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MONENSIN AND NITROGEN UTILIZATION BY STEERS FED CONCENTRATE DIETS

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MONENSIN AND NITROGEN UTILIZATION
BY STEERS FED CONCENTRATE DIETS

by

Russell Brian Muntifering

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Russell Brian Muntifering

entitled Monensin and Nitrogen Utilization by Steers Fed Concentrate Diets

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

C. Brent Theurer — April 21, 1980

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STATEMENT BY AUTHOR

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SIGNED: Russell Brian Muntifering

To my parents,

to whom I owe everything

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ABSTRACT

Monensin was fed at levels of 0 and 33 ppm in a series of experiments to determine its effect on nitrogen (N) utilization by steers fed diets high in sorghum grain (76%, steam-processed, flaked) and corn (90%, whole shelled).

Total ruminal volatile fatty acid (VFA) concentrations measured 3 hr postprandially were not affected by monensin addition to either diet. Molar proportion of acetic acid decreased ($P < .05$) and that of propionic acid increased ($P < .05$) in response to monensin when fed with the corn-based diet; a similar trend ($P > .05$) was observed with monensin addition to the sorghum grain-based diet.

Monensin had no effect on apparent total tract digestibility of dry matter or energy in either diet, but consistently improved apparent total tract digestibility of crude protein (CP) in both diets. Improvement was greater ($P < .05$) for the sorghum grain-based diet (4%); less improvement (2 to 3%) was observed for the corn-based diet ($P > .05$). Retention of N tended ($P > .05$) to improve in response to monensin with the sorghum grain-based diet (24 _vs_ 20% of N intake; 41 _vs_ 36% of N absorbed).

Ruminal ammonia concentrations measured 3 hr postprandially were decreased ($P < .10$) by monensin with the

corn-based diet, reflecting decreased ($P > .10$) ruminal digestion of feed N (44 *vs* 51%) for steers fed monensin. Abomasal passage of ammonia N was decreased ($P < .10$) by monensin addition to the corn-based diet.

Monensin decreased ($P < .10$) ruminal true digestion of organic matter (OM_C, corrected for *de novo* bacterial cell synthesis) with the corn-based diet (39 *vs* 50%), and could be almost entirely accounted for by decreased ($P < .10$) ruminal digestion of starch (61 *vs* 50%). Monensin had no effect on apparent total tract digestibility of OM or starch. Monensin decreased ($P < .07$) the contribution of bacterial N (52 *vs* 58%) and increased ($P < .06$) that of ruminally undegraded feed (bypass) N (46 *vs* 40%) to total abomasal N, but had no effect on passage of total abomasal N or amino acids. Efficiency of bacterial protein synthesis was unchanged, and averaged 15 g bacterial CP/100 g ruminally digested OM_C.

Monensin decreased ($P < .05$) the contribution of bacterial N (42 *vs* 50%) and increased ($P < .05$) that of ruminally undegraded feed (bypass) N (58 *vs* 50%) to total N digested postruminally, resulting in less ($P < .10$) bacterial N (23 *vs* 28 g/day) and a tendency ($P > .10$) for more feed bypass N (32 *vs* 27 g/day) to be digested in the intestines of steers fed monensin with the corn-based diet.

That monensin caused a greater proportion of feed OM and N to be digested and absorbed in the intestines than in the rumen (with possibly greater resultant metabolic efficiency) may account for some of the benefit of feeding this compound with high grain diets, as losses incurred in the ruminal transformation of feed nitrogen to bacterial nitrogen appear to be partially eliminated.

CHAPTER 1

INTRODUCTION

The ruminant animal occupies a unique niche in agricultural systems by virtue of its symbiotic relationship with the anaerobic microflora of the reticulo-rumen. Hungate (1966) has likened rumen microbial fermentation to a continuous, anaerobic microbial culture possessing all the characteristics of a continuous system: substrate supply, end product removal, buffering capacity, temperature control and growth rate (dilution) regulation. By means of rumen microbial fermentation, β-linked polysaccharides may be degraded into substrates useable by the host animal. Nitrogen from non-protein sources may be incorporated into microbial cells. The ruminant system clearly provides a means of producing human food from materials that cannot be used by man directly. Unfortunately, the system is also a delimiter of agricultural production because of the anaerobic nature of rumen microbial fermentation.

Anaerobiosis entails obligatory losses of energy as heat of fermentation and as methane. Stoichiometric estimates indicate that the recoverable energy (ATP) obtained from the anaerobic fermentation of hexose represents only 10 to 12% of the aerobic potential to derive free energy

(Hungate, 1966). Accompanying this energy loss is a loss of amino-nitrogen as ammonia. In exchange for maintaining the rumen microbial capacity to produce food from fibrous feeds and non-protein nitrogen (NPN), productive efficiency of the host is compromised. Because the ever-increasing demand for meat and milk from ruminants will likely continue, manipulation of rumen fermentation could provide a means of optimizing animal productivity. The strategies employed must consider the extent to which rumen fermentation can provide nutrients to the host as well as the merits of regulating and/or bypassing rumen fermentation to obtain most efficient utilization of feedstuffs.

Fermentation of glucose and pyruvate to volatile fatty acids (VFA) in the rumen is accompanied by the production of hydrogen. Disposal of hydrogen and regeneration of reduced cofactors are both essential for obtaining maximum efficiency from ruminal energy transactions. Most metabolic hydrogen in the rumen is used in the formation of propionic and butyric acids and in the reduction of carbon dioxide to methane (Leng, 1970). Because the energetic efficiencies of converting hexose to acetate, propionate and butyrate are 62, 109 and 78%, respectively, the energy recoverable as VFA from hexose may be increased by diverting hydrogen normally reposited in methane into propionate and butyrate. A variety of chemical agents have been used to increase propionate and butyrate production at the expense of methanogenesis by this mechanism, most notably chloral starch (Chalupa, 1975) and halomethane analogs (Trei, Singh and Scott, 1970). Interest in these compounds as fermentation modulators has declined in recent years, largely due to inconsistent responses in performance trials with sheep and cattle (Chalupa, 1977).

Loss of potential energy from anaerobic fermentation is accompanied by loss of amino-nitrogen as ammonia. The ruminal microflora degrades dietary protein to amino acids which are in turn fermented to ammonia, carbon dioxide and organic acids (Hungate, 1966). Ammonia nitrogen that is not utilized to manufacture new microbial protein represents a loss to the host animal, as it is largely absorbed from the rumen and excreted by the kidneys as urea. At a given energy input, the output of microbial protein may be insufficient to meet the requirements of highly productive animals. Increasing the outflow and balance of amino-nitrogen from the rumen might then involve minimizing ruminal degradation of dietary protein and maximizing the capacity to synthesize protein from NPN.

Regulation of microbial proteases and deaminase activity has been accomplished by various antibiotics (Schelling et al., 1973) and by diaryl iodonium compounds (Chalupa, Chow and Parish, 1976). Nevertheless, use of these materials has not consistently improved nitrogen utilization. Attempts to maximize NPN utilization by controlling rates of ammonia release with urease inhibitors or with frequent urea feeding have produced variable and conflicting results (Chalupa, 1972; Pitzen et al., 1973). Remaining interest in these compounds as possible modulators of ruminal nitrogen transactions is largely academic.

Monensin sodium was approved by the Food and Drug Administration in 1975 as an additive for cattle fed in confinement for slaughter at levels of 5.5 to 33 mg/kg of complete feed or 50 to 360 mg/animal/day. A biologically active compound produced by a strain of *Streptomyces cinnamonensis* (Haney and Hoehn, 1967), monensin has consistently improved the efficiency of feed utilization in cattle by 8 to 12% when incorporated into complete feeds at 33 ppm (Anonymous, 1975). While the exact mechanism(s) by which this compound elicits its effect is unknown, it is the only approved chemical agent to date which has been shown to modulate various biochemical transactions occurring in rumen fermentation.

It is generally accepted that monensin improves energetic efficiency of rumen fermentation by increasing the production of propionic acid while decreasing the production of acetic and butyric acids (Richardson et al., 1976), but this does not satisfactorily account for all of the improvement in feed efficiency due to this compound (Raun et al., 1976). Increased propionate may also lower heat increment (Blaxter, 1962), spare amino acids that would normally be used for gluconeogenesis (Reilly and Ford, 1971) and stimulate body protein synthesis (Eskeland, Pfander and Preston, 1974).

Recent work suggests that monensin reduces ruminal protein degradation and/or amino acid deamination, resulting in improved utilization of dietary protein in some instances. Poos, Hanson and Klopfenstein (1979) observed monensin decreased outflow of bacterial nitrogen and increased passage of ruminally undegraded feed nitrogen when fed with diets containing extremes of supplemental high bypass protein and NPN. Monensin has also been observed to improve crude protein digestibility and nitrogen retention by steers fed forage (Dinius, Simpson and Marsh, 1976). The effect of monensin on ruminal conversion of feed protein to microbial protein and on subsequent utilization of absorbed nitrogen by concentrate-fed steers has not been established. Such information is critical because concentrates (i.e., cereal grains) furnish most of the crude protein equivalence (CPE) in typical growing-finishing diets for feedlot cattle, especially in the Southwest.

The investigations reported herein were conducted to determine the effect of monensin on various aspects of nitrogen utilization by steers fed concentrate diets, including (1) crude protein digestibility and post-absorptive utilization of nitrogen, (2) site (ruminal vs postruminal) and extent of protein digestion and (3) efficiency of bacterial protein synthesis in vivo. These studies constitute one of the first definitive attempts to identify the role of monensin in the nitrogen economy of beef cattle fed high grain diets.

CHAPTER 2

LITERATURE REVIEW

Monensin and Nitrogen Utilization

Beef Cattle Performance

That monensin may improve nitrogen utilization was first indicated in performance studies with growing-finishing steers and heifers (Potter, Raun et al., 1976). In three separate experiments, cattle were fed 0, 5.5, 11, 22, 33 and 44 ppm monensin in pre-conditioning diets containing 11.9 to 13.0% crude protein (CP) on a dry matter (DM) basis. Across all experiments and levels of monensin, carcass composition as well as gains in fat, lean and bone were similar. Cattle fed monensin consumed less digestible energy (DE) and CP than controls ($P < .01$), hence retained a greater percentage of dietary energy (7 to 14%) and protein (9 to 19%). Since monensin did not alter carcass composition, the improvements in feed efficiency observed in these studies (10 to 17%) were not the result of altered energy density in the carcass, but reflect a more efficient utilization of feed energy for carcass gain. The real significance of the improved protein utilization is not readily interpretable since the dietary CP levels were certainly not expected to limit animal performance.

In another trial (Potter et al., 1978), steers were factorially assigned to receive the following levels of monensin in isocaloric and isonitrogenous corn or barley rations with or without a single implant of diethylstilbesterol (DES): 0 ppm, 33 ppm, 11 ppm for 7 days then 33 ppm, or 11 ppm for 21 days then 33 ppm. Monensin did not appear to interact with either DES or grain source, and improved rate of gain ($P = .02$), feed efficiency ($P < .0001$) and carcass energy deposition ($P = .016$) as a percentage of dietary energy intake. Carcass protein retention as a percentage of dietary protein intake was significantly ($P = .01$) improved by DES, and tended to be greater for steers fed monensin.

Byers and Moffitt (1977) investigated possible growth pattern modifications with DES and monensin. Steers previously wintered on low or moderate nutritional planes were factorially assigned to receive no treatment, DES implant (24 mg) or monensin (200 mg/head/day). Haylage and shelled corn were fed *ad libitum* with either a basal protein-mineral supplement or .9 kg soybean meal. In contrast to results of previous studies, monensin altered composition of gain across all experimental factors, primarily by increasing fat percentage (4%). DES altered composition of gain by increasing rate of protein deposition by 37%. A slight improvement in rate of protein deposition for steers fed monensin was entirely accounted for by slightly heavier carcasses at slaughter, as carcass protein percentage was unchanged. Whether protein deposition as a percentage of protein intake was improved by monensin could not be discerned, as feed intakes were not reported in this study.

The available data suggest that monensin might alter composition of growth and/or efficiency of converting dietary protein to carcass protein. This may explain why monensin improves rate of gain in forage-fed cattle without affecting intake (Potter, Cooley et al., 1976). It would not appear that monensin acts by the same mechanism of action as anabolic agents (e.g., DES) which increase the biological potential for protein deposition.

In addition to the studies previously discussed in which monensin was theorized to possibly spare dietary protein on the basis of altered composition and/or growth patterns, most performance trial data supporting this contention have indicated interactions of monensin with levels and/or sources of dietary protein.

Davis and Erhart (1976) conducted a 120-day finishing trial to determine whether monensin had a urea-sparing effect. Steers were fed a corn and corn silage ration from which supplemental urea was withdrawn after 0, 42, 84 or 120 days. On a DM basis, urea-supplemented rations contained 11.5% CP, whereas those without urea contained 9.5% CP. Monensin levels were 0 ppm (120 days), 11 ppm for 7 days then 33 ppm for 113 days, 11 ppm for 21 days then 33 ppm for

99 days, and 33 ppm (120 days). Rate or efficiency of gain was not appreciably altered by urea unless monensin was fed. Monensin improved efficiency of urea-containing rations by an average of 18% and of rations without urea by 8%. While the data do not indicate an unequivocal urea-sparing effect of monensin, they did show that steers fed monensin gained more rapidly and efficiently when their rations contained urea. The authors concluded that urea was more efficiently utilized when fed with monensin, but that the efficiency of urea utilization may have been more closely related to absolute consumption of crude protein.

A 116-day growth trial was conducted by Gates and Embry (1977) to evaluate the effect of monensin on utilization of an unsupplemented corn and corn silage ration containing 10.5% CP (DM basis) or the same ration supplemented to 13.1% CP (DM basis) with soybean meal or to 12.6% CP (DM basis) with urea. Monensin additions improved feed efficiency in all treatments, but the magnitude of improvement was greater with no supplemental protein (19%) and urea (18%) than with soybean meal (10%). Protein source effects were somewhat confounded by different protein intakes in this trial, so definitive conclusions could not be made. Urea was more efficiently utilized when fed with monensin, but in contrast to the findings of Davis and Erhart (1976), steers fed monensin with the basal ration performed no better when supplemented with urea.

