EFFECT OF MONENSIN SUPPLEMENTATION ON RUMINAL AND POSTRUMINAL DIGESTION IN SHEEP AND ON ADAPTATION OF RUMINAL MICROBES

by

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(ABSTRACT)

Three experiments were conducted to examine the effects of an ionophore, monensin sodium, on digestion in sheep. The first experiment was concerning alterations induced by long-term supplementation and subsequent withdrawal of the ionophore. The diet was a pelleted mixture of 43% native prairie hay, 34% corn grain and 21% lupin grain plus 100 g wheat straw. Monensin (33 ppm) was added to the diet of four wethers, and four other animals served as controls and consumed an identical diet without monensin. Monensin supplementation increased ($P < .05$) ruminal propionate while decreasing ($P < .05$) acetate levels throughout the 146-d experiment. Withdrawal caused acetate to return to control levels, but decreased ($P < .05$) propionate. During ionophore supplementation, the digestibilities of organic matter (OM) and dry matter (DM) were increased ($P < .05$) by an average of 8 and 9%, respectively. Apparent digestibility of N was increased from 75 to 77% after 19 d of supplementation. There was an augmentation ($P < .05$) in the flow of bacterial N at the duodenum

after 96 d of supplementation. This effect disappeared with ionophore withdrawal.

In a second experiment, the effect of monensin on postruminal digestion was examined in three trials with six wethers. There were three treatments: control, dietary monensin and monensin infused into the duodenum. The diets were the same as in the previous experiment. Dietary monensin caused the same changes in ruminal VFA as in the previous experiment. Infused monensin had no effect on VFA. Dietary monensin increased ($P < .05$) trypsin activity at the ileum. Dietary ionophore did not alter nutrient digestibilities, but shifted ($P < .05$) the site of OM and DM digestion from the cecum to before the terminal ileum by 14 and 10%, respectively.

In an in vitro experiment rumen contents from monensin-adapted and nonadapted sheep were compared with and without additional ionophore in a 6 h incubation system. Rumen contents from adapted sheep did not differ from contents of nonadapted sheep in the quantity of microbial N synthesized. With monensin addition to the incubation, microbial synthesis dropped by 49% in nonadapted microorganisms. In adapted contents the decrease in synthesis was only 9%. Monensin-adapted microbes degraded ($P < .01$) more protein substrate than those which were not adapted.

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CHAPTER I

INTRODUCTION

Ionophores are compounds used in the livestock industry as growth promotants and(or) coccidiostats. The most commonly used ionophore is monensin-sodium, marketed under the tradename of Rumensin (Rumsey, 1984). Monensin has been in use in agriculture for over 10 yr but its mode of action in ruminants is still unknown. Several theories have been proposed to explain the modifications in rumen function but none completely account for all the phenomena seen with ionophore supplementation (Schelling, 1984). In addition to research concerned with elucidating the mode of action of these additives, there is interest in determining the duration of their effects on fermentation.

Adaptation is a common event in ruminant nutrition. Attempts to modify rumen fermentation using chemical agents generally fail after a relatively short time because of bacterial adaptation to the compounds (Hogan and Weston, 1969). One of the intriguing aspects of the ionophores is their apparent ability to alter rumen fermentation and maintain these changes over long periods of time. Another point of interest, presently receiving attention, is whether the changes induced by these compounds are continued after withdrawal from the feed. If withdrawal did not modify animal response, it should be possible to remove the ionophores from the diet before slaughter. This would allow

producers to minimize costs by decreasing expenditures for the compounds, and would also diminish possible inadvertent contamination of the food chain.

Avoparcin, a non-ionophore feed additive, has been shown to increase amino acid N absorption in sheep (MacGregor and Armstrong, 1984). There is little information available in the literature concerning the postruminal effects of the ionophores. Ilan et al. (1981) reported that monensin supplementation increased fecal concentrations of amylase and decreased those of trypsin in young calves.

In the first experiment reported here, animal and microbial adaptation to the ionophore, monensin sodium, was studied. This experiment was designed to examine if the action of monensin diminishes over time. After this long period of supplementation, the effect of ionophore withdrawal was observed. Another study was conducted to examine the possibility that monensin may act postruminally in sheep. Metabolism by rumen microbes from monensin adapted and non-adapted sheep was studied in an in vitro experiment.

CHAPTER II

REVIEW OF LITERATURE

Adaptation

Animal Response. Concerning the whole animal, monensin and the other ionophores generally induce an increase in weight gain and(or) a decrease in feed intake. Embry and Swan (1975), using both high-forage and high-concentrate diets, report that the reduction in feed intake caused by monensin, continued throughout the trials (110 and 106 d for the high-roughage and high-concentrate diets, respectively). Kargaard and Van Niekerk (1980), however, noted that the reduced intake of a corn meal based diet was most severe just after monensin was added to the diet. During the first 4 wk monensin depressed intake 32%, compared to the controls. As the trial progressed, the intake of monensin-fed cattle gradually approached that of the controls until only a 5% depression remained, suggesting an adaptation to monensin, even though intake remained reduced. Davis and Erhart (1976), using a corn-based diet, also found that monensin decreased intake most dramatically during the first 21 d. Intakes then increased to approach those of the controls.

Utley et al. (1978) report that grazing heifers consumed less corn supplement than the controls, but the quantity consumed increased with time, from 38% less to 25%, 21% and 17% less for d 0 to 28, 28 to 56, 56

to 84 and d 84 to 112, respectively. Whether these decreases in intake are due to palatibility problems caused by the inclusion of monensin or alterations in rumen fermentation followed by adaptation or a combination of these two factors is difficult to determine. In all these trials there were no significant differences in weight gain of animals fed monensin and control diets.

Calhoun et al. (1979), working with feedlot lambs, reported that during the first 14 d monensin supplemented lambs gained less than the controls (.136 vs .167 kg/ d, $P < .05$). By d 28 monensin had decreased intake (1.31 kg/d vs 1.43, $P < .05$) but increased feed efficiency (8.0 vs 7.4, $P < .01$). The response to monensin was greatest at the beginning of the 70-d trial for gain and feed efficiency but the response gradually decreased as time on trial increased. The opposite effect, however, was seen with intake, which remained depressed for the entire trial.

Anthony et al. (1975) indicated that in grazing yearling cattle monensin supplementation had no effect on weight gains in a 112-d experiment. Cattle were weighed every 28 d with no significant differences between the means, indicating that time had no effect on the response to monensin. In contrast to these studies where monensin decreased or had no effect on growth, Boling et al. (1977) reported monensin increased growth rate to the largest extent during d 56 to 112 in a 140-d steer grazing trial. Horn et al. (1982) also found the ability of monensin to increase weight gains of steers, wintering on dormant range pasture, changed with time. The ionophore increased gains during the first 61 d ($P < .01$) but had no effect during d 62 to 120 ($P < .05$).

Another ionophore, lasalocid, significantly increased daily gains 14.7% during the first 140 d of a steer grazing trial (Horton et al., 1984). The overall increase in gain for the entire 238 d study was only 11.8%, indicating the effect of lasalocid diminished with time. When lasalocid was fed to fattening bulls in France the effect on feed efficiency was greatest during the first 4 wk of the experiment and decreased with time (Geay et al., unpublished data). In a trial using yearling cattle fed salinomycin, another ionophore, McClure et al. (1980) found that increases in daily gain and feed efficiency were established early in the 126-d study and persisted until the end. In contrast, Nakashima et al. (1982) stated that there was a trend for the feed efficiency of supplemented steers to decrease as time on salinomycin increased.

It is difficult to resolve these conflicting reports on the effects of monensin and the other ionophores on growing and fattening animals. Undoubtedly, different diets, species, and animal ages explain some of the differences. Whether it is palatibility that initially decreases feed intake or the increase in rumen retention time, which generally follows ionophore administration (Schelling, 1984) or another unelucidated mechanism is impossible to determine without further research. Avoparcin, a non-ionophore feed additive, also favorably affects feed efficiency but this effect, like that sometimes seen with the ionophores, tends to diminish with time (Pichler, 1980).

Digestive Parameters. The ionophores are also capable of affecting digestive parameters in ruminants. Poos et al. (1979) reported that after 10 d of monensin supplementation, dry matter (DM) and acid detergent

fiber (ADF) digestibilities were decreased ($P < .05$) in lambs fed a roughage-based diet, but by d 40 these changes had disappeared. However, the monensin x time interactions were not significant and the authors stated it was not possible to conclude there was adaptation to the compound. Monensin had no effect on apparent digestibility of N in either of the two periods. There was no direct or adaptative effects of monensin or lasalocid on N and cellulose digestibilities measured in sacco on d 1, 4, 7, 10, and 13 of the adaptation period to the ionophores (Ricke et al., 1983). In vivo, these workers found no day x treatment interaction for DM digestibility, which indicates there was no adaptation during the 12 d of monensin or lasalocid addition to the feed, in contrast to the results of Poos et al. (1979).

Volatile Fatty Acids. Changes in VFA concentrations are the most utilized indicators of ionophore action on rumen fermentation. Considering short-term adaptation, monensin had no effect on total VFA concentrations but there were significant changes in propionate and acetate levels in a beef steer fed forage (Simpson et al., 1979). During the first 4 d of adaptation, the values for the acetate:propionate ratio fell from 3.37 to 1.87. For d 8 to 11 and 15 to 18, the ratios were 2.29 and 2.51. The authors concluded that during the first few days of monensin administration the concentration of propionate increases with a corresponding decrease in acetate levels. In that study propionate concentrations rose through d 4 but had plateaued by d 8.

Potchoiba et al. (1984) reported that addition of monensin caused a decrease in total VFA concentrations on d 3, 5 and 7 ($P < .01$) in

grazing cows during one trial and on d 3 during a second. In the second trial monensin-fed cows had increased total VFA on d 5 ($P < .01$). During both trials monensin decreased acetate levels ($P < .01$) on d 3, 5, 7, and 9, while propionate concentrations were increased during the same times ($P < .01$), except during trial 2 when monensin addition increased propionate on d 1, also ($P < .01$). The ionophore decreased butyrate concentrations on d 3 and 5 ($P < .01$), but the effect was not apparent on d 7 and 9. Carlson et al. (1983), in a similar experiment, found monensin did not affect total VFA levels from d 3 to d 9 ($P < .05$). Propionate increased ($P < .01$), while acetate and butyrate decreased on all days monensin was administered. Overall, monensin increased propionate concentrations by 9% and decreased those of acetate and butyrate by 5%.

Darden et al. (1984) studied the effects of monensin, lasalocid and avoparcin on rumen fermentation in beef steers fed a corn- based diet. Monensin and lasalocid decreased total VFA ($P < .10$) after 11 d, while avoparcin had no effect. None of the additives, however, affected individual VFA levels, which the authors attributed to the 12 times daily feeding regimen.

Merchen and Berger (1984) reported salinomycin had no effect on total VFA concentrations, but increased propionate, while decreasing acetate and butyrate concentrations. These shifts in VFA levels occurred rapidly and were complete within 4 d after ionophore addition.

It appears that the ionophores establish their alterations in VFA concentrations soon after addition to the diet, but the question of whether

they are capable of maintaining the effects has also generated considerable interest. Poos et al. (1979) reported that monensin increased propionate ($P < .05$) while decreasing acetate ($P < .05$) for the entire 46 d lamb trial. Utley et al. (1976) found no pretreatment differences between control and monensin-fed heifers but after 84 d monensin had reduced acetate levels by 12% and butyrate concentrations by 25% and increased propionate by 60%. The same treatments were continued for an 84 d finishing trial. Monensin-induced changes in VFA levels were stable for 168 d of supplementation. Similar results were reported by Potter et al. (1976). Monensin supplementation increased propionate concentrations and decreased those of acetate and butyrate in cattle fed green chop or in grazing animals. There was no significant effect on total VFA levels. The day x treatment interaction was not significant, indicating that monensin established its effect early in the trials, approximately 112 d in length, and maintained this effect throughout.

Richardson et al. (1976) indicated that monensin had no effect on total VFA but decreased acetate, butyrate, isovalerate and valerate concentrations and increased propionate levels in feedlot cattle. Although individual VFA levels varied with sampling day, the day x treatment interaction was not significant. De Jong and Berschauer (1983) reported no signs of adaptation to monensin during a 42-d trial with sheep fed a mixed diet. They noted that addition of monensin to the diet caused a sudden decrease in the acetate:propionate ratio. Vijchulata et al. (1980) reported that monensin had no effect on total VFA, butyrate or valerate concentrations. The compound increased isovalerate but decreased acetate

levels. There was no time x treatment interaction for any of these VFA. In contrast, the authors indicated there was a significant time x treatment interaction for propionate concentrations. Monensin increased propionate levels to 17.8 moles/100 g on d 63, which dropped to 14.1 moles/100 g on d 130, suggesting ruminal adaptation to the ionophore. Thonney et al. (1981) studied effects of both monensin and lasalocid during a 98-d experiment in growing cattle. Although there was no significant day x treatment interaction, the acetate:propionate ratio tended to increase for both ionophores as the trial progressed. Values for the ratio were 2.9 on day 22, which increased to 3.21 on day 79. The authors suggested possible microbial adaptation.

Spears and Harvey (1984), working with grazing steers fed lasalocid, noted that the compound increased propionate ($P < .05$), decreased butyrate and valerate ($P < .10$) and had no effect on isobutyrate, isovalerate and total VFA concentrations. There were no day x treatment interactions for these VFA. Acetate, however, was significantly affected by sampling time ($P < .05$), as was the acetate:propionate ratio. Lasalocid significantly decreased acetate only on d 28. On d 56 and 112 the ionophore had no effect on acetate levels. The acetate:propionate ratio followed the same pattern, being significantly depressed only on d 28. Hixon et al. (1982) found monensin decreased acetate on d 76 and 310 of monensin supplementation. Perry et al.(1983) reported that in growing steers monensin diminished total VFA on d 56 but had no effect on d 140. The compound also increased propionate on d 56 but this effect had also disappeared by d 140. Monensin did not affect acetate or

butyrate concentrations throughout the trial, in contrast to much of the literature.

Nakashima et al. (1982) reported that after 28 d of salinomycin supplementation in steers, there were significant decreases in acetate ($P < .05$) and butyrate concentrations. Propionate levels were increased significantly. At 140 d the effect on acetate was more pronounced (54.9 vs 65.6 moles/ 100 moles) but there was less of an effect on butyrate. Propionate concentrations continued to be elevated. After 280 d, salinomycin no longer had any effect on acetate or butyrate levels, and the effect on propionate was also diminished ($P < .05$). These results suggest that there is microbial adaptation to salinomycin which could diminish its effectiveness as a feed additive.

Other Ruminal Parameters. The ionophores also alter other rumen fermentation characteristics but in a less conclusive manner than the VFA. Results in the literature are often conflicting, depending on the ionophore, species and diet. Carlson et al. (1983) observed that monensin decreased ruminal NH_3 levels in grazing cows on d 4, 7 and 9, but did not affect rumen pH. Poos et al. (1979) also reported that the pH did not change with monensin supplementation on d 11 and 40 in a lamb feeding trial. Monensin significantly depressed rumen NH_3 the entire length of the trial. Similar results were reported by Perry et al. (1983) for rumen NH_3 on d 56 and 140 in a steer trial. There was no significant change in NH_3 depression with time. In contrast, the ionophore, lasalocid, had no effect on rumen NH_3 in a 126 d steer trial (Spears and Harvey, 1984). Salinomycin had no effect on rumen pH but reduced NH_3

levels ($P < .05$) only in the middle of a 280 d steer trial (Nakashima et al., 1982). There were no significant differences in NH_3 between the controls and ionophore-fed steers at the beginning or end of the experiment. Bartley et al. (1983) reported lasalocid sucessfully prevented grain bloat for 60 d, with no apparent microbial adaptation to the compound.

Blood Parameters. No significant monensin x time interaction was noted for plasma urea levels by Poos et al. (1979), but the compound significantly increased urea levels. Potter and coworkers (1976) also reported monensin increased blood urea nitrogen (BUN) ($P < .05$), but there was no effect of time. Similar results were reported by Perry et al. (1983). Lasalocid (Spears and Harvey, 1984) and salinomycin (Nakashima et al., 1982) had no effect on plasma urea nitrogen.

The results are less conclusive for plasma glucose values. Potter et al. (1976) reported monensin increased glucose levels ($P < .05$) and tended to increase plasma insulin throughout a 112-d trial. In contrast, Perry et al. (1983) reported that monensin significantly increased blood glucose on d 56 but had no effect on d 140 which follows the results these workers found for ruminal propionate. Spears and Harvey (1984) reported that lasalocid decreased plasma glucose on d 28 and 56 but had no effect on d 112. There were no significant changes in the concentration of propionate with time in this trial. Nakashima et al. (1982) observed that salinomycin had no effect on any of the measured blood parameters over a 280-d period.

Microbial Populations. The changes induced in rumen fermentation by the ionophores are probally due to several factors, including changes

in microbial populations. Addition of 30 µg monensin/ g to in vitro cultures of rumen bacteria caused the proportion of resistant bacteria to rise from 5 to 15 % of the total viable count to 60 to 90% (Anonymous, 1981). Concentrations of monensin above this level slightly increased the percentage of resistant bacteria but sensitive strains were never totally eliminated from the cultures. Both cellulolytic ruminococci and lactic acid bacteria, each known to be sensitive to monensin, were able to adequately adapt to the ionophore.

Chen and Wolin (1979) reported that species differences exist for monensin sensitivity. Monensin and lasalocid inhibited gram positive bacteria more than gram negative species. Growth of Ruminococcus flavefaciens, Ruminococcus albus and Bacteroides fibrisolvens were inhibited by 2.5 µg ionophore/ ml. Selenomonas ruminantium (5 strains) exhibited no growth inhibition in the presence of monensin or lasalocid (40 µg/ ml). Bacteroides ruminicola and Bacteroides succinogenes initially did not grow with the ionophores present but growth was detected after 24 h of incubation. Strains of Bacteroides developed resistance to the ionophores.

Herod and coworkers (1979) found that monensin or lasalocid addition to nonadapted rumen cultures decreased microbial protein synthesis, total gas and methane (CH_4) production. Neither ionophore affected total VFA but both increased propionate and decreased acetate levels. With inocula from animals adapted to the ionophores, neither ionophore had an effect on microbial protein synthesis or gas production. Short (1978) observed that addition of monensin (1 mg/ kg) to nonadapted

semi-continous cultures of rumen microorganisms decreased cell yield and cellulose digestion. When the inocula were obtained from animals consuming the ionophore, monensin had no effect on either parameter. De Jong and Berschauer (1983) also worked with adapted and nonadapted microorganisms. The adapted cultures were obtained from sheep fed monensin for 32 d. Monensin decreased ($P < .025$) total VFA production when both types of inocula were grown on cellulose. The drop was most dramatic with the nonadapted cultures. Monensin increased ($P < .05$) NH_3 with nonadapted cultures but decreased ($P < .025$) it when added to adapted fermenters. The authors attributed the increase to an inhibitory effect of monensin on microbial protein synthesis in the unadapted microorganisms. Cellulose digestion was not affected by the ionophore addition to adapted fermenters, but with nonadapted cultures, degradation was severely inhibited.

There are two theories concerning how monensin supplementation increases the relative proportions of propionate to acetate. Both theories concern the inter-species transfer of hydrogen gas (H_2) in the rumen. Monensin has been reported to decrease ruminal CH_4 production (Ushida et al., 1982). Dellinger and Ferry (1984) reported monensin inhibited CH_4 formation when Methanobacterium formicicum was grown on formate but not on H_2/CO_2. Bacteria grown on formate with monensin present produced more H_2 than CH_4. A decrease in H_2 utilization by methanogenic bacteria may allow the accumulation of H_2 in the rumen. An increase in the partial pressure of H_2 may inhibit the enzymatic processes by which certain bacterial species produce both acetate and H_2 (Wolin, 1981). A

drop in ATP formation by these bacteria would render them less competitive and perhaps favor substrate utilization by propionate producing microorganisms. Alternatively, this increase in H_2 could be utilized for production of propionate itself. Henderson (1980) reported Bacteroides ruminicola and Selenomonas ruminantium were able to utilize extracellular H_2 to produce succinate which could then be decarboxylated to propionate.

Dawson and Boling (1983) conducted a study designed to examine the changes in VFA proportions in relationship to the percentage of monensin-resistant bacteria. Monensin supplementation immediately caused an increase in total VFA and a decrease in the acetate:propionate ratio but had no effect on bacterial total viable counts. There were no significant differences in the percentage of resistant bacteria in the controls and monensin-fed heifers until d 8 of monensin supplementation. By d 14, however, monensin-fed animals had significantly more resistant bacteria than the controls. The difference between the two groups peaked on d 24 when monensin-fed heifers had 50% more resistant bacteria. One of the more interesting facts from this study was the lack of a significant correlation between the percentage of resistant bacteria and the observed VFA changes. One possibility is that monensin itself was responsible for at least some of the metabolic changes associated with monensin supplementation.

Dellinger and Ferry (1984) reported that CH_4 production by Methanobacterium formicicum JF-1, a bacterium isolated from sewage sludge, was inhibited when cells were grown on formate. Growth on

formate was also inhibited by the ionophore. However, with bacteria grown on H_2/CO_2 there was a lag period in both growth and methanogenesis and after 4 d monensin addition no longer had any effect. This recovery was not due to the development of resistance, in contrast to the results of Dawson and Boling (1983). Methanobacterium did not inactivate the ionophore.

Mackie et al. (1984) observed that the adaptation of bacteria to monensin was influenced by the sodium ion (Na+) concentration of the growth media. Increasing the Na+ concentration progressively decreased cell yields in comparison to control values when monensin was added to the media. The authors reported that both gram positive and gram negative bacteria were able to adapt to monensin. Dawson and Boling (1984) also reported that adaptation to monensin or lasalocid was influenced by the mineral concentration of the media, in particular by the amount of K. Increasing the quantity of K in the media decreased the sensitivity of bacteria to monensin or lasalocid. The authors suggested that this phenomenon was due to the inability of the ionophore to deplete the intracellular K levels in a high K environment, the suggested mode of action of the ionophores (Harold and Baarda, 1967). In contrast to these results, it has been reported that administration of monensin or lasalocid for 30 d did not establish resistant strains of lactate producing bacteria (Nargarga et al., 1981).

Poos et al. (1979) found that monensin decreased protozoa numbers ($P < .05$) for the entire length of a 46 d lamb trial. Dinius et al. (1976)

reported, however, that monensin had no effect on the protozoal population after 21 d of supplementation.

Withdrawal and its Effects

Short-term. Another factor of interest concerning the ionophores is whether their effects continue and for how long after withdrawal of the compound from the diet. Concerning weight gain in grazing cattle fed a monensin supplement, there was no difference between feeding the supplement daily or on alternate days (Muller et al., 1980). Similar results were reported for lasalocid in cattle grazing tropical pastures with the supplement offered daily or three times/ wk (Horton et al., 1983). Shepard and Calhoun (1983) found that less frequent feeding of monensin tended to increase crude protein digestibility. The maximum response was with alternate day supplementation. Machen et al. (1983) report the frequency of monensin supplementation affected DM digestibility, with most improvement when monensin was fed every day. Potter et al. (1980), studying ruminal VFA, noted that alternate day dosing with monensin provided similar VFA response as daily dosing. Rodriguez et al. (1986) studied the effect of monensin addition on ruminal amino acid (AA) and NH_3 levels in steers fed a diet composed of corn and soybean meal. The source of roughage was cottonseed hulls. After a 14 d adaptation period to monensin, rumen contents were sampled before the morning meal and 1, 2, 3, 4, 6, 8, 10, and 12 h afterwards. In strained contents monensin depressed total NH_3 throughout the entire sampling period. There was

a nonsignificant decrease in the quantities of total AA. In the particulate portion of the rumen contents monensin had no effect on either the levels of NH_3 or total AA throughout the 12 h. The authors concluded that monensin does not decrease the quantity of rumen NH_3 by reducing the rate of AA deamination.

Two and 4 h after feeding, both monensin and lasalocid tended to decrease butyrate and increase propionate concentrations (Ricke et al., 1984). Neither ionophore had an effect on total VFA, acetate or NH_3 levels. By 6 h post-feeding the two ionophores had significantly increased propionate and decreased NH_3 concentrations ($P < .05$). Acetate levels were slightly diminished while total VFA concentrations tended to be increased at this time. Overall, the ionophores tended to diminish acetate, butyrate and NH_3 levels while increasing that of propionate. Quantities of total VFA were unchanged.

Concerning total pool sizes, Ricke et al. (1984) reported that at 2 h post-administration the ionophores decreased acetate and total VFA pools ($P < .06$). At 4 h there was still a tendency for acetate quantities to be depressed but only the acetate:propionate ratio was reduced ($P < .06$) in ionophore-fed animals. At 6 h both ionophores tended to increase the propionate pool size, while lasalocid slightly increased the acetate pool. By 8 h post-feeding the ionophores no longer had an effect on the acetate:propionate ratio but monensin still tended to diminish the quantity of acetate which lasted through 12 h post-feeding. In contrast, lasalocid promoted slight increases in the quantities of both acetate and propionate at 8 h, and by 12 h these effects were significant. Monensin continued

to depress the NH_3 pool until 12 h post-feeding while lasalocid tended to increase it from 4 to 12 h.

Dinius et al. (1976) reported monensin tended to uniformly depress acetate, and increase propionate across all sampling times from 0 to 8 h post-feeding. Monensin had no postprandial effect on percentages of butyrate, isobutyrate, valerate or isovalerate. In steers, fed dry range grass, the monensin-induced depression in acetate was present at 4 h post-feeding, was most severe from 10 to 16 h and then decreased at 22 h (Lemenager et al., 1978). A similar pattern was observed for an increase in propionate. The ionophore had no significant effect on butyrate but significantly decreased total VFA at 10 and 22 h Vijchulata et al. (1980) reported monensin significantly increased propionate concentrations at 28 h post-supplementation. Of the other VFA, only acetate and isovalerate were affected by monensin at that time; both VFA were depressed.

All these results support the concept that the consequences of ionophore supplementation on rumen fermentation last for at least 24 h post-administration. The lack of difference between daily or alternate d ionophore supplementation of cattle also supports this conclusion.

Long-term. Very little information is available concerning the long-term effects of ionophore withdrawal. Three d after monensin withdrawal, acetate and propionate levels were still significantly affected by the ionophore in grazing cows. The effect on total VFA and butyrate concentrations, however, was variable. Both were changed in one trial but not in a second (Potchoiba et al., 1984). Rumen NH_3 also remained

depressed 3 d after monensin withdrawal (Carlson et al., 1983). Bartley et al. (1983) report lasalocid was effective in diminishing grain bloat in cattle for approximately 7 d after its withdrawal. Ushida et al. (1982) found that the monensin induced decreases in total gas, CO_2 and CH_4 productions disappeared 7 d after withdrawal. Dawson and Boling (1982) reported that the depression in the acetate:propionate ratio caused by monensin supplementation had vanished by d 10 of withdrawal. The percentage of monensin-resistant bacteria, however, remained elevated for more than 18 d after withdrawal of the ionophore. The authors concluded that changes in bacterial populations cannot account for all observed alterations in rumen fermentation.

Conclusions. The ionophores appear to quickly establish their effects on animal performance, increasing weight gains and feed efficiency and(or) decreasing feed intake. Depending on the species, diet and animal age, these alterations appear to continue or tend to slowly diminish as time progresses. The effects of the ionophores on digestibility apparently disappear a short time after administration. Alterations in ruminal VFA and NH_3 levels are quickly established and can be maintained for long periods of time. The effect on bacterial populations appears more slowly. Modifications in blood parameters are variable.

Alternate day ionophore supplementation of cattle causes no apparent difference in animal performance. This conclusion is supported by the continuation of ionophore-induced changes in rumen fermentation for at least 24 h after their withdrawal. By d 7 to 10 of withdrawal, fermentation in the rumen has returned to control values. The modifica-

tions in the microbial population are among the last parameters to change after withdrawal.

Postruminal Monensin Effects

Volatile Fatty Acids. Most of the research on the ionophores and related compounds has been concentrated on its effects in the rumen. However, there is some evidence that these feed additives may also exert an influence on postruminal digestion. Korniewicz et al. (1983) reported that the addition of monensin to the diet of fattening lambs had no effect on the length of the small intestine. Raun et al. (1976) report that monensin supplementation of sheep caused a decrease in the cecal concentrations of acetate, butyrate and valerate. There were corresponding increases in the levels of propionate and isovalerate. Yokoyama and coworkers (1985) reported that in steers fed a corn grain/corn silage diet, addition of monensin (250 $mg \cdot head^{-1} \cdot d^{-1}$) caused an increase in cecal pH (6.99 vs 7.18). The proportions of VFA, however, were unaltered in the cecum.

Digestion. Zinn et al. (1980) fed cattle an 80% corn-based diet with monensin at 150 $mg \cdot head^{-1} \cdot d^{-1}$. These authors found that monensin supplementation had no effect on the intestinal digestion of protein or fiber. Tolbert et al. (1979) reported that feeding monensin (33 ppm) to steers on a 70%-concentrate diet, had no effect on the postruminal digestion of OM but increased the postruminal digestibility of N (31.1% for monensin and 20.9% for control). Darden and coworkers (1984), using

steers fed a 60%-concentrate diet, studied the effect of feeding avoparcin, lasalocid and monensin on postruminal digestion. None of the compounds changed the intestinal digestion of DM, organic matter (OM), starch, neutral detergent fiber (NDF) and ADF. Lasalocid, however, decreased the amount of N absorbed postruminally (lasalocid-49, avoparcin-57, monensin-59, control-57 g N absorbed postruminally/d).

Funk et al. (1986) reported that feeding lasalocid shifted the digestion of starch to the small intestine in lambs fed a high-concentrate diet containing 20 ppm lasalocid. The authors suggested this effect could explain the improvement in animal performance seen with ionophore supplementation. Thivend and Jouany (1983) studied the action of lasalocid on digestion in sheep. They reported that lasalocid addition in excess of 21 ppm to a diet based on sugar beet pulp, decreased the postruminal digestion of OM (control-54.8, 21 ppm-49.0, 43 ppm-49.0, 63 ppm-45.9%). Lasalocid, however, did not have an effect on the postruminal digestion of N. Muntifering et al. (1981), working with steers fed 33 ppm monensin in a 90% whole shelled corn diet, reported that monensin supplementation had no effect on OM and starch digested postruminally. Monensin also had no effect on postruminal digestion of crude protein. Monensin decreased the amount of bacterial N in the total N digested postruminally (42 vs 50%). Monensin also increased ($P<.05$) the quantity of feed N escaping ruminal digestion by 8%, which led to its being digested in the intestines. Another ionophore, ICI 139603, was studied in sheep by Rowe and coworkers (1982). This additive had no effect on the postruminal digestion of N.

Goetsch and Owens (1986a) infused two antibiotics, neomycin sulfate and bacitracin into the terminal ileum of four heifers fed a high-concentrate diet. Infusion of the antibiotics tended to decrease digestion of OM and starch in the hindgut and total tract. There was also an increase in the amount of feed N entering the duodenum. In a similar study using a high-forage diet, Goetsch and Owens (1986b) reported that ileal infusion of the two antibiotics tended to increase the flow of N from the rumen while tending to decrease ruminal digestion of OM, ADF and starch.

Intestinal Enzymes. Galitzer et al. (1983) studied the effects of lasalocid and monensin toxicity in cattle. They report that at lethal doses both ionophores affect the secretory pancreas. In cattle dying within 72 h of administration of either ionophore, there were no zymogen granules in pancreatic acinar cells. The authors suggested that at lower doses the compounds may increase the secretion of pancreatic enzymes into the small intestine. Van Hellen et al. (1977) reported that monensin caused an increase in the activity of pancreatic amylase in cattle.

In contrast, Johnson et al. (1986) found that supplementing 22 ppm monensin for 15 d to sheep fed an 80%-corn diet had no effect either on the bile-pancreatic flow or amylase activity. Ilan and coworkers (1981) studied the effects of monensin supplementation (75 mg/ 100 kg body weight) on fecal enyzme activity in young calves. After 15 d monensin had no effect on the activities of amylase, lipase, trypsin or chymotrypsin. On d 30 monensin had caused an increase in amylase activity from 18.4 to 24.4 activity units/ g DM. Lipase levels were not af-

fected, but both trypsin and chymotrypsin activities were depressed ($P<.06$) from 32.5 to 13.7 units/ g DM and from 36.2 to 21 units/ g DM, respectively. Despite the drop in proteolytic enzyme activity, protein digestibility was not affected in this trial. The inconclusive nature of the study by Johnson et al. (1986) could be due to the brief duration of that experiment (15 to 17 d) since Ilan et al. (1981) found no differences until 30 d after ionophore addition.

Using a non-ionophore feed additive, avoparcin, MacGregor and Armstrong (1982) studied its effect on AA uptake in the small intestine. The diet was composed of 70% dried grass and 30% barley alone or with 45 ppm avoparcin. The compound did not affect the flow of AA-N into the small intestine, but its presence did cause an increase in the efficiency of AA-N uptake (control, .597 vs .687 for avoparcin). In another study the same authors infused a solution of 45 mg avoparcin$\cdot$head$^{-1}\cdot$d^{-1} into the rumen or proximal duodenum of four wethers. When the feed additive was infused into the duodenum, the efficiencies of total AA-N, essential AA-N and nonessential AA-N uptakes were increased by 7.6, 10.8 and 9.7%, respectively. If avoparcin was infused into the rumen there were also increases in AA-N uptakes (9.1% total AA, 10.5% essential AA and 11% nonessential AA). The authors concluded that the effect of avoparcin on AA uptake is independent of its mode of action in the rumen (MacGregor and Armstrong, 1984). Parker et al. (1984) studied the effects of supplementing rats with 0, 5, 10, 15, 20, 30, or 60 ppm for 14 d. Avoparcin did not alter the length of the small intestine (overall mean 82 cm). Increasing the concentration of avoparcin in the diet caused a

significant rise in the activity of a mucosal dipeptidase in the small intestine. The authors suggest that if the hydrolysis of dipeptides is the rate limiting step in the uptake of AA-N, then the growth promoting ability of avoparcin could be explained by an increase in the amount of AA-N reaching the animal.

Conclusions. The ionophores have varied effects on postruminal digestion. They appear to retain enough activity after passage to the cecum to alter the VFA produced by cecal bacteria, mimicing changes often seen in the rumen. The ionophores appear to be able to shift the digestion of starch to the small intestine, although how this is accomplished is not clear. It could be due to reduced bacterial activity in the rumen. In other experiments, however, the addition of these compounds has had no effect on starch digestion. The effect of the feed additives on postruminal N digestion is also confused. These conflicting reports can be explained by species differences, the diets used and the age of the animals. Pancreatic enzyme activities may also be subject to alterations caused by the presence of these compounds. Certain authors reported increases in activity while others find no differences. Avoparcin does appear to alter AA-N uptake in the sheep small intestine, but how it does this is still unknown.

One note of caution is necessary before concluding that the ionophores and other feed additives have or do not have an effect postruminally. Except for the experiment by MacGregor and Armstrong (1984) all these trials have involved feeding the experimental compound. Since these additives have already been shown to alter rumen fermentation

and the rate of nutrient passage, the nutrient composition of the flow entering the duodenum will be different in the control and additive-fed animals. Whether the alterations in rumen function caused by the feed additives can account for all the changes seen at the small intestine is difficult to determine. The possible postruminal effects of the ionophores and related compounds are not well known. This field merits further research to elucidate the mechanism of action of the ionophores.

CHAPTER III

JOURNAL ARTICLE I

THE EFFECT OF LONG-TERM MONENSIN SUPPLEMENTATION AND ITS SUBSEQUENT WITHDRAWAL ON DIGESTION IN SHEEP

Summary

The effects of long-term addition of monensin sodium and its subsequent withdrawal were studied in sheep. Monensin was added to the diet for periods of 19 and 96 d. A period to examine the effects of withdrawal was conducted 27 d after the halt of ionophore feeding. Monensin supplementation increased ($P < .05$) dry matter (DM) and organic matter (OM) digestibilities during supplementation. There was a corresponding increase in ruminal OM digestion. All of these effects disappeared with ionophore withdrawal. Monensin had no effect on postruminal digestion of OM. The ionophore decreased total volatile fatty acid (VFA) concentrations after 96 d. Monensin addition decreased acetate and butyrate while increasing propionate levels. After withdrawal, monensin-fed animals had lower ($P < .05$) propionate levels than the controls. In VFA samples taken at weekly intervals monensin established its effects on acetate, propionate and butyrate within 7 d of administration. After withdrawal, these VFA returned to control values in 24 h.

Ninety-six d of supplementation eliminated Enoploplastron protozoa from the rumen. The ionophore increased (P <.05) apparent digestibility of N after 19 d, but this effect had disappeared by d 96. Monensin supplementation and withdrawal had no effect on ruminal alpha-amino N levels. There was, however, an increase in ruminal protease activity (35%) after 19 d of supplementation. Monensin addition and withdrawal had no effect on ruminal deaminase activity. The compound did not alter N flow at the duodenum, but did decrease that of NH_3. After withdrawal, NH_3 flow returned to control levels. The flow of bacterial N was increased after 96 d of supplementation. The ionophore, however, did not affect the efficiency of microbial synthesis. There were no treatment effects on ruminal liquid phase turnover rate or distribution volume of the liquid phase marker. Monensin increased (P <.01) plasma glucose, but depressed plasma insulin levels.
(Key Words: Monensin, Adaptation, Withdrawal, Digestibilities, Sheep.)

Introduction

Monensin is used in cattle feeding programs to improve the efficiency of gain (Goodrich et al., 1984). This type of usage is frequently for long periods. Perry et al. (1983) reported that the effect of monensin supplementation on VFA levels tended to disappear between d 56 and 140 in a steer trial. Little data are available concerning the duration of monensin-induced alterations in ruminants. Poos et al. (1979) studied adaptation to monensin in sheep but this experiment was relatively short

(46 d). These workers reported that 10 d of monensin supplementation caused a decrease in DM and acid detergent fiber (ADF) digestibilities, but there was no effect after 40 d of ionophore feeding. Potchioba et al. (1984) reported that monensin-induced changes in propionate concentrations continued for 3 d after ionophore withdrawal. Dawson and Boling (1982), however, found that the percentage of monensin-resistant bacteria was elevated until 18 d after monensin withdrawal. The depression in the acetate:propionate ratio remained for 10 d. Little consensus exists in the literature concerning the duration of alterations in rumen fermentation after administration of monensin ceases. The purpose of this experiment was to examine the effect of time of administration of monensin and monensin withdrawal on digestion parameters in the ruminant.

Materials and Methods

Eight Texel wethers, equipped with rumen fistulas and simple duodenal cannulae, were assigned to two groups of four sheep each. One group received the monensin treatment while the other served as the control. The sheep were fed twice daily. Water and a salt lick were continously available. The animals were individually housed indoors in 1 x 1.5 m pens.

All dietary components were ground, mixed and pelleted (table 1). The pelleted diet was fed at approximately 45 g/ kg metabolic body weight ($wt^{.75}$). In addition, 100 g of straw were fed daily to aid in maintaining

Table 1. COMPOSITION OF BASAL DIET

Item	Percent[a]
Ingredient composition	
Prairie hay, first cutting, prebloom	43.3
Corn grain	34.4
Lupin grain	20.7
Mineral mix[b]	1.6
Chemical composition	
Organic matter	92.8
Nitrogen	2.3
Composition of mixture A	
Durieux cellulose	76.4
Aluminum sulfate	.76
Cr_2O_3	22.9

[a] Dry basis.

[b] 55.6% $CaHPO_4$, 26.3% NaCl, 1.1% S, 9.2% $MgSO_4$ 7 H_2O, 7.1 Na_2SO_4 10 H_2O, .32% $ZnSO_4$ 7 H_2O, .16% $MnSO_4$ H_2O, .03% $CuSO_4$ 5 H_2O, .15% $FeSO_4$ 7 H_2O, 7 ppm $CoSO_4$ 7 H_2O, 12 ppm $Ca(IO_3)_4$ 6 H_2O, 3.3 ppm Na_2SeO_3 5 H_2O, vitamins (A-130000, D_3-73000, E-3625 IUnits/ kg).

rumen function and rumination. Lupin grain was chosen as the protein source to examine the effects of monensin on very soluble proteins. For the additive-containing diet, monensin sodium[1] was incorporated at the rate of 36 ppm. With the addition of the straw, the level of monensin was approximately 33 ppm, the amount most commonly used. Two more diets were also formulated; duplicates of the monensin and control diets, except for the addition of chromic oxide (Cr_2O_3) on cellulose paper. These diets contained 10 g mixture A/ kg diet (table 1). The Cr_2O_3 was used as the solid phase marker in the digestibility and digesta turnover sections of the experiment.

The experiment, which lasted 240 d, consisted of four experimental periods (figure 1). The first period served to establish control values for the animals, during which all sheep were fed the control diet. At the end of this sampling period, the sheep in treatment 1 were transfered to the monensin-containing diet and those in treatment 2 continued to receive the control diet throughout the experiment to account for normal variations in digestion parameters. The second period started 19 d after starting monensin supplementation. This period was to measure any short-term changes in digestion caused by the ionophore. The third period started after 96 d of monensin addition and was used to determine if the ionophore was capable of maintaining its effect on digestion or if ruminal adaptation to the compound occurred. Afterwards (period 4), sheep in treatment 1 were returned to the control diet to examine the

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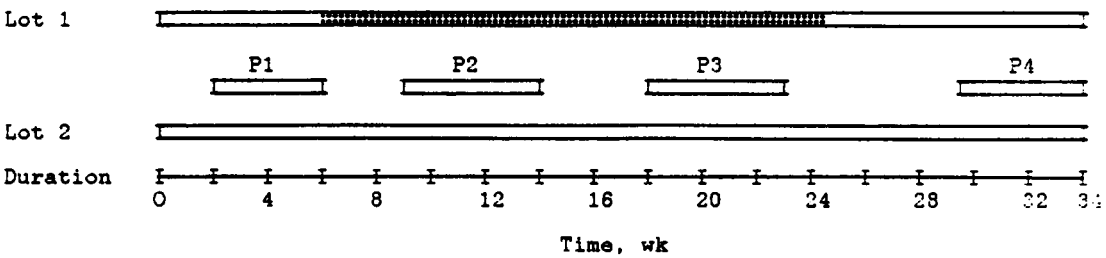
[1] Rumensin, Eli Lilly-France, St-Cloud, France.



Control diet

Monensin diet

P1, P2, P3, P4 : Experimental periods 1, 2, 3 and 4

Figure 1. Experimental design for experiment 1.

effect of monensin withdrawal after 146 d of ionophore supplementation. The fourth sampling period was 27 d after withdrawal. Sheep were adapted to all dietary changes during 48 h.

Five days before sampling began, all animals were switched to the respective Cr_2O_3 containing diets, and an infusion of a 10% (w/v) polyethylene glycol (PEG) 4000 solution into the rumen was started. A 250 ml priming dose of a 20% (w/v) PEG solution was administered at the beginning of the adaptation period to shorten the amount of time required to reach ruminal steady state conditions for the PEG infusion. Overall digestibilities were determined from feces obtained during an 8-d total collection period which followed the 5 d of adaptation. All samples were stored at -20 C unless otherwise noted.

Duodenal contents were then sampled for the next 48 h for the determination of intestinal flows, using the dual marker system (Faichney, 1975). Digesta was sampled every 4 h with a 2 h lag time between days so that a sample was obtained for each 2-h period. The samples were pooled for each sheep and stored at -20 C until analyzed. Polyethylene glycol and Cr_2O_3 were used to mark the liquid and solid phases, respectively. After the conclusion of duodenal sampling, the administration of both PEG and Cr_2O_3 was stopped to determine rumen retention times for both the liquid and solid phases. Samples were taken from the duodenum at 0, 2, 4, 6, 12, 24, and 24 h after the end of marker administration.

Protozoa numbers were determined for 3 d in ruminal digesta samples taken 1.5 h after the morning feeding. Five milliliters of rumen

contents were treated with 7 to 10 drops of 30% (w/v) formaldehyde and stored at 4 C until counted, using a binocular microscope. Samples of ruminal fluid were obtained 1.5 and 3 h after feeding for the determination of alpha- amino N. The samples were centrifuged at 35,000 x g for 30 min. The supernant was then acidified with concentrated H_2SO_4 and stored at -20 C (Whetstone et al., 1981). Rumen contents were sampled before eating and every 1.5 h afterwards for 7.5 h on two consective days. Each 450 ml sample was filtered through a 1 mm metal mesh and pooled by animal. The samples were preserved with 30% (w/v) formaldehyde and stored at 4 C. A portion of this filtrate was conserved for NH_3 and VFA determinations. For NH_3, 1 ml of filtrate was diluted with 4 ml of 12.5% (w/v) NaCl solution. Samples for VFA determination (9 ml) were preserved with 1 ml of a solution of 1% (w/w) mercuric chloride and 5% (v/v) orthophosphoric acid (Jouany, 1982). The remainder of the filtrate was used for the isolation of rumen bacteria by differential centrifugation (Jouany and Thivend, 1972). Rumen samples were obtained once per week for VFA determinations to monitor any changes in fermentation induced by monensin or microbial adaptation to the ionophore.

Ruminal cellulolytic activity was examined by an in sacco method using straw as the substrate (Jouany and Senaud, 1982). The activity of bacterial deaminases was determined in vitro using 1 ml of filtered (1 mm mesh) rumen contents (Erfle et al., 1982).

Ruminal protein degradation was investigated in two manners. In situ proteolysis was examined using the in sacco method of Mehrez and

Orskov (1977). Approximately 3 g of ground lupin grain (3 mm screen) were incubated for 2, 4, 6, 12, and 24 h in 5 x 10 cm bags (internal dimensions). The bag pore size was 100 µm. This pore size was chosen after a preliminary experiment comparing 50 and 100 µm pore sizes. An in vitro analysis of protease activity in total rumen contents was also performed (Brock et al., 1982).

Blood samples were taken by jugular puncture for 5 d, once before eating and 2 and 6 h after the morning meal. The plasma samples were obtained using sodium heparin Venoject[2] tubes. These samples were taken during the second, third and fourth periods.

Percent dry matter was determined by heating the samples for 48 h at 80 C. The quantity of mineral matter was determined by dry ashing at 500 C for 5 h. Nitrogen content was determined on fresh samples using the Kjeldahl method. The method of Michel (1971) was used for the analysis of NH_3. Volatile fatty acids were determined by gas chromatography (Jouany, 1982). Total alpha-amino N was determined by the method of Moore and Stein (1954) with the modifications suggested by Whetstone et al. (1981). The method of Thivend et al. (1965) was used to determine starch content of the samples.

Diaminopimelic acid (DAPA) and purine bases were used as microbial markers. The quantities of DAPA in the duodenal and isolated bacterial samples were determined by ion-exchange chromatography. The quantity of purine bases in the samples was determined by high pressure liquid

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[2] Terumo Company

chromatography (HPLC) (Ushida et al., 1985). Chromic oxide was determined using an automated analysis chain (Mathieson, 1970), and PEG was analyzed by the method of Hyden (1955). Glucose and insulin were determined in plasma samples. Glucose was analyzed using glucose oxidase in an automated-analysis chain (Michel, 1971). Insulin was determined with a SB-INSI-1-M radioimmunoassay kit, using ovine insulin as the standard.

The statistical analysis was performed using methods of SAS (1985). The experiment was a split-plot design with the measurements repeated in time. The model included the effects of treatment, animals nested within treatment group, period and treatment x period interaction. The effect of treatment was tested using animals nested in treatment group as the error term. If the two groups of animals differed for a parameter in period 1, a covariance statistical analysis was performed. The values obtained in the first period were used as covariates in this analysis. These parameters were duodenal flows of non-ammonia N (NAN), bacterial N, starch and bacterial OM; ruminal digestion of starch; ruminal acetate (moles/ 100 moles); ruminal liquid phase retention time; and true ruminal digestion of OM. For blood parameters sampling time was added to the model. For the weekly VFA analyses, week was substituted for period in the model. Differences between the treatment groups were tested using a t-test where the denominator was calculated with the mean square error term from the analysis and the mean square of the animal effect. The overall experimental error rate was $P < .05$.

Results and Discussion

Monensin supplementation increased (P <.01) apparent DM digestibility in periods 2 and 3 by 11 and 7%, respectively (table 2). After 27 d of withdrawal, there was no longer an effect of the ionophore. These results are in contrast to those of Poos and coworkers (1979) who reported that monensin supplementation decreased DM digestibility initially, but had no effect after 40 d. Beede et al. (1980), however, reported monensin supplementation of steers fed a low protein diet increased DM digestibility from 65.8 to 68.8%. Thornton and Owens (1981) reported monensin tended to increase DM digestibility in growing steers. In the present study the period x treatment interaction was significant, suggesting the effect of the ionophore changed with time. The 4% decrease observed between periods 2 and 3 may indicate possible adaptation to the ionophore, although the effect on DM digestibility was still significant in period 3.

As would be expected, the increase in DM digestibility was mirrored in apparent OM digestibility with increases of 10 and 7% in periods 2 and 3, respectively. After withdrawal of the ionophore, there was still a significant increase in digestibility. However, this difference (2.7%) was similar to that of the first period (2%). Faulkner et al. (1985) reported that monensin supplementation of beef steers fed high-forage diets increased OM digestibility. In the present study the period x treatment interaction was significant, suggesting adaptation to monensin may have occurred. This increase in OM digestion appeared to occur mainly in the

Table 2. THE EFFECT OF MONENSIN ADDITION AND SUBSEQUENT WITHDRAWAL ON ORGANIC MATTER DIGESTION IN AND AFTER THE RUMEN

	Treatment by periods								
	1		2		3		4		
Item	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	SE[b]
DM digestibility, %	64.8	66.9	62.7*	69.6	64.4*	68.9	64.8	66.7	.8
Organic matter									
Intake, g/ d	998	949	980	947	989	956	984	944	4
Apparent ruminal digestion[c]	44.8	51.0	42.4*	50.1	41.0*	52.6	37.0	42.7	1.9
Duodenal flow, g/ d	544	463	563*	469	583*	454	620	535	19
Postruminal digestion[c]	22.9	18.6	22.8	21.8	26.0	19.0	30.3	26.1	1.8
Digestibility[c]	67.7	69.7	65.3*	71.9	67.0*	71.5	67.3*	70.0	.8

[a] Con-control; Mon-monensin.
[b] Standard error of means.
[c] Percentage of intake.
* Monensin and control groups differ within that period ($P < .05$).

rumen. However, a large nonsignificant difference (14%) existed between the two groups in period 1. In period 2 the groups differed by 18%, while in period 3 the difference was 28%. After withdrawal the effect of monensin supplementation on ruminal OM degradation disappeared. With increased ruminal digestion there was a corresponding decrease in the flow of OM at the duodenum on a g/ d or percentage of intake basis. Despite the drop in OM flow during periods 2 and 3, there was no significant effect of dietary monensin on the postruminal digestion of OM. There were, however, numerical decreases which would be expected due to the drop in OM flow to the intestines. Monensin supplementation or withdrawal had no effect on the flow of ash at the duodenum (table 3), although Kirk et al. (1985) reported monensin could affect mineral absorption in sheep. Starch digestibility was slightly decreased ($P < .05$) in period 3, in contrast to the results of Muntifering et al. (1981) who observed no effect of the ionophore on the total tract digestibility of this nutrient. The 1% decrease in starch digestibility was unlikely to have significantly affected energy metabolism in the wethers. Monensin addition and withdrawal had no other effect on the digestion of starch. Muntifering et al. (1981) reported that monensin tended to increase the intestinal digestion of starch. The authors attributed the improvement observed in animal performance to this rise in available energy due to higher intestinal digestion of starch.

Monensin addition tended to numerically decrease total VFA levels (table 4). In period 3 monensin significantly decreased the quantity of total VFA. This decrease was due more to a larger increase in the control

Table 3. THE EFFECT OF MONENSIN ADDITION AND SUBSEQUENT WITHDRAWAL ON ASH FLOW AND STARCH DIGESTIBILITY

	Treatment by periods								
	1		2		3		4		
Item	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	SE[b]
Ash duodenal flow, g/ d	146	135	148	128	145	131	137	132	2.9
---------- Starch ----------									
Intake, g/ d	201	193	204	273	203	280	189	180	1.9
Ruminal digestion, % of intake	82.1	89.4	87.5	94.6	87.2	95.3	84.8	87.9	1.3
Duodenal flow, g/ d	36.7	20.6	25.4	14.9	25.9	13.4	29.2	21.7	2.8
Digestibility, %	99.0	99.0	98.9	99.4	98.4*	97.3	98.5	98.6	.3

[a] Con-control; Mon-monensin.
[b] Standard error of means.
* Monensin and control groups differ within that period ($P < .05$).

Table 4. THE EFFECT OF MONENSIN ADDITION AND WITHDRAWAL ON RUMINAL VFA LEVELS

	Treatment by periods								
	1		2		3		4		
Item	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	SE[b]
Total VFA[c]	70.2	69.2	61.8	52.9	113.9*	89.1	55.2	62.2	4.2
Acetate:propionate ratio	2.77	3.21	2.38*	1.71	3.3*	2.15	2.97*	3.77	.15
Moles/ 100 moles									
Acetate	60.2	63.9	59.8*	54.9	64.5*	58.9	61.8	63.6	.9
Propionate	22.4	20.1	25.8*	32.4	19.6*	27.5	20.8*	17.1	1.2
Isobutyrate	.89	.9	.82	.8	.78	.91	.95	.97	.05
Butyrate	12.7	11.1	10.2*	8.2	11.7*	9.0	12.8	11.9	.6
Isovalerate	1.7	2.2	1.7	2.5	1.6	2.1	1.9	2.5	.3
Valerate	1.6	1.7	1.5	1.3	1.4	1.4	1.5	1.5	.1
Caproic acid	.55	.58	.18	.11	.4	.16	.26	.38	.08

[a] Con-control; Mon-monensin.
[b] Standard error of means.
[c] Mmoles/ ml.
* Monensin and control groups differ within that period (P <.05).

animals than to an effect in monensin-fed sheep. The presence of the ionophore decreased the percentage of acetate in both periods 2 and 3 by approximately 8%. After withdrawal, acetate levels rose to those found in the controls. Monensin administration increased ($P < .05$) the amount of propionate by 26% in period 2 and 40% in period 3. After ionophore withdrawal, monensin-fed wethers had lower ($P < .05$) levels of propionate, compared to the controls. In period 3 there was a trend for monensin to increase ruminal isobutyrate levels from .78 to .91 moles/ 100 moles. Monensin-fed sheep had decreased levels of butyrate in periods 2 and 3. The ionophore tended to decrease isovalerate levels, but no significant differences were detected between the groups within periods. Richardson et al. (1976) reported similar results in a cattle trial. In the present experiment monensin maintained its effects on ruminal VFA over the entire 146 d. There were no treatment effects on valerate or caproic acid concentrations. The A:P ratio was decreased ($P < .05$) in both periods 2 and 3 by monensin supplementation. After withdrawal, monensin-fed sheep had a significantly higher A:P ratio, compared to the controls, due to decreased propionate and higher acetate concentrations. Dawson and Boling (1982) reported that the monensin-induced depression in the A:P ratio disappeared by d 10 of withdrawal.

Monensin decreased total VFA levels in the weekly samples, but the differences were not always significant (figure 2). There appeared to be no pattern as to when the two groups differed. Acetate levels (moles/ 100 moles) differed ($P < .05$) within 1 wk after ionophore addition to the diet (figure 3). Acetate remained depressed in supplemented sheep until

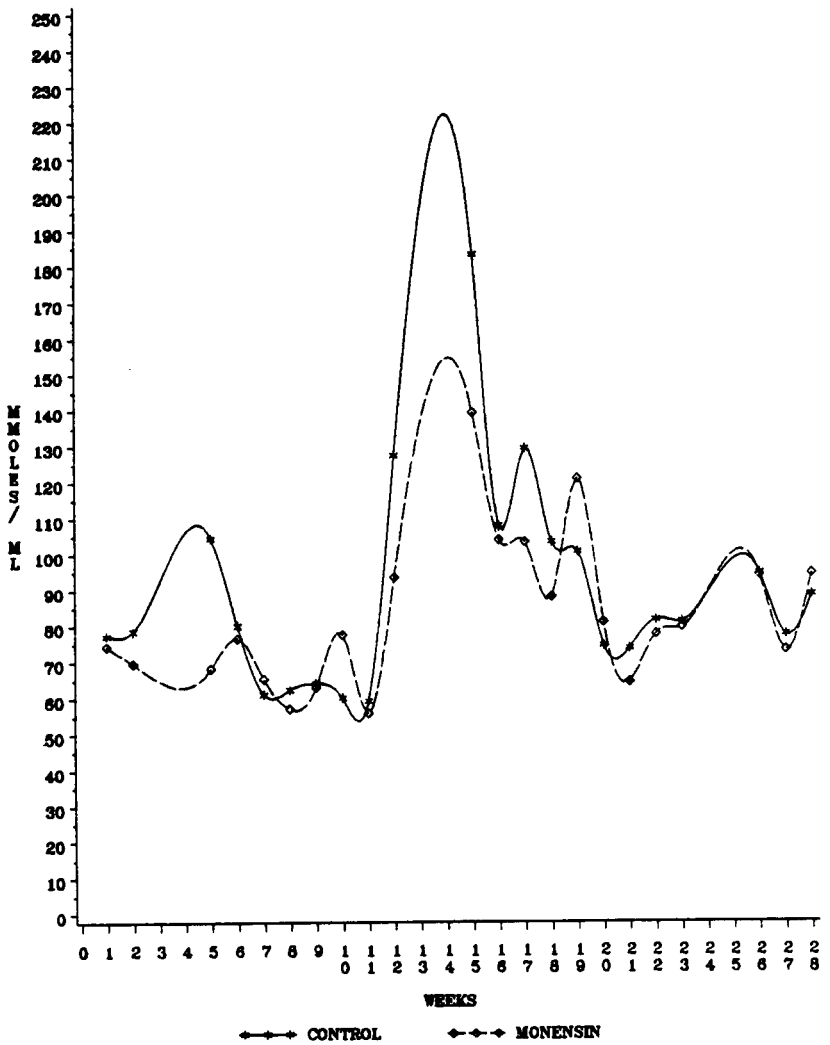

FIGURE 2. EFFECT OF MONENSIN SUPPLEMENTATION AND WITHDRAWAL ON TOTAL VOLATILE FATTY ACID CONCENTRATIONS.

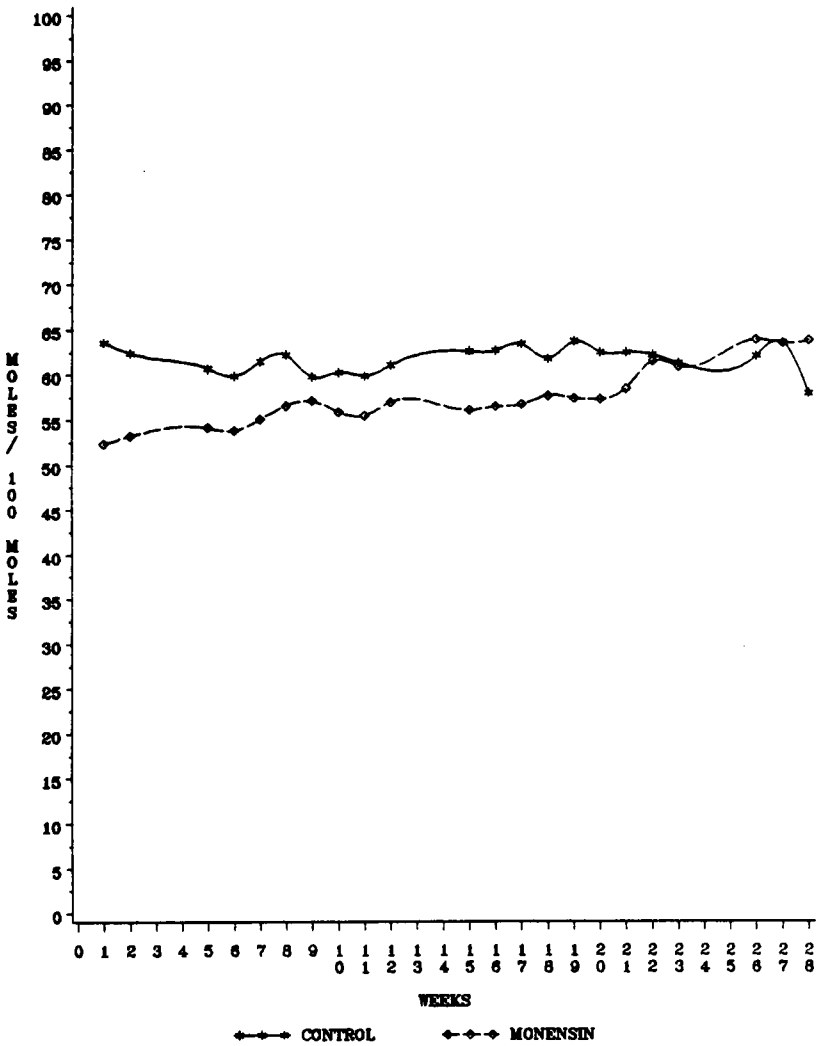

FIGURE 3. EFFECT OF MONENSIN SUPPLEMENTATION AND WITHDRAWAL ON RUMINAL ACETATE LEVELS.

monensin was withdrawn from the diet during wk 21. Levels returned to control values within approximately 24 h after withdrawal. Utley et al. (1976) reported similar results in that monensin maintained its effects on acetate and propionate concentrations throughout a 168 d cattle trial. The same pattern was observed for propionate, butyrate and the A:P ratio (figures 4, 5 and 6). Propionate levels were decreased by 43% before withdrawal. Lemenager et al. (1978b) also observed that the effect of monensin supplementation tended to disappear by 22 h post-administration.

The effect of monensin on the protozoal population was dependent on the species (table 5). Polyplastron and Entodinium numbers were not affected by the administration or withdrawal of the ionophore. The percentage of Polyplastron in the total number of protozoa, however, increased during period 2, but returned to control levels in period 3. Isotricha numbers increased in monensin-fed animals during periods 2 and 3, but the increase was significant only in period 3. This rise in Isotricha numbers during monensin supplementation may indicate that this protozoal species is better able to adapt to the metabolic changes induced by the ionophore. After withdrawal, Isotricha returned to control values. With the increases in both Polyplastron and Isotricha, Entodinium counts were reduced. Entodinium made up the largest proportion of the protozoal population. In period 3 of the experiment the ionophore eliminated Enoploplastron protozoa from the rumen. The presence of monensin caused a decrease of 70 (period 2) and 50% (period 3) in total protozoal numbers, even though the effect of the ionophore was not significant.

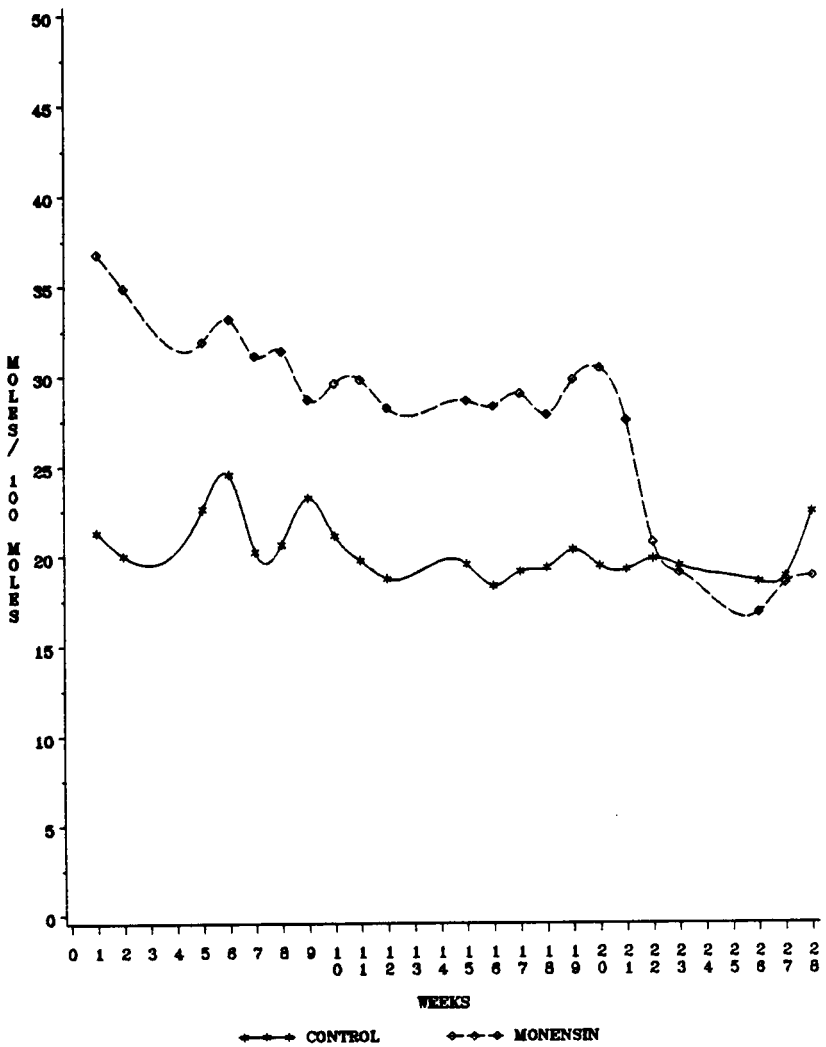

FIGURE 4. EFFECT OF MONENSIN SUPPLEMENTATION AND WITHDRAWAL ON RUMINAL PROPIONATE LEVELS.

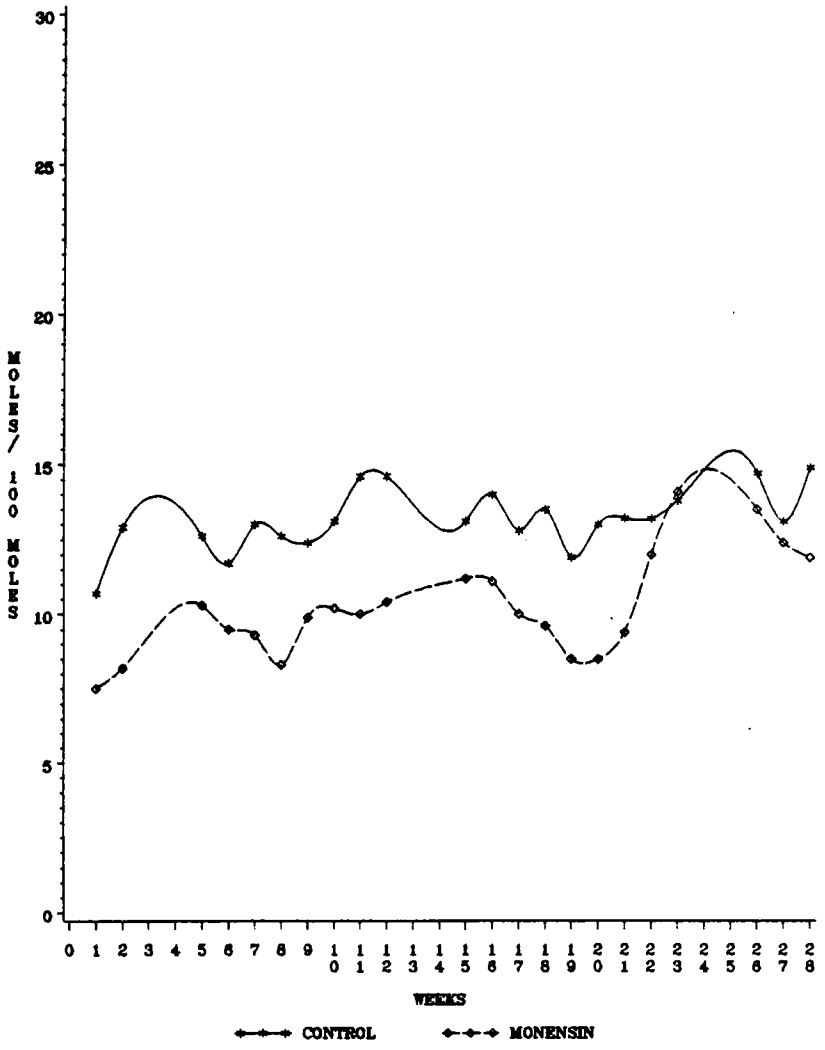

FIGURE 5. EFFECT OF MONENSIN SUPPLEMENTATION AND WITHDRAWAL ON RUMINAL BUTYRATE LEVELS.

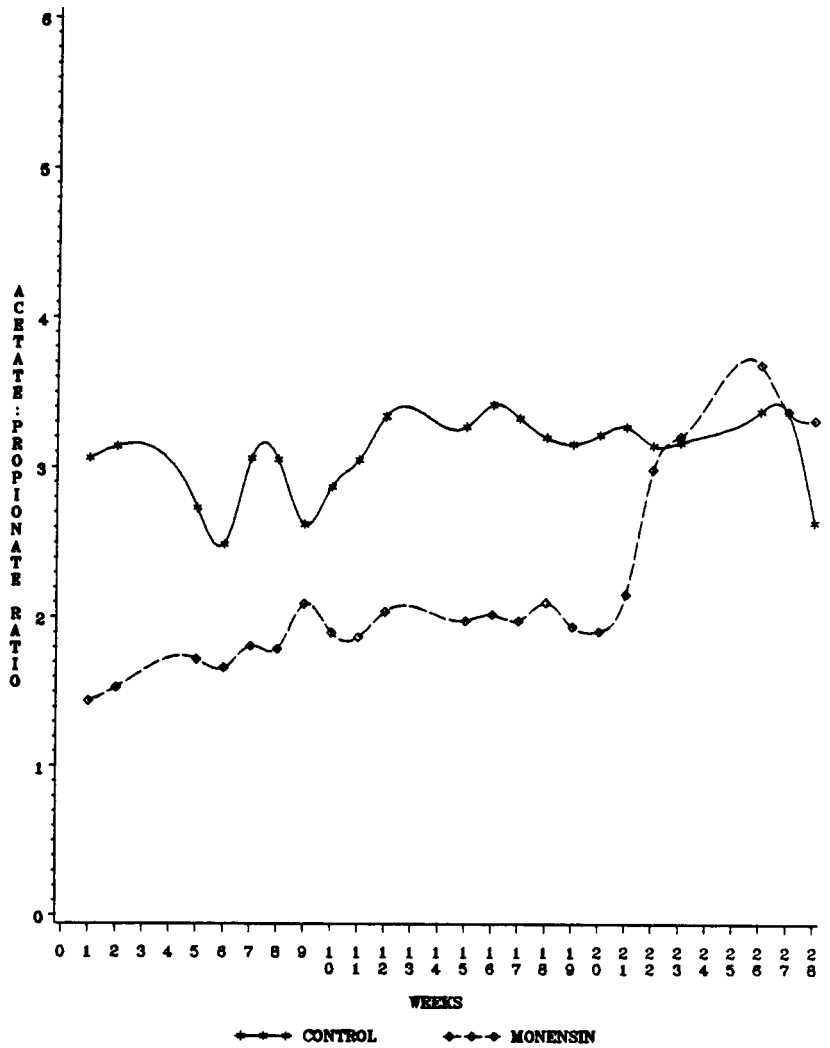

FIGURE 6. EFFECT OF MONENSIN SUPPLEMENTATION AND WITHDRAWAL ON THE ACETATE:PROPIONATE RATIO.

Table 5. THE EFFECT OF MONENSIN SUPPLEMENTATION AND WITHDRAWAL ON THE COMPOSITION OF THE PROTOZOAL POPULATION IN THE RUMEN

Item	Treatment by periods 1 Con[a]	1 Mon[a]	2 Con[a]	2 Mon[a]	3 Con[a]	3 Mon[a]	4 Con[a]	4 Mon[a]	SE[b]
				Numbers					
Isotricha x 10^1	353	547	595	735	698*	1604	625	702	316
Polyplastron x 10^1	470	540	820	769	827	528	1628	2508	220
Enoploplastron x 10^2	0	19	13	3	448	0	35	0	52
Entodinium x 10^3	360	227	379	102	243	128	74	112	63
Total protozoa x 10^3	366	254	394	117	304	149	101	142	60
				Percentage					
Isotricha	1.54	3.44	1.71	7.25	2.74*	10.6	6.71	4.05	2.1
Polyplastron	1.85	2.46	2.39*	7.8	2.64	3.62	17.6	18.8	2.0
Enoploplastron	0	.86	.36	.34	13.64	0	.94	0	1.2
Entodinium	96.9	93.2	95.5	84.5	81.0	85.8	74.7	77.2	5.4

[a] Con-control; Mon-monensin.
[b] Standard error of means.
* Monensin and control groups differ within that period ($P < .05$).

Poos et al. (1979) reported monensin supplementation diminished protozoal numbers in a lamb trial. Itabashi and coworkers (1984) also found that monensin fed to goats decreased numbers by 50%. Leng et al. (1984), however, reported that the ionophore had no effect on protozoal pool sizes or species composition.

Monensin decreased the concentration of N in bacteria during period 3 (table 6). Faulkner et al. (1985) also reported that monensin supplementation decreased bacterial protein content in whole rumen contents. In the present study, the ionophore also decreased the amount of bacterial ash in period 2, but this effect disappeared in period 3. The drop in mineral content could be due to the ion transporting abilities of monensin. The return to control values could indicate that the bacteria had found a method of successfully counteracting this ion movement. The increase in bacterial ash content after monensin withdrawal may indicate that the mechanism with which the bacteria compensated for the decrease in ash caused by monensin in period 2 was still active, leading to this increase in mineral content. This possibility is unlikely since 27 d should have been sufficient time for bacterial adaptation to the absence of the ionophore. Another possibility is that monensin selected for species changes in the ruminal bacterial population. These species may normally have a higher mineral content. Dawson and Boling (1982) reported that the percentage of monensin resistant-bacteria remained elevated for more than 18 d after ionophore withdrawal. In the present study rumen pH varied ($P < .01$) with administration of monensin. When control levels dropped in period 3 the presence of the ionophore stabilized the pH. When

Table 6. THE EFFECT OF MONENSIN SUPPLEMENTATION ON RUMINAL MICROBIAL PARAMETERS

	Treatment by periods								
	1		2		3		4		
Item	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	SE[b]
Bacterial N, % DM	6.9	7.3	7.2	7.5	6.9*	6.1	7.0	6.8	.2
Bacterial ash, % DM	23.3	24.0	26.1*	20.1	25.2	25.4	23.2*	25.8	.9
Ruminal pH	6.2	6.17	6.26	6.34	6.07*	6.33	6.04	5.99	.04
Flow bacterial OM, g/ d	198	130	200	150	146*	230	223	177	19
Efficiency[c]	44	26	49	30	36	36	60	40	4
True ruminal OM digestion, %	24.2	37.2	21.1	34.2	26.0	29.0	14.2	23.8	3.5

[a] Con-control; Mon-monensin.
[b] Standard error of means.
[c] Efficiency of bacterial synthesis: g bacterial N synthesized/ kg OM apparently digested in the rumen.
* Monensin and control groups differ within that period (P <.05).

monensin was withdrawn, ruminal pH in these animals returned to control levels.

Monensin had no effect on cellulose digestion as measured by the in sacco technique with 20 μm pore size (table 7). This small pore size would prevent most protozoal species from entering and degrading the cellulose. These bags thus, represent the bacterial contribution to cellulose breakdown in the rumen. Dinius et al. (1976) reported monensin to have no effect on cellulose degradation in steers fed a forage diet. Similar results were reported by Lemenager and coworkers (1978a) in steers fed concentrates. Cellulose digestion was decreased in period 2 after 19 d of monensin supplementation. This decrease occurred exclusively in the 100 μm bags which measured both protozoal and bacterial contributions to cellulose degradation. This decrease was probably due to shifts in protozoal numbers and(or) species, since no effect was observed in the 20 μm bags which measured the bacterial contribution. During this period there was an increase in Polyplastron as a percentage of total protozoal numbers. Jouany and Senaud (1982) reported that Polyplastron species were involved in ruminal cellulose digestion.

Monensin addition to the diet increased ($P < .05$) apparent N digestibility from 74.6 to 76.6% in period 2 (table 8). In periods 3 and 4 there were no differences in apparent N digestibility. Poos et al. (1979) reported monensin to have no effect on the digestibility of N in a lamb trial. Beede et al. (1980), however, found monensin supplementation improved N digestion from 41.7 to 49.3%. Shqueir et al. (1982) also reported that monensin increased ($P < .05$) the digestion of crude protein in lambs

Table 7. THE EFFECT OF MONENSIN SUPPLEMENTATION ON RUMINAL IN SACCO PARAMETERS

Item	Treatment by periods 1 Con[a]	1 Mon[a]	2 Con[a]	2 Mon[a]	3 Con[a]	3 Mon[a]	4 Con[a]	4 Mon[a]	SE[b]
Cellulose degradation									
20 um sacs, % DM loss	22.6	20.3	27.7	25.6	26.1	22.9	22.8	21.7	1.3
100 um sacs, % DM loss	36.2	35.6	41.9*	36.0	42.3	39.1	--	--	1.3
Lupin degradation									
DM degradation									
Immediately soluble, %	38.1	37.4	36.8	38.0	39.7	41.4	38.0	32.5	1.9
Potentially degradable, %	61.4	62.6	61.8	62.0	60.3	58.7	62.0	66.6	2.0
Rate[c]	.092	.07	.086	.083	.095	.086	.087	.103	.008
N degradation									
Immediately soluble, %	61.3	64.2	59.0*	62.8	63.4	66.4	66.8	66.5	1.2
Potentially degradable, %	38.5	35.8	41.0*	37.2	36.6	33.6	33.2	33.5	1.1
Rate[c]	.13	.094	.123	.112	.143	.121	.123	.123	.008

[a] Con-control; Mon-monensin.
[b] Standard error of means.
[c] % disappearance/ h.
* Monensin and control groups differ within that period ($P < .05$).

Table 8. THE EFFECT OF IONOPHORE ADDITION AND WITHDRAWAL ON APPARENT DIGESTION OF NITROGEN

	Treatment by periods								
	1		2		3		4		
Item	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	SE[b]
Intake	23.6	22.6	23.2	21.1	24.3	21.4	24.7	23.5	.05
Duodenal N flow, g/ d	26.3	23.2	26.4	23.7	25.9	23.7	26.2	24.1	.6
NH_3-N flow, g/ d	2.0	2.3	2.3*	1.5	2.0*	1.6	2.0	1.8	.13
NAN[c] flow, g/ d	24.4	20.8	24.1	22.3	23.9	22.1	24.1	22.3	.69
% of intake	103.4	92.4	103.9	106.6	98.4*	103.5	97.7	94.6	3.0
DAPA flow, g/ d	18.0	12.4	19.2	14.0	13.5*	18.5	20.6	15.8	1.5
Feed and endogenous N flow, g/ d	6.5	8.4	5.4	8.3	10.4*	3.6	3.6	6.5	1.2
Apparent digestibility of N, %	71.3	72.7	70.6*	74.6	74.0	75.8	75.6	76.1	.8

[a] Con-control; Mon-monensin.
[b] Standard error of means.
[c] Non-ammonia N.
* Monensin and control groups differ within that period (P <.05).

fed ryegrass straw. The lack of difference in period 3 in the present study may indicate adaptation to the ionophore after 96 d of supplementation.

Despite the improvement in N digestibility in period 2, the addition or withdrawal of monensin had no effect on the production of alpha-amino N in the rumen, either for units/ time or percentage basis (table 9). In contrast to these results Matsumoto et al. (1984) reported that monensin supplementation decreased the levels of ruminal free amino acids. Horton (1979), however, reported that monensin increased ruminal free amino acids in steers fed rolled barley. In agreement with the results reported here, Lemenager and coworkers (1978a) reported the ionophore to have no effect on alpha-amino N levels in the rumen. Rodriguez et al. (1986) also found monensin did not affect free amino acid concentrations in the rumen.

Monensin addition or withdrawal had no effect on the amount of immediately soluble or potentially degradable DM of lupin grain measured in sacco (table 7). There was also no effect on the rate of DM degradation. Monensin supplementation increased ($P < .05$) by 6.4% the amount of the immediately soluble N fraction of lupin grain measured in sacco. The effect had disappeared by period 3 when the difference between the two groups was almost identical to that which was present in the first period (4.4, period 3 vs 4.7%, period 1). Withdrawal of the ionophore had no effect on this parameter. With this increase in the immediately soluble N fraction there was a corresponding drop ($P < .05$) in the quantity of potentially degradable N in lupin grain in period 2. Monensin

Table 9. THE EFFECT ON MONENSIN SUPPLEMENTATION AND WITHDRAWAL ON RUMINAL PARAMETERS

	Treatment by periods								
	1		2		3		4		
Item	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	SE[b]
Amino N, mg[c]	10.2	13.3	12.1	12.6	12.7	9.6	6.5	6.6	2.5
Amino N, %[d]	21	20	20	33	19	17	10	11	5.0
Protease[e]	12.0	9.6	11.2*	15.1	12.8	13.4	11.0*	13.7	.9
Deaminase[f]	4.4	3.6	5.9	5.9	6.2	6.4	5.9	6.3	.4
Ruminal NH_3, ug/ ml	145	181	160	132	238	196	181	234	16

[a] Con-control; Mon-monensin.
[b] Standard error of means.
[c] Mg glycine equivalents disappearing/ h/ 100 ml rumen fluid.
[d] Percentage of the initial amount.
[e] Mg azocasein hydrolyzed/ h/ g DM rumen contents.
[f] umoles NH_3 produced/ ml rumen fluid/ h.
* Monensin and control groups differ within that period ($P < .05$).

supplementation after period 2 and its withdrawal had no further effect. Neither monensin nor its withdrawal changed the rate of N degradation. The alterations in the amount of the immediately soluble N fraction may have been an indirect effect of monensin supplementation. Changes in solubility would be related to differences in rumen fluid composition. Monensin has been reported to decrease the turnover of the liquid phase in the rumen (Lemenager et al., 1978a), which could lead to the changes in solubility observed in sacco.

Monensin addition in period 2 increased ($P < .05$) ruminal protease activity by 35% (table 9). In period 3 there ceased to be a significant difference between the two groups, which could indicate possible microbial adaptation to the ionophore. This increase in protease activity could possibly be due to the microorganisms using protein as an energy source in an effort to compensate for the energy depletion caused by monensin. The ionophore had no effect on ruminal deaminase activity. Protease activity was measured in total rumen contents while deaminase was analyzed using filtered fluid. The differences in the microbial populations could explain the discrepancy between the two analyses. Barao et al. (1983) noted that the ionophore depressed both protease and deaminase activity in steers fed an 80% corn diet.

