

MONENSIN FOR COWS GRAZING LOW QUALITY DRY WINTER
RANGE GRASS: EFFECTS ON FORAGE INTAKE, RUMEN
TURNOVER, VOLATILE FATTY ACIDS,
LACTATION, AND PERFORMANCE

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CHAPTER I

INTRODUCTION

The ruminant animal is unique in that it can convert large quantities of cellulose into high quality human food. This is possible due to the large population of microorganisms in the rumen. Because of this capability, ruminants will occupy an even more important role in world food production in the future, especially in areas where land is not suitable for crop production. However, the need for increased animal efficiency and/or performance is becoming more critical as the food supply becomes more limited, and as the demand for grain and other plant products for human consumption increases.

Monensin, a biologically active compound produced by Streptomyces cinnamomensis has been shown to improve feed efficiency when added to high concentrate feedlot rations. Monensin-fed feedlot cattle tend to consume less feed but gain at a similar rate as non-monensin fed cattle. When fed to cattle grazing green forage, monensin has increased both gain and efficiency. Since forage is the principle feed source for the beef cow, any compound which could alter efficiency and/or performance of grazing cows could affect the profitability of the beef cow enterprise. Information is not available, however, on the effectiveness of monensin when fed to range beef cows grazing low quality range grass.

The purposes of this study were: 1) to evaluate the effect of monensin on milk production and milk composition for lactating range

cows; 2) to evaluate the effect of monensin on forage and supplement intake and 3) to estimate the effect of monensin on rumen turnover rates and rumen components.

CHAPTER II

REVIEW OF LITERATURE

Introduction

Plant energy sources, primarily cellulose and starch, are hydrolyzed by rumen microbes to simple sugars and then to volatile fatty acids (VFA's) which supply much of the energy to the ruminant. Although this system allows the ruminant to utilize feedstuffs largely unavailable to non-ruminants, fermentation in the rumen results in considerable energy loss to the host animal. Any method of altering VFA production which could reduce energy loss during fermentation would improve efficiency of feed utilization providing total energy yield and bacterial protein synthesis are not inhibited. Monensin has been shown to shift the microbial fermentation in the rumen and increase the propionate to acetate and butyrate ratio. This review will deal with the apparent mode of action of monensin and its effect on forage intake, rumen turnover, and lactation.

Monensin

Monensin is a biologically active compound produced by Streptomyces cinnamomensis (Haney and Hoehn, 1967) and belongs to the general class of compounds termed polyethers. Monensin has been observed to: increase the molar proportion of propionate in vitro and in vivo (Richardson et al., 1976); be effective in preventing coccidiosis in poultry (Shumard

and Callender, 1967) and ruminants (Fitzgerald and Mansfield, 1973); and have moderate in vitro activity against gram-positive organisms (Haney and Hoehn, 1967).

The mode of action of monensin for improvement of feed efficiency has been attributed to a shift in the microbial population and a subsequent increase in production of propionate and decrease in production of acetate and butyrate. These shifts in the end products of fermentation result in an energy savings since propionate fermentation is energetically more efficient than either acetate or butyrate fermentations (Wolin, 1960; Hungate, 1966). Richardson et al., (1976) calculated energy savings associated with a 10 moles/100 moles increase in ruminal propionate of approximately 5.6%. In experiments conducted by Raun et al., (1976), monensin increased the molar proportions of propionate 5 to 10 moles/100 moles.

Raun et al., (1976) concluded that a 3 to 6% increase in the metabolizable energy content of the ration due to altered VFA ratios could not account for the total feed efficiency improvement observed. A secondary savings of energy may occur in animals fed monensin, if propionate is used more efficiently by the ruminant tissues than acetate. Smith (1971) has reviewed the literature dealing with the efficiency of utilization of acetate, propionate and butyrate. Some studies have indicated the three principal fatty acids are used with equal efficiency, while others suggest that propionate is utilized more efficiently. If the latter is true, then monensin could theoretically improve the efficiency of energy utilization beyond the percentage savings calculated as occurring in the rumen fermentation. Other potential savings resulting from a shift in VFA's may include: 1) lower heat increment, especially on

above maintenance rations (Armstrong and Blaxter, 1957; Blaxter, 1962); 2) protein sparing effect, since propionate is a precursor of glucose (Leng et al., 1967) and normally ruminants obtain some of their glucose from amino acids (Reilly and Ford, 1971); 3) stimulation of protein synthesis in the animal (Eskeland et al., 1974); and 4) changes in the composition of ingesta reaching the lower tract and increases in extent of digestion in the lower tract.

Feed Intake

For ruminants, the bulky and fibrous nature and low digestible energy content of forage diets cause rumen distention to limit voluntary feed intake. For diets consisting mainly of roughages, voluntary intake is limited by the capacity of the reticulorumen and by the rate of disappearance of digesta from this organ (Balch and Campling, 1962). Voluntary intake appears directly related to rate of disappearance of digesta from the reticulorumen. The addition of dilute solutions of urea to the reticulorumen of cows offered oat straw improved digestibility, reduced retention time of food residues in the tract and increased voluntary intake by 39% (Campling et al., 1962). The importance of the level of nitrogen in the diet to microbial digestion of cellulose and to voluntary intake was demonstrated by Moir and Harris (1962) and Elliott and Topps (1963). More recent evidence suggests that voluntary intake of low protein roughages may increase with improved protein status of the animal, i.e., to a metabolic effect (Egan, 1965 and Weston, 1966b).

The decrease in the rate of disappearance from the reticulorumen of digesta derived from roughage caused by adding cereal grain to a diet

of hay, and was directly related to a reduction in the voluntary intake of hay by cows (Campling, 1966). This was attributed to reduced cellulolytic activity of the rumen microflora causing an increased time of retention of hay residue in the gut. Another way to experimentally increase the time of retention of roughage residues and therefore depressing voluntary intake is to restrict rumination by using a closely fitted muzzle (Pearce and Moir, 1964). The breakdown of roughages by chewing during rumination speeds reduction in particle size in the reticulorumen (Pearce, 1967) and thus allows particles to pass more rapidly from this organ. Results presented by Troelsen and Campbell (1968) support the concepts that physical bulk limits intake of coarse roughages and that voluntary intake and the size and shape of roughage particles passing through the reticulo-omasal orifice are closely related.

The control of voluntary intake of low-protein roughages such as straws is controlled partly by physical factors, and especially by the rate of breakdown of digesta in the rumen, and possibly by metabolic factors arising from the protein status of the animal. With highly digestible diets which do not fill the reticulorumen to capacity (e.g., concentrates and immature forage) it is unlikely that physical or bulk factors limit voluntary intake.

Several authors have proposed the concept of palatability, that is the hedonic response of an animal to a food which depends on taste, smell, flavor, and texture (Young, 1967), to explain the regulation of voluntary intake of roughage diets by ruminants (Blaxter et al., 1961; Campling et al., 1962; and Weston, 1966a). This suggestion was made because of the convincing evidence for physical control. Greenhalgh and Reid (1967) challenged this conclusion and reported results from an

experiment involving oral or intra-ruminal feeding of dried grass or straw. They suggested that digestibility and palatability were of about equal importance in determining voluntary intake of these roughages by sheep.

Monensin theoretically will increase the energy content of the diet by increasing the ratio of propionate to acetate and butyrate. Forage-fed cattle, as a general rule, ate to bulk fill and any increase in usable energy caused by monensin should be reflected in weight gain. In feeding trials, with monensin, where high roughage diets are fed in drylot, improvement in average daily gains have been variable. Several experiments have shown similar gains, but lower feed intakes, when cattle were fed rations containing 60 to 75% roughage at various levels (0 to 44 ppm) of monensin (Raun et al., 1976; Embry and Swan, 1975 and Hale et al., 1975). However, some (Sherrod et al., 1975; Bolsen et al., 1975; Utley et al., 1975) have obtained increases in average daily gains from 5 to 12% and lower feed intakes with similar diets and monensin levels.

No research to date has reported forage intake by grazing animals when monensin was fed, however, several researchers have reported increased daily gains with monensin feeding on grass clover pastures (Cambett et al., 1973), Coastal bermudagrass (Oliver, 1975) and orchardgrass, alfalfa, brome and ladino clover (Potter et al., 1976). The greatest response to monensin was observed at intakes of 100-200 mg/hd/day level. In contrast, Anthony and Brown (1975) and Harris et al., (1976) found no effect on daily gain when steers were from 0 to 200 mg of monensin for steers grazing on Coastal bermudagrass. It appears that the response to monensin is highly variable and dependent on level of monensin and forage quality.

Little research has reported forage intake of cows fed maintenance intakes, however, Turner et al., (1977) reported that monensin-fed cows outgained control cows with less meadow grass intake. If similar results could be obtained with cows grazing low quality winter range grass, the net result could be a savings in forage plus increased reproductive efficiency.

Rumen Turnover

The relationships between rumen volume, fermentation rate, rate of turnover, and extent of digestion (Mitchell, 1942) indicate the importance of turnover rate in the economy of feed utilization by the ruminant. More complete digestion of forages will be obtained with a longer retention time in the rumen. Long retention is correlated with a large rumen volume, slow fermentation rate and high digestibility (Hungate, 1966). In a number of studies, however, increased turnover and feed intake have counterbalanced the loss in digestibility, with no net difference in total efficiency of the ration (Blaxter and Graham, 1956; Forbes et al., 1925). Under other conditions, such as increased activity during grazing, the faster turnover may prove advantageous (Phillips et al., 1960). Since the rate of digestion of a given piece of forage particle diminishes with residence time in the rumen, increases in turnover rate will not cause a proportional decrease in digestibility (Hungate, 1966).

No research to date has been reported in the literature showing the effect of monensin on rumen turnover rate and its relationship with feed intake. It is possible that decreased rumen turnover rate could partially, if not totally, account for decreased feed intakes with monensin

feeding observed by Turner and Raleigh (1977), Raun et al., (1976), Embry and Swan (1975) and Hale et al., (1975).

Lactation

Milk secretion and biosynthesis of milk lipids have received extensive review. Reviews have dealt with the physiology (Linzell, 1959) and the biochemistry (Jones, 1969) of the lactating mammary gland, as well as the nutritional requirements for the synthesis of milk constituents (Rook and Storry, 1964) and the origin of milk lipid precursors (Garton, 1963).

Ruminant milk lipids are characterized by the presence of substantial quantities of short and medium chain fatty acids. This attribute is a result of an active de novo synthesis from the simple metabolites, acetate and β -hydroxybutyrate, which are supplied to the mammary gland (Dimick et al., 1970). Popják et al., (1951) demonstrated the contribution of acetate to milk fatty acids and Shaw and Knatt (1941) established the utilization of β -hydroxybutyrate by the lactating gland. Considerable attention has focused on the metabolic pathways and quantitative significance of these simple precursors. It is now accepted that the blood precursors of ruminant-milk fat are glucose, acetate, β -hydroxybutyrate, and triglycerides associated with blood serum β -lipo-proteins.

The effect that monensin may have on milk production and milk composition when the propionate:acetate ratio is changed has been studied by Randel and Rouquette (1976). They found that monensin had little effect on milk production or butterfat, solids and protein content with Coastal bermudagrass hay fed to Brahman crossbreds. What effect monensin

may have on low producing cows grazing low quality winter range has not been reported in the literature.

CHAPTER III

MONENSIN, FORAGE INTAKE AND LACTATION OF BEEF

COWS GRAZING LOW QUALITY DRY

WINTER RANGE GRASS

Summary

Two trials were conducted to evaluate the effect of monensin on intake and lactation of cows grazing low quality dry winter range grass. In trial 1 cows were fed a 30% protein soybean meal supplement with 0, 50 or 200 mg of monensin per cow per day. In trial 2 cows were fed the same supplement with 0 or 200 mg of monensin.

Cow weight change was similar when 200 mg of monensin was added to the control supplement in both trial 1 and 2. In trial 1, cows fed the 50 mg level lost more weight ($P < .05$) than either the control or 200 mg monensin treatments. Relative forage intake in trial 1 was reduced 13.6% ($P < .05$) and 19.6% ($P < .05$) when 50 and 200 mg of monensin were fed respectively. Similarly, grazing time in trial 2 was reduced 14.6% when monensin was fed.

Ruminal acetate and butyrate were consistently decreased and propionate was consistently increased when 200 mg of monensin was fed. Milk yield and milk composition were not appreciably affected by monensin, but calves reared by monensin fed cows gained weight more rapidly ($P < .05$), suggesting that calves reared by cows fed monensin utilized milk and/or forage more efficiently when they consumed monensin contain-

ing supplement.

Introduction

Monensin increases the energy content of a diet by increasing the ratio of propionate to acetate and butyrate. Forage fed cattle supposedly eat to bulk fill; consequently, an increase in usable energy by monensin feeding should increase weight gain. But in drylot feeding trials, monensin added to high roughage diets has had variable effects on gain. Several experiments have shown unchanged gain, but lower feed intake (Raun et al., 1975; Embry and Swan, 1975; and Hale et al., 1975), while others report increased gain and lower feed intake (Sherrod et al., 1975; Bolsen et al., 1975; and Utley et al., 1975). Influence of monensin on forage intake of cows fed near maintenance intakes has received little study. Turner et al. (1977) reported that monensin fed cows outgained control cows while consuming less meadow-grass hay.

Lipids in the milk of ruminants contain substantial quantities of short and medium chain length fatty acids. This is the result of active de novo synthesis from the simple metabolites, acetate and β -hydroxybutyrate, which are supplied to the mammary gland (Dimick et al., 1970; Popják et al., 1951; and Shaw and Knott, 1941). Milk fat levels typically decrease as the ruminal acetate to propionate ratio decreases (Davis and Brown, 1969). Randel and Rouquette (1976) fed monensin to Brahman crossbred cows and found monensin had little effect on milk production or levels of butterfat, solids and protein.

The purposes of this study were: 1) to evaluate the effect of monensin on forage and supplement intake and 2) to evaluate the effect of monensin on weight change, milk production and milk composition of

range beef cows grazing dry native winter range grass.

Experimental Procedures

Two winter trials were conducted in central Oklahoma on native tall-grass range with climax vegetation of little bluestem (Andropogon scoparius), big bluestem (Andropogon gerardi), Indian grass (Sorghastrum nutans) and switch grass (Panicum virgatum). Ingredient make-up of supplements fed in the trials are shown in Table I.

Trial 1

Sixty-nine mature open Hereford cows were employed in a 123 day trial from November 13 to March 15. Cows were stratified by weight to 23 simultaneous 3 x 3 Latin squares and randomly allotted to one of three treatments. The treatments were 0, 50 or 200 mg additions of monensin per cow daily to 30% natural crude protein supplements. All cows were allowed to graze in a common pasture, corralled daily and individually stall fed 1.25 kg of their respective supplement once daily, six days per week. Feed refusals were recorded daily.

Cows were weighed at the beginning and end of each 41 day period. On the last day of each period, cows were fed their respective supplements and allowed to graze for approximately 3 hr, after which rumen samples were taken for VFA analysis. On day 40, 82 and 123 of the trial 10, 7 and 8 randomly selected cows per treatment were ruminally sampled for VFA analysis. Samples were taken by stomach tube by the system developed by Raun and Burroughs (1962). Microbial action was stopped by adding 5 gm of meta-phosphoric acid per 50 ml rumen fluid and VFA's were analyzed according to Supelco Bulletin 749 using SP-1200/H₃PO₄ packing.

TABLE I
INGREDIENT MAKEUP OF PROTEIN SUPPLEMENT

Item	International Reference Number	% in Supplement
Corn, yellow	4-02-915	22.77
Soybean meal	5-04-604	58.25
Alfalfa hay, grd	1-99-118	10.00
Molasses, cane	4-04-696	5.00
Solium phosphate, monobasic	6-04-287	2.50
Calcium phosphate, dibasic	6-01-080	.75
Sodium sulfate	6-04-292	.68
Trace mineral mix		.05
Vitamin A palmitate	7-05-143	22,000 IU/kg

Relative forage intake of cows grazing dry native range grass was estimated in December, February and March using 20g chromium sesquioxide daily as an external indicator. The chromium sesquioxide was administered with one-half the daily allocation of supplement at 8:00 a.m. and 4:00 p.m. during the six day preliminary and five day fecal collection periods. Fecal grab samples were dried at 60°C, prepared by the method of Williams et al. (1962), and analyzed for chromium content by atomic absorption spectroscopy. Three esophageally cannulated cows were used to obtain representative forage samples to estimate forage digestibility by in vitro dry matter disappearance techniques. The IVDMD procedure used inocula mixed from four steers adapted to harvested dry winter range grass and fed the non-monensin supplement.

Trial 2

Seventy-two mature lactating Hereford cows were used in a 152-day wintering trial from December 5 to May 6. Cows were randomly allotted, after blocking by weight and calving date, to two treatments with two replications per treatment. Cows were placed on four pastures and rotated among pastures at 14 day intervals to minimize pasture and location effects. The two treatments were 30% natural crude protein supplement with 0 or 200 mg of monensin added per cow per day. Table I shows the ingredient make-up of the supplement. The supplements were self-fed at a rate of 1.45 kg/cow/day with salt added to limit intake. Cows calved from October 11 to December 3 with a mean calving date of November 1 and November 2 for the control and monensin supplement respectively.

Four 24-hr pasture observations were conducted in January, February

and March to estimate grazing time and frequency of supplement intake. Grazing time was estimated by observing each cow at 15-min intervals and recording whether she was or was not grazing. The supplement feeder was continuously observed and frequency and duration of supplement intake were recorded for 24 hours. Each of the treatment replications were observed during the winter supplementation period. Immediately following the pasture observations, rumen samples were taken from 10 randomly selected cows per treatment. Preservation and analytical procedures were the same as in trial 1.

Estimates of 24-hr milk productions were obtained by the calf weigh-suckle-weigh technique each 6-hr for all calves on days 62, 83, 110 and 150 of the trial.

Milk composition of all cows was estimated on days 95 and 139 of the trial. Cows were IM injected with 8 cc of Sparine tranquilizer (50 mg/cc, Wyeth) approximately 45-min prior to milking and IV injected with 1 cc of oxytocin (20 USP units/cc) immediately preceding milking. Cows were milked as completely as possible with a milking machine and representative milk samples were taken for analysis. Samples on day 95 were analyzed for butterfat only while samples on day 139 were analyzed for butterfat and total milk solids and solids-not-fat was calculated.

Statistical Analysis

To estimate carry-over effects of supplements from one period to the next, the selection of Latin squares were balanced so that each treatment was administered an equal number of times following each other treatment. Therefore, cow weight change and relative forage intake in trial 1 were analyzed as 23 simultaneous 3 x 3 Latin squares by the

procedure of Lucas (unpublished notes) to facilitate testing for carry-over effects. All VFA data from trial 1 and 2, and cow grazing observations in trial 2, were analyzed as a completely randomized design. One cow fed the control supplement in trial 2 lost her calf early in the experiment, therefore that cell was estimated by procedures outlined in Cochran and Cox (1957) for randomized block designs. Cow weight change, milk yield, milk composition and calf gain data, in trial 2, were analyzed as a randomized block design. Post-partum interval data of cows and frequency and duration of supplement intake by calves were analyzed by least-square procedures. Number of cows exhibiting estrus, conception rate and number of calves consuming supplement were analyzed by Chi-square procedures.

Results and Discussion

Trial 1

Performance of cows grazing dry winter range grass is shown in Table II. Average daily supplement intakes were equal among treatments. Weight loss of cows fed the 0 and 200 mg treatments were similar. Cows fed 50 mg of monensin lost more weight than cows on the other treatments ($P < .05$). Carry over effects were not significant ($P > .50$) suggesting that previous treatment did not affect subsequent treatments as cows proceeded through the Latin square. These results would suggest that 50 mg is less useful than 0 or 200 mg monensin daily for cows grazing poor quality winter range. That the 200 mg level of monensin does not alter cow weight change under dry winter range conditions agree with earlier findings of Lemenager et al. (1975).

TABLE II
 PERFORMANCE OF COWS DURING WINTER SUPPLEMENTATION IN TRIAL 1

Item	Monensin, mg/cow/day			S.E.
	0	50	200	
Cows, number	69	69	69	
Daily supplement, kg	1.25	1.25	1.25	
Initial cow wt, kg	409.78	408.41	408.91	
Cow wt change kg	-6.76 ^b	-9.76 ^a	-6.43 ^b	1.00
direct effects, kg	-6.75 ^b	-9.98 ^a	-6.62 ^b	2.47
carry over effects, kg	.02	- .64	.62	3.31

^{a,b} Means in a row with different superscripts differ statistically (P < .05).

Relative forage dry matter intakes are shown in Table III. Forage intake was depressed from 9.61 kg on the control to 8.30 kg ($P < .05$) and 7.73 kg ($P < .05$) for the 50 and 200 mg treatments respectively. Carry over effects were not significant ($P > .20$). Dinius et al. (1976) reported no change in in vivo digestibilities of dry matter, crude protein, hemicellulose, and cellulose of orchardgrass hay when monensin was added. Based on this assumption, IVDM results of esophageal samples taken during the intake trials were used to calculate relative forage intakes. The estimated forage digestibilities were 41.7%, 38.2% and 39.8% for December, February and March intake trials, respectively.

Total and molar percentages of volatile fatty acids are shown in Table IV. Rumens fluid from cows fed 200 mg supplement daily had less acetate and butyrate ($P < .05$) and more propionate ($P < .05$) than rumens fluid from cows fed the control supplement. Rumens fluid from cows fed the 50 mg supplement was intermediate in concentration of VFA's between the control and 200 mg treatments, but not consistently different from the control. The failure of the 50 mg level of monensin to consistently alter acetate, propionate and butyrate further indicate it is not as good a level of monensin for cows grazing dry native range. Total molar concentration of VFA's was not significantly affected by the addition of monensin.

Trial 2

Results of cow performance in trial 2 are shown in Table V. Average daily supplement and salt intakes and calving dates were approximately equal on the two treatments. Weight changes of cows were similar during the dry grass portion of the trial, but cows fed monensin appeared to

TABLE III
 RELATIVE FORAGE INTAKE OF COWS GRAZING DRY WINTER
 RANGE GRASS IN TRIAL 1

Item	Monensin, mg/cow/day			S.E.
	0	50	200	
Forage intake, kg	9.61 ^a	8.30 ^b	7.73 ^c	.09
direct effects, kg	9.56 ^a	8.29 ^b	7.80 ^c	.10
carry over effects, kg	.15	-.04	.19	.14

^{a,b,c} Means in a row with different superscripts differ statistically (P < .05).

TABLE IV
 TOTAL AND MOLAR PERCENTAGES OF VOLATILE FATTY ACIDS
 IN RUMEN FLUID OF COWS IN TRIAL 1

Item	Monensin, mg/cow/day			S.E.
	0	50	200	
Acetate, molar %	78.46 ^a	78.43 ^a	73.68 ^b	.72
Propionate, molar %	15.86 ^b	16.85 ^b	21.92 ^a	.40
Butyrate, molar %	5.71 ^a	4.70 ^b	4.12 ^c	.19
Total, mM/l	32.11	31.77	30.42	2.55

^{a,b,c} Means in a row with different superscripts differ statistically ($P < .05$).

TABLE V
 PERFORMANCE OF COWS DURING WINTER SUPPLEMENTATION IN TRIAL 2

Item	Monensin, mg/cow/day		S.E.
	0	200	
Cows, number	36	36	
Ave daily supplement, kg	1.50	1.45	
Ave daily salt intake, kg	.58	.51	
Ave calving date	Nov 1	Nov 1	
Initial cow wt, kg	428.5	427.4	
Total wt change, kg	- 6.0	+ .8	3.28
Dry grass wt change, kg	-41.0	-44.2	5.26
Green grass wt change, kg	+35.0	+45.1	5.12
Cows exhibiting estrus, number	26	29	
Post-partum interval, days ^a	110.0	98.6	6.43
Conception rate, % ^b	50.0	58.6	

^aPPI of cows that exhibited estrus during the trial.

^bConception rate of cows that exhibited estrus.

recover weight more rapidly the last 45 days of the trial after green grass began to grow and be grazed.

The number of cows exhibiting estrus, post-partum interval and conception rate of cows during the trial (Table V) were not significantly different. However, the post-partum interval of cows that exhibited estrus during the trial averaged 11.4 days less ($P < .20$) for monensin fed cows which agrees with results obtained by Turner et al. (1977).

Grazing behavior and supplement intake of cows are shown in Table VI. Cows fed the monensin supplement grazed 6.9%, 28.2% and 6.0% less time in observations 1, 2 and 3 ($P < .10$, $P < .01$, $P < .25$) than control cows. When averaged over the first three grazing observations, monensin-fed cows grazed 14.6% less than control cows. These results are in agreement with results obtained in trial 1. In observation 4, after green grass appeared, cows fed the monensin supplement grazed 5.6% more ($P < .20$) than control fed cows. This may partially explain why monensin fed cows tended to gain faster than control cows after green grass appeared.

Frequency and duration of supplement intake were similar when averaged over the four grazing observations. When averaged over the four grazing observations, cows consumed 5.0 and 4.6 supplement meals per day, with each meal having a duration 7.3 and 8.0 minutes for control and monensin treatments respectively. Cows consumed both supplements with equal approximately frequency except in observation 3, when control cows ate supplement more frequently than monensin fed cows ($P < .05$). Duration of supplement intakes varied from one grazing observation to the next. In observation 1 and 3, monensin fed cows consumed supplement for a longer duration than control cows ($P < .05$), but in observation 2,

TABLE VI
GRAZING BEHAVIOR AND SUPPLEMENT INTAKE OF COWS IN TRIAL 2

Item	Monensin, mg/cow/day		S.E.
	0	200	
Grazing Observation 1 (January 23)			
Grazing time, %	30.5 ^a	23.4 ^b	.84
Freq. of supp. intake, times/day	5.5	5.2	.36
Duration of supp. intake, min.	7.6 ^c	10.3 ^d	.80
Grazing Observation 2 (February 13)			
Grazing time, %	36.2 ^e	26.0 ^f	1.24
Freq. of supp. intake, times/day	5.2	5.2	.27
Duration of supp. intake, min.	11.1 ^a	7.8 ^b	1.42
Grazing Observation 3 (February 20)			
Grazing time, %	29.8	28.0	.97
Freq. of suppl. intake, times/day	4.2 ^c	3.3 ^d	.23
Duration of supp. intake, min.	4.6 ^d	7.5 ^c	.63
Grazing Observation 4 (March 19)			
Grazing time, %	41.0	42.3	1.27
Freq. of supp. intake, times/day	5.1	4.7	.28
Duration of supp. intake, min.	6.0	6.5	.85

^{a,b} Means in a row with different superscripts differ significantly (P < .10).

^{c,d} Means in a row with different superscripts differ significantly (P < .05).

^{e,f} Means in a row with different superscripts differ significantly (P < .01).

control cows consumed supplement for a longer duration than monensin fed cows ($P < .10$). In observation 4, duration of supplement intake was similar between treatments. The statistical differences observed between treatments for frequency and duration of supplement intake may possibly be explained on the basis of social interaction between cows within a given pasture and not necessarily treatment differences.

Total and molar percentages of VFA's are shown in Table VII. The addition of monensin decreased acetate ($P < .01$) and increased propionate ($P < .01$) in observations 1 through 4. Butyrate was decreased in observation 1 and 4 with similar trends in observations 2 and 3. Total molar concentration was statistically depressed ($P < .01$) only in observation 1.

Milk yield and milk composition results are shown in Table VIII. Milk yield was not appreciably altered ($P > .50$) by monensin feeding when estimated by either the calf weigh-suckle-weigh technique or the milking by machine, however, estimated milk production was somewhat lower by the calf suckle technique. Butterfat, solids and solids-not-fat concentrations were not significantly affected ($P > .50$) by the addition of monensin to range supplements, in agreement with results of Randel and Rouquette (1976).

Performance of calves from cows fed monensin is shown in Table IX. Total calf gain was greater ($P < .05$) by calves reared by monensin fed dams. Calves reared by monensin-fed cows gained .63 kg/day compared to .59 kg/day for calves reared by control cows. Dividing results into the dry grass and green grass periods, calves reared by monensin-fed cows tended to gain faster in both phases of the trial.

During the first grazing observation it was noted that some calves

TABLE VII
 TOTAL AND MOLAR PERCENTAGES OF VOLATILE FATTY ACIDS IN
 RUMEN FLUID OF COWS IN TRIAL 2

Item	Monensin, mg/cow/day		S.E.
	0	200	
Observation 1			
Acetate, molar %	77.54 ^a	63.84 ^b	.69
Propionate, molar %	17.87 ^b	32.91 ^a	.68
Butyrate, molar %	4.59 ^a	3.25 ^b	.22
Total, mM/l	33.01 ^a	41.92 ^b	2.71
Observation 2			
Acetate, molar %	79.37 ^a	71.78 ^b	1.09
Propionate, molar %	16.11 ^b	24.43 ^a	.92
Butyrate, molar %	4.51 ^c	3.79 ^d	.30
Total, mM/l	38.81	40.60	2.49
Observation 3			
Acetate, molar %	80.73 ^a	68.79 ^b	1.29
Propionate, molar %	15.42 ^b	27.65 ^a	1.27
Butyrate, molar %	3.85	3.55	.32
Total, mM/l	38.91	38.90	4.49
Observation 4			
Acetate, molar %	74.77 ^a	71.21 ^b	.54
Propionate, molar %	16.82 ^b	21.53 ^a	.59
Butyrate, molar %	8.42 ^a	7.26 ^b	.18
Total, mM/l	46.02	45.07	1.38

a,b Means in a row with different superscripts differ significantly (P < .01).

c,d Means in a row with different superscripts differ significantly (P < .10).

TABLE VIII
 YIELD AND COMPOSITION OF MILK FROM COWS GRAZING DRY
 NATIVE RANGE GRASS IN TRIAL 2

Item	Monensin, mg/cow/day		S.E.
	0	200	
Milk production, kg ^a	4.0	4.0	.31
Milk production, kg ^b	6.2	6.0	.59
Butterfat, %	3.2	3.1	.13
Milk solids, %	12.8	12.9	.07
Solids-not-fat, %	9.6	9.8	.10

^aMean of four 24-hour milk productions by calf weigh-suckle-weight technique.

^bMean of milk production by milking machine technique.

TABLE IX
PERFORMANCE OF CALVES FROM COWS FED MONENSIN IN TRIAL 2

Item	Monensin, mg/cow/day		S.E.
	0	200	
Calves, number	35	36	
Initial calf wt, kg	106.5	106.6	
Total calf gain, kg	89.9 ^b	95.7 ^a	2.06
Dry grass gain, kg	52.4	55.9	1.61
Green grass gain, kg	37.5 ^d	39.7 ^c	.84

a,b Means in a row with different superscripts differ significantly (P < .05).

c,d Means in a row with different superscripts differ significantly (P < .07).

from each treatment were consuming supplement; therefore, in the second and subsequent grazing observations calf behavior was also observed. Frequency and duration of supplement intake of calves reared by monensin fed cows are shown in Table X. No consistent pattern in number of calves consuming supplement, frequency or duration of supplement intake by calves was detected. When averaged over the three pasture observations, 14.3 and 15.0 calves consumed supplement daily with 2.5 supplement meals per day, and meal durations of 3.5 and 3.3 minutes for control and monensin treatments respectively. The 6.8% increase in calf gain observed when monensin was fed may be attributed to increased efficiency of use by the calf of milk and/or forage consumed.

TABLE X
 FREQUENCY AND DURATION OF SUPPLEMENT INTAKE OF CALVES
 REARED BY COWS FED MONENSIN IN TRIAL 2

Item	Monensin, mg/cow/day	
	0	200
Grazing Observation 2 (February 13)		
No. of calves eating supplement	17	16
Freq. of supp. intake, times/day	3.5 ± .44	3.0 ± .46
Duration of supp. intake, min.	3.6 ± .46	3.1 ± .48
Grazing Observation 3 (February 20)		
No. of calves eating supplement	17 ^c	13 ^d
Freq. of supp. intake, