nolan , 1993)

The end products of dietary carbohydrate, structural and non-structural are VFAs. The principle VFAs are acetate, propionate and butyrate. As far as the microbes are concerned, VFAs are the waste products, but to their host, they represent the major source of absorbed energy. The majority of the VFAs produced in the rumen are lost by absorption across the rumen wall. The concentration of VFAs in the rumen at any given time reflects the balance between the rate of production and the rate of absorption. The concentration of total VFAs is usually between 60 and 150 mM. The concentration is highly variable, depended on both dietary composition and

the time of feeding. According to Whitelaw (1970), maximal concentration usually occurs 2-4 hours after each meal, the individual VFA concentration proportion is determined by the microbial population which intern affected by the basal diet. High-fiber diet rations encourage the growth of acetate-producing bacterial species and the acetate:propionate:butyrate molar proportion would typically be in the region of 70:20:10, whereas starch-rich concentrate diets favor the development of propionate-producing bacterial species and give those VFAs proportion in the region of 50:40:10.

As mention above, the action of monensin directly inhibits certain microorganism species. It is widely acceptable that cows treated with monensin have lower ratio of acetate to propionate. This change in ratio presumes that propionate is produced at the expense of acetate. Ruminal butyrate concentration is also decreased with monensin (Schelling, 1984). The interaction of dietary types with the actions of monensin results in the optimum monensin action. The addition of monensin increases the percentage of propionate and decreases that of acetate. The effects are more pronounced with the low forage diet than with the high forage diet (Richardson, 1976; Zinn et al., 1994; Ramanzin, 1997).

In the pathway of nitrogen transaction, monensin supplementation affects microbial growth and its proteolytic and deamination activities. Microbial growth effect of monensin depends on the microbial adaptibility. It is clear from the previous study that monensin depresses microbial protein flows and increases more dietary protein to the lower gut (Poos et al., 1979). The study on the effects of monensin on the microbial growth in sheep shows that the urinary allantoin excretion is not changed from the control group (Dewhurst and webster, 1992). The levels of plasma allantoin, which correspond to ruminal microbe nucleic acid levels or growth, are correlated to the excretion of allantoin in milk and urine (Giesecke et al., 1994). There

are no interaction between dietary types and urinary allantoin excretion. By reducing the ruminal degradation of dietary protein, rumimal ammonia and bacterial nitrogen reaching the abomasum, monensin increases dietary protein reaching the abomasum (Schelling, 1984).

The other ruminal modes of action of monensin are the reduction of lactic acid and methane productions. Monensin can inhibit most lactate-producing bacteria which rapidly grows when the diet is abruptly changed from forage to concentrate. The total methane emission, from cellulytic-methanogenic microorganisms should be reduce at least 6% when given the cows monensin (KirchgeBner, 1995). Monensin can also reduced the incidence of bloat caused by both grazing legume pasture and feeding high grain diet (Lowe et al., 1990).

Nutrients, hormones determining milk synthesis and their interactions with monensin

Milk production of the individual cow depends on two major factors. Nutrient supply of the gland is the first one. The latter is the capacities and the population of active mammary cells which depend on the stage of lactation and the hormonal status. Changing these two factors in each stage of lactation may affect the synthetic activity of the mammary cell.

Lactose is proposed to be the major milk composition determinants of milk volume due to its osmotic effect. Because glucose is accounted for 60-85% of lactose yield (Chaiyabutr et al., 1980) and the rate of lactose synthesis with a Km of 1.5 mM exceeds the glucose concentration within the cells (Kuhn et al., 1980), it is the glucose avaibility that plays an important role on the lactose synthetic activity. Study on the

relationship between milk yield and plasma glucose shows the positive correlation between physiological plasma glucose concentration, approximately 3.0 mM and milk yield (Kronfeld, 1982). The abomasum glucose infusion, which increases plasma glucose concentration from 3.66 mM to 3.9 mM also, increases milk yield (Frobish and Davis, 1976). Since the presence of steep gradient of glucose across the plasma membrane and the occurrence of different types of the glucose transporter on the mammary cells , especially the glucose transporter type one (GLUT1) which its activity does not depend on the concentration of insulin (Zhao et al., 1993; Zhao et al., 1996), glucose availability may be the main physiological process which determines the synthetic activity of lactose and milk production.

Successful lactation requires the mammary cells to develop and maintain the biochemical and cytological machineries to synthesize and secret milk. The processes are accomplished by the complex interaction among many hormones and the mammary cell differentiation patterns.

For dairy cows, prolactin is necessary for the process of lactogenesis, but at the stage of maintenance milk production (galactopoiesis), growth hormone (GH) provides a homeorhetic control that shifts the partitioning of nutrients in a lactating cow. With bovine somatotropin (Bst), glucose production by the liver increases and its oxidation by body tissues decreases (Schams, 1995). It has been demonstrated that the galactopoietic effect of GH involves an increase in the synthetic capacity of the mammary gland (Nielsen and Riis, 1993).

Addition of monensin does not change the levels of both GH and insulin (Duff. et al., 1994). The concentration of glucose is not consistently increased (Abe et al.. 1994; Hayes et al., 1996; Ramanzin et al., 1997). Studies of different diets, from

high and low rations of concentrate to forage with monensin on the nutrients in the circulation show that the portal flux of glucose tends to be greater with monensin whereas there are indifferences in the hepatic flux and systemic concentration of glucose (Harmon and Avery, 1989; Harmon et al., 1993).

Along with these data, the increased production of propionate with monensin feeding which spares gut glucose utilization, tends to increase the concentration of glucose in the portal circulation, however, the concentration of glucose in the systemic circulation is not constantly affected.

The stage of lactation and the response to monensin

The lactation cycle of dairy cow begins with a period of mammary development, which is followed by lactogenesis. Milk synthesis and secretion increase to a peak production and are maintained in the period of galactopoiesis until the time of involution occurs.

Secretory cell proliferation occurs mainly during pregnancy and continues during the first few weeks postpartum, but cell numbers do not increase significantly after the third week of lactation. Mammary cell differentiation occurs after parturition. At the time of peak milk yield, the gland normally contains a maximum number of highly differentiated mammocytes.

By measuring the activities of cell differentiated marker enzymes; acetyl-CoA carboxylase, fatty acid synthetase and galactosyltransferase, it is found that the cellular activities continue to increase even when the milk yield begins to decline (Wilde and Knight, 1989).

It has been shown that the rate of DNA synthesis, as measured by the incorporation of [^{3}H] thymidine into the explants, remains low throughout the declining phase of lactation. This may be due to the balance between the rate of decreasing in the existing cells and the replacement of new population of the differentiated ones. In the late stage of lactation, the declining in milk yield is associated with a decrease in the total DNA content of the mammary parenchyma. The cellular apoptosis and the enzymes of the plasmin-plasminogen system are involved in this stage of lactation (Hurley, 1989; Politis, et al., 1987).

The experiments on dairy cows and monensin supplement are mostly conducted in the early stage of lactation. With the use of the slow-releasing monensin preparation, which has one hundred days of action, the treated cows are supplemented with monensin from the early to the middle lactation stages. During these periods of lactation, monensin can increase feed efficiency. The total milk yield is increased in pastured cows (Lowe et al., 1990.; Lean et al., 1994; Hayes et al., 1996) but not in feedlot cows(Baile et al., 1982.; Sauer et al., 1989; Abe et al., 1994). There has been no experiment studying the effects of monensin on milk production in the late stage of lactation.

The potential role of monensin contributing to milk production

It is well acceptable that monensin treatment has the positive effect on feed efficiency and increases milk volume per feed intake. The potential of monensin to increase total milk production or daily milk volume depends on its ability to increase glucose availability to the mammary gland. Increasing plasma glucose concentration is the intermediary process in the action of monensin. The plasma glucose depends not

only from the production of ruminal propionate resulted from the ruminal action of monensin but also depends on dietary type and the balance between the production and the utilization of glucose from many organs.

In the early stage and the middle stage of lactation, mammary gland is the first priority organ for utilizing glucose, the fractional extraction of glucose of the mammary gland is 25-30% (Laarveld et al., 1981; Laarveld et al., 1985) depending on the stage of gestation and milk yield. The response of mammary glands by increasing milk synthesis is due to the peak increase of their activities and cell numbers. Using monensin in these periods will certainly have the positive effect on milk production.

Because of the declining in the active mammary cell population in the late stage of lactation, the glucose utilization is changed. Using monensin supplementation in order to increase feed efficiency and milk production in the late stage of lactation has not been experimented. Since the mammary cell activity is also maintained through the late stage of lactation, monensin supplementation may have its effects on milk production similarity to those during the early and middle stage of lactation.