The disagreement in the literature concerning the effect of monensin on ruminal protein degradation probably results from dietary differences. In the present study a very soluble, readily degradable protein source, lupin grain, was used. The ease with which lupin is degraded is most likely why monensin had no effect on any of the de-

gradation parameters studied, except for protease. The lack of alteration in the rate of lupin degradation in sacco correlates well with the amino-N levels and deaminase activity found in the rumen. The increase in protease activity is puzzling, but may be related to an energy deficiency experienced by the microbial populations. The rise in protease may also be related to alterations in the microbial populations, such as decreased protozoal numbers. Orpin and Letcher (1983) reported that elimination of protozoa increased bacterial numbers in rumen fluid by 480%. Protease may have been increased because soluble proteins are attacked primarily by bacteria (Siddons and Paradine, 1981). The increase in the amount of immediately soluble N, measured in sacco, could be related to monensin induced alterations in rumen fluid composition due to a possible decrease in the liquid phase turnover rate.

Monensin administration decreased ruminal NH_3 levels, although no significant differences were detected within periods. Levels returned to control values after ionophore withdrawal. If large changes had occurred in ruminal N degradation one would expect to see alterations in ruminal NH_3 levels. Poos et al. (1979) and Muntifering and coworkers (1981) also reported that the ionophore decreased ruminal NH_3.

Ionophore supplementation had no effect on the flow of N at the duodenum (table 8). Even though monensin had no effect on the levels of ruminal NH_3, the flow of NH_3 was reduced at the duodenum. This effect was the most noticeable for period 2 (35%) ($P < .05$), but still existed as the experiment progressed (20%, period 3). After withdrawal, NH_3 flow in monensin-fed animals returned to control levels. Muntifering

and coworkers (1981) noted that monensin supplementation of steers decreased the abomasal flow of NH_3. Despite the drop in NH_3 flow, there were no significant changes in the flow of NAN due to monensin. However, when expressed on a percentage of N intake, monensin addition increased ($P < .05$) the amounts of NAN during period 3. During this period there was an increased outflow of bacterial N from the rumen, which could account for the increase in NAN flow. In agreement with Poos et al. (1979), monensin had no significant effect on apparent digestibility of N in period 3, suggesting the increase in bacterial N was digested in the intestines or fermented in the lower gut.

The flow of DAPA, a bacterial marker, was significantly altered by the addition of monensin. The ionophore increased bacterial N flow in period 3, expressed as g/ d or a percentage of NAN flow basis. The augmentation of bacterial flow suggests that by period 3 the microorganisms had adapted to the presence of the compound. There was also a corresponding increase in the flow of bacterial OM from the rumen. The alterations in bacterial ash content reported earlier also support the hypothesis of adaptation. No treatment effects were observed in period 2 or after withdrawal, in contrast to the findings of Poos et al. (1979) who reported that monensin supplementation decreased bacterial N flow. Moore et al. (1980) found monensin had no effect on the flow of microbial protein reaching the abomasum. In the present experiment the increase in bacterial N flow led to a decrease in the flow of feed N from the rumen in period 3. The drop could be explained by an increase in bacterial metabolic activity needed to support higher levels of growth. There was,

however, no change through the entire trial in the quantity of bacterial N synthesized (g/ kg OM apparently fermented in the rumen) (table 6). Both Muntifering et al. (1981) and Moore et al. (1980) found monensin supplementation did not affect the efficiency of microbial N synthesis. In the present study there was also no treatment effect on the amount of OM truly fermented in the rumen. Due to differences in purine bases between the two groups of sheep in the first period, interpretation of the results is difficult (appendix C).

Monensin supplementation tended ($P < .07$) to decrease the flow of DM at the duodenum as measured by Cr_2O_3, the solid phase marker, in both periods 2 and 3 by 17 and 21%, respectively (table 10). The drop in DM flow supports the conclusion that more OM was degraded in the rumen with monensin supplementation. Monensin withdrawal allowed an increase to control levels. Monensin addition and withdrawal had no effect on the recovery of Cr_2O_3. Due to difficulties with Cr_2O_3 it was impossible to determine ruminal solid phase turnover times. After administration of Cr_2O_3 was halted, there was a plateau in marker concentrations which lasted for approximately 12 h. This plateau made it impossible to determine solid phase turnover times.

Feeding monensin or withdrawal had no significant effect on the flow of liquid and DM from the rumen. There was a numerical decrease in period 2, supporting the conclusion that alterations in liquid phase turnover may have changed the composition of the ruminal fluid. The ionophore had no effect on liquid phase retention time or on the distribution volume of PEG. There were, however, numerical increases in

Table 10. THE EFFECT OF MONENSIN ADDITION AND SUBSEQUENT WITHDRAWAL ON RUMINAL TURNOVER RATES AND DUODENAL FLOWS OF LIQUID AND SOLID CONTENTS

	Treatment by periods								
	1		2		3		4		
Item	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	SE[b]
Chromic oxide									
Flow, g/ d	709	602	700*	578	722*	574	765	684	20.5
Recovery, %	97	92	104	102	99	102	94	96	2.2
Polyethylene glycol 4000									
Flow, kg/ d	15.1	14.0	15.5	12.8	13.9	13.1	13.2	12.8	.5
Retention time, h	14.2	16.7	14.8	20.8	11.2	18.3	14.0	15.5	.8
Distribution volume, liters	8.6	9.6	10.1	11.0	6.3	10.2	7.3	8.0	.8
Recovery, %	83.5	82.3	77.6	80.8	88.5	87.7	79.4	78.8	2.2

[a] Con-control; Mon-monensin.
[b] Standard error of means.
* Monensin and control groups differ within that period (P <.05).

retention time during periods 2 and 3. The retention of liquids in the rumen could explain the decrease in the flow of liquid and DM observed in period 2. Lemenager et al. (1978a) reported ionophore supplementation of steers decreased liquid phase turnover by 30%.

The presence of ruminal monensin increased ($P < .01$) plasma glucose levels, although no differences were detected within each period (table 11). Sampling time also significantly affected glucose levels. Glucose concentrations dropped slightly from from 59.2 before feeding to 58.6 mg/ dl 2 h post-feeding. At 6 h post-feeding there was an increase to 61.9 mg/ dl. Despite the increases in plasma glucose levels, the ionophore diminished ($P < .01$) the amount of insulin in the blood. Again, no differences were detected between groups within each period. This drop in hormone levels could explain the rise in plasma glucose. The mode of action for this decrease is not known since increases in ruminal propionate, which are often reported with ionophore supplementation, are normally expected to increase plasma insulin levels (Bines and Hart, 1984). Insulin concentrations also varied significantly with sampling time. Insulin increased from 55.3 before feeding to 70.7 µunits/ ml 2 h post-feeding. By 6 h post-feeding insulin decreased to 63.3 µunits/ ml. Potter at al. (1976) reported monensin supplementation increased plasma glucose, although these authors also found insulin levels to be increased. Wahle and Livesey (1985) reported dietary monensin decreased plasma insulin in sheep fed both high roughage and concentrate diets.

Table 11. THE EFFECT OF MONENSIN ADDITION AND WITHDRAWAL ON PLASMA GLUCOSE AND INSULIN

	Treatment by periods						
	2		3		4		
Item	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	SE[b]
Glucose, mg/ dl	58.4	60.6	57.0	60.5	60.9	62.0	5.1
Insulin, uU/ ml	69.2	47.4	68.7	58.2	71.6	63.4	2.7

[a] Con-control; Mon-monensin.
[b] Standard error of means.

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CHAPTER IV

JOURNAL ARTICLE II

THE EFFECT OF FEEDING AND INTESTINAL INFUSION OF MONENSIN ON INTESTINAL DIGESTION IN SHEEP

Summary

Monensin was supplied in two fashions, fed in the diet or infused into the duodenum. Neither monensin treatment had an effect on amylase activity at the ileum but fed ionophore increased trypsin activity ($P < .05$). Infused monensin increased plasma glucose levels ($P < .05$) over those found in the controls. Infused ionophore had no effect on ruminal volatile fatty acids (VFA). None of the treatments had an effect on total VFA levels. Fed monensin decreased the quantities of acetate in the rumen while increasing those of propionate by 47% ($P < .05$). Ruminal monensin decreased ($P < .05$) butyrate concentrations. There was no significant monensin x day interaction. None of the treatments altered VFA levels at the ileum, although dietary monensin tended to increase propionate. Neither monensin treatment altered the digestibility of any constituent. No significant differences were recorded in nutrient flow at the ileum. There was no treatment effect on extent or site of N digestion. Fed monensin, however, altered the sites of dry matter (DM) and organic

matter (OM) digestion. Monensin feeding increased (P <.05) the amount of DM (7%) and OM (14%) digested before the cecum.
(Key Words: Monensin, Intestinal Digestion, Glucose, Sheep.)

Introduction

There are many reports concerning the effects of the ionophores on rumen fermentation. However, there are very little data available on any possible postruminal action of these compounds. Monensin is thought to act in ruminants primarily by affecting rumen fermentation (Schelling, 1984), but there are reports that the ionophores may also affect animal physiology (Chew et al., 1978). MacGregor and Armstrong (1984) reported that avoparcin, a non-ionophore feed additive which has many similar effects of the ionophores, caused an increase in the uptake of amino acids from the lumen of the small intestine in sheep. Parker et al. (1984) reported that in the rat, avoparcin caused an increase in the activity of intestinal dipeptidase in the gut muscosa. These reports suggest that this compound could have a direct effect on the small intestine itself.

The ionophores when fed to monogastrics are reported as improving growth. This effect is attributed to the coccidiocidial action of the compounds (Anonymous, 1987). It is possible, however, that the ionophores may also act on intestinal digestion. Ilan et al. (1981) reported that the addition of monensin sodium to the diet of young calves increased or left unchanged the activities of some intestinal enzymes (amylase and lipase) while decreasing the activity of others (trypsin and chymotrypsin). This

project was designed to examine possible effects of ionophore addition on digestion in the small intestine of sheep. The ionophore chosen was monensin sodium since most of the reports in the literature deal with this compound.

Materials and Methods

Six adult Texel wethers, equipped with flexible rumen cannula, simple duodenal and re-entrant cannulae in the terminal ileum, were used in three trials. The duodenal cannula was installed upside down in order to facilitate the infusion of liquids. The animals were individually housed indoors in 1.5 X 1.0 m pens under natural lighting. The wethers were collared and chained to the front of their pens. The chains were of sufficient length to allow the animals free movement, but made it impossible for them to turn completely around. The sheep had continuous access to both water and a salt lick. The animals were fed twice daily at 0800 and 1600 h.

Two diets were used in this experiment, a control containing no monensin and an identical diet except for the addition of 36 ppm of monensin (table 12). The monensin was added in the form of Rumensin[3], the commercial form of the ionophore. Both diets were pelleted and fed at 50 g/ kg metabolic body weight ($wt^{.75}$). In addition, 100 g of straw were fed per day to aid in maintaining rumen function. With the

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[3] Eli Lilly France, St-Cloud, France

Table 12. COMPOSITION OF BASAL DIET

Item	Percent[a]
Ingredient composition	
Prairie hay, first cutting, prebloom	43.3
Corn grain	34.4
Lupin grain	20.7
Mineral mix[b]	1.6
Chemical composition	
Organic matter	92.8
Nitrogen	2.3

[a] Dry basis.

[b] 55.6% $CaHPO_4$, 26.3% NaCl, 1.1% S, 9.2% $MgSO_4$ 7 H_2O, 7.1 Na_2SO_4 10 H_2O, .32% $ZnSO_4$ 7 H_2O, .16% $MnSO_4$ H_2O, .03% $CuSO_4$ 5 H_2O, .15% $FeSO_4$ 7 H_2O, 7 ppm $CoSO_4$ 7 H_2O, 12 ppm $Ca(IO_3)_4$ 6 H_2O, 3.3 ppm Na_2SeO_3 5 H_2O, vitamins (A-130000, D_3-73000, E-3625 IUnits/ kg).

addition of the straw, the actual level of monensin in the diet was 33 ppm. These diets were also formulated containing .229% (w/w) Cr_2O_3 in cellulose paper for use in determining digestibility.

For each trial the six wethers were allotted to three treatments: 1)basal diet, 2)monensin sodium fed, 3)basal diet, with monensin sodium infused into the inverted duodenal cannula. One wether was removed because of large feed refusals in period 2. Thus, the data were calculated on five animals per treatment. The experiment consisted of three experimental periods. For each trial the animals were rerandomized to the treatments, with the restriction that no animal could receive the same treatment twice. Sheep consuming monensin in the feed would have altered rumen digestion due to the effect of the ionophore on the ruminal microbial population. By infusing monensin into the duodenum, this added source of variation was eliminated. Only effects due to the ionophore would cause changes in intestinal absorption, which could then be compared to the control animals (a negative control) or to the monensin-fed wethers. The quantity of monensin sodium infused was calculated to provide the same amount of active material as that which the sheep consumed in the monensin diet. Monensin in the salt form was not soluble in water. The ionophore was dissolved in a solution of ethanol and water (1.1:1.6, v/v) and this mixture was infused at a rate which was proportional to the body weight of the sheep (.82 $ml \cdot kg^{-1} \cdot d^{-1}$). The infusion was maintained continuously during the trial. The animals not on the monensin infusion treatment were infused with a solution of ethanol and water to eliminate possible effects of the alcohol on intestinal digestion.

Each trial consisted of a 20-d adaptation to allow any effects of the previous treatment to dissipate and to allow adaptation to the current treatment, a total fecal collection period, an ileal sampling period, and blood sampling.

Five days before the start of collection the animals were switched to their respective Cr_2O_3-containing diets. During the last period there were insufficient quantities of the control diet, so the animals were fed the Cr_2O_3 diet continuously. The animals were housed in conventional metabolism crates during this time. The crates also allowed continuous access to both water and a salt lick. The amount of time for the total collection of feces was either 8 or 10 d. If the re-entrant ileal cannula of an animal became blocked, the digestibility period was extended to allow normal gut motility to resume. All samples were frozen at -20 C unless otherwise noted.

After the fecal collection, samples of ileal contents were collected every 6 h for 3 d for the determination of nutrient flow at the ileum. Sampling for each day was delayed by 2 h so that samples were taken for every 2 h interval. Ileal contents were pooled by animal and frozen. Blood samples were obtained over 3 d by jugular puncture using sodium heparin Venoject tubes. Each day blood was obtained before eating the morning meal, and 2 and 6 h after eating. The whole blood was centrifuged to obtain plasma.

Samples were taken from the rumen for VFA analysis. These samples were obtained on d 7 and 16 of the adaptation period and on the last day of ileal sampling. The samples were taken 1.5 h after the morning

meal. Nine milliliters of filtered rumen fluid (1 mm mesh) were mixed with 1 ml of 1% (w/w) mercuric chloride in 5% (v/v) orthophosphoric acid (Jouany, 1982).

Dry matter was determined by heating the samples at 80 C for 48 h and ash content by ashing at 500 C for 5 h in a muffle furnace. Nitrogen was analyzed on fresh samples using the automated Tecator Keltec system based on the Kjeldahl method. Ammonia was determined by the method of Michel (1971). Starch was analyzed by the procedure developed by Thivend et al. (1965). Chromic oxide was used as the digestive marker and was determined by a colorimetric procedure (Mathieson, 1970). The analysis of VFA was by gas chromatography (Jouany, 1982). The activities of amylase (Peyraud, 1983), trypsin and chymotrypsin (Nitsan et al., 1974) were determined on total ileal contents. The procedure of Nitsan (1974) was modified by use of a .05 M Tris-chloride buffer, pH 7.6, containing .02 M $CaCl_2$ (Erlanger et al., 1966). Trypsin was analyzed using benzoyl arginine p-nitroanilide hydrochloride dissolved in dimethylsulfoxide. The procedure was identical to that used for chymotrypsin. Plasma was analyzed for glucose using glucose oxidase (Michel, 1971). Nutrient flow at the ileum was determined using Cr_2O_3 as the marker.

The experiment was statistically analyzed as a switchback design with treatments balanced to periods such that any residual effects of previous periods could be eliminated in the analysis. The design was analyzed using the general linear model program of SAS (1985). The model was y = treatment + period + animal. For plasma glucose time of

day was added to the model. Day was added to the model for the analysis of ruminal VFA. Comparisons between treatment least square means were made using the test of Tukey (1953).

Results and Discussion

Neither monensin supplementation nor the site of administration had a significant effect on intestinal amylase activity (table 13). There was a numerical increase in enzyme activity with monensin present in the feed, but due to individual variation among the sheep, the difference was not significant. Ilan et al. (1981) reported monensin increased amylase activity in young calves, but Johnson et al. (1986) found that the ionophore had no effect on amylase activity in sheep after 15 d of supplementation.

Presence of the ionophore in the feed resulted in a higher ($P < .05$) level of trypsin activity at the terminal ileum, compared to the monensin infusion treatment. Control animals exhibited intermediate levels of activity. In contrast, when young calves were fed the ionophore, decreased trypsin activity in the feces was observed by Ilan and coworkers (1981). The depression in activity in the calves could have been due to microbial degradation of the enzyme in the large intestine.

Neither monensin treatment affected ileal NH_3 levels, compared to the controls (table 14). Muntifering et al. (1981) reported that in steers fed monensin there was a tendency for abomasal concentrations of NH_3 to be depressed. In the present study there was no treatment effect on the flow of ash at the ileum or the amount of ash present in total ileal

Table 13. EFFECT OF MONENSIN ADMINISTRATION ON INTESTINAL ENZYMES PRESENT IN TOTAL ILEAL CONTENTS

	Treatment			
		Monensin		
Item	Control	Fed	Infused	SE[a]
Amylase[b]	75.7	98.6	58.4	27.9
Trypsin[c]	3.52[d,e]	4.68[d]	3.14[e]	.35

[a] Standard error of means.
[b] ug maltose liberated/ ml.
[c] Trypsin activity/ ml.
[d,e] Means with different superscripts differ (P <.05).

Table 14. EFFECT OF SITE OF MONENSIN SUPPLEMENTATION ON ILEAL AND FECAL PARAMETERS

	Treatment			
		Monensin		
Item	Control	Fed	Infused	SE[a]
Ileum				
NH3, ug/ml	157	133	171	23.0
DM, %	8.26	7.78	8.42	.2
Ash flow, g/d	87.7	86.4	85.5	5.3
Ash, %	19.6	20.1	19.9	.5
Feces				
DM, %	34.6	34.1	32.4	1.0
Ash, %	16.3[b,c]	15.6[b]	16.9[c]	.19
Chromic oxide recovery, %	90.2	89.1	88.3	4.9

[a] Standard error of means.
[b,c] Means with different superscripts differ (P <.05).

contents. The presence of monensin in the rumen, however, decreased ($P < .05$) fecal ash levels by approximately 7% in comparison with its infusion. Control values were intermediate between the two groups. Monensin was reported to affect mineral absorption in sheep (Kirk et al., 1985). Increased absorption or decreased excretion of minerals into the gastro-intestinal tract could explain this decrease in fecal ash. Treatments did not affect ileal or fecal DM levels. Yokoyama et al. (1985) reported that monensin supplementation in cattle increased cecal DM (10.07 vs 10.38%).

Infused monensin increased ($P < .05$) plasma glucose from 60.1 for the controls to 62.3 mg/ dl (figure 7). There was no significant difference between monensin-fed (60.7 mg/ dl) and control animals. Across treatments, there was a significant effect of sampling time. Glucose levels were stable or decreased slightly from 0 to 2 h and then increased at 6 h post feeding. The monensin x sampling time interaction was not significant, indicating that the effect of monensin was relatively constant during the day. Previous results are conflicting concerning the effect of monensin on plasma glucose levels. Johnson et al. (1986) in sheep and Matsumoto et al. (1982) in cattle reported that monensin had no effect on plasma glucose. However, Wahle and Livesey (1985) and Austin and Wilde (1985) found the ionophore increased glucose levels. The increase seen with monensin infusion may have been due to the greater quantity of the ionophore present in the intestine. Potter et al. (1976) reported that plasma glucose rose as the level of supplemented monensin increased

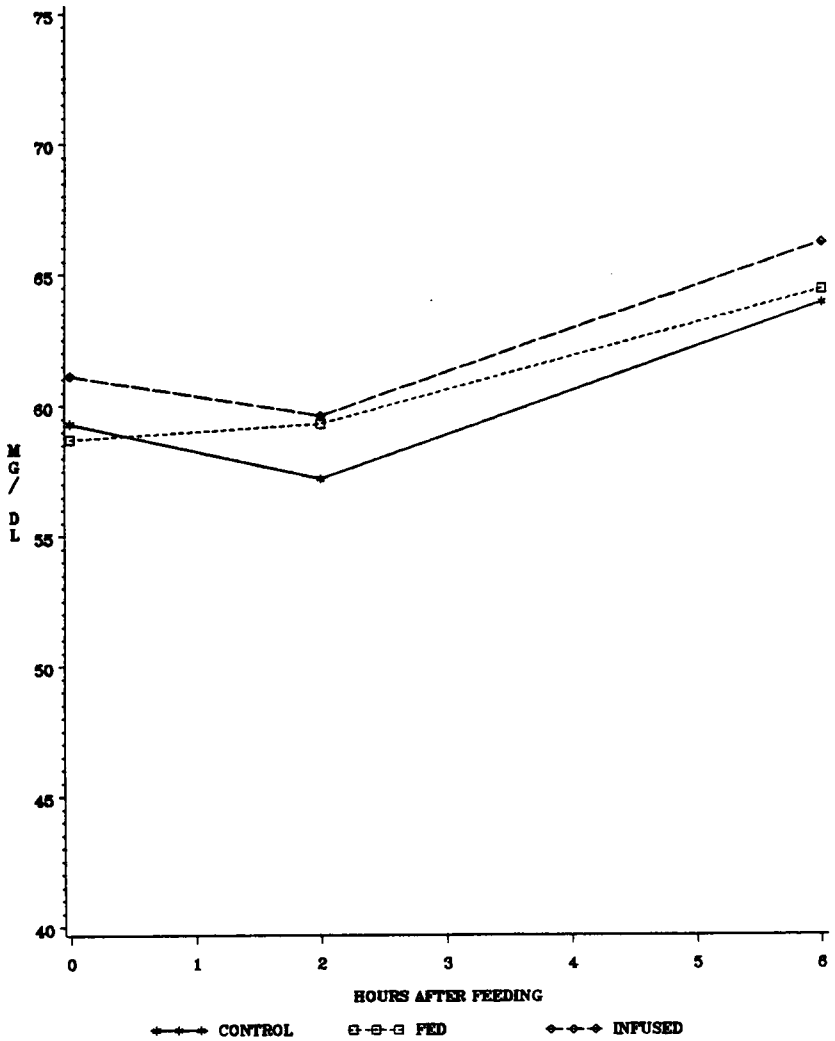

FIGURE 7. EFFECT OF SITE OF MONENSIN ADMINISTRATION ON PLASMA GLUCOSE LEVELS.

in grazing cattle. The highest glucose concentration was obtained with 200 mg•head^{-1}•d^{-1}.

Only the presence of monensin in the feed had a significant effect on ruminal VFA levels (table 15). There appears to be no recycling of monensin back to the rumen since infusion of the ionophore had no effect on VFA. Monensin exhibited its normal action on VFA levels. There was a depression in acetate with a corresponding increase in propionate. Total VFA levels were not affected by treatment. Ruminal concentrations of isobutyrate in monensin-fed sheep were decreased ($P < .05$), compared to the infused ionophore, but did not differ from control values. Feeding monensin depressed ($P < .05$) butyrate concentrations. Treatment had no effect on isovalerate or caproic acid concentrations. Feeding monensin decreased valerate concentrations ($P < .05$), when compared with the infusion treatment. There was no difference from the controls. There was no day x treatment interaction for the VFA, which indicates that the fed monensin established its effects by d 9 of supplementation and maintained the effects until completion of the trial.

None of the treatments caused any changes in total VFA levels at the terminal ileum (table 16). No caproic acid was detected in any of the sheep. There was a trend ($P < .07$) for the monensin-fed animals to have increased concentrations of propionate in the ileum. This increase would be expected since there would be higher levels of propionate entering the small intestine. Monensin fed to cattle had no effect on cecal levels of acetate, propionate or butyrate (Yokoyama et al., 1985). In contrast, Raun et al. (1976) mentioned that monensin supplementation of sheep

Table 15. EFFECT OF MONENSIN ADMINISTRATION ON RUMINAL VFA LEVELS

	Treatment			
		Monensin		
Item	Control	Fed	Infused	SE[a]
Total VFA[b]	73.5	72.9	78.9	3.6
Moles/ 100 moles				
Acetate	60.45[c]	55.32[d]	62.77[c]	.88
Propionate	20.64[c]	28.67[d]	18.43[c]	.87
Isobutyrate	.88[c,d]	.74[c]	1.01[d]	.06
Butyrate	12.73[c]	10.56[d]	13.51[c]	.43
Isovalerate	1.68	1.69	1.95	.11
Valerate	1.83[c,d]	1.61[c]	2.03[d]	.07
Caproic acid	1.71	1.36	.34	.89

[a] Standard error of means.
[b] Mmoles/ ml.
[c,d] Means with different superscripts differ (P <.05).

Table 16. EFFECT OF MONENSIN ADMINISTRATION ON ILEAL VFA LEVELS

Item	Treatment			SE[a]
	Control	Monensin		
		Fed	Infused	
Total VFA[b]	4.6	5.4	5.8	.8
Moles/ 100 moles				
Acetate	89.4	86.02	86.5	1.1
Propionate	6.6[c]	9.3[d]	8.4[c,d]	.6
Isobutyrate	.28	.67	.69	.2
Butyrate	3.2	3.2	3.3	.2
Isovalerate	.49	.51	.37	.37
Valerate	.05	.37	.79	.25

[a] Standard error of means.
[b] Mmoles/ ml.
[c,d] Means with different superscripts differ (P <.05).

depressed cecal concentrations of acetate and butyrate while increasing that of propionate.

There was no treatment effect on the recovery of Cr_2O_3 in the feces (table 14). Monensin, either infused or fed, had no effect on the apparent digestibilities of DM, OM, N, starch or fiber. Muntifering et al. (1981) reported that monensin supplementation of steers had no effect on the apparent digestibilities of OM, starch or CP. Zinn and coworkers (1980) also found that monensin had no effect on apparent digestibilities of fiber and protein. In the present study no difference was detected concerning the flow of lignin at the ileum. There was also no effect on the flows or digestion sites of N, starch, NDF and ADF fractions (tables 17 and 18). Poos et al. (1979) reported that in growing lambs monensin initially depressed DM and ADF digestibilities, but after 40 d of adaptation there was no effect. The lambs had been fed monensin for 10 d when the first trial was completed. In the present study the animals were on their respective treatments for 20 d which could have allowed adaptation to monensin. In agreement with this trial, Poos et al. (1979) found no effect of monensin on N digestibility.

Although total digestibilities and ileal flows for DM were not affected by treatment, the sites of digestion were altered (table 19). The presence of monensin in the feed decreased the amount of DM digested after the ileum by 24%. Infusion of the ionophore also decreased the quantity of DM digested post-ileally, and this difference was significant. The same results were observed when the quantity of DM was expressed as percentage of digestible DM. Feeding monensin increased ($P < .05$)

Table 17. EFFECT OF MONENSIN ADMINISTRATION ON SITE AND QUANTITY OF STARCH AND APPARENT NITROGEN DIGESTIBILITIES

Item	Treatment			
	Control	Monensin		SE[a]
		Fed	Infused	
Nitrogen				
Intake, g/d	21.6	20.9	21.9	.3
Ileal flow, g/d	7.77	7.79	7.97	.51
Digestibility, %	73.2	73.7	73.3	2.1
Pre-ileal digestion				
% of intake	60.9	63.0	62.7	1.4
% of digestible N	80.3	86.5	81.0	3.6
Post-ileal digestion				
% of intake	14.7	10.3	14.3	3.0
% of digestible N	19.7	13.5	19.0	3.6
Starch				
Intake, g/d	221	221	211	15
Ileal flow, g/d	5.6	6.7	6.5	.68
Digestibility, %	99.5	99.5	99.4	.04
Pre-ileal digestion				
% of intake	96.5	96.9	96.8	.51
Post-ileal digestion				
% of intake	3.4	2.5	3.2	.5

[a] Standard error of means.

Table 18. EFFECT OF MONENSIN ADMINISTRATION ON SITE AND QUANTITY OF CELL WALL DIGESTIBILITY

Item	Treatment			
	Control	Monensin		SE[a]
		Fed	Infused	
NDF				
Intake, g/d	226	221	211	15
Ileal flow, g/d	236	232	232	19
Digestibility, %	53.9	56.9	56.3	2.3
Pre-ileal digestion				
% of intake	40.0	39.7	42.5	2.3
Post-ileal digestion				
% of intake	17.3	16.5	18.0	.9
ADF				
Intake, g/d	222	225	230	7
Ileal flow, g/d	143	141	139	10.7
Digestibility, %	58.2	56.0	58.5	2.6
Pre-ileal digestion				
% of intake	36.8	37.0	40.6	2.3
Post-ileal digestion				
% of intake	14.9	19.5	17.2	2.1

[a] Standard error of means.

the amount of DM digested before the ileum, when expressed as a percentage of the digestible DM, but not as a percentage of intake. Monensin-fed animals digested prececally approximately 7% more of the total digestible DM.

Monensin-fed sheep digested ($P < .05$) 87.6% of the digestible OM before the cecum, compared with 78.4 and 76.9 for monensin infused and control animals, respectively. There was no significant difference between the infusion and control treatments (table 20). The presence of monensin in the feed decreased ($P < .05$) the amount of OM digested postileally, both as a percentage of OM intake or digestible OM. No effect was observed for monensin infusion.

The increase in DM and OM digestion before the cecum could explain the improvement in animal performance reported when the ionophore is fed to cattle. Muntifering et al. (1981) reported that monensin decreased ruminal digestion of OM which may have led to increased digestion in the small intestine. However, if the increase in digestion occurred in the rumen, the animal would not profit from it other than from benefits associated with an increase in microbial numbers or fermentation. The rise in plasma glucose with the infusion of monensin indicates that the ionophore may have postruminal effects. The increase in glucose could be due to increased propionate absorption from the small intestine, which seems unlikely since most of the VFA are absorbed across the rumen wall. Monensin may also act directly on the pancreas, perhaps affecting insulin secretion, as was suggested by Galitzer et al. (1983).

Table 19. EFFECT OF MONENSIN ADMINISTRATION ON SITE AND AMOUNT OF DRY MATTER DIGESTIBILITY

	Treatment			
		Monensin		
Item	Control	Fed	Infused	SE[a]
Intake, g/d	976	966	976	3.7
Ileal flow, g/d	482.9	424.2	473.9	25.3
Digestibility, %	68.8	71.0	68.0	1.3
Pre-ileal digestion				
% of intake	50.3	56.0	51.9	2.1
% of digestible DM	72.0[b]	79.1[c]	74.5[d]	.5
Post-ileal digestion				
% of intake	19.5[b]	14.8[c]	17.8[d]	.2
% of digestible DM	28.0[b]	20.9[c]	25.5[d]	.5

[a] Standard error of means.
[b, c, d] Means with different superscripts differ ($P < .05$).

Table 20. EFFECT OF MONENSIN ADMINISTRATION ON QUANTITY AND SITE OF ORGANIC MATTER DIGESTIBILITY

	Treatment			
		Monensin		
Item	Control	Fed	Infused	SE[a]
Intake, g/d	903	893	902	3.7
Ileal flow, g/d	389.5	338.9	380.1	23.9
Digestibility, %	71.9	71.3	71.1	1.8
Pre-ileal digestion				
% of intake	56.4	61.9	58.1	2.2
% of digestible OM	76.9[b]	87.6[c]	78.4[b]	.9
Post-ileal digestion				
% of intake	16.7[b]	9.0[c]	15.7[b]	1.0
% of digestible OM	23.1[b]	12.4[c]	21.6[b]	.9

[a] Standard error of means.
[b,c] Means with different superscripts differ (P <.05).

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CHAPTER V

JOURNAL ARTICLE III

EFFECT OF MONENSIN ADDITION ON IN VITRO FERMENTATION OF IONOPHORE ADAPTED AND NONADAPTED RUMEN CONTENTS

Summary

Ionophore adapted and unadapted rumen contents were incubated with four different N sources, with and without supplemental monensin. The adapted contents were obtained from sheep which had been fed monensin for 131 d. The N sources were fish and soybean meals, lupin grain and $(NH_4)_2SO_4$. Monensin addition had no significant effect on fermentor pH. Type of rumen contents and monensin addition did not alter total VFA levels. Lupin grain produced ($P < .05$) the highest amount of total VFA, with $(NH_4)_2SO_4$ producing the least. Ionophore adapted rumen contents significantly decreased acetate levels. Nitrogen source had no effect on acetate concentrations. Added monensin depressed ($P < .05$) acetate by 6%. Adapted rumen contents produced 57% more propionate overall. Monensin increased propionate concentrations by 32% with unadapted contents while the increase was only 4% in adapted cultures. Of the N sources lupin grain produced the highest levels of butyrate. Adaptation to the ionophore decreased butyrate production by 49%, overall

compared to nonadapted rumen contents. Monensin addition further decreased ($P < .05$) butyrate by an additional 20%. Monensin addition also decreased ($P < .05$) by 4% the quantity of organic matter fermented. Adapted rumen contents produced significantly less gas than nonadapted cultures. Ammonia fermentors had the lowest levels of total gas production while lupin fermentors produced the most. Added ionophore decreased total gas by 7.5% in nonadapted cultures and 1.2% with adapted microbes. There was an increase ($P < .05$) in the CO_2:CH_4 ratio with adapted rumen contents. Source of N had no effect on this parameter. Addition of monensin caused a further increase in the ratio. Type of rumen contents had no effect on the amount of microbial synthesis. The amount of microbial N synthesized was highest with lupin grain (93.7 mg N) as the N source and lowest in the NH_3 fermentors (81.9) ($P < .05$). Added ionophore decreased microbial N synthesis by 31%. Incubation of the protein sources with adapted rumen contents significantly increased degradibility. Lupin was the most (60%) while fish meal was the least (25%) degradable. Monensin addition decreased protein degradation by 25%. Ruminal microbial populations do adapt to the presence of monensin. After adaptation is complete, the microorganisms are less subject to the metabolic disturbances caused by the ionophore, compared to nonadapted microbes. Hence, precautions should be used when working with nonadapted microbes.

(Key Words: Monensin, In Vitro, Protein Degradation, Adaptation.)

Introduction

The effect of feed additives on ruminal fermentation is often studied in vitro, which allows the screening of large numbers of compounds. However, differences exist in the literature concerning the effect of ionophore addition to in vitro cultures. Bartley and coworkers (1979) reported monensin and lasalocid decreased microbial protein synthesis. Herod et al. (1979) found that neither ionophore affected microbial protein synthesis when adapted inocula were used. In another study cellulose degradation by nonadapted microorganisms was severely inhibited by the addition of monensin, while little effect was observed in inocula obtained from sheep fed the ionophore for 32 d (de Jong and Berschauer, 1983). Short (1978) also reported that the effect of monensin on cellulose degradation changed if the microbial population was adapted to the presence of monensin. This experiment was undertaken in an effort to determine the relative response in vitro of monensin adapted and nonadapted microbes to monensin addition.

Materials and Methods

This study was conducted with sheep from a previous experiment. Eight Texel wethers with rumen fistula and simple duodenal canula were housed individually in 1.5 X 1.0 m pens with continuous access to water and a salt lick. Animals were fed twice daily at 0800 and 1600 h. The

day before sampling for an in vitro experiment the sheep were denied water overnight.

The sheep were fed a pelleted diet (table 21) at the level of 45 g/ kg metabolic body weight ($wt^{.75}$), and were allotted to monensin levels of 0 and 36 ppm. The animals also received 100 g of straw per day to aid in maintaining rumination. The straw lowered the level of monensin in the total diet to approximately 33 ppm. The animals were fed their respective diets for 131 d before the start of the in vitro incubations to assure that the rumen microbes were completely adapted to the ionophore.

The in vitro system (figure 8) used to study protein degradation and microbial synthesis was based on that of Jouany and Thivend (1986). Briefly, rumen contents are incubated with a known quantity of energy (starch) in the presence of the protein to be studied. The amount of energy present in the protein was accounted for so that all the fermentors had the same amount of energy available. The incubation was for 6 h with samples of fluid and gas removed periodically to measure the rate and extent of fermentation. Both the liquid and solid phases from the rumen ingesta were used in the incubation to ensure that the microbial populations are similar to those in the rumen. The fermentation parameters measured included pH and production of NH_3, VFA and gas. The protein sources used in this trial were selected because of different solubilities. The proteins, in order of decreasing solubility, were ground lupin grain (the protein source in the animal diet), soybean meal and fish meal (the most insoluble protein used). A control fermentor was also included using $(NH_4)_2SO_4$ as the N source. Monensin sodium was added

Table 21. COMPOSITION OF BASAL DIET

Item	Percent[a]
Ingredient composition	
Prairie hay, first cutting, prebloom	43.3
Corn grain	34.4
Lupin grain	20.7
Mineral mix[b]	1.6
Chemical composition	
Organic matter	92.8
Nitrogen	2.3

[a] Dry basis.

[b] 55.6% $CaHPO_4$, 26.3% NaCl, 1.1% S, 9.2% $MgSO_4$ 7 H_2O, 7.1 Na_2SO_4 10 H_2O, .32% $ZnSO_4$ 7 H_2O, .16% $MnSO_4$ H_2O, .03% $CuSO_4$ 5 H_2O, .15% $FeSO_4$ 7 H_2O, 7 ppm $CoSO_4$ 7 H_2O, 12 ppm $Ca(IO_3)_4$ 6 H_2O, 3.3 ppm Na_2SeO_3 5 H_2O, vitamins (A-130000, D_3-73000, E-3625 IUnits/ kg).

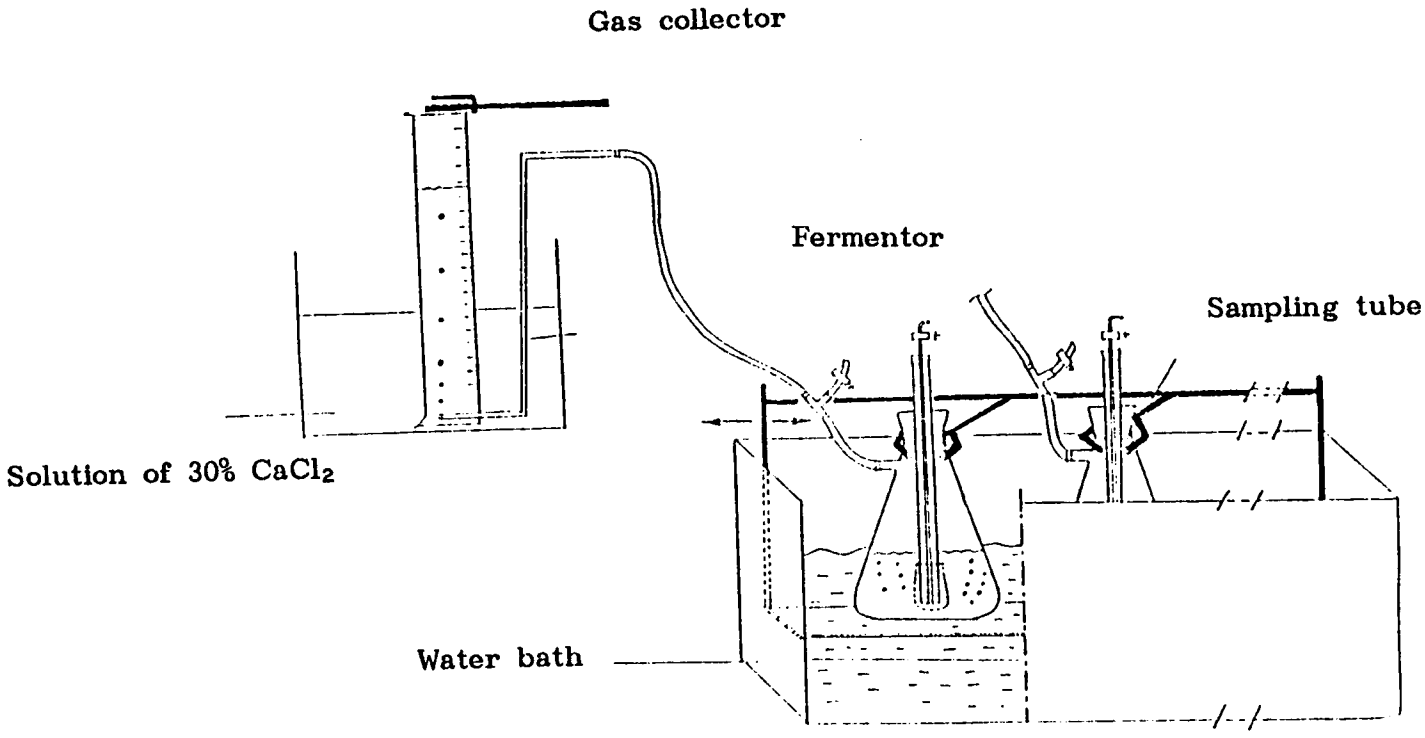

Figure 8. In vitro system for the incubation of rumen contents.

to the designated fermentors at the rate of 1 mg/ fermentor which was approximately 69.4 µg/ g subtrate. When compared to the level of the ionophore fed to the animals, the amount of compound in the incubations was twice that present in the rumen (69.4 µg/ g for incubation vs 33 µg/ g feed).

Levels of NH_3 produced in the fermentors were determined using the method of Michel (1971). Analysis of VFA was by gas chromatography (Jouany, 1982). The pH was measured electrometrically. Gas composition was determined as described by Jouany and Senaud (1978). The amount of organic matter fermented was calculated using a formula based on VFA production (Demeyer and Van Nevel, 1979).

The statistical analysis was accomplished using the general linear model system of the SAS package (1985). The experiment was a split-plot design with adapted or nonadapted rumen fluid being the whole plot. Type of protein and the presence or absence of added monensin were arranged in a factorial fashion within each whole plot. The model for the analysis included the type of ruminal ingesta, replication, protein source and monensin addition. Replication of the experiment was used to test the source of rumen fluid. Differences between the protein sources were tested using Tukeys (1953) test on the least square means.

Results and Discussion

The presence of rumen fluid from sheep that were adapted to monensin tended to decrease ($P < .059$) fermentor pH (table 22). Monensin

Table 22. ALTERATIONS IN FERMENTER PH AND TOTAL VOLATILE FATTY ACID PROFILES DUE TO MICROBIAL ADAPTATION TO THE PRESENCE OF MONENSIN AND DIFFERENT NITROGEN SOURCES

Protein source	Type of rumen contents: Nonadapted, No monensin	Nonadapted, Added monensin	Monensin adapted, No monensin	Monensin adapted, Added monensin	SE[a]
			pH[b]		
Control[c]	5.24	5.34	5.2	5.17	.02
Fish[c]	5.26	5.28	5.23	5.16	.02
Soybean[d]	5.18	5.22	5.16	5.12	.01
Lupin[c]	5.25	5.32	5.16	5.2	.02
Mean	5.23	5.29	5.19	5.16	.01
			Total VFA, mmoles/ ml		
Control[c]	119.9	119.0	113.2	120.6	2.0
Fish[d]	126.8	131.2	128.1	129.9	2.0
Soybean[d,e]	133.5	133.3	138.9	126.0	1.9
Lupine[e]	137.2	136.0	142.2	136.2	1.5
Mean	129.4	129.9	130.6	128.2	1.5

[a] Standard error of means.

[b] Rumen contents x monensin ($P < .001$) and protein x monensin ($P < .03$) interactions were significant.

[c,d,e] Overall means for the protein sources with different superscripts differ ($P < .05$).

addition had no significant effect on pH, similar to the results of Stewart et al. (1979). Protein solubility altered pH with fermentors containing soybean meal having lower ($P < .05$) pH (5.17), compared with the other N sources (5.23). The effect of ionophore addition on pH varied with the N source. Monensin addition overall had no effect on fermentor pH with soybean meal but decreased it if fish meal was the N source (5.25 absent vs 5.22 present). Monensin increased the pH with both NH_3 and lupin grain. Ionophore addition to adapted rumen fluid caused ($P < .01$) the pH to drop from 5.19 to 5.16. With nonadapted inoculum monensin increased the pH from 5.23 to 5.29. These small changes in pH, although significant, are unlikely to exert any effect on rumen fermentation in vivo.

Only N source had any effect ($P < .01$) on the amount of total VFA produced in the fermentors. The control fermentor with only NH_3 available, had lower ($P < .05$) levels of total VFA (118 mmoles/ ml), which was probably due to less fermentation occurring. Lupin grain had the highest amount of total VFA (138 mmoles/ ml). Soybean and fish meals were intermediate, with 133 and 129 mmoles/ ml, respectively. The decrease in fermentation with only NH_3 as the N source may indicate that rumen microorganisms need some preformed amino acids to ferment at maximum rate. Monensin in this study did not affect total VFA levels. However, Whetstone and coworkers (1981) report that the presence of monensin decreased total VFA in cultures adapted to the ionophore. De Jong and Berschauer (1983) published similar results for both adapted and nonadapted microorganisms. Bartley et al. (1979) found monensin had no ef-

fect on total VFA levels produced by nonadapted microbes. The results of Whetstone et al.(1981) and de Jong and Berschauer (1983) were obtained with cultures dependent on fiber digestion for energy, while data for the present study and that of Bartley et al. (1979) were obtained using a more readily degraded energy source. Chen and Wolin (1979) showed that some cellulolytic bacteria species were susceptible to monensin inhibition.

Acetate production was 14% lower for monensin adapted ruminal fluid (76.7 vs 66.0 mmoles/ ml) (table 23). When acetate levels were expressed as a percentage of total VFA, a similar decrease in acetate was noted, indicating that it was a real decrease in acetate and not due to lowered fermentation activity. Added ionophore depressed acetate concentrations by 6% (mmole, ($P < .05$)) and by 5% (moles/ 100 moles, ($P < .05$)). Decreased acetate production is characteristic of ionophore supplementation in ruminants. De Jong and Berschauer (1983) found that monensin addition (100 µg/ 350 g cellulose substrate) decreased acetate levels with nonadapted microbes. Adapted microorganisms, however, produced more acetate (51 vs 55 moles/ 100 moles) with ionophore addition. Whetstone et al. (1981), working with adapted rumen fluid, report that monensin depressed acetate production. In the present study lupin grain gave significant increases in acetate levels when compared to NH_3 (76.4 lupin vs 66.7 NH_3, mmoles). However, when acetate was expressed as a percentage, equalizing the fermentation differences between N sources, no significant effect of N remained.

Table 23. ALTERATIONS IN ACETATE AND PROPIONATE CONCENTRATIONS[a] DUE TO MICROBIAL ADAPTATION TO THE PRESENCE OF MONENSIN AND DIFFERENT NITROGEN SOURCES

	Type of rumen contents				
	Nonadapted		Monensin adapted		
Protein source	No monensin	Added monensin	No monensin	Added monensin	SE[b]
			Acetate[c,d]		
Control	60.6	58.4	53.7	52.8	.9
Fish	60.0	58.0	53.4	52.4	.8
Soybean	60.5	58.0	52.5	39.8	3.4
Lupin	60.1	57.9	52.2	51.5	1.0
Mean	60.3	58.1	53.0	49.1	1.5
			Propionate[c,d,e]		
Control[f]	21.2	27.6	37.7	39.1	1.9
Fish[f,g]	20.6	27.6	37.1	39.0	1.9
Soybean[f,g]	20.8	27.4	37.2	38.9	1.9
Lupin[g]	20.6	27.0	37.2	37.8	1.9
Mean	20.8	27.4	37.3	38.7	.2

[a] Moles/ 100 moles.
[b] Standard error of means.
[c] Overall effect of type of rumen contents ($P < .05$).
[d] Overall effect of monensin addition to the fermentor ($P < .05$).
[e] Rumen contents x monensin interaction was significant ($P < .001$).
[f,g] Overall means for the protein sources with different superscripts differ ($P < .05$).

When adapted rumen contents were used the amount of propionate produced was 57% higher ($P < .05$). This increase was maintained when propionate was expressed as a percentage of total VFA (38 vs 24.1%). Use of all protein sources resulted in more mmoles of propionate, compared to NH_3. However, on the basis of moles/ 100 moles, the NH_3 flasks had the highest quantities (31.4%) and lupin the lowest (30.6%). This inversion was due to correcting for the amount of fermentation which occurred.

Addition of ionophore also changed ($P < .01$) the amount of propionate. This VFA increased by 12.7% (mmoles) and from 29.1 to 33.1 moles/ 100 moles with monensin. The effect of monensin addition on propionate production varied with the type of inoculum used. Monensin caused an increase of 32% with nonadapted rumen fluid, but in adapted cultures the increase was only 4%. Another common finding in the literature concerning monensin supplementation, is an increase in propionate concentrations (Schelling, 1984). The large rise in propionate production in nonadapted fermentors with added ionophore in the present study suggests that the microbial populations in the two types of rumen contents were different. Chen and Wolin (1979) indicated that monensin may increase propionate production by selecting for succinate-producing bacteria. The succinate is then decarboxylated to form propionate. Monensin may also affect bacterial metabolism to augment propionate production (Dawson and Boling, 1984). It is more probable, however, that its major effect in adapted inoculum would be species selection, which would allow a larger augmentation in propionate levels. The smaller rise in production

with added ionophore to adapted ruminal contents could be due to the fact that the bacterial population already approached maximal production of the VFA, so further monensin addition would have little effect. Similar results were reported by de Jong and Berschauer (1983). A significant increase in propionate secretion was seen with added monensin to nona-dapted cultures, while little altering occurred with adapted microorganisms.

Use of adapted ruminal fluid resulted in a 21% decrease ($P < .01$) in levels of isobutyrate produced (table 24). More isobutyrate was produced with lupin grain as the N source. Levels (moles/ 100 moles) for the four N sources were: .53 for lupin, .46 for soybean, .46 for fish, and .39 for NH_3. Monensin decreased ($P < .05$) isobutyrate production approximately 15%. Monensin addition decreased the production of this VFA by 15% with lupin meal but only by 6.6% in the NH_3 fermentor. Once the differences in fermentation were accounted for there was no interaction between monensin addition and N source. The type of rumen contents also interacted with N source concerning the production of isobutyrate. Adapted fluid caused a drop of 21.6 and 24.9% in the mmolar quantities present in NH_3 and lupin fermentors, respectively. The differences in isobutyrate levels between the different N substrates were less than those changes observed when monensin was added to the fermentors. When expressed on a moles/ 100 moles basis, the difference between the two N sources was larger, a drop of 19.4% for the NH_3 and 26.3% for lupin. This depression in isobutyrate production using adapted cultures when monensin supplementation had no effect (moles/ 100 moles)

Table 24. ALTERATIONS IN ISOBUTYRATE AND BUTYRATE CONCENTRATIONS[a] DUE TO MICROBIAL ADAPTATION TO THE PRESENCE OF MONENSIN AND DIFFERENT NITROGEN SOURCES

	Type of rumen contents				
	Nonadapted		Monensin adapted		
Protein source	No monensin	Added monensin	No monensin	Added monensin	SE[b]
	Isobutyrate[c,d,e]				
Control[f]	.46	.4	.35	.34	.01
Fish[g]	.58	.45	.42	.38	.02
Soybean[g]	.57	.43	.44	.4	.02
Lupin[h]	.68	.54	.45	.45	.03
Mean	.57	.46	.42	.39	.01
	Butyrate[c,d,e]				
Control[f]	15.3	11.2	6.3	6.0	1.0
Fish[f,g]	15.8	11.2	6.8	6.1	1.0
Soybean[f,g]	15.2	11.4	7.3	6.8	.9
Lupin[g]	15.2	11.5	7.5	7.5	.8
Mean	15.4	11.3	7.0	6.6	.1

[a] Moles/ 100 moles.
[b] Standard error of means.
[c] Effect of type of rumen contents ($P < .05$).
[d] Effect of monensin addition to the fermentor ($P < .05$).
[e] Rumen contents x monensin ($P < .001$) and rumen contents x protein ($P < .01$) interactions were significant.
[f,g,h] Overall means for the protein sources with different superscripts differ ($P < .05$).

supports the idea that changes occurred in the microbial populations present in the rumen. There was a smaller decrease in isobutyrate concentrations with monensin when adapted rumen fluid was used. Nonadapted contents with monensin addition had a 20.3% reduction while with adapted contents the decrease was only 7.5%. Slyter (1979) also reported that monensin would decrease production of isobutyrate by rumen bacteria.

Adapted rumen contents produced approximately 49% less butyrate (table 24). Nitrogen source also affected butyrate production, with lupin grain producing the highest level. The lowest amounts were found in NH_3 fermentors. Monensin addition decreased the levels of this VFA by 20%. Short (1978) reported that monensin decreased butyrate production/g substrate fermented but Whetstone et al. (1981) found monensin had no effect on this VFA. Both groups worked with adapted microorganisms. When amounts of butyrate were expressed as a percentage of the total VFA, adapted rumen fluid decreased production with all N sources. The drop was largest for the NH_3 fermentors (54.1%) and smallest for lupin (43.4%). The effect of added monensin was the least when adapted rumen contents were used in the incubation. The presence of monensin decreased production by 4 percentage units with nonadapted microbes, but by only .4 units with an adapted population. De Jong and Berschauer (1983), working with both adapted and nonadapted rumen fluids, reported that supplemental ionophore had no effect on butyrate production.

As with butyrate, the use of adapted rumen contents resulted in lower production of isovalerate by a small but significant amount (table

Table 25. ALTERATIONS IN ISOVALERATE AND VALERATE CONCENTRATIONS[a] DUE TO MICROBIAL ADAPTATION TO THE PRESENCE OF MONENSIN AND DIFFERENT NITROGEN SOURCES

Protein source	Type of rumen contents: Nonadapted, No monensin	Nonadapted, Added monensin	Monensin adapted, No monensin	Monensin adapted, Added monensin	SE[b]
			Isovalerate[c]		
Control[e]	1.05	1.15	.99	1.01	.02
Fish[f]	1.32	1.35	1.27	1.13	.04
Soybean[f]	1.37	1.31	1.36	1.21	.02
Lupin[g]	1.72	1.69	1.47	1.52	.04
Mean	1.37	1.37	1.27	1.22	.02
			Valerate[c,d]		
Control[e]	.91	.86	.77	.73	.02
Fish[f]	1.05	1.0	.94	.85	.02
Soybean[h]	1.1	1.02	1.01	.94	.02
Lupin[g]	1.18	1.11	1.02	1.05	.02
Mean	1.06	1.0	.93	.89	.01

[a] Moles/ 100 moles.
[b] Standard error of means.
[c] Effect of type of rumen contents ($P < .05$).
[d] Effect of monensin addition to the fermentor ($P < .05$).
[e,f,g,h] Overall means for protein source with different superscripts differ ($P < .05$).

25). Lupin grain fermentors contained the highest levels of this VFA, with NH_3 incubations producing the least. Addition of monensin had no effect on the quantities of isovalerate produced. When levels were expressed on a mmolar basis, the addition of monensin caused isovalerate levels to increase in NH_3 fermentors, but levels decreased in fermentors containing natural protein sources. Monensin depressed concentrations by approximately 2% with lupin and fish meal while those of soybean meal dropped by 11.9%.

Use of adapted rumen contents resulted in an 11% decrease ($P < .05$) in mmoles of valerate. All N sources differed in the quantities of valerate produced. Lupin grain fermentation had the highest levels, followed by soybean and fish meals. Ammonia fermentors contained the least. Monensin addition caused a slight but significant depression in valerate levels.

Type of rumen contents had no effect on the amount of OM fermented in the incubations (table 26). Monensin addition decreased the amount of OM fermented overall by approximately 4%, in contrast to its effect on total VFA. Organic matter was fermented to the largest extent (4.79 g) with lupin grain while the control degraded the least (4.09). Soybean and fish meals were intermediate (4.5 and 4.47 g). The effect of monensin addition varied with the source of N. Monensin increased the amount fermented with NH_3, there was no effect with fish meal, but the ionophore decreased the amount of OM fermented with lupin grain and soybean meal.

Use of adapted ruminal fluid resulted in approximately 10% less total gas ($P < .01$) produced (table 27). Ammonia fermentors produced the least

Table 26. AMOUNT OF ORGANIC MATTER FERMENTED[a] AS AFFECTED BY MICROBIAL ADAPTATION TO THE PRESENCE OF MONENSIN AND DIFFERENT NITROGEN SOURCES

	Type of rumen contents				
	Nonadapted		Monensin adapted		
Protein source	No[b] monensin	Added[b] monensin	No[b] monensin	Added[b] monensin	SE[c]
Control[d]	4.36	4.17	3.8	4.04	.08
Fish[e]	4.62	4.6	4.32	4.36	.08
Soybean[e]	4.84	4.68	4.7	3.78	.08
Lupin[f]	4.95	4.76	4.82	4.62	.08
Mean	4.69	4.55	4.41	4.2	.08

[a] g

[b] Effect of monensin addition to the fermenter ($P < .05$).

[c] Standard error of means.

[d, e, f] Overall means for the protein sources with different superscripts differ ($P < .05$).

Protein x monensin interaction was significant ($P < .05$).

amount of gas, suggesting that the microbial population was less active or there was higher methane production since 4 moles of hydrogen gas are required to produce 1 mole of CH_4. The N source producing the most was lupin grain with soybean and fish meals being intermediate between lupin and NH_3 fermentors. Monensin addition decreased the amount of gas produced by 4.6% which was less than the drop observed with adapted rumen contents. Both Bartley et al. (1979) and Stewart and Edwards (1979) reported monensin supplementation decreased total gas production by rumen microbes. In the present study the effect of monensin addition was more severe with nonadapted rumen contents, a 7.5% decrease, than with adapted organisms where only a 1.2% drop was observed. Herod et al. (1979) published similar results.

Use of monensin adapted rumen contents resulted in an increase in the ratio of carbon dioxide to methane (CO_2:CH_4) from 3.85 to 5.05, indicating that more CO_2 was produced and(or) that the quantity of CH_4 was decreased (table 27). Methanogenic bacteria are not inhibited by the presence of monensin or lasalocid (Chen and Wolin, 1979). However, Wolin (1981) suggested that monensin may act on CH_4 production by decreasing the amount of H_2 produced which would lead to a drop in CH_4. He postulated that the decrease in H_2 was due to species selection by the ionophore. Dellinger and Ferry (1984) reported that monensin had no effect on CH_4 production by Methanobacterium formicicum grown with H_2/CO_2, but decreased methanogenesis when the cells were grown on formate. The ionophore also inhibited growth on formate, but after 4 d had no effect on cellular growth rate with H_2/CO_2. Nitrogen source did

Table 27. CHANGES IN TOTAL GAS PRODUCTION[a] AND $CO_2:CH_4$ RATIO DUE TO MICROBIAL ADAPTATION TO THE PRESENCE OF MONENSIN AND DIFFERENT NITROGEN SOURCES

Protein source	Type of rumen contents: Nonadapted — No monensin	Nonadapted — Added monensin	Monensin adapted — No monensin	Monensin adapted — Added monensin	SE[b]
			Total Gas[c,d,e]		
Control[f]	1580	1465	1362	1355	25
Fish[g]	1665	1527	1442	1417	26
Soybean[i]	1707	1560	1495	1457	25
Lupin[h]	1730	1625	1517	1517	26
Mean	1671	1544	1454	1437	7
			$CO_2:CH_4$[c,d]		
Control	3.29	4.32	5.02	5.4	.23
Fish	3.6	4.42	5.05	5.25	.23
Soybean	3.42	3.75	4.85	4.81	.23
Lupin	3.74	4.26	4.9	5.1	.17
Mean	3.51	4.19	4.95	5.14	.13

[a] Milliters.
[b] Standard error of means.
[c] Effect of type of rumen contents ($P < .05$).
[d] Effect of monensin addition to the fermentor ($P < .05$).
[e] Rumen contents x monensin interaction was significant ($P < .001$).
[f,g,h,i] Overall means for the protein sources with different superscripts differ ($P < .05$).

not affect the CO_2:CH_4 ratio. Ionophore addition further increased the ratio by 10%. Stewart and Edwards (1979) reported that monensin decreased CH_4 production but had no effect on CO_2. There was a trend for monensin addition to have the largest effect with nonadapted contents. The ratio rose 19.4% for nonadapted and 3.8% for adapted contents.

The type of rumen contents had no significant action on the amount of microbial synthesis occurring in the fermentors (table 28). Nitrogen source did affect microbial synthesis, probably due to their different degradabilities. Lupin grain supported the highest numerical level of synthesis (93.7 mg N/ fermentor). Soybean and fish meals followed with 90.6 and 87.5 mg, respectively. The NH_3 fermentor had the lowest amount of synthesis (81.9). Monensin addition decreased ($P < .05$) the amount of new microbial growth from 104.7 to 72.2, a decrease of 31%. Bartley et al. (1979), with nonadapted cultures, and Whetstone et al. (1981), with adapted microorganisms, observed that monensin decreased microbial protein synthesis. However, Short (1978), working with adapted microbes, reported that monensin had no effect on cell yields. There appears to be adaptation to the ionophore since with adapted contents the drop in synthesis with monensin was only 9.3% (93.6 to 84.9 mg). However, with nonadapted contents the decrease was much more severe, from 115.8 to 59.4 (approximately 49%). Herod et al. (1979) report similar results using in vitro cultures of rumen fluid from cattle.

Monensin adapted rumen contents increased ($P < .01$) the overall degradability of the proteins from 37.7 to 41.6 g degradable N/ 100 g total N, reflecting a possible uncoupling of fermentation from growth since

Table 28. ALTERATIONS IN MICROBIAL SYNTHESIS[a] DUE TO MICROBIAL ADAPTATION DUE TO THE PRESENCE OF MONENSIN AND DIFFERENT NITROGEN SOURCES

	Type of rumen contents				
	Nonadapted		Monensin adapted		
Protein source	No[b] monensin	Added[b] monensin	No[b] monensin	Added[b] monensin	SE[c]
Control[d]	107.7	55.6	85.9	78.4	5.0
Fish[d,e]	115.4	58.7	92.6	83.4	5.5
Soybean[e,f]	119.0	59.6	97.1	86.5	5.8
Lupin[f]	121.0	63.8	98.9	91.1	5.5
Mean	115.8	59.4	93.6	84.9	1.6

[a] Mg N/ fermenter.
[b] Effect of monensin addition to the fermentor ($P < .05$).
[c] Standard error of means.
[d,e,f] Overall means for the protein source with different superscripts differ ($P < .05$).
Rumen contents x monensin interaction was significant ($P < .001$).

Table 29. CHANGES IN PROTEIN DEGRADABILITIES[a] DUE TO MICROBIAL ADAPTATION TO THE PRESENCE OF MONENSIN AND DIFFERENT NITROGEN SOURCES

	Type of rumen contents[b]				
	Nonadapted		Monensin adapted		
Protein source	No[c] monensin	Added[c] monensin	No[c] monensin	Added[c] monensin	SE[d]
Fish[e]	32.38	13.84	30.3	22.64	1.3
Soybean[g]	38.47	23.33	42.37	31.93	1.17
Lupin[f]	66.05	52.1	61.65	60.96	1.12
Mean	45.63	29.75	44.77	38.51	1.38

[a] g N/ 100 g total N.
[b] Effect of type of rumen contents ($P < .05$).
[c] Effect of monensin addition to the fermentor ($P < .05$).
[d] Standard error of means.
[e,f,g] Overall means for the protein sources with different superscripts differ ($P < .05$).
Rumen contents x monensin interaction was significant ($P < .002$).

microbial synthesis was similar in the two types of rumen contents (table 29). The increase, however, occurred only with soybean meal as the protein source. Similar results were reported for nonadapted rumen contents (Van Nevel and Demeyer, 1977). In their study, however, monensin decreased microbial synthesis and left substrate fermentation unchanged. This difference may be related to their use of a nonadapted microbial population. Protein sources did differ in their degradabilities. As would be expected from the VFA concentrations, lupin grain was degraded to the greatest extent, 60.2% of the total N, which was calculated using the production of NH_3 from protein degradation and dividing it by the total amount of N present in the fermentor. Differences in degradabilities would be expected since bacteria, which make up the most metabolically active portion of the microbial population (Russell and Hespell, 1981), attack soluble proteins the most readily (Siddons and Paradine, 1981). Soybean meal contained 34.0% fermentable N and fish meal 24.8%. The low value for fish meal would be expected since it is considered a protein source which normally by-passes the rumen. These differences in protein degradation are supported by the levels of isovolatile fatty acids measured in the fermentors. Levels of these VFA are generally related to the amount of protein degraded by the microbes. The addition of monensin decreased ($P < .01$) protein degradability by 25%. Using casein as the N source, Whetstone et al. (1981) also found that monensin decreased .degradation even with an adapted microbial population. The depression in N degradation was greatest for nonadapted contents (35 vs 14%). Adapted rumen microbial populations are appar-

ently able to better compensate for the metabolic disturbances caused by ionophore addition.

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CHAPTER VI

General Discussion

In both in vivo experiments the length of adaptation to the presence of monensin was approximately the same, 19 d for period 2 in experiment 1 and 20 d in experiment 2. Monensin supplementation in both studies produced the typical effect of the ionophore on VFA production. It did not affect total VFA levels, although there was a decrease in period 3 of the first experiment. There was a similar depression (8%) in acetate concentration (moles/ 100 moles) in both experiments. The increase in propionate was smaller in experiment 1 (26 vs 47%). Butyrate levels were depressed in the two experiments. Richardson et al. (1976) reported similar effects when monensin was fed to cattle.

Infusion of monensin at the duodenum did not produce these alterations in VFA levels, which indicates that the ionophore was not recycled back into the rumen after absorption from the gastro-intestinal tract. Donoho et al. (1978) reported monensin was excreted from the body via the bile.

Monensin increased apparent N digestibility by 5.7% in experiment 1 (period 2) in contrast with the results of Poos et al. (1979) who observed no effect. Nitrogen digestibility was unchanged in experiment 2, despite an increase in the activity of trypsin at the ileum. Data from the first experiment suggest that monensin had little influence on ruminal

degradation of a readily-soluble protein source. Although there was no significant effect of monensin on ruminal NH_3 levels within each period, the ionophore did depress ($P < .05$) the flow of NH_3 at the duodenum. Muntifering et al. (1981) also observed a decrease in the abomasal flow of NH_3. Although less NH_3 may have entered the duodenum, ileal concentrations did not differ (experiment 2). None of the treatments changed the extent or site of starch degradation to any significant degree in these experiments, although monensin decreased digestion by 1% in experiment 1 (period 3). In contrast, Muntifering et al. (1981) suggested that the increase in animal performance with ionophore addition to the diet was due to an increase in the intestinal digestion of starch. These researchers, however, used a diet based on corn grain.

In the first experiment monensin increased the DM and OM digestibilities by 9 and 8%, respectively. These results are in contrast with much of the earlier work, in which it was generally reported that the ionophore decreased or left unchanged nutrient digestibilities. Beede et al. (1980) reported similar findings to ours, however, in cattle fed a low-protein diet. In the present study the site of the increase in digestion was apparently the rumen, since there was an increase in apparent ruminal degradation of OM. In experiment 2 there was no ionophore effect on the apparent DM or OM digestibility, but the sites of digestion were changed from the cecum and large intestine to before the terminal ileum. The results from the first experiment indicate that the increase in OM digestion may have occurred in the rumen. The animal would benefit from

this increase in digestibility from the changes in products resulting from the augmentation in microbial fermentation activity.

In both experiments monensin supplementation increased plasma glucose levels. In the second experiment only the infused ionophore caused this increase. The rise in glucose with monensin infusion was not associated with an increase in the amount of propionate in the rumen. Most authors believe that it is the increase in propionate concentrations which triggers the rise in plasma glucose (Schelling, 1984). The increase observed with infused ionophore may indicate that monensin acts directly on the pancreas or liver in altering glucose metabolism. Monensin supplementation appears to cause most of its alterations in ruminal microbial metabolism. However, there appear to be some postruminal effects, such as the increase in trypsin activity. The augmentation in plasma glucose levels with infused ionophore indicates a possible direct effect on the animal itself.

Adaptation does occur with ionophore supplementation. The ability of the adapted rumen contents to resist the 49% drop in microbial synthesis seen with nonadapted microorganisms and added ionophore supports this conclusion. The increase in bacterial N flow in period 3 of the first experiment suggests that bacterial adaptation also occurs in vivo. Bacteria in monensin-fed wethers initially had depressed levels of ash (20.1, period 2 vs 25.4% period 3). The recovery in period 3 may indicate that the microbes were able to develop a mechanism to counteract the ion transporting ability of the ionophore.

Total protozoal numbers were depressed an average of 60% by monensin supplementation in the first experiment. This decrease in numbers suggests that protozoa may be less able to adapt to the metabolic changes induced by monensin. This effect, however, appears to be species dependent, since the percentage of Isotricha increased during ionophore addition to the diet.

Even though adaptation occurred, many of the effects associated with ionophore supplementation continued to be expressed, such as production of increased levels of propionate and a depression in acetate and butyrate levels. These alterations were found in vivo after 146 d of supplementation. The increases in OM and DM digestibilities also continued throughout ionophore administration. Adapted rumen contents did have increased proteolytic activity in vitro. This effect was not seen in vivo since none of the parameters used to measure protein degradation in the rumen were altered after 96 d of supplementaion. It is possible that these measures of protein breakdown do not adequately reflect actual protein degradation in the rumen, which is still poorly understood.

The rapid return (within 24 h) of VFA levels to control values in the weekly VFA samples after monensin withdrawal may indicate that the ionophore has a threshold concentration which must be maintained if it is to alter microbial fermentation. Twenty-four hours after withdrawal, there still should have been a certain quantity of monensin present in the rumen, since it is improbable that rumen turnover was sufficiently fast to completely clear the rumen of the ionophore. Despite these residual amounts of monensin, VFA concentrations returned to control levels.

However, in the in vitro experiment rumen contents from monensin-fed sheep still exhibited the effects of ionophore supplementation. Rumen contents were obtained after fasting the animals overnight (16 h). Sufficient quantities of the ionophore remained to continue its effects in vitro. Once the contents were placed in the fermentors, there would be no further removal of monensin and alterations in microbial fermentation would be maintained.

If the two types of rumen contents used in the in vitro experiment are analyzed separately as would occur if nonadapted contents were the sole inoculum used, several discrepancies between adapted and nonadapted populations are revealed. Use of nonadapted rumen contents would indicate that both monensin and protein source significantly influenced fermentor pH and acetate concentrations (moles/ 100 moles). However, no effect would be seen with adapted microbes. Monensin addition to a nonadapted population masks the effect of protein source on propionate and butyrate levels (moles/ 100 moles). Isobutyrate concentrations would be affected by monensin only in nonadapted fermentors. For total gas production and the CO_2:CH_4 ratio, use of nonadapted microorganisms would indicate an effect of monensin addition while none exists in the adapted populations. For microbial synthesis the addition of the ionophore would hide the contribution of N solubility to the changes in this parameter. There were no differences between the two populations in total VFA production, isovalerate concentration and degradability of the protein sources. Several authors have also reported that results obtained with monensin added to in vitro cultures of rumen contents can vary

dependent on whether or not the inocula are adapted to the ionophore (Short, 1978; Herod et al., 1979 and de Jong and Berschauer, 1983).

These changes in the results of the different factors studied in this experiment suggest that care must be taken in interpreting the results of in vitro experiments when ionophores are studied using nonadapted microbial populations.

Microbial adaptation to monensin occurs in ruminal microbial populations. Effects of monensin supplementation on bacteria in vivo tended to disappear or be diminished as time progressed. The lack of effect of monensin addition on the amount of microbial N synthesis in vitro in adapted rumen digesta supports this conclusion. The withdrawal of the ionophore in vivo resulted in the return of fermentation parameters to control levels, suggesting that monensin must be present to maintain some of its alterations. The ionophore causes few alterations in postruminal digestion. There was, however, an increase in plasma glucose levels associated with infusion of monensin at the duodenum. Adaptation of microbial populations used in artificial culture systems can affect the results obtained in vitro. The differences are often in the intensity of the response, with adapted microbes being less susceptible to monensin-induced metabolic disturbances.

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Appendix A. Comparison of Markers for Microbial Nitrogen Flow

The samples from period 1 in the first experiment were used to compare two types of markers for the microbial populations present in the rumen. The markers chosen were diaminopimelic acid (DAPA), a marker for rumen bacteria, and the purine bases, markers present in both bacteria and protozoa. The purine bases were analyzed by two methods. One analysis was the manual spectrophotometric method, which was originally proposed by Zinn and Owens (1980), and then modified by Ushida et al. (1985). This method gives the total of the purine bases present in the sample. The second analysis was using high performance liquid chromatography (HPLC) (Ushida et al., 1985) which allows the individual quantification of each purine base. The double marker system (Faichney, 1975) was used in this experiment to calculate the flow of microbial N at the duodenum. This method corrects for unrepresentative sampling of digesta contents, which can occur when simple cannulae are used. In addition, flow values were calculated using Cr_2O_3 as the single marker. The interest in using a single marker system is due to the long and complicated analysis for DAPA, where only two samples can be completed each day on one ion exchange column. The double marker system requires the analysis of the liquid and solid phases of digesta in order to calculate flow, which effectively doubles the number of samples to be analyzed. Use of a single marker requires only one analysis of the

digesta, which is important if large numbers of samples need to be processed.

The samples used to make these comparisons were taken before any of the sheep received monensin. There were seven wethers from which it was possible to obtain all the necessary samples, total duodenal digesta and an isolated fraction of liquid phase bacteria. The statistical analysis was accomplished using SAS (1985). The model included marker, animal, type of calculation and marker x calculation interaction. The markers were compared using Tukeys (1953) test.

The system of calculation had no effect ($P > .15$) on microbial flow at the duodenum. The lack of difference between the two methods indicates that the samples were representative of the digesta passing at the duodenum. There was also no interaction ($P > .90$) between the markers and method of calculation. Comparison of DAPA with the purine bases is not technically correct since DAPA accounts only for bacteria, while the purines are markers for both bacteria and protozoa. However, DAPA was chosen as the standard for these comparisons. None of the purine markers, manual method, individual bases (adenine, guanine, thymine and cytosine) or the total of the HPLC-determined bases differed significantly from values calculated using DAPA (table 30). Cytosine consistently gave higher values ($P < .05$) than adenine and guanine. Guanine gave the lowest estimates of microbial flow at the duodenum.

The similarity of results using the purine values to DAPA suggests that the flow of protozoa from the rumen in this study was not sufficient to cause an increase in microbial flow. Since only approximately 20% of

Table 30. LEAST SQUARE MEANS FOR MICROBIAL NITROGEN FLOW[a]

		HPLC Purine Bases					
DAPA	Man[b]	Adenine	Cytosine	Guanine	Thymine	Total	SE
14.3[c]	14.5[c]	13.1[cd]	16.1[ce]	12.5[cd]	14.1[c]	14.3[c]	.47

[a] g/ d.
[b] Purine bases determined with method of Ushida et al., 1985.
[c,d,e] Means with different superscripts differ (P <.05).

the protozoal population leave the rumen (Jouany, personal communication) it is possible that their contribution to microbial N at the duodenum would be small. There was also little feed contamination of the duodenal samples. One problem with the use of purine bases as markers is that they are present in some dietary ingredients. If a sufficient quantity of feed bypasses rumen fermentation, it could lead to an overestimation of microbial-N flow. This factor did not appear to be a problem in this study.

Cytosine has been proposed as a microbial marker (Schelling et al, 1984), but our results show that high values for microbial N flow are obtained with this marker. Guanine appears to underestimate microbial N, as may adenine, although to a lesser extent. Although use of thymine gives a good estimate of microbial N, thymine is often difficult to resolve from other peaks in the HPLC analysis (B. Lassalas, personal communication). The manual spectrophotometric method and total HPLC purine bases are in close agreement with each other and with DAPA values. One point that should be noted is that the effect of animal was significant in this study. Some authors use standard equations to calculate microbial flow instead of isolating microorganisms from the rumen. Since animal did have an effect on calculated flows, this suggests that it is necessary to isolate rumen microbes from individual animals rather than use standard equations to avoid under- or overestimating microbial N flow.

Appendix B. Examples of Analysis of Variance

Appendix Table 1. ADAPTATION AND WITHDRAWAL EXPERIMENT

Source	DF
Group	1
Animals within group	6
Period	3
Group x Period	3
Error	15
Total	28

Group was tested using animals within group as the error term.

Appendix Table 2. POSTRUMINAL DIGESTION EXPERIMENT

Source	DF
Period	2
Animals	4
Treatment	2
Error	6
Total	14

Appendix Table 3. IN VITRO EXPERIMENT

Source	DF
Rumen contents	1
Batch within rumen contents	6
Nitrogen source	3
Monensin	1
Nitrogen x monensin	3
Contents x nitrogen	3
Contents x monensin	1
Contents x nitrogen x monensin	3
Error	42
Total	63

Rumen contents were tested using batch within rumen contents as the error term.

Appendix C. Miscellaneous Tables

Appendix Table 4. THE EFFECT OF MONENSIN SUPPLEMENTATION AND WITHDRAWAL ON DUODENAL FLOW OF MICROBIAL NITROGEN CALCULATED USING PURINE BASES

	Treatment by periods								
	1		2		3		4		
Item	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	Con[a]	Mon[a]	SE[b]
Flow, g/ d									
Cytosine	20.6	13.7	16.2	16.2	20.6	17.9	17.7	20.1	1.5
Guanine	14.7	11.1	13.0	11.1	15.2	12.1	16.2	16.4	1.1
Thymine	15.7	13.0	15.6	13.0	18.4	20.9	10.3	15.3	1.4
Adenine	17.5	11.2	14.4	14.1	14.7	14.1	15.5	15.1	1.7
Total	17.7	12.4	14.9	13.9	17.7	15.8	15.6	17.5	1.2

[a] Con-control; Mon-monensin.
[b] Standard error of means.

Appendix Table 5. THE EFFECT OF MONENSIN ADDITION AND WITHDRAWAL ON ACETATE LEVELS[a] IN WEEKLY RUMEN SAMPLES

Week	Treatment		SE[b]
	Monensin	Control	
1	52.3	63.6*	.6
2	53.2	62.4*	.6
3	--	--	
4	--	--	
5	54.1	60.7*	.6
6	53.8	59.9*	.6
7	55.1	61.5*	.6
8	56.6	62.2*	.7
9	57.1	59.8*	.6
10	55.9	60.3*	.6
11	55.5	59.9*	.6
12	57.0	61.1*	.6
13	--	--	
14	--	--	
15	56.1	62.6*	.7
16	56.5	62.7*	.6
17	56.7	63.4*	.6
18	57.7	61.8*	.6
19	57.4	63.7*	.6
20	57.3	62.5*	.6
21	58.5	62.5*	.6
22	61.5	62.2	.6
23	60.9	61.3	.6
24	--	--	
25	--	--	
26	63.9	62.1	.7
27	63.5	63.6	.7
28	63.8	57.9	.7

[a] Moles/ 100 moles.
[b] Standard error of means.
* Monensin and control groups differ that wk ($P < .05$).
These data are also presented graphically in figure 3.

Appendix Table 6. THE EFFECT OF MONENSIN SUPPLEMENTATION AND WITHDRAWAL ON PROPIONATE LEVELS[a] IN WEEKLY RUMEN SAMPLES

Week	Treatment		SE[b]
	Monensin	Control	
1	36.8	21.3*	.8
2	34.9	20.0*	.8
3	--	--	
4	--	--	
5	31.9	22.6*	.8
6	33.2	24.5*	.8
7	31.1	20.2*	.9
8	31.4	20.6*	.8
9	28.7	23.2*	.8
10	29.6	21.1*	.8
11	29.8	19.7*	.8
12	28.2	18.7*	.9
13	--	--	
14	--	--	
15	28.6	19.5*	.8
16	28.3	18.3*	.8
17	29.0	19.1*	.8
18	27.8	19.3*	.8
19	29.8	20.3*	.8
20	30.4	19.4*	.8
21	27.5	19.2*	.8
22	20.7	19.8	.8
23	19.0	19.4	.8
24	--	--	
25	--	--	
26	16.8	18.5	.9
27	18.4	18.8	1.0
28	18.8	22.4	1.0

[a] Moles/ 100 moles.
[b] Standard error of means.
* Monensin and control groups differ that wk ($P < .05$).
These data are also presented graphically in figure 4.

Appendix Table 7. THE EFFECT OF MONENSIN ADDITION AND WITHDRAWAL ON BUTYRATE LEVELS[a] IN WEEKLY RUMEN SAMPLES

Week	Treatment		SE[b]
	Monensin	Control	
1	7.5	10.7*	.5
2	8.2	12.9*	.5
3	--	--	
4	--	--	
5	10.3	12.6*	.5
6	9.5	11.7*	.5
7	9.3	13.0*	.5
8	8.3	12.6*	.5
9	9.9	12.4*	.5
10	10.2	13.1*	.5
11	10.0	14.6*	.5
12	10.4	14.6*	.5
13	--	--	
14	--	--	
15	11.2	13.1*	.5
16	11.1	14.0*	.5
17	10.0	12.8*	.5
18	9.6	13.5*	.5
19	8.5	11.9*	.5
20	8.5	13.0*	.5
21	9.4	13.2*	.5
22	12.0	13.2	.5
23	14.1	13.8	.5
24	--	--	
25	--	--	
26	13.5	14.7	.5
27	12.4	13.1	.6
28	11.9	14.9	.6

[a] Moles/ 100 moles.
[b] Standard error of means.
* Monensin and control groups differ that wk ($P < .05$).
These data are also presented graphically in figure 5.

Appendix Table 8. THE EFFECT OF MONENSIN SUPPLEMENTATION AND WITHDRAWAL ON TOTAL VFA CONCENTRATIONS[a] IN WEEKLY RUMEN SAMPLES

Week	Treatment		SE[b]
	Monensin	Control	
1	74.3	77.3	4.9
2	69.6	78.6	4.9
3	--	--	
4	--	--	
5	68.3	104.5*	4.9
6	76.5	80.0	4.9
7	65.3	61.0	4.9
8	57.1	62.3	5.3
9	62.9	64.1	4.9
10	77.6	60.0*	4.9
11	55.8	59.3	4.9
12	93.5	127.3*	4.9
13	--	--	
14	--	--	
15	139.3	183.1*	5.3
16	103.8	107.7	4.9
17	103.2	129.2*	4.9
18	87.9	103.3*	4.9
19	120.8	100.6*	4.9
20	80.8	74.4	4.9
21	64.4	73.6*	4.9
22	77.5	81.5	4.9
23	79.4	81.0	4.9
24	--	--	
25	--	--	
26	93.9	94.4	5.3
27	73.0	77.3	5.7
28	94.3	88.4	5.7

[a] Mmoles/ ml.
[b] Standard error of means.
* Monensin and control groups differ that wk (P <.05).
These data are also presented graphically in figure 2.

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