Perry et al. (1979) conducted two separate trials to study monensin, ammonia-mineral-suspension (AMS) silage and protein level effects. In Trial 1, heifers were fed limited corn silage, ensiled high-moisture corn and three different levels of a protein supplement with 0 or 150 mg monensin/head/day. Monensin improved feed efficiency by 11% across all protein levels and did not appear to interact with level of crude protein intake (445, 536 or 626 g/day), which ranged below and above the recommended level of 600 g/day for 250-kg heifers gaining 0.8 kg per day (NRC, 1976). In a subsequent trial in which steer calves were fed either AMS silage plus corn or regular corn silage plus corn and a protein supplement, monensin at 200 mg/head/day improved feed efficiency more when fed with AMS silage than with supplemented regular corn silage. Crude protein intakes were approximately equal between treatments. The authors concluded that the differential response of silage types to monensin was due to the nature of the NPN in the rations (ammonium lactate for AMS vs urea in the protein supplement). While these trials failed to establish a true protein sparing effect owing to monensin, they served to demonstrate the capacity for monensin to interact with different types of protein supplementation. Other studies have shown that urea-supplemented diets are not as efficiently utilized as natural protein-supplemented diets when fed with monensin.

In a 127-day growth study, Hanson and Klopfenstein (1979) evaluated the performance response to monensin when supplementing corn silage-corn husklage diets with two levels and two sources of protein. Growing cattle were factorially assigned to receive 0 or 200 mg monensin per head daily in diets supplemented to either 10.5 or 12.5% CP (DM basis) with brewers dried grains or urea. Cattle fed brewers dried grains with monensin gained faster and were more efficient than cattle fed brewers dried grains without monensin, but the improvement was significant ($P < .05$) only for the lower protein group (10.5%). Monensin addition to either level of urea supplementation had no effect on rate or efficiency of gain. These data suggested a protein sparing effect by monensin in low protein diets containing natural protein supplements, but contradicted earlier studies involving monensin-urea combinations (Davis and Erhart, 1976). The authors contended that the apparent urea-sparing effect observed by Davis and Erhart (1976) was in fact a natural protein sparing effect because the diets used in the earlier trials contained substantial amounts of plant protein and less than 1% urea (DM basis). Urea content of diets fed by Hanson and Klopfenstein (1979) were 1.3 or 2.1% of dry matter. Since the natural protein supplements supported improved beef cattle performance while the urea supplements did not, protein reaching the lower gastrointestinal tract may have been a limiting factor for gain and feed efficiency with urea-supplemented diets.

In another 120-day trial of Hanson and Klopfenstein (1979), growing steers were fed 0 or 33 ppm monensin in a corn silage-high moisture corn diet supplemented to either 11.1 or 13.1% CP (DM basis) with soybean meal. Monensin supported 8.1% improvement in feed efficiency with the 11.1% CP diet, but only a 3.2% improvement with the 13.1% CP diet. This reinforced their earlier contention that monensin spares protein in natural protein-supplemented diets at lower levels of dietary protein.

The superiority of natural or preformed protein over NPN supplements fed in combination with monensin was also demonstrated in a range beef cow maintenance study (Lemenager, Owens and Totusek, 1978). Seventy-eight mature Angus and Hereford cows were maintained on native tallgrass range in central Oklahoma for 107 days, and were fed 0 or 200 mg monensin per head daily with a 15 or 30% CP supplement based on soybean meal or a 30% CP supplement based on extruded urea-grain (Starea 44). Monensin did not affect weight loss of cows, but tended to partially offset weight loss in the soybean meal-supplemented group, suggesting a possible protein sparing by monensin at low levels of dietary protein containing predominantly natural or preformed protein.

Monensin offset loss of gain and performance efficiency in a supplemental protein withdrawal trial conducted by Dartt, Boling and Bradley (1978). In a 168-day trial with growing steers, monensin was fed at 0 or 200 mg/head/day with a diet of corn silage, ground shelled corn and soybean meal. On day 84, soybean meal was withdrawn and replaced with ground shelled corn for half of the steers in each monensin treatment. Steers fed monensin during the soybean meal withdrawal phase tended ($P > .05$) to gain weight more rapidly and were more efficient ($P < .01$) than steers not receiving monensin during this period, and approached a level of gain and feed efficiency for the entire trial that was comparable to that of steers maintained on protein supplement without monensin. An apparent sparing of corn silage and corn grain protein occurred during the soybean meal withdrawal phase for these steers.

The protein sparing action of monensin, in addition to being somewhat specific for low protein diets based primarily on natural or preformed protein sources, also appears to vary in magnitude depending on the degree of grain processing.

In a 155-day feeding trial (Gill et al., 1977) designed to evaluate possible monensin and protein level interactions, cattle were fed whole shelled corn rations supplemented with soybean meal to provide 9.5, 10.3, 11.2 or 12.3% CP (DM basis). An interaction of protein level and monensin was apparent, with monensin improving feed efficiency and rate of gain at the lower protein levels.

When three protein levels (9, 11 and 13%, DM basis) and three monensin levels (0, 16 and 33 ppm) were fed in ensiled, rolled high moisture corn diets in a later study (Gill et al., 1978), maximal improvement in feed efficiency owing to monensin occurred at the higher protein levels. The results of these trials indicate that the magnitude of protein sparing is greatest at lower protein levels when corn diets have received little or no processing, and at higher protein levels when diets are based on high moisture corn that has been ensiled and rolled. Monensin benefits with high moisture corn appear to diminish if protein levels are marginal. Differences in response to monensin between these two processing methods may have been due, more importantly, to differences in protein solubility between the two grain sources.

In summary, a protein sparing action of monensin has been suggested on the basis of data from beef cattle performance studies conducted at various experiment stations throughout the country. The magnitude of response appears to be related to the types of diets fed, level and source of dietary protein, and the extent of processing in the case of cereal grains. The response has been consistent and has had some very definite consequences in practical feeding situations.

Digestion and Metabolism

Dinius et al. (1976) published the first report suggesting a monensin response by host animal digestion and metabolism of nitrogen. Four Holstein steers (259 kg) were used in a 4 x 4 Latin square design to evaluate the effect of 0, 11, 22 and 33 ppm monensin in a 90% orchardgrass hay diet (12.8% CP, DM basis) on digestibility of dietary components and on nitrogen balance. Dry matter intake as a percentage of body weight for the treatments listed was 3.72, 3.86, 3.81 and 3.61, respectively ($P > .10$). Digestibility of dry matter, hemicellulose and cellulose were not affected by monensin, but CP digestibility tended ($P > .05$) to increase for steers fed monensin. Nitrogen retention (percentage of nitrogen intake) was highly variable, but was 51% greater ($P > .05$) for steers fed monensin.

The effect of 0 or 33 ppm monensin on digestibility and nitrogen utilization of a sorghum grain-based diet (14.6% CP, DM basis) was investigated by Tolbert and Lichtenwalner (1978) using growing steers (300 kg). Daily intakes of dry matter (5 kg), nitrogen (117 g) and water (3.2 kg) were unaffected by monensin ($P > .05$). While monensin decreased digestibility of crude fiber by 7%, it increased digestibility of DM, CP, ether extract and nitrogen-free extract by 7, 5, 16 and 5%, respectively. Surprisingly, nitrogen retention (g/day, percentage of intake and percentage of absorbed) was markedly decreased by monensin. This was due primarily to increase in urinary nitrogen output by steers fed monensin (41 vs 28 g/day), a possible reflection of higher blood urea nitrogen often observed with monensin feeding.

Beede et al. (1978) fed a low protein diet (8.5% CP, DM basis) with or without monensin (23 ppm) to growing goats (average trial weight, 15 kg). The dietary protein level was intended to be slightly deficient to facilitate detection of a possible protein sparing effect by monensin. Monensin improved CP digestibility (56.5 vs 47.9%; $P < .001$), but had no effect on digestibility of DM or gross energy, or on nitrogen balance. Monensin-fed goats excreted more urinary nitrogen than controls ($P < .05$).

Several metabolism trials have been conducted to evaluate possible interactions of monensin with dietary protein levels and/or grain processing methods as they may affect digestion and metabolism. Glenn et al. (1978) fed three levels of dietary protein (10, 13 and 16%, DM basis) with or without 16.5 ppm monensin in a balance trial with lambs (25 kg). Nitrogen digestibility was increased by monensin for lambs fed the 10 and 13% CP diets (10.0 and 11.5% improvement, respectively). Nitrogen retention was increased by monensin only with the 13% CP diet (1.25 vs .90 g/day, but was decreased with the 10% CP diet (-.23 vs .39 g/day) and the 16% CP diet (.35 vs .57 g/day).

Beede et al. (1979) used two levels of dietary protein (8.3 and 17.5%) and two levels of monensin (0 and 23 ppm) in a nitrogen balance trial with growing goats (average trial weight, 20 kg). Monensin improved nitrogen digestibility at both protein levels ($P < .05$), and improved ($P < .05$) retention of nitrogen at the higher protein level (3.1 vs 2.8 g/day). Nitrogen retention was unaffected by monensin at the lower protein level, which was unexpected by the authors.

Rust et al. (1978) fed 0 or 33 ppm monensin in whole shelled corn diets containing 9.4 or 12.2% CP (DM basis) to growing steers (284 kg) in a series of digestion and metabolism trials. Digestibility of dry matter, starch and protein were noticeably improved (4.4, 3.5 and 3.5%, respectively) by monensin in the low protein diet, but only slightly improved (1.5, 0.2 and 2.4%, respectively) in the high protein diet. Monensin improved protein true digestibility in the low protein diet (93.5 vs 90.8%), but not in the high protein diet (95.2 vs 95.1%). Monensin decreased intake by 9% without affecting daily nitrogen retention, suggesting a more efficient use of dietary protein by steers fed monensin. The authors proposed that enhanced digestibility with monensin feeding could possibly be attributed to reduced intake or meal size or longer ruminal retention time.

In another trial by Rust et al. (1979), effects and interactions of corn moisture level, dietary protein level and monensin were examined using growing steers (278 kg) in a series of digestion and metabolism trials. Rations were based on dry whole shelled or high moisture corn (11.0 and 23.0% moisture), two protein levels with added soybean meal (9.3 and 12.3% CP, DM basis) and 0 or 33 ppm monensin. Monensin decreased ($P < .025$) DM intake, and increased digestibility of organic matter ($P < .025$), starch ($P < .10$) and nitrogen ($P < .10$) across protein and corn moisture levels. Across corn moisture levels, monensin decreased nitrogen retention (g/day as well as percentage of nitrogen intake) with the low protein level diets, but increased retention with the high protein diets. Monensin appeared to benefit the dry corn diets more than the high moisture diets, supporting the results of feedlot trials previously conducted at the same station (Gill et al., 1977; Gill et al., 1978).

In two separate lamb trials, Poos et al. (1979) examined adaptation and digestibility responses to monensin by growing lambs. For Trial 1, lambs (35 kg) were fed a corn cob and sorghum grain diet supplemented to 11.5% CP (DM basis) with brewers dried grains or urea and with 0 or 30 mg monensin per head daily. The six-day digestion trial (days 11 to 17) was preceded by a very short dietary adaptation period of 10 days in which intakes were held constant between experimental groups. Monensin decreased ($P < .05$) DM digestibility in both protein source groups, but had no effect ($P > .05$) on nitrogen digestibility. Urinary nitrogen (g/day) was increased ($P < .05$) by monensin in both protein groups, resulting in lower nitrogen retention ($P < .05$) for lambs fed monensin. For Trial 2, lambs (32 kg) were fed the brewers dried grains diet with 0, 22 or 38 ppm monensin for the same period of time as in Trial 1 (10 days of dietary adaptation), and continued to be fed for an additional 30 days. Digestion and retention responses to monensin by days 11 to 17 were almost identical to those observed in Trial 1, but were not apparent by days 40 to 46. After the longer feeding period, digestion and retention values between monensin and control lambs were similar. Although not significant, the observable monensin by period interaction suggested that digestion and metabolism responses to monensin may be influenced by time on feed.

In summary, digestion and metabolism responses to monensin have been variable, and are seemingly influenced by a number of factors. It would not appear that protein sparing observed in feedlot studies can be attributed to consistent monensin improvements in digestibility and metabolism.

Blood Urea Nitrogen and Amino Acids

In two separate dose response experiments (Raun et al., 1976), steers and heifers were fed 0, 2.2, 5.5, 11, 22, 44 and 88 ppm monensin in preconditioning and finishing diets containing 9.2 to 11.8% CP and 12.6% CP (DM basis), respectively. Across both experiments, all levels of monensin decreased the molar proportion of ruminal acetate, butyrate and valerate and increased the molar proportion of propionate and isovalerate. Monensin significantly increased ($P < .05$) blood urea nitrogen (BUN) at all levels except 88 ppm. If monensin had allowed more energy to be available to the animals without changing the supply of amino acids, the BUN would have been expected to decrease. Since the opposite occurred, monensin may have increased the supply of amino acids relative to the supply of energy. Another explanation is that propionic acid increased the efficiency of cellular nitrogen utilization. Such a response to increased propionic acid is supported by Eskeland et al. (1974), who observed greater nitrogen retention and higher BUN levels in propionic acid-infused lambs than were observed in lambs infused with acetic acid.

Steen et al. (1978) investigated the effects of monensin levels on performance, feed efficiency and plasma metabolites in growing-finishing steers. Steers received corn silage *ad libitum* and were limit-fed a corn-soybean meal mixture plus 0, 100 mg, 200 mg or increasing levels (depending on air-dry feed consumption) of monensin/head/day. Monensin increased daily gains ($P < .01$) and improved feed efficiency ($P < .01$). No consistent effect of monensin on plasma amino acids was observed, but BUN tended to increase ($P > .05$) in response to monensin at all levels as well as with time on experiment.

In a similar trial at the same station (Pendlum, Boling and Bradley, 1980), growing steers were fed corn silage *ad libitum* and a soybean meal or urea supplement with 0 or 200 mg monensin/head/day. Plasma urea nitrogen (PUN) was higher ($P < .05$) for steers fed urea on days 29 and 106 of the study. Steers fed monensin had higher PUN than controls on both sampling days, but the difference was significant ($P < .05$) only on day 29. Plasma concentrations of valine, methionine, isoleucine and lysine were lower ($P < .05$) for monensin-fed steers on day 29, and all remaining essential amino acids showed a similar trend ($P > .05$). On day 106, essential individual amino acid concentrations were generally similar to controls or higher in steers fed monensin, with threonine and methionine being higher ($P < .05$) in those fed monensin. Plasma serine, glutamic acid, glutamine, alanine and ornithine were higher ($P < .05$) in steers fed monensin, and a trend toward an increase in the remaining individual nonessential amino acids was noted.

In two lamb feedlot trials (Glenn, Ely and Deweese, 1977), monensin was incorporated into diets containing 11 and 13% CP (DM basis) at 0, 5.5, 11 and 22 ppm. Plasma urea nitrogen tended to decrease with increasing levels of monensin in the 13% CP diet, and to increase with time on experiment. Poos et al. (1979) observed significant increase ($P < .01$) in PUN of lambs fed increasing levels of monensin (0,22 and 38 ppm) with brewers dried grain- or urea-supplemented diets containing 11.5% CP (DM basis). No time on experiment effect was observed in their studies.

In a subsequent study (Poos et al., 1979), Angus steers were fed 7 kg of a corn cob and sorghum grain diet supplemented to 11.5% CP (DM basis) with brewers dried grains or urea. Each ration was fed with 0 or 200 mg monensin per head daily. Plasma amino acids were not influenced either by supplemental nitrogen source or by addition of monensin. The short dietary adaptation period (10 days) used in this study should be noted.

While it is generally accepted that monensin elevates BUN, effects on concentrations of plasma amino acids are less conclusive. Possible sources of variability may relate to monensin interactions with types of diets fed, levels of dietary protein, types and levels of protein supplementation, time on feed and physiological state.

Ruminal Ammonia Nitrogen

Dinius et al. (1976) used four ruminally fistulated Holstein steers in a 4 x 4 Latin square to evaluate the effect of monensin on rumen fluid parameters. Steers were limit-fed a 90% orchardgrass hay diet with different levels of monensin for 21 days, after which ruminal fluid samples were taken hourly for 8 hours after the morning feeding. Ruminal fluid ammonia values pooled over all sampling times were 26.0, 22.7, 17.3 and 20.3 mg/100 ml for steers receiving 0, 11, 22 and 33 ppm monensin, respectively. Since cattle receiving no monensin tended ($P > .10$) to have higher ruminal ammonia levels, this may have resulted in a greater transfer of ammonia into the blood and a subsequently greater loss of urinary nitrogen than for cattle fed monensin. This may explain the tendency ($P > .05$) for improved nitrogen retention by steers fed monensin in a companion study (Dinius, et al., 1976).

A 130-day growth trial was conducted by Hanson and Klopfenstein (1979) to evaluate beef cattle response to monensin in corn silage-husklage diets supplemented with various sources and levels of protein. Steers were fed brewers dried grains or urea supplements at two levels of dietary protein (10.5 or 12.5%, DM basis) with two levels of monensin (0 or 200 mg/head/day). Ruminal ammonia nitrogen measured 5 hr postfeeding on days 127 through 130 in randomly gate-cut steers was lowered by monensin ($P < .10$) for all levels and sources of protein. The authors concluded that this indicated a possible reduction of rumen microbial proteolytic activity.

Poos et al. (1979) studied the effect of monensin on ruminal parameters in two separate lamb trials. In Trial 1, wether lambs were fed 0 or 30 mg monensin per head daily in corn cob-sorghum grain diets supplemented to 11.5% CP (DM basis) with brewers dried grains or urea. Lambs were fed for 17 days and then sampled for rumen contents 5 hr postfeeding on day 18. Monensin significantly reduced ruminal ammonia levels in both supplement groups ($P < .05$). In Trial 2, the brewers dried grains-supplemented diet was fed to wether lambs for 47 days, except that monensin was provided at levels of 0, 22 and 38 ppm. Ruminal ammonia nitrogen measured 5 hr postfeeding on days 18 and 47 was significantly reduced by monensin ($P < .01$), with no apparent sampling day interaction. Hence, the capacity for monensin to reduce ruminal ammonia nitrogen levels is apparently not affected by length of time on feed.

Whether monensin lowers ruminal ammonia by one or more of several possible mechanisms of action has not been clearly elucidated _in vivo_. In addition to decreased consumption of monensin diets, these mechanisms may include possible effects on microbial urease activity, absorption of ammonia from the rumen, protein degradation, amino acid deamination and microbial protein synthesis. Several _in vitro_ studies have been conducted in these areas.

Tolbert, Lichtenwalner and Broderick (1977) conducted an _in vitro_ trial to determine the effect of monensin on proteolytic activity. Monensin was added at 0 or 33 ppm to finely ground sorghum grain supplemented to contain 12.5% CP with soybean meal or urea. Incubations containing monensin had an average of 9.4% greater free amino acid and 25.3% lower free ammonia concentrations in the rumen fluid supernatant. Monensin depressed the free ammonia levels of the soybean meal incubations to a lesser extent than in the urea incubations. Free amino acid concentrations were decreased by 3.9% in the soybean meal incubations containing monensin, but were elevated by 16.1% in urea incubations containing monensin. These data indicated that monensin may inhibit microbial deamination of amino acids.

Using rumen inoculum from a sheep previously adapted to monensin at 22 ppm, Short et al. (1978) observed a dose-dependent ($P < .005$) reduction in ammonia nitrogen and increase in non-ammonia non-microbial nitrogen by additions of 1, 2 and 4 ppm monensin to culture flasks containing mixed rumen microorganisms. They concluded that the data supported a possible protein sparing effect of monensin.

Van Nevel and Demeyer (1977) studied the effect of monensin in the metabolism of carbohydrate and protein substrates by mixed rumen microorganisms. Monensin was added to incubation flasks at 0, 5 or 25 ppm. In incubations of cellobiose and maltose to which ammonium carbonate had been added as a nitrogen source, monensin at 5 and 25 ppm significantly lowered ($P < .01$) ammonia production by 41 and 45%, respectively. In incubations of isoelectric casein as the sole substrate, monensin lowered protein disappearance ($P < .05$) and resulted in a slightly higher ($P < .01$) accumulation of amino-nitrogen and a considerable depression ($P < .05$) in ammonia production. On the basis of these data, it was concluded that monensin inhibits deaminase activity to a greater extent than it does proteolysis. Proteolysis in the rumen proceeds faster than deamination of amino acids, hence the normal accumulation of amino-nitrogen as an intermediate (Blackburn, 1975).

The effect of protein solubility and monensin on ruminal microbial use of ammonia was investigated by Dinius (1978). Monensin was added at 0, .1, .5, 1.0 or 3.0 ppm to incubation tubes containing soybean meal or blends of fishmeal, casein or urea with starch to provide isonitrogenous substrates (66 mg N/g). Monensin levels of .5, 1.0 and 3.0 ppm reduced ammonia accumulations in the natural protein incubations, but had no effect on assimilation of ammonia into microbial protein at all levels of monensin and for all substrates. Whether the extent of ammonia production inhibition by monensin varied with protein solubility was not reported.

Observations of reduced ruminal ammonia nitrogen accumulations owing to monensin have been very consistent, suggesting that monensin may have a direct effect on nitrogen utilization.

Microbial Protein Synthesis and Feed Bypass

The depression of ruminal ammonia nitrogen accompanying monensin feeding has been interpreted as a reduction of microbial proteolytic and deaminase activity. Since ammonia is the primary nitrogen substrate with which rumen microbes produce new cellular protoplasm (Hungate, 1966), reduced ammonia nitrogen levels might limit the total extent and/or productive efficiency of rumen microbial protein synthesis.

Van Nevel and Demeyer (1977) examined the effect of monensin on total and net growth of mixed rumen bacteria *in vitro*. Monensin was added at levels of 0, 0.5, 1.0, 5.0 and 25.0 ppm to rumen fluid incubations in which cellobiose, maltose and nitrogen as ammonium bicarbonate served as growth substrates. Total microbial growth determined from ^{32}P incorporation was greater ($P < .05$) for control incubations than for those containing monensin at all levels (.97 vs .61 mg nitrogen incorporated into microbial material per 100 μmol hexose fermented). Net microbial growth determined from ammonia nitrogen incorporation appeared to be inhibited by all levels of monensin (.10 vs .26), the extent of inhibition being greater for net growth. Since total amount of hexose fermented was not altered by monensin, the resultant energy normally used for microbial maintenance and growth may have been unavailable for these functions; i.e., monensin may have uncoupled growth from fermentation. Since monensin is an ionphore, the authors proposed that monensin has an inhibitory effect on peptide transport into microbial cells.

Monensin and ruminal protein utilization was investigated *in vivo* by Owens et al. (1978). Steers (500 kg) equipped with permanent abomasal cannulae were fed a 17% CP (DM basis), 85% concentrate ration with 0 or 33 ppm monensin in a crossover design. Steers were fed 3 kg of ration twice daily during the first four days of each period, and were manually fed 500 g of ration every 2 hr (to stabilize non-ammonia nitrogen flow) for the last 3 days of each period. While monensin increased ruminal bypass of protein by 22%, total microbial protein production was unchanged. Because monensin decreased ruminal dry matter digestion by 16%, the efficiency of microbial protein synthesis (g microbial protein per 100 g DM digested in the rumen) was increased (12.1 vs 9.7). Monensin lowered ruminal liquid turnover rate by 24%, but had no effect on solids turnover rate. This does not agree with earlier observations by Lemenager, Owens, Shockey et al. (1978) that monensin lowers both ruminal liquid and solids turnover rates. If the latter were true, monensin should have increased the extent of ruminal DM digestion and decreased the efficiency of microbial protein synthesis in the trial of Owens et al. (1978). Decreasing ruminal turnover rates generally increases residence time of feed materials and decreases microbial growth rate, thus increasing digestion but compromising bacterial growth efficiency.

Tolbert et al. (1979) conducted three trials with abomasally fistulated steers to determine the effect of monensin on chemical and physical aspects of digestion. Steers were fed a 70% concentrate diet (12.5% CP, DM basis) with 0 or 33 ppm monensin and sampled for abomasal contents on two consecutive days at 0, 4, 8 and 12 hr post am feeding. Monensin increased ruminal bypass of protein by 11% ($P < .10$). In contrast to the study of Owens et al. (1978), monensin decreased bacterial protein output from the rumen by 25%. Monensin increased ruminal dry matter disappearance by 9% and decreased ruminal solids turnover rate by 17%. Values for efficiency of bacterial protein synthesis were not reported in this study, and could not be calculated because DM intakes were not given.

Monensin and microbial synthesis as affected by protein source (natural, preformed vs NPN) was investigated by Poos et al. (1979). Abomasally-fistulated steers (262 kg) were fed 7 kg of a corn cob and sorghum grain diet supplemented to 11.5% CP (DM basis) with brewers dried grains or urea. Each diet was fed with 0 or 200 mg monensin per head daily. Abomasal samples were taken twice daily for three consecutive days. Monensin increased ($P > .05$) ruminal dry matter digestion with the urea-supplemented diet, but not with the brewers dried grains diet. Nitrogen flow to the abomasum exceeded nitrogen intake in both supplement groups. Monensin decreased ($P < .05$) bacterial protein output by 33% and increased ($P < .05$) ruminal bypass of plant protein by 37 and 55%, respectively, for the brewers dried grains and urea supplemented groups, indicating a decrease in efficiency of bacterial protein synthesis. Daily abomasal nitrogen flow in excess of nitrogen intake was also observed by Owens et al. (1978), but not by Tolbert et al. (1979).

It appears that monensin consistently increases the passage of ruminally undegraded (bypass) protein. With one exception (Owens et al., 1978), this has been accompanied by a decrease in bacterial protein passage. The magnitude of this effect is undoubtedly related to the nature of dietary protein. Solubility, extent of degradability and propensity to liberate ammonia nitrogen in ruminal fluid incubations have all been demonstrated to affect the magnitude of proteolytic and deaminative inhibition by monensin *in vitro* (Tolbert et al., 1977; Dinius, 1978).

Available data regarding the effect of monensin on efficiency of microbial protein synthesis is quite variable and somewhat inconclusive. Much of this variability between studies has a readily-observable biological basis. Level of feed intake (Coelho da Silva et al., 1972), level (Hume, Moir and Sornes, 1970) and source (Hume, 1970) of dietary protein, concentrate to roughage ratios (Cole et al., 1976) and processing methods (Prigge et al., 1978) have all been clearly shown to affect efficiency of microbial protein synthesis. Experimental protocol (animal size and numbers, sampling interval and numbers, etc.) may be another source of variation. A possibly greater source may lie in the various methods used to estimate digesta flow rates and microbial protein synthesis *in vivo* (Theurer, 1979).

The effect of digesta flow markers on estimates of microbial protein synthesis has not been clearly elucidated. They must necessarily influence the estimation of efficiency of microbial protein synthesis, because ruminal digestion coefficients for organic matter and nitrogen are based on ratios of flow markers to these nutrients. Markers are generally used to estimate flow of digesta particulate matter (e.g., lignin, Cr_2O_3, Dy, Ru), liquid phase (e.g., polyethylene glycol, ^{51}Cr-EDTA) or both (dual phase).

Theurer (1979) has shown that ruminal nutrient digestion coefficients determined by reference to lignin usually exceed those obtained by reference to chromium-containing compounds (Cr_2O_3, ^{51}Cr-EDTA). Specifically in the case of protein, ruminal outflow values are generally less than that ingested when lignin is used as the reference marker, and often exceed that ingested when reference is made to some form of chromium. These differences have resulted in a greater apparent efficiency of microbial protein synthesis by reference to Cr_2O_3 than to lignin (Theurer, 1979). A dual-phase system (Faichney, 1975) does not completely overcome this problem. According to Theurer (1979), efficiency estimates may be quite variable depending on which combinations of particulate and liquid phase markers are used (e.g., lignin + polyethylene glycol vs ^{103}Ru + ^{51} Cr-EDTA).

The studies of Owens et al. (1978) and Poos et al. (1979) employed a dual-phase system of digesta flow markers, including Cr_2O_3 and polyethylene glycol (PEG). Abomasal nitrogen flow exceeded nitrogen intake in these trials. This was not observed in the studies of Tolbert et al. (1979), in which ytterbium (Yb), a rare earth element, was employed as a solids flow marker. The rare earth elements have been shown to bind to plant material and flow through the gastrointestinal tract with undigested feed residues (Ellis and Huston, 1968). Ruminal outflow of dry matter was less than that ingested in all the monensin studies discussed previously. Marker differences may have affected estimates of microbial protein synthesis insofar as estimates of abomasal nitrogen flow may have been affected.

Markers for estimating the contribution of microbial nitrogen to digesta have included α,ε-diaminopimelic acid (DAP, a bacterial cell wall constituent), 2- aminoethylphosphonic acid (AEP, a protozoal amino acid) nucleic acids (RNA, DNA or both), ^{35}S and ^{15}N labels. These five components are commonly regarded as the most useful markers presently available, yet each has inherent disadvantages.

Use of RNA has been shown to yield greater estimates (4 to 9%) of the contribution of microbial nitrogen to digesta than those based on ^{35}S incorporation (Ling and Buttery, 1978). This may be due to the presence of small amounts of RNA in feedstuffs and to interference by DNA in some analytical determinations. Smith et al. (1978) proposed the use of "modified RNA" values (RNA x .85) to bring estimates into reasonable agreement with those based on DAP and ^{32}P incorporation. Ratios of RNA-N:total N are generally less variable than DAP-N:total N for pure bacterial cultures (Smith, 1975), but this may not be the case for duodenal digesta (Ling and Buttery, 1978). The use of RNA also theoretically accomodates protozoal contributions to digesta nitrogen (Ling and Buttery, 1978; Smith, 1975).

Protozoa lack DAP, and for this reason the use of DAP as a satisfactory microbial marker has been criticized. It has been shown to yield values 30% lower than those based on ^{35}S incorporation used simultaneously (Ling and Buttery, 1978), and to agree reasonably with those based on a simultaneous use of ^{32}P (Smith, 1975). In other studies (Siddons et al., 1979), use of either RNA or DAP grossly overestimated (two-fold) the contribution of microbial protein to digesta compared to ^{35}S incorporation. In the case of DAP, this may be due to the possible presence of this amino acid in feed (Rahnema, 1977) and/or to a preferential degradation of cell contents (DAP being present in the cell wall) in the process of rumen bacterial cell turnover (Siddons et al., 1979). Clearly, microbial estimates based on RNA vs DAP under similar conditions may vary considerably.

Cost and complexity of the use of ^{15}N have limited its application. The use of AEP as a protozoal marker has been jeopardized by its recent discovery in feed materials (Ling and Buttery, 1978) and by a possibly erroneous confusion with a different amino acid possessing similar elution characteristics on sulfonated polystyrene ion-exchange resins (Rahnema, 1977).

In the monensin studies discussed previously, estimates of the proportion of digesta nitrogen that was microbial in nature ranged from 11 to 48%. This range is not surprising in view of the range of estimates possible by use of different microbial markers. Tolbert et al. (1979) did not report how estimates of bacterial nitrogen were made. Bacterial nitrogen averaged 48% of total abomasal nitrogen on the basis of DAP in the trial of Poos et al. (1979), and 36% on the basis of RNA (F. N. Owens, personal communication) in the trial of Owens et al. (1978). Marker differences among these trials may have been further exaggerated by the extremes of feed intake (body weight basis) employed, as resultant differences in ruminal turnover have been demonstrated to affect extent and efficiency of microbial protein synthesis (Cole et al., 1976). Furthermore, the assumption by Owens et al. (1978) that the RNA-N:total N ratio is constant (.10) may have invalidated otherwise meaningful between-treatment comparisons, since monensin decreased ruminal liquid turnover rate in their study. Decreased dilution rate is associated with a significant reduction in the contribution of nucleic acid nitrogen to total nitrogen in bacterial cells, and is reflective of slower growth rate (Bergen and Yokoyama, 1977). For this reason, use of universal constants (e.g., .10) to estimate bacterial protein by ratio technique should be avoided if experimental treatment effects on ruminal turnover are suspected.

In summary, only a limited number of studies have addressed themselves to possible monensin effects on microbial protein synthesis and on passage of microbial vs ruminally undegraded feed (bypass) protein *in vivo*. Upon initiation of this research, no data were available with concentrate (i.e., cereal grains) feeding regimens. For this reason, the following series of experiments were conducted to assess the role of monensin in the nitrogen economy of beef cattle fed high grain diets.

CHAPTER 3

EFFECT OF MONENSIN ON NITROGEN UTILIZATION AND DIGESTIBILITY OF CONCENTRATE DIETS BY STEERS

Summary

Monensin was fed at levels of 0 and 33 ppm in a series of digestion and metabolism trials to determine its effect on utilization of high grain diets and on ruminal parameters in yearling steers. Monensin had no effect ($P > .05$) on apparent digestibility of dry matter (DM), gross energy (GE) or starch with a 90% corn diet (10.5% CP, DM basis). Monensin in the corn-based diet tended to increase crude protein (CP) digestibility (63.4 vs 61.3%) and decrease ruminal ammonia concentration (2.5 vs 6.5 mg/100 ml) measured 3 hr postprandially ($P > .05$). In metabolism trials with a 76% sorghum grain diet (11.7% CP, DM basis), monensin improved apparent digestibility of CP ($P < .05$), but not DM or GE ($P > .05$). Retention of nitrogen (N), expressed as a percentage of N intake, tended to improve (24 vs 20%) in response to monensin addition to the sorghum grain-based diet ($P > .05$); a similar trend was observed ($P > .05$) for N retention expressed as a percentage of N absorbed (41 vs 36%). Total ruminal volatile fatty acid (VFA) concentrations measured 3 hr postprandially were not altered by monensin when fed with either diet. Molar proportion of acetic acid

decreased ($P < .05$) and that of propionic acid increased ($P < .05$) in response to monensin when fed with the corn-based diet. Monensin did not appreciably alter molar proportions of VFA when fed with the sorghum grain-based diet. Results suggest a possible improvement in N utilization may account for some of the benefits of feeding monensin with high grain diets.

Introduction

Monensin, a biologically active compound produced by a strain of *Streptomyces cinnamonensis* (Haney and Hoehn, 1967), is known to alter rumen fermentation and improve feed efficiency in growing-finishing steers. While the influence of monensin on animal performance has been well established (Perry, Beeson and Mohler, 1976; Raun et al., 1976; Boling, Bradley and Campbell, 1977), the mechanism by which this compound elicits its effect is not completely understood.

It is generally accepted that monensin improves energetic efficiency of rumen fermentation by increasing the molar proportion of propionic acid while decreasing molar proportions of acetic and butyric acids (Richardson et al, 1976). This very likely reflects an alteration of ruminal VFA production rates (Prange, Davis and Clark, 1978; Van Maanen et al, 1978). Nevertheless, shifts in molar proportions of VFA do not satisfactorily account for all the improvement in feed efficiency associated with monensin (Richardson et al., 1976). Observed ruminal responses to monensin have also included depression of methanogenesis (Thornton et al., 1976; Van Nevel and Demeyer, 1977), slower liquid and solids turnover rates (Lemenager, Owens, Shockey et al., 1978) and reduced ammonia levels (Dinius et al, 1976; Tolbert et al., 1977), indicating decreased microbial proteolysis of dietary protein and/or altered site of protein digestion (Poos et al., 1979). Other possible effects of monensin on host animal metabolism, other than those on rumen fermentation directly, have been reviewed (Richardson et al., 1976; Raun et al., 1976).

Several feeding trials have shown that monensin may spare dietary protein (Hanson and Klopfenstein, 1979; Gill et al., 1977). While monensin has shown a tendency to improve nitrogen utilization by steers fed forage (Dinius et al., 1976), a response by steers fed concentrate diets has not been established. For this reason, a series of digestion and metabolism trials was conducted to evaluate the effect of monensin on utilization of nitrogen and other dietary components by steers fed high grain diets. Preliminary report of part of this study has been made (Muntifering and Theurer, 1978).

Experimental Procedures

Digestion Trials (Corn-Based Diet)

Two digestion trials were conducted as a crossover experiment to determine the effect of monensin on digestibility of a 90% whole shelled corn diet (Table 1) containing 10.5% crude protein (dry matter basis). Eight yearling steers, initial weight 400 kg, were randomly assigned to two treatment groups to receive 0 or 33 ppm monensin in the experimental diet. In each phase of the experiment, the diet was fed *ad libitum*, twice daily, to all steers for 3 weeks. This adjustment period was followed by a 10-day preliminary phase and a 6-day total fecal collection period. Steers were housed in individual pens (2.4 x 4.9 m) and daily fecal collections were made from the concrete floors of the pens.

For preliminary and collection periods within each trial, daily intake for each steer was adjusted to 7 kg air-dry feed (approximately 90% of *ad libitum* intake). During collection periods, a 5% aliquot of daily fecal output from each steer was dried at 50 C for 48 hr in a forced-air oven. Feed samples were taken daily and dried similarly. At the end of the collection period, feed and fecal samples were each pooled to yield individual animal composites for chemical analysis.

Dry matter and nitrogen in feed and feces were determined by AOAC procedures (1970). Gross energy content of

Table 1. Diet composition (Digestion trials)[a,b]

Ingredient	Internat'l Ref. No.	%
Corn, dent yellow, grain, whole shelled	4-02-931	90.0
Cottonseed hulls	1-01-599	4.9
Soybean meal, solv-extd.	5-04-604	2.5
Limestone, grnd.	6-02-632	1.1
Alfalfa hay, s-c, mid-bloom, grnd.	1-00-063	.9
Molasses, cane	4-04-696	.3
Salt		.3
Chemical analysis:	%	
Dry matter, %	87.9	
Crude protein, %[c]	10.5	
Starch, %[c]	58.9	
Gross energy, kcal/kg[c]	4400	

[a]Air-dry basis.

[b]Vitamin A added to furnish 2,200 IU/kg.

[c]Dry matter basis.

feed and feces was determined in an adiabatic bomb calorimeter. Starch in feed and feces was determined by the acid hydrolysis method of Osman et al., (1970) as modified by Kartchner and Theurer (1980).

On the day following the termination of each collection period, a sample of rumen contents was obtained via stomach tube from each steer 3 hr after the am feeding. Rumen fluid was separated from whole digesta using four layers of cheesecloth, and microbial activity was terminated by addition of saturated mercuric chloride solution (1 ml/100 ml rumen fluid). Rumen fluid was immediately analyzed for ammonia nitrogen by the micro-diffusion technique of Seligson and Seligson (1951). A portion of rumen fluid was deproteinized with 25% w/v meta phosphoric acid (1 ml/5 ml rumen fluid), centrifuged at 1000 rcf for 10 min, filtered through no. 1 Whatman paper and analyzed for VFA by gas chromatography (Bristol, 1972). Data were analyzed as a crossover design by analysis of variance (Steel and Torrie, 1960).

Metabolism Trials (Sorghum Grain-Based Diet)

Four metabolism trials were conducted as two crossover experiments to determine the effect of monensin on nitrogen utilization by steers fed a 76% sorghum grain diet (Table 2) containing 11.7% crude protein (dry matter basis). Eight yearling steers, initial weight 259 kg, were randomly assigned to two treatment groups to receive 0 or 33 ppm

Table 2. Diet composition (Metabolism trials)[a,b]

Ingredient	Internat'l Ref. No.	%
Sorghum grain, steam-processed, flaked	4-04-444	75.8
Alfalfa hay, s-c, mid-bloom, grnd.	1-00-063	16.0
Molasses, cane	4-04-696	5.0
Tallow	4-04-409	2.0
Salt		.5
Limestone, grnd.	6-02-632	.4
Dicalcium phosphate	6-01-080	.3
Chemical analysis:	%	
Dry matter, %	85.5	
Crude protein, %[c]	11.7	
Gross energy, kcal/kg[c]	4450	

[a]Air-dry basis.

[b]Vitamin A added to furnigh 2,200/IU/kg.

[c]Dry matter basis.

monensin in the experimental diet. Feeding protocol during adjustment periods was identical to that of the digestion trials. For preliminary and collection periods, daily intake for each steer was adjusted to approximately 90% of the daily voluntary intake measured during the last week of the 3-week adjustment period. Steers were maintained in metabolism crates for both the 10-day preliminary phase and the 6-day quantitative collection of urine and feces. Two steers became injured after Trial 1, so only six were crossed over to opposite treatments for Trial 2.

This experiment was repeated with another group of eight steers, initial weight 257 kilograms. One steer was unable to proceed into the second half of the crossover due to sickness, and was replaced by another steer. Combining all four trials from the two crossover experiments resulted in a total of 15 animal observations per treatment. Sampling protocol and analytical procedures for determination of proximate components in animal composites of feed and feces were identical to those employed in the digestion trials. Urine was collected in plastic containers to which 25 ml hydrochloric acid diluted 1:2 with water had been added. Daily urine collections from each steer were filtered through cheesecloth and a 2% aliquot was saved and refrigerated. Urine samples from each steer were pooled and analyzed for Kjeldahl nitrogen. Rumen fluid sampling protocol and VFA determinations were as previously outlined. Data from all trials were pooled and analyzed by analysis of variance using the method of least squares (Steel and Torrie, 1960).

Results and Discussion

Digestion Trials (Corn-Based Diet)

Monensin had no effect (P > .05) on apparent digestibility of dry matter, gross energy or starch in the corn-based diet (Table 3). Digestibility of crude protein tended to be higher for steers fed monensin, but the difference was not significant (P > .05). In other trials with corn-based diets (Rust et al., 1978), digestibilities of dry matter, crude protein and starch were markedly improved by monensin when fed with a low protein diet (9.4%, dry matter basis), but were only slightly improved when fed with a high protein diet (12.2%, dry matter basis). Our inability to detect a significant improvement in digestibility due to monensin in these studies may have been related to the level of crude protein in the diet as well as to the low level of dry matter and protein intake by both treatment groups (Table 3). In our studies, feed intake was maintained at a constant level between treatment groups to avoid possible confounding of digestibility with feeding level, whereas cattle fed monensin consumed 9% less feed than controls in the trial of Rust et al., (1978). In that trial, the capacity for monensin to

Table 3. Mean dry matter intake and apparent digestibility by steers fed a corn-based diet[a]

Item	Dietary monensin, ppm 0	33	SD
Dry matter intake/day			
Total, g	6218	6224	6.4
Total, $g/W_{kg}^{.75}$	68.9	67.7	.9
Apparent digestibility, %			
Dry matter	76.3	75.9	2.7
Energy	74.6	74.9	2.8
Crude protein	61.3	63.4	3.3
Starch	90.4	90.6	1.8

[a]All values are means of eight observations.

improve digestibility may have been related in part to the lower feed intake by cattle fed monensin, and to a possibly longer ruminal retention time (Lemenager, Owens, Shockey et al., 1978).

Gill et al. (1977) found that improvement in utilization of whole corn diets due to monensin varied with the level of dietary crude protein, with maximal response occurring when the protein level was 10.3% (dry matter basis). Since the protein percentage in our trial was similar to that of Gill et al. (1977), it would not appear that improvement in performance by monensin at this percentage of dietary protein can be attributed to a consistent improvement in ration digestibility. Total protein intake as it relates to animal performance may be a more important consideration.

Total ruminal VFA concentration was not altered by monensin (Table 4), although molar proportion of acetic acid decreased ($P < .05$) and that of propionic acid increased ($P < .05$). This shift in molar fractions of VFA is in agreement with results of other trials with whole shelled corn diets (Gill et al., 1977). Propionic acid is a major precursor of glucose in the ruminant animal, and an increase in ruminal propionate may spare amino acids that would otherwise be deaminated in body tissues for gluconeogenesis (Eskeland et al., 1974; Van Maanen et al., 1978). Ruminal ammonia levels, although not significantly different between treatments ($P > .05$), tended to be lower for steers fed

Table 4. Ruminal VFA and ammonia nitrogen values for steers fed a corn-based diet[a,b]

Item	Dietary monensin, ppm 0	33	SD
VFA			
Total, μmole/ml	70.2	72.1	18.1
C_2, %	53.6	50.2*	2.7
C_3, %	25.3	31.9*	3.6
C_4, %	12.4	11.6	2.9
Ammonia, mg/100 ml	6.5	2.5	3.5

[a]All values are means of eight observations.

[b]Measured 3 hr postprandially.

*Significantly different from control ($P < .05$).

monensin (Table 4). This is consistent with observations that monensin may exert an inhibitory effect on deamination of dietary protein by ruminal microflora (Tolbert et al., 1977). Conservation of nitrogen in host animal tissues and in the rumen by these mechanisms may explain the improved nitrogen utilization by steers fed monensin with whole shelled corn diets in other trials (Rust et al., 1978). A more active tissue protein synthesis could also be a factor, as indicated by the trend for improved protein digestibility.

Metabolism Trials (Sorghum Grain-Based Diet)

Within all trials, steers fed monensin initially experienced a slight depression in feed intake during the 3-week adjustment periods, but invariably consumed more daily dry matter ($P < .08$) and nitrogen ($P < .05$) than control steers during subsequent preliminary and collection periods. Similar intake adjustments have been observed in feedlot cattle fed monensin with sorghum grain-based diets (Hale et al., 1976).

Monensin had no effect ($P > .05$) on apparent digestibility of dry matter or gross energy (Table 5), but did improve apparent digestibility of crude protein ($P < .05$). The magnitude of improvement in crude protein digestibility (4.4%) was similar to that observed in the digestion trials with the corn-based diets (3.4%). Correcting for metabolic fecal nitrogen (MFN) by the equation MFN = 10.9 g/wt_{kg} fecal

Table 5. Mean dry matter intake, apparent digestibility and nitrogen balance by steers fed a sorghum grain-based diet[a]

Item	Dietary monensin, ppm 0	33	SD
Dry matter intake/day			
Total, g	4985	5168	292
Total, $g/W_{kg}^{.75}$	72.3	75.6	4.6
Apparent digestibility:			
Dry matter	77.6	78.2	1.0
Energy	76.0	76.5	.9
Crude protein	56.5	59.0*	2.3
Nitrogen balance, g/day			
Intake	92.7	98.2*	5.6
Fecal	40.4	40.3	3.4
Urinary	33.6	34.3	4.2
Retained	18.6	23.6	4.8
% of intake	20.4	24.1	4.3
% of absorbed	36.2	40.6	7.2

[a]All values are means of 15 observations.

*Significantly different from control ($P < .05$).

dry matter (Coleman and Barth, 1977) yielded protein true digestibility estimates of 69.2 and 71.0% for steers fed 0 and 33 ppm monensin, respectively ($P < .05$). Tolbert and Lichtenwalner (1978) observed a similar improvement when monensin was fed with a sorghum grain-based diet containing 14.6% crude protein (dry matter basis) at similar intakes between control and monensin steers.

Monensin has shown a tendency to improve apparent crude protein digestibility by steers fed orchardgrass hay (Dinius et al., 1976) or corn-corn cob diets (Anonymous, 1975). Results may not be entirely comparable between these observations and those reported herein because of differences in types of diets (forage vs concentrate) and level of intake (low, restricted vs *ad libitum*) employed by various investigators. Nevertheless, the available data suggest that improved digestibility of dietary components, especially protein, may be a slight benefit of monensin when fed with growing and finishing diets.

Steers fed monensin with the sorghum grain-based diet retained more nitrogen ($P < .06$) than control steers (23.6 vs 18.6 g/day; Table 5). This may be due in part to the unintentionally higher nitrogen intake ($P < .05$) by steers fed monensin over controls (98.2 vs 92.7 g/day), since fecal and urinary nitrogen were very similar between treatments. Among all steers, nitrogen retention was highly variable whether expressed as a percentage of nitrogen intake

(C.V. = 28%) or of absorbed nitrogen (C.V. = 25%), but tended to improve in response to monensin ($P > .05$). Correcting for endogenous urinary nitrogen (EUN) by the equation $EUN = .43_{kg}^{.5}$ (Coleman and Barth, 1977) and for MFN yielded nitrogen retention estimates of 59.3 and 61.1% of absorbed nitrogen for steers fed 0 and 33 ppm monensin, respectively ($P > .05$). Similar trends for improvement have been observed in balance trials with forages (Dinius et al., 1976), and this may partially account for the improved liveweight gain of cattle fed monensin with forage (Potter, Cooley et al., 1976). Our results do not agree with the balance trial data of Tolbert and Lichtenwalner (1978), who observed markedly lower nitrogen retention (whether expressed as a percentage of ingested or absorbed nitrogen) by steers fed monensin with a sorghum grain-based diet.

Neither total ruminal VFA concentration nor molar proportions of VFA were altered ($P > .05$) by monensin (Table 6). Monensin has generally been observed to increase molar proportion of propionic acid while decreasing molar proportions of acetic and butyric acids in rumen fermentation, but this pattern has not been consistent in several studies with beef cattle fed sorghum grain-based finishing diets (Anonymous, 1975; Hale et al., 1975). In one of these trials (Hale et al., 1975), monensin did not alter molar proportions of individual VFA, but did increase total ruminal VFA concentrations. Shell et al. (1979) observed an increase in

Table 6. Ruminal VFA values for steers fed a sorghum grain-based diet[a,b]

Item	Dietary monensin, ppm		SD
	0	33	
VFA			
Total, μmole/ml	78.2	81.6	16.6
C_2, %	47.1	45.6	3.6
C_3, %	40.3	41.9	5.9
C_4, %	8.3	8.6	2.1

[a]All values are means of 15 observations.

[b]Measured 3 hr postprandially.

production of acetic and propionic acids by monensin addition to a diet similar to the one used in this study, but this was not apparent on the basis of molar proportions of the individual VFA.

On the basis of the available data, it appears that a slight improvement in nitrogen utilization may account for some of the benefit of feeding monensin with concentrate diets.

CHAPTER 4

MONENSIN EFFECTS ON SITE AND EXTENT OF WHOLE CORN DIGESTION AND BACTERIAL PROTEIN SYNTHESIS IN BEEF STEERS

Summary

Monensin was fed at 0 and 33 ppm in a crossover trial to determine its effect on ruminal vs postruminal utilization of a corn-based diet (10.0% crude protein, dry matter basis) and bacterial protein synthesis in abomasally-fistulated steers. Monensin decreased ($P < .10$) ruminal true digestion of organic matter (OM_c, corrected for bacterial cell synthesis) by 21%, but had no effect on apparent total tract digestion of organic matter. Apparent ruminal and total tract digestibilities of crude protein were unchanged. Monensin decreased ($P < .07$) the contribution of bacterial N (52 vs 58%) and increased ($P < .06$) that of ruminally undegraded feed N (46 vs 40%) to total abomasal N, but had no effect on total N or amino acids recovered from the abomasum. Efficiency of bacterial protein synthesis (g bacterial CP/100 g ruminally digested OM_c) was unchanged. Monensin decreased ($P < .05$) the contribution of bacterial N (42 vs 50%) and increased ($P < .05$) that of ruminally undegraded feed N (58 vs 50%) to total N digested postruminally. That monensin caused a greater proportion of feed OM and N to be digested in the intestines than in the rumen (with possibly greater

resultant metabolic efficiency) may account for some of the benefit of feeding this compound with high grain diets.

Introduction

Monensin, a biologically active compound produced by a strain of *Streptomyces cinnamonensis* (Haney and Hoehn, 1967) has been generally accepted to improve energetic efficiency of rumen fermentation by increasing the molar proportion of propionic acid while decreasing the molar proportions of acetic and butyric acids (Richardson et al., 1976). More recently, monensin has been shown to spare dietary protein when fed to growing-finishing beef cattle (Gill et al, 1977; Hanson and Klopfenstein, 1979), and to improve crude protein digestibility and nitrogen retention by growing steers fed high grain diets (Muntifering et al., 1980). Poos et al. (1979) observed monensin decreased ruminal outflow of bacterial nitrogen and increased ruminally undegraded feed (bypass) nitrogen in trials with steers. Similarly, inhibition of bacterial protein synthesis (Van Nevel and Demeyer, 1977) and ruminal protein degradation (Tolbert et al., 1977) by monensin have been demonstrated *in vitro*.

Depending upon dietary protein quality, consequences of reduced ruminal proteolysis and of bacterial growth inhibition may be critical to satisfying amino acid requirements of highly productive animals in practical feeding situations. While monensin has been shown to affect the conversion of feed protein to microbial protein with diets representing extremes of high bypass protein and of nonprotein nitrogen (Poos et al., 1979), no data is available with diets containing high levels of cereal grains. Such information is critical because cereal grains usually furnish most of the crude protein equivalence in growing-finishing diets for beef cattle.

The objectives of these trials were to determine the effect of monensin on the site and extent of digestion of a corn-based diet and on bacterial protein synthesis in abomasally-fistulated steers.

Experimental Procedures

Two trials were conducted as a crossover experiment to determine the effect of monensin on 1) the extent of ruminal vs postruminal digestion of a 90% whole shelled corn diet (Table 7) containing 10.0% crude protein (dry matter basis) and on 2) bacterial protein synthesis *in vivo*.

Six steers, initial weight 225 kg, were randomly assigned to two treatment groups to receive 0 or 33 ppm monensin in the experimental diet for Trial 1. Steers were surgically fitted with permanent abomasal cannulae by a modification of the method of Dougherty (1955), and were allowed 4 to 6 weeks for recovery and adaptation to management. Steers were crossed over to opposite experimental

Table 7. Diet composition (Abomasal passage trials)[a,b]

Ingredient	Internat'l Ref. No.	%
Corn, dent yellow, grain, whole shelled	4-02-531	90.0
Cottonseed hulls	1-01-599	5.0
Soybean meal, solv-extd.	5-04-604	2.5
Limestone, grnd.	6-02-632	1.1
Alfalfa, aerial part, dehy meal	1-00-023	.9
Potassium chloride		.3
Chromic oxide		.2
Chemical analysis:	%	
Dry matter	88.1	
Organic matter[c]	97.3	
Ash[c]	2.7	
Crude protein[c]	10.0	

aAir-dry basis.

[b]Vitamin A added to provide 2,200 IU/kg.

[c]Dry matter basis.

treatments for Trial 2. In addition, another four steers (body weight 228 kg) were randomly assigned to the two treatment groups. One of these steers lost its cannula and was removed from the experiment, resulting in a total of 7 and 8 animal observations for control and monensin treatments, respectively.

In each trial of the experiment, the diet was fed *ad libitum*, twice daily, to all steers for 3 weeks. Each of the adjustment periods was followed by a 10-day preliminary phase and a 6-day period of total fecal collection and abomasal spot sampling (12 hr interval advanced 2 hr daily). Sham collections of abomasal contents were made from all steers for the last 4 days of each 10-day preliminary phase. Steers were housed in individual pens (2.4 x 4.9 m) and daily fecal collections were made from the concrete floors of the pens.

For preliminary and collection periods within each trial, daily intake for each steer was adjusted to approximately 90% of the daily voluntary intake measured during the last week of the three week adjustment period. Further adjustments were made as necessary to equalize intake between experimental treatments. All feed ingredients except corn and cottonseed hulls were pelleted through a .48 cm die and fed as a top dressing. Chromic oxide powder (Cr_2O_3) was incorporated into the pelleted ingredients during each 10-day preliminary phase and 6-day collection period.

A 5% aliquot of daily fecal output was dried at 50 C for 48 hr in a forced-air oven. Feed samples were taken daily and dried similarly. Abomasal contents were collected in plastic bags, placed immediately on dry ice and stored at -20 C. Individual samples of abomasal digesta were lyophilized and pooled on an equal dry weight basis for each steer. Feed and fecal samples were each pooled to yield individual animal composites for chemical analysis.

Dry matter, ash and nitrogen in feed, feces and abomasal digesta were analyzed by AOAC procedures (1970). Chromic oxide content of feed, feces and abomasal digesta was determined by the perchloric acid oxidation procedure of Kimura and Miller (1957). Starch in feed, feces and abomasal digesta was determined by the acid hydrolysis method of Osman et al. (1970), as modified by Kartchner and Theurer (1980). Feces were analyzed for acid detergent-insoluble nitrogen by the method of Mason (1969). Abomasal digesta was analyzed for ammonia nitrogen by distillation over MgO (AOAC, 1970) and for amino acids, including diaminopimelic acid (DAP), by ion-exchange chromatography (Moore, Spackman and Stein, 1958) modified to enable separation of DAP from methionine (Rahnema, 1977). Bacterial nitrogen in samples of abomasal digesta was estimated from lysine and leucine ratios by summation equations derived from those reported by Ely et al. (1967) and Potter, McNeill and Riggs (1971). Undigested bacterial nitrogen in feces was calculated as the difference between total nitrogen and acid detergent-insoluble nitrogen content. Ruminal bypass of feed nitrogen was calculated as the difference between non-ammonia nitrogen and bacterial nitrogen content of abomasal digesta (includes protozoal and endogenous nitrogen).

Ruminal digestion coefficients for organic matter and crude protein based on nutrient-indicator ratios were corrected to represent 100% recovery of chromic oxide in feces. Ruminal true digestion of organic matter (OM_C) was estimated by further correction for *de novo* bacterial cell synthesis. Postruminal digestion coefficients for organic matter and crude protein fractions were calculated on the basis of amounts present in abomasal digesta (presumably entering the small intestine) and appearing in the feces.

In a separate experiment, the effect of monensin on amino acid composition and DAP:total nitrogen ratio of mixed rumen bacteria was evaluated. A mature rumen fistulated Hereford steer was fed the experimental diet containing 0 or 33 ppm monensin for a 3-week period. On two consecutive days at the end of each period, a sample of rumen fluid was obtained 3 hr postprandially. Washed bacterial cell suspensions were prepared by a modification of the procedure of Ibrahim, Ingalls and Bragg, (1970). These were dried at 50 C on glass plates, pulverized and stored in glass vials. On a dry matter basis, these samples contained an average of 9.3% ash and 49.7% crude protein (microkjeldahl N x 6.25).

Data were analyzed as a crossover design by analysis of variance, using the method of least squares (Steel and Torrie, 1960).

Results and Discussion

Monensin reduced ($P < .10$) ruminal true digestion of organic matter (OM_C) by 21% (Table 8). This is in good agreement with the data of Owens et al. (1978), who observed a 16% reduction in apparent ruminal dry matter digestion by monensin with steers fed a 57% corn diet. In other trials with steers fed harvested forage (Lemenager, Owens, Shockey et al., 1978), monensin lowered ruminal turnover rates for liquid by 31% and for solids by 44%, thus increasing ruminal residence time of feed materials and expectedly the extent of ruminal digestion. Monensin lowered ruminal liquid turnover rate with concentrate-fed steers in the same study. Monensin decreased ruminal solids turnover rate in the trial of Tolbert et al. (1979), and increased the extent of apparent ruminal dry matter disappearance by approximately 9% with steers fed a 70% concentrate diet. The reason(s) for these inconsistencies is unclear, but may reflect variation among trials owing to use of different flow marker systems and dietary regimens. Fecal recovery of chromic oxide averaged 89.6 ± 3.2 (mean ± SD) in our study, and was unaffected by treatment.

Table 8. Monensin effect on site and extent of organic matter digestion[a]

Item	Dietary monensin, ppm 0	33	SD
Intake, g	3822	3814	185
Intake, $g/W_{kg}^{.75}$	62.9	64.3	3.2
Ruminal digestion, %[b]	49.8	39.3†	9.2
Ruminal digestion, g[b]	1906	1543	408
Entering duodenum, g	2428	2711	317
Postruminal digestion, %[c]	73.6	73.6	5.5
Postruminal digestion, g[c]	1818	2064	337
Total digestion, %	84.0	83.1	1.7
Total digestion, g	3212	3168	118

[a]All values are means of seven (0 ppm) and eight (33 ppm) observations.

[b]Corrected for 512 (0 ppm) and 440 (33 ppm) g bacterial organic matter synthesized.

[c]Based on amount in abomasal digesta (presumably entering duodenum).

†Different from control ($P < .10$).

Decreased ruminal digestion of OM_c by monensin was almost entirely accounted for by a 19% decrease ($P < .10$) in extent of ruminal starch digestion (Table 9). Since monensin had no effect on total tract or postruminal digestion coefficients for organic matter or starch, more organic matter (2064 vs 1818 g/day) and starch (1117 vs 873 g/day) tended ($P > .10$) to be digested in the intestines of steers fed monensin. This may be interpreted as an added benefit of feeding monensin with high concentrate diets, as resultant glucose from intestinal starch digestion should be more efficiently utilized by the host animal than would VFA arising from ruminal fermentation of organic matter (Orskov, 1977). Since pancreatic amylase secretion apparently increases in response to increasing amounts of dietary starch escaping rumen fermentation (Clary, Mitchell and Little, 1967), our observations may explain the significant increase of amylase activity by monensin in the study of Van Hellen et al. (1977). Elevated plasma glucose and serum insulin levels have been observed in feedlot steers fed monensin (Raun et al., 1976).

Monensin had no effect on apparent ruminal, postruminal or total tract digestion of crude protein (Table 10), nor did it affect protein true digestibility. Abomasal recovery of crude protein exceeded crude protein intake in both treatment groups, resulting in negative apparent ruminal digestion coefficients for this component. This is not

Table 9. Monensin effect on site and extent of starch digestion[a]

Item	Dietary monensin, ppm 0	33	SD
Intake, g	2424	2418	117
Ruminal digestion, %	61.1	49.5†	12.3
Ruminal digestion, g	1474	1219	336
Entering duodenum, g	950	1200	275
Postruminal digestion, %[b]	90.2	90.8	4.5
Postruminal digestion, g[b]	873	1117	289
Total digestion, %	96.8	96.7	.9
Total digestion, g	2347	2336	114

[a]All values are means of seven (0 ppm) and eight (33 ppm) observations.

[b]Based on amount in abomasal digesta (presumably entering duodenum).

†Different from control ($P < .10$).

Table 10. Monensin effect on site and extent of crude protein digestion[a]

Item	Dietary monensin, ppm 0	33	SD
Intake, g	392	392	19
Ruminal digestion, %	-22.4	-19.6	9.4
Ruminal digestion, g	-88	-75	35
Entering duodenum, g	480	467	28
Postruminal digestion, %[b]	74.5	74.7	1.9
Postruminal digestion, g	358	350	30
Total digestion, %	68.8	70.1	1.7
Total digestion, g	270	275	20
Protein true digestibility, %[c]	95.4	95.5	.2

[a]All values are means of seven (0 ppm) and eight (33 ppm) observations.

[b]Based on amount in abomasal digesta (presumably entering duodenum).

[c]Based on acid detergent-insoluble nitrogen content of feces.

an uncommon observation (Theurer, 1979), and is probably related to an influx of endogenous nitrogen and/or an underestimation of ruminal organic matter digestion by reference to chromic oxide (Drennan, Holmes and Garrett, 1970); thus abomasal flow of organic matter (including crude protein) would be overestimated. Nevertheless, this should not invalidate between-treatment comparisons.

Analysis of washed bacterial cell suspensions revealed that DAP:total nitrogen ratios were not altered by monensin, in agreement with Poos et al. (1979). The DAP nitrogen content of bacterial cells averaged .54% of total nitrogen, a relatively low value within the range (.5 to 1.1%) reported in a review of this topic (Smith, 1975). Preliminary estimates of the contribution of bacterial nitrogen to total abomasal nitrogen based on the DAP ratio technique (Hutton, Bailey and Annison, 1971) were interpreted as being unreasonably high (73 vs 82%) for monenson and control treatments, respectively; $P < .10$). This phenomenon has been observed by other investigators (Nikolic and Jovanovic, 1973) and has been attributed to a possible preferential degradation of cell contents (DAP being present in the cell wall) in the process of rumen bacterial turnover (Siddons et al., 1979). For this reason, it was felt that the best estimate of bacterial nitrogen to total abomasal nitrogen might be accomplished by the summation equation technique (Ely et al., 1967) based on the leucine and lysine content of feed, bacteria and abomasal digesta. This method has been satisfactorily employed for sorghum grain diets (Potter et al., 1970; Rahnema et al., 1979).

Monensin decreased ($P < .07$) the proportion of abomasal nitrogen that was of bacterial origin (52 vs 58%) and increased ($P < .06$) that of ruminally undegraded feed origin (46 vs 40%; Table 11). These values agree with the data of Cole et al. (1976), in which microbial and feed nitrogen comprised 51 and 43%, respectively, of abomasal nitrogen for steers fed a whole shelled corn diet containing 7% roughage. Bacterial nitrogen also represented a smaller proportion of abomasal nitrogen in steers fed monensin compared to controls in a recent study by Tolbert et al. (1979). That monensin lowered ($P < .10$) daily abomasal passage of ammonia nitrogen (Table 12) may reflect a depression of ruminal ammonia levels (Dinius et al., 1976; Tolbert et al., 1977) resulting from inhibition of microbial proteolytic activity (Poos et al., 1979).

Monensin reduced ($P < .10$) daily abomasal passage of bacterial nitrogen by 14% (Table 12). This was accompanied by a 15% increase ($P > .10$) in daily passage of ruminally undegraded feed nitrogen. Poos et al. (1979) observed monensin to reduce bacterial nitrogen by approximately 33% in steers fed diets supplemented with either brewers dried grains or urea, whereas feed bypass nitrogen was increased by 37 and 55%, respectively, for the two sources of

Table 11. Monensin effect on fractionation of abomasal nitrogen[a]

Item[b]	Dietary monensin, ppm 0	33	SD
	--------%--------		
Bacterial N	58.1	51.8†	3.7
Feed N[c]	39.7	46.2†	3.6
Non-ammonia N	97.8	98.1	.2
NH_3-N	2.2	1.9	.2

[a]All values are means of seven (0 ppm) and eight (33 ppm) observations.

[b]Percent of total abomasal N represented in each fraction.

[c]Includes protozoal and endogenous N.

†Different from control ($P < .10$).

Table 12. Monensin effect on passage of abomasal nitrogen fractions and efficiency of bacterial protein synthesis[a]

Item	Dietary monensin, ppm 0	33	SD
	----(g/day)----		
N intake	62.8	62.7	3.1
Abomasal N	76.8	74.7	4.4
Bacterial N	44.8	38.5†	3.6
Feed N[b]	30.3	34.8	4.9
Non-ammonia N	75.1	73.3	4.4
NH_3-N	1.7	1.4†	.2
Bacterial CP/100 g OM digested in rumen[c]	16.1	14.7	10.3

[a]All values are means of seven (0 ppm) and eight (33 ppm) observations.

[b]Includes protozoal and endogenous N.

[c]Corrected for 512 (0 ppm) and 440 (33 ppm) g bacterial organic matter synthesized in the rumen.

†Different from control ($P < .10$).

supplemental nitrogen. Monensin had no effect on daily amounts of bacterial nitrogen in the trial of Owens et al. (1978), but increased feed bypass nitrogen by 22%. The extent to which monensin alters the relative proportions of bacterial and feed bypass nitrogen in abomasal digesta appears to be related to the nature of the dietary protein. Solubility, extent of degradability and propensity to liberate ammonia nitrogen in ruminal fluid incubations have been demonstrated to affect the magnitude of proteolytic and deaminative inhibition by monensin *in vitro* (Tolbert et al., 1977; Dinius, 1978).

Efficiency of bacterial protein synthesis (Table 12) was relatively unchanged by monensin (16.1 vs 14.7 g bacterial CP/100 g ruminally digested OM_c). While monensin effects on this parameter *in vivo* have not been reported elsewhere, Van Nevel and Demeyer (1977) observed monensin to significantly inhibit synthesis of bacterial cells *in vitro*. This may be of extreme importance in applied ruminant feeding situations where NPN supplies an appreciable portion of the dietary crude protein equivalence (CPE). Monensin had no beneficial effect on beef cattle performance in the trial of Hanson and Klopfenstein (1978) for diets containing approximately 40% of their CPE as urea, but was effective in cattle finishing diets where approximately 17% of the CPE was in the form of urea (Davis and Erhart, 1976).

Passage of essential and non-essential amino acids (Table 13) was not affected by monensin. This was expected on the basis of the small changes (15%) in proportions of feed and bacterial nitrogen in abomasal digesta between treatments. In other trials where monensin effects on these proportions were more dramatic (Poos et al., 1979), passage of essential and non-essential amino acids tended to increase for diets supplemented with a natural protein source, but not for those supplemented with urea. Monensin decreased ($P < .10$) passage of DAP by 12% (2.2 vs 2.5 g/day), a reflection of the 14% depression in amount of bacterial nitrogen for this treatment.

Ruminal digestion of feed nitrogen (Table 14) was calculated to be 51 and 44% for control and monensin treatments, respectively. Postruminal digestion coefficients for feed nitrogen and for bacterial nitrogen were not different between treatments, and averaged 91 and 61%, respectively. Monensin decreased ($P < .05$) the contribution of bacterial nitrogen (42 vs 50%) and increased ($P < .05$) that of ruminally undegraded feed nitrogen (58 vs 50%) to total nitrogen digested and absorbed postruminally; hence, steers fed monensin tended to digest more feed nitrogen and less bacterial nitrogen in the intestines than controls. The net effect of altering the proportions of feed and bacterial nitrogen digested postruminally in this fashion may be an added benefit of feeding monensin, as losses incurred in the ruminal

Table 13. Monensin effect on abomasal amino acid flow[a]

	Dietary monensin, ppm		
	0	33	SD
	-----(g/day)-----		
Essential:			
Isoleucine	20.5	20.4	2.6
Leucine	40.6	42.5	7.0
Lysine	24.8	23.6	2.8
Valine	24.4	24.0	3.3
Phenylalanine	21.2	21.8	3.4
Threonine	17.7	17.4	2.4
Methionine	8.4	7.9	1.4
Histidine	9.2	9.6	1.5
Arginine	18.3	18.1	2.6
Total	185.0	185.1	26.7
Non-essential:			
Glycine	21.3	20.8	2.7
Glutamic acid	61.6	68.8	14.3
Serine	15.9	16.0	2.8
Alanine	29.6	29.5	4.5
Tyrosine	16.8	16.8	2.2
Aspartic acid	39.1	37.8	4.8
Cystine	.8	1.1	.5
Proline	23.4	24.8	5.5
Total	208.7	215.6	34.6
Diaminopimelic acid	2.5	2.2[†]	.3

[a]All values are means of seven (0 ppm) and eight (33 ppm) observations.

[†]Different from control ($P < .10$).

Table 14. Monensin effect on site and extent of feed and bacterial nitrogen digestion[a]

Item	Dietary monensin, ppm 0	33	SD
Ruminal digestion			
Feed N, g	32.5	28.0	5.2
Feed N, %	51.3	43.6	8.6
Postruminal digestion			
Feed N, g[b]	27.4	31.9	4.8
Feed N, %[b]	90.2	91.1	1.3
Feed N, % of total N digested postruminally	50.0	58.1*	3.9
Bacterial N, g[c]	28.2	22.6†	3.1
Bacterial N, %[c]	61.4	59.9	5.5
Bacterial N, % of total N digested postruminally	50.0	41.9*	3.9

[a]All values are means of seven (0 ppm) and eight (33 ppm) observations.

[b]Based on amount in abomasal digesta and recovered as fecal acid detergent-insoluble N.

[c]Based on amount in abomasal digesta and recovered as fecal acid detergent-soluble N.

*Different from control ($P < .05$).

†Different from control ($P < .10$).

transformation of feed nitrogen to bacterial nitrogen would be partially eliminated. These losses include ammonia absorbed from the rumen, nucleic acid nitrogen in microbial cells and a lower postruminal digestibility of microbial cells compared to ruminally undegraded feed proteins (Satter and Roffler, 1976). Although not evident in this study, changes in proportions of feed and bacterial protein for intestinal digestion may explain monensin improvements in total tract apparent crude protein digestibility observed in other trials with whole corn diets (Rust et al., 1978; Muntifering et al., 1980).

These data suggest that monensin may be an effective tool for altering the nature of nitrogenous compounds available for digestion and absorption in the ruminant intestines. As interest in bypassing rumen fermentation and increasing ruminal dilution rates increases, appreciation for qualitative aspects of feed proteins for ruminants must assume greater significance if amino acid requirements are to be satisfactorily met.

CHAPTER 5

CONCLUSIONS

Based on data presented in the previous chapters and in the appendices to follow, the following conclusions are made with regards to monensin and nitrogen utilization by beef cattle fed diets high in sorghum grain (76%, steam-processed, flaked) and corn (90%, whole shelled).

1. Monensin had no effect on total ruminal VFA concentrations with either diet. Molar proportion of acetic acid decreased ($P < .05$) and that of propionic acid increased ($P < .05$) in response to monensin when fed with the corn-based diet; a similar trend ($P > .05$) was observed with monensin addition to the sorghum grain-based diet. Shifts in molar proportions of VFA cannot account for all the improvement (8 to 12%) in feed efficiency typically observed with beef cattle fed monensin, suggesting other possible benefits of feeding this compound with high grain diets.

2. Monensin had no effect on apparent total tract digestibility of dry matter or energy in either diet.

3. Apparent total tract digestibility of crude protein was slightly, but consistently, improved by monensin addition to either diet. Improvement was greater ($P < .05$) for the sorghum grain-based diet (4%); less improvement

(2 to 3%) was observed for the corn-based diet (P > .05). Nitrogen retention tended (P > .05) to improve in response to monensin with the sorghum grain-based diet (24 vs 20% of nitrogen intake; 41 vs 36% of nitrogen absorbed).

4. Monensin decreased (P < .10) ruminal ammonia concentrations with the corn-based diet, and lowered (P < .10) daily abomasal passage of ammonia nitrogen to the small intestine. This suggests that monensin inhibits ruminal protein degradation and/or amino acid deamination, as evidenced by the tendency (P > .10) for ruminal digestion of feed nitrogen to be lower (44 vs 51%) in steers fed monensin with the corn-based diet.

5. Ruminal digestion of organic matter (corrected for de novo bacterial cell synthesis) was reduced (P < .10) by monensin in the corn-based diet (39 vs 50%), and could be almost entirely accounted for by decreased (P < .10) ruminal digestion of starch (61 vs 50%). Monensin tended (P > .10) to increase the amounts of energy-yielding nutrients that were digested and absorbed in the intestines compared to ruminal digestion, which could result in improved metabolic efficiency.

6. Reasonable estimation of bacterial protein was made by the use of lysine and combinations of lysine x leucine or DAP x leucine in simultaneous equations. Estimates based on DAP alone or DAP x lysine were interpreted as being unreasonably high, and may have resulted from the presence

of DAP (or another amino acid possessing similar elution characteristics) in all feed materials analyzed (corn, cottonseed hulls and pelleted supplement).

7. Monensin decreased ($P < .07$) the contribution of bacterial N (52 vs 58%) and increased ($P < .06$) that of ruminally undegraded feed (bypass) N to total nitrogen passing the abomasum with the corn-based diet, resulting in decreased ($P < .10$) passage of bacterial N (38 vs 45 g/day) and a trend ($P > .10$) for increased passage of bypass N (35 vs 30 g/day) to the small intestine. Because of the magnitude of changes (15%) in proportions of feed vs bacterial nitrogen in abomasal digesta between treatments, passage of individual amino acids was unchanged. Monensin had no effect on efficiency of bacterial protein synthesis, which averaged 15 g bacterial CP/100 g organic matter digested (true) in the rumen.

8. Monensin decreased ($P < .05$) the contribution of bacterial N (42 vs 50%) and increased ($P < .05$) contribution of bypass feed N (58 vs 50%) to total nitrogen digested postruminally, resulting in less ($P < .10$) bacterial N 23 vs 28 g/day) and a trend ($P > .10$) for more feed bypass N (32 vs 27 g/day) digestion in the intestines of steers fed monensin. This is interpreted as an added benefit of feeding monensin, as losses incurred in the ruminal transformation of feed nitrogen to bacterial nitrogen may have been partially eliminated.

APPENDIX A

DETERMINATION OF THE CONTRIBUTION OF MICROBIAL PROTEIN TO ABOMASAL DIGESTA

Experimental Procedures

Samples of abomasal digesta were collected from abomasally fistulated steers fed a 90% whole shelled corn diet containing 0 or 33 ppm monensin. Collection procedures and diet composition are described in Chapter 4. Bacteria were separated from rumen contents of a mature rumen fistulated Hereford steer fed the experimental diet by the procedure of Ibrahim et al. (1970), except that bacterial precipitates were washed with small volumes of deionized water instead of acetate-phosphate buffer.

Dry matter, ash and nitrogen (microkjeldahl) content of feed, bacteria and abomasal samples were determined by AOAC procedures (1970). Samples were also analyzed for amino acids, including diaminopimelic acid (DAP), by ion-exchange chromatography (Moore et al., 1958) modified to enable separation of DAP from methionine (Rahnema, 1977). Bacterial protein in samples of abomasal digesta was estimated from amino acid ratios by summation equations derived from those reported by Ely et al. (1967). Several amino acids used singly or in two-acid combinations were compared.

Results and Discussion

Amino acid compositions (mg amino acid nitrogen/g total nitrogen) of bacteria and dietary ingredients are presented in Table A-1. These values are in good agreement with values previously reported from this station (Rahnema, 1977). Percent crude protein in four samples of rumen bacteria ranged from 39.4 to 56.4%, and averaged 49.7% of dry matter. Ash content of mixed rumen bacteria ranged from 8.0 to 11.8% (average 9.3% of dry matter), which is somewhat lower than the range of 18 to 27% reported by Smith (1975) and the average value (25%) reported by Rahnema (1977). Differences in methods for preparing bacterial cell suspensions undoubtedly contribute to some of this discrepancy (e.g., washing precipitates with water vs buffer).

All samples analyzed showed the presence of DAP (or another amino acid possessing similar elution characteristics) in variable amounts, ranging from 1.7 (corn) to 5.4 (rumen bacteria) mg DAP-N per g total N. That feed ingredients contain DAP is contrary to the popular belief that this amino acid is present only in bacteria, but supports earlier observations from this station (Rahnema, 1977).

The use of uncorrected DAP (disregarding the DAP present in feed) resulted in the highest estimation of bacterial protein in abomasal digesta for the 33 ppm level of monensin (Table A-2). All other estimates using DAP were adjusted for feed concentrations of this amino acid.
DAP x lysine gave the highest value for bacterial protein in the 0 ppm monensin group. In general, two-acid combinations appeared to yield the highest estimates for both groups.

Leucine alone gave unrealistic values with standard deviations larger than their means. Similar patterns were observed by Rahnema (1977).

All methods ranked the two levels of monensin in the same order. Estimates based on DAP alone were interpreted as being unreasonably high, a phenomenon observed by other investigators (Nikolic and Jovanovic, 1973; Siddons et al., 1979). Lysine x leucine appeared to yield more reasonable, less variable results, and was the method of choice in the studies of Potter et al. (1970) for estimating bacterial protein in abomasal digesta of steers fed sorghum grain. For these reasons, the summation equation technique based on the content of these amino acids (lysine and leucine) in feed, bacteria and abomasal digesta was used in this study (Chapter 4).

Table A-1. Amino acid composition of bacteria and feed ingredients

Item	Bacteria	Corn	Cottonseed Hulls	Pelleted Supplement
No. Samples	4	2	2	2
	-------mg amino acid N/g total N-----------			
Amino Acid				
Lys	71.8	31.1	43.9	62.1
His	18.5	41.1	34.8	36.8
Arg	63.0	77.2	114.1	118.9
Asp	55.9	36.1	49.7	62.9
Thr	28.1	19.0	19.7	21.7
Ser	22.7	26.3	27.8	28.1
Glu	49.8	96.2	90.9	91.6
Pro	13.6	50.1	25.3	26.9
Gly	49.6	37.1	41.7	41.2
Ala	54.0	59.7	36.9	36.1
Cys	.8	1.3	.2	2.5
Val	35.1	32.7	32.7	33.3
Met	9.8	8.5	3.6	5.2
DAP	5.4	1.7	3.9	2.1
Ile	25.8	19.9	20.3	25.9
Leu	34.7	63.3	34.0	41.8
Tyr	16.7	12.7	10.7	14.7
Phe	19.3	21.8	21.8	22.8

Table A-2. Percent bacterial protein in abomasal digesta of steers fed 0 or 33 ppm monensin in a whole corn diet as determined by various amino acid ratios[a,b]

Methods	Dietary monensin, ppm 0	33
	Mean ± SD	
DAP (uncorrected)	88.0 ± 9.9	82.5 ± 10.4†
DAP (corrected)	81.8 ± 15.1	73.4 ± 15.9†
Lysine	72.6 ± 22.2	70.7 ± 30.5
Leucine	19.1 ± 32.8	.49± 45.1
DAP x lysine	96.1 ± 43.4	77.5 ± 38.2
DAP x leucine	70.3 ± 11.4	60.0 ± 9.9†
Lysine x leucine	58.1 ± 8.6	51.8 ± 12.4†

[a]Each value is the mean of 7(0 ppm) and 8(33 ppm) observations.

[b]For detailed composition of diet, refer to Chapter 4.

†Different from control ($P < .10$)

APPENDIX B

TABULAR DATA, DIGESTION TRIALS

Table B-1. Percent dry matter, crude protein, starch and gross energy (Mcal/kg) per steer in feed and feces by treatment (Digestion trial 1)[a]

	Treatments							
	Control				Monensin			
Steer No.:	16	8	6B	15	18	17	5B	2
Dry matter[b]								
Feed	93.71	93.71	93.71	93.71	93.79	93.79	93.65	93.64
Feces	93.21	93.89	93.49	92.58	94.60	94.42	94.44	93.50
Crude Protein								
Feed	10.56	10.56	10.56	10.56	10.53	10.53	10.47	10.44
Feces	17.39	14.66	17.70	17.93	15.79	15.13	17.77	14.14
Starch								
Feed	58.65	58.65	58.65	58.65	58.44	58.44	58.67	58.67
Feces	21.85	28.76	27.57	25.37	27.40	24.00	7.73	23.82
Gross energy (Mcal/kg)								
Feed	4.41	4.41	4.41	4.41	4.40	4.40	4.41	4.41
Feces	4.73	4.70	4.76	4.81	4.59	4.43	4.67	4.62

[a]Dry matter basis.

[b]Dry matter values were obtained by vacuum drying at 100 C, previously partially dried samples of feed and feces.

Table B-2. Percent dry matter, crude protein, starch and gross energy (Mcal/kg) per steer in feed and feces by treatment (Digestion trial 2)[a]

	Treatments							
	Control				Monensin			
Steer No.:	18	17	5B	2	16	8	6B	15
Dry matter[b]								
Feed	93.61	93.60	93.59	93.61	93.59	93.63	93.59	93.52
Feces	92.06	91.12	91.81	91.76	93.30	91.22	92.67	91.04
Crude protein								
Feed	10.42	10.46	10.45	10.42	10.43	10.52	10.48	10.55
Feces	18.28	16.94	18.85	17.09	15.43	15.65	16.52	18.30
Starch								
Feed	58.84	59.06	58.83	58.84	58.84	58.83	59.11	59.50
Feces	25.21	23.71	8.31	21.44	23.99	28.86	20.05	22.53
Gross energy (Mcal/kg)								
Feed	4.40	4.41	4.38	4.40	4.39	4.39	4.41	4.39
Feces	4.67	4.69	4.69	4.63	4.46	4.59	4.63	4.71

[a] Dry matter basis.

[b] Dry matter values were obtained by vacuum drying at 100 C, previously partially dried samples of feed and feces.

Table B-3. Body weight, intake and output of dry matter (DM) per steer (Digestion trials)

Item	Steer Number							
	18	16	17	8	5B	6B	2	15
Trial 1								
Body weight, kg	407	393	396	387	409	389	382	407
DM intake, g	6170	6148	6170	6148	6148	6148	6125	6148
DM output, g	1587	1198	1495	2114	1137	1626	1615	1519
Trial 2								
Body weight, kg	411	415	407	430	436	425	412	449
DM intake, g	6288	6288	6288	6290	6288	6259	6288	6218
DM output, g	1421	1565	1548	1877	1039	1405	1321	1288

APPENDIX C

TABULAR DATA, METABOLISM TRIALS

Table C-1. Percent dry matter, crude protein and gross energy (Mcal/kg) of feed and feces and crude protein of diluted urine per steer by treatment (Metabolism trial 1)[a]

Item	Treatments							
	Control				Monensin			
Steer No.:	5A	20	6A	14A	30	25	10	19
Dry matter[b]								
Feed	93.56	93.56	93.56	93.56	93.94	93.94	93.94	93.94
Feces	92.90	92.00	91.92	93.72	93.29	92.80	92.91	93.26
Crude protein								
Feed	11.52	11.52	11.52	11.52	11.51	11.51	11.51	11.51
Feces	23.45	23.03	24.24	21.38	20.30	24.00	21.63	20.31
Diluted urine	1.34	1.28	1.90	.80	1.66	1.62	1.80	2.47
Gross energy (Mcal/kg)								
Feed	4.47	4.48	4.49	4.47	4.45	4.45	4.45	4.45
Feces	4.57	5.09	4.99	4.70	4.69	4.70	4.72	4.71

[a]Dry matter basis, except for diluted urine (volume basis).

[b]Dry matter values were obtained by vacuum drying at 100 C, previously partially dried samples of feed and feces.

Table C-2. Percent dry matter, crude protein and gross energy (Mcal/kg) of feed and feces and crude protein of diluted urine per steer by treatment (Metabolism trial 2)[a]

Item	Treatments					
	Control			Monensin		
Steer No.:	30	25	19	5A	20	6A
Dry matter[b]						
Feed	92.51	93.14	92.51	92.50	92.50	92.50
Feces	91.10	93.98	02.69	92.50	91.94	92.03
Crude protein						
Feed	11.55	11.41	11.55	11.76	11.76	11.76
Feces	22.13	22.81	21.68	22.84	21.31	22.92
Diluted urine	1.92	2.79	1.76	1.66	2.09	2.50
Gross energy (Mcal/kg)						
Feed	4.52	4.47	4.52	4.54	4.54	4.54
Feces	4.79	4.87	4.76	4.81	5.10	4.82

[a]Dry matter basis, except for diluted urine (volume basis)

[b]Dry matter values were obtained by vacuum drying at 100C, previously partially dried samples of feed and feces

Table C-3. Percent dry matter, crude protein and gross energy (Mcal/kg) of feed and feces and crude protein of diluted urine per steer by treatment (Metabolism trial 3)[a]

Item	Treatments							
	Control				Monensin			
Steer No.:	14B	8	16	2	5B	15	6	17
Dry matter[b]								
Feed	96.20	96.20	96.20	96.20	96.70	96.34	96.34	96.34
Feces	92.82	92.55	92.44	92.14	93.66	92.11	92.32	92.02
Crude protein								
Feed	11.44	11.44	11.44	11.44	11.79	11.75	11.75	11.75
Feces	20.88	20.97	20.66	19.92	21.64	22.80	20.46	21.23
Diluted urine	4.70	2.89	3.84	2.55	1.88	1.64	3.74	4.27
Gross energy (Mcal/kg)								
Feed	4.43	4.43	4.43	4.43	4.39	4.45	4.45	4.45
Feces	4.79	4.69	4.66	4.61	4.74	4.78	4.72	4.73

[a]Dry matter basis, except for diluted urine (volume basis).

[b]Dry matter values were obtained by vacuum drying at 100 C, previously partially dried samples of feed and feces.

Table C-4. Percent dry matter, crude protein and gross energy (Mcal/kg) of feed and feces and crude protein of diluted urine per steer by treatment (Metabolism trial 4)[a]

Item	Treatments							
	Control				Monensin			
Steer No.:	5B	15	6B	17	18	8	16	2
Dry matter[b]								
Feed	93.28	93.28	93.28	93.28	94.20	94.20	94.20	94.20
Feces	90.72	90.55	90.03	88.69	89.84	91.58	91.27	90.83
Crude protein								
Feed	12.06	12.06	12.06	12.06	12.52	12.52	12.52	12.52
Feces	23.38	24.66	25.07	25.09	25.31	24.66	23.82	23.67
Diluted urine	3.04	1.86	2.12	2.47	1.94	2.34	2.71	2.48
Gross energy (Mcal/kg)								
Feed	4.44	4.44	4.44	4.44	4.44	4.44	4.44	4.44
Feces	4.94	4.93	4.87	4.90	5.15	4.98	4.83	4.83

[a]Dry matter basis, except for diluted urine (volume basis).

[b]Dry matter values were obtained by vacuum drying at 100C, previously partially dried samples of feed and feces.

Table C-5. Body weight, intake and output of dry matter (DM) and diluted urine volume per steer (Metabolism trials)

	Steer Number							
Item	10[a]	14A[a]	6A	5A	30	20	25	19
Trial 1								
Body weight, kg	253	276	247	272	240	246	281	255
DM intake, g	5361	5439	4677	5530	5432	3862	5310	5506
DM output, g	1074	1422	1052	1329	1320	855	1065	1352
Diluted urine volume, ml	10000	20808	10333	15500	10000	10000	10000	10000
Trial 2								
Body weight, kg			288	318	276	290	321	290
DM intake, g			5398	5583	5473	5339	5159	5925
DM output, g			1321	1239	1505	1192	1084	1411
Diluted urine volume, ml			10000	16500	10000	10000	10000	15000
	Steer Number							
	5B	17	2	6B	16	8	15	14B[b]
Trial 3								
Body weight, kg	255	256	242	247	255	264	274	274
DM intake, g	4641	4726	4732	4726	4732	4211	4726	4760
DM output, g	892	1106	1120	1070	1177	894	1050	939
Diluted urine volume, ml	10000	5167	6667	5000	5000	5000	11250	5000

Table C-5. Continued

Item	Steer Number								
	5B	17	2	6B	16	8	15	14B[b]	18[c]
Trial 4									
Body weight, kg	324	310	311	318	317	329	348		312
DM intake, g	5182	5182	5195	4731	5195	5195	5182		5195
DM output, g	909	1080	1051	945	1127	1014	1066		1011
Diluted urine volume, ml	10000	10000	10000	10000	10000	10000	11667		10000

[a]Missing from Trial 2.

[b]Missing from Trial 4.

[c]Missing from Trial 3.

APPENDIX D

TABULAR DATA, ABOMASAL PASSAGE TRIALS

Table D-1. Percent dry matter, ash and chromic oxide of feed, abomasal digesta and feces per steer by treatment (Abomasal passage trial 1)[a]

Item	Treatments					
	Control			Monensin		
Steer No.:	257	262	258	263	261	264
Dry matter[b]						
Feed	92.92	92.92	92.92	92.91	92.91	92.91
Abomasal digesta	94.32	92.51	92.49	93.84	92.27	94.75
Feces	91.82	92.29	92.66	92.23	92.90	91.81
Ash (DM basis)[b]						
Feed	2.45	2.45	2.45	2.36	2.36	2.36
Abomasal digesta	10.02	6.40	19.89	5.27	7.87	15.92
Feces	8.38	7.59	11.07	11.65	8.36	7.31
Chromic oxide						
Feed	.234	.234	.234	.225	.225	.225
Abomasal digesta	.554	.319	.263	.336	.256	.152
Feces	1.26	1.13	1.24	2.06	1.42	.98

[a]Organic matter basis.

[b]Dry matter values were obtained by vacuum drying at 100C, previously partially dried samples of feed, feces and abomasal digesta.

Table D-2. Percent nitrogen fractions in feed, abomasal digesta and feces per steer by treatment (Abomasal passage trial 1)[a]

Item	Treatments					
	Control			Monensin		
Steer No.:	257	262	258	263	261	264
<u>Crude protein</u>						
Feed	10.26	10.26	10.26	10.28	10.28	10.28
Abomasal digesta	30.56	18.50	14.94	18.56	14.81	11.13
Feces	20.31	17.06	19.81	27.38	20.25	15.94
<u>Ammonia nitrogen</u>						
Abomasal digesta	.10	.05	.05	.06	.06	.03
Abomasal nitrogen, % of	2.11	1.82	2.09	1.89	2.36	1.63
<u>Non-ammonia nitrogen</u>						
Abomasal digesta	4.79	2.91	2.34	2.91	2.31	1.75
Abomasal nitrogen, % of	97.89	98.18	97.91	98.11	97.64	98.37
<u>Bacterial protein</u>[b]						
Abomasal protein, % of	56.53	50.04	45.64	49.19	46.45	26.47
<u>ADF-protein</u>[b]						
Feces	3.42	2.39	3.04	4.90	3.32	2.51

[a]Organic matter basis

[b]Nitrogen x 6.25

Table D-3. Percent dry matter, ash and chromic oxide of feed, abomasal digesta and feces per steer by treatment (Abomasal passage trial 2)[a]

Item	Treatments								
	Control				Monensin				
Steer No.:	259	263	261	264	257	267	265	262	258
Dry matter[b]									
Feed	92.02	92.02	92.02	92.02	92.02	92.02	92.02	92.02	92.02
Abomasal digesta	94.18	93.01	93.60	94.08	92.82	93.32	91.60	92.18	92.99
Feces	89.64	91.78	91.25	90.79	91.40	90.74	91.32	90.74	90.86
Ash (DM basis)[b]									
Feed	2.87	2.87	2.87	2.87	2.92	2.92	2.92	2.92	2.92
Abomasal digesta	8.84	7.66	11.04	9.61	11.22	10.74	10.32	5.74	9.62
Feces	10.61	9.99	12.17	8.72	7.69	11.04	7.68	7.94	8.30
Chromic oxide									
Feed	.223	.223	.223	.223	.224	.224	.224	.224	.224
Abomasal digesta	.327	.334	.342	.243	.595	.255	.325	.231	.277
Feces	1.35	1.45	1.61	1.06	1.04	1.39	.88	1.16	1.15

[a]Organic matter basis.

[b]Dry matter values were obtained by vacuum drying at 100C, previously partially dried damples of feed, feces and abomasal digesta.

Table D-4. Percent nitrogen fractions in feed, abomasal digesta and feces per steer by treatment (Abomasal passage trial 2)[a]

Item	Treatments: Control				Monensin				
Steer No.:	259	263	261	264	257	267	265	262	258
Crude protein									
Feed	10.31	10.31	10.31	10.31	10.32	10.32	10.32	10.32	10.32
Abomasal digesta	21.94	20.69	22.12	17.06	30.44	17.00	18.94	16.00	15.81
Feces	22.56	20.94	22.06	18.88	15.12	21.94	14.31	18.00	18.63
Ammonia nitrogen									
Abomasal digesta	.08	.09	.08	.06	.10	.05	.07	.04	.05
Abomasal nitrogen, % of	2.25	2.68	2.31	2.01	2.11	1.76	2.18	1.50	2.06
Non-ammonia nitrogen									
Abomasal digesta	3.43	3.22	3.46	2.67	4.77	2.67	2.96	2.52	2.48
Abomasal nitrogen, % of	97.75	97.32	97.69	97.99	97.89	98.24	97.82	98.50	97.94
Bacterial protein[b]									
Abomasal protein, % of	66.39	63.91	68.57	55.96	67.51	55.30	49.93	61.65	57.93
ADF-protein[b]									
Feces	3.16	3.59	3.48	2.18	2.61	3.18	1.71	2.31	2.66

[a]Organic matter basis.
[b]Nitrogen x 6.25.

Table D-5. Body weight, intake and output of organic matter (OM) per steer (Abomasal passage trials)

	Steer Number								
Item	257	267[a]	259[a]	263	265[a]	262	261	258	264
Trial 1									
Body weight, kg	248			225		211	220	216	227
OM intake, g	3618			3787		3618	3449	3618	3621
OM output, g	617			415		695	471	627	782
Trial 2									
Body weight, kg	267	224	246	261	215	234	241	237	250
OM intake, g	4444	3760	3762	4275	3760	3760	3933	3931	3933
OM output, g	876	535	547	574	740	677	444	676	766

[a]Missing from Trial 1.

Table D-6. Percentage of chromic oxide recovery per steer (Abomasal passage trials)

	Steer Number								
Item	257	267[a]	259[a]	263	265[a]	262	261	258	264
Trial 1	91.71			99.88		92.65	85.99	91.94	93.79
Trial 2	91.75	88.48	87.91	87.13	77.41	93.44	81.42	88.42	92.53

[a]Missing from Trial 1.

APPENDIX E

ANALYSES OF VARIANCE

All data were analyzed at the University of Arizona Computer Center on the CDC 6400 Computer. Statistical Package for the Social Sciences (SPSS Version 7.0 -- June 27, 1977) is distributed by Vogelback Computing Center, Northwestern University, Chicago, IL. Program MULTIVARIANCE (Univariate and Multivariate Analysis of Variance, Covariance and Regression -- Version 5) is distributed by National Educational Resources, Inc., 215 Kenwood Avenue, Ann Arbor, MI.

Digestion trial data were analyzed as a crossover design by analysis of variance (SPSS). Data from metabolism and abomasal passage trials included missing cells and unequal subclass numbers, and were analyzed by analysis of variance using the method of least squares (MULTIVARIANCE).

Table E-1. Mean squares for dietary and ruminal component parameters (Digestion trials)

	Mean Squares			
Parameters	Trial	Animal	Treatment	Error
Degrees of freedom	1	7	1	6
Dietary component				
Dry matter				
Intake, g	72630**	50.5	132	40.6
Intake, $g/W_{kg}^{.75}$	15.5**	3.7*	5.5*	.86
Digestibility, %	18.2	32.4*	.52	7.2
Gross energy				
Digestibility, %	23.0	35.5*	.36	7.9
Starch				
Digestibility, %	9.8	26.5**	.14	3.3
Nitrogen				
Digestibility, %	6.4	41.7†	17.9	11.1
Ruminal Component				
VFA				
Total, M	423	203	14.6	326
C2, %	62.4*	49.7*	46.9*	7.4
C3, %	119*	200**	172*	13.0
C4, %	1.1	28.8†	2.5	8.7
Ammonia-N, mg %	1.6	3.2	39.2†	9.5

†($P < .10$), *($P < .05$), **($P < .01$).

Table E-2. Mean squares for dietary and ruminal component parameters (Metabolism trials)

Parameters	Mean Squares					
	Experiment	Trial Within Experiment	Animal Within Experiment	Treatment	Experiemnt x Treatment Interaction	Error
Degrees of freedom	1	2	15	1	1	9
Dietary component						
Dry matter						
Intake, g	1140625**	649999*	133028	321473†	56300	85638
Intake, $g/W_{kg}^{.75}$	524**	59.3	37.1	64.2	10.7	21.6
Digestibility, %	35.2**	9.8**	5.8**	1.1	.06	.99
Gross energy						
Digestibility, %	28.5**	4.8*	5.4**	1.3	.50	.85
Nitrogen						
Intake, g	105†	502**	46.1	255*	3.5	31.5
Digestibility, %	117**	1.7	17.2*	35.4*	7.8	5.1
Fecal, g	291**	88.1*	28.7†	1.0	2.9	11.8
Fecal, %	178**	1.7	17.2*	35.6*	7.8	5.1
Urinary, g	9.8	303**	37.8	22.9	3.6	17.7
Urinary, %	48.1	126*	32.0	.30	7.2	23.1
Retained, g	13.8	90.7†	33.3	104†	30.3	22.6
Retained, % of intake	40.8	149**	38.0	42.4	30.0	18.7
Retained, % of absorbed	7.7	433**	92.9	49.2	61.4	52.4
Ruminal component						
VFA						
Total, M	3380**	102	315	74.1	288	274
C2, %	.04	50.7†	24.5	1.7	7.0	12.9
C3, %	.02	170*	31.2	1.5	35.6	35.2
C4, %	1.7	40.0	2.0	1.7	13.6†	4.2

†(P < .10), *(P < .05), **(P < .01).

Table E-3. Mean squares for organic matter and nitrogen fraction parameters (Abomasal passage trials)

Parameters	Mean Squares: Period	Animal	Treatment	Error
Degrees of freedom	1	8	1	4
Organic matter				
Intake, g	397737*	51820	.75	34202
Intake, $g/W_{kg}^{.75}$	13.3	5.7	.52	10.4
Output, g	8009	25723	2523	7944
Apparent total digestion				
Total collection, %	.20	17.3*	.92	2.8
Total collection, g	292638**	67144†	2581	13969
True ruminal digestion, %	492†	555*	404†	85.2
True ruminal digestion, g	1324717*	944995*	457861	166190
Entering small intestine, g	39146	708928*	342732	100335
Postruminal digestion, %	29.7	203*	2.1	30.1
Postruminal digestion, g	82568	702168*	286443	113678
Nitrogen fraction				
Crude protein intake, g	5398*	558	.08	369
Crude protein intake, $g/W_{kg}^{.75}$	.29	.06	.01	.11
Crude protein output, g	65.9	260*	18.8	22.9
Crude protein				
Apparent total digestion				
Total collection, %	13.0†	14.3†	1.9	2.9
Total collection, g	4271*	508	21.3	392
Ruminal digestion, %	36.9	329	.55	87.6
Ruminal digestion, g	127	4902†	4.1	1248

Table E-3. Continued

Parameters	Mean Squares Period	Animal	Treatment	Error
Degrees of freedom	1	8	1	4
Crude protein				
Entering small intestine, g	3868†	4893*	3.0	759
Postruminal digestion, %	2.8	9.4	.56	3.8
Postruminal digestion, g	2924	3664†	6.8	9.0
Protein true digestibility, %	2.03**	.33*	.07	.04
Ammonia nitrogen				
Entering small intestine, g	.15†	.06	.12†	.02
Percent of abomasal nitrogen	.05	.10	.18	.05
Non-ammonia nitrogen				
Entering small intestine, g	91.4†	123*	.08	19.5
Percent of abomasal nitrogen	.05	.10	.18	.05
Feed Nitrogen				
Ruminal digestion, %	1579**	324†	198	74.8
Ruminal digestion, g	927**	145†	62.1	27.4
Entering small intestine, g	347*	113	63.5	24.1
Percent of abomasal nitrogen	832**	90.9*	88.7†	13.0
Postruminal digestion, %	13.8†	10.8†	.14	1.9
Postruminal digestion, g	336*	115†	61.2	22.9
Percent of total nitrogen digested postruminally	1512*	175*	112*	15.6
Bacterial nitrogen				
Entering small intestine, g	796**	64.7†	62.6†	12.9
Percent of abomasal nitrogen	818**	88.5*	82.4†	13.7
Postruminal digestion, %	1065**	110	63.4	30.0
Postruminal digestion, g	708**	63.9*	49.6†	9.6
Percent of total nitrogen digested postruminally	1512**	175*	112*	15.6

†($P < .10$), *($P < .05$), **($P < .01$)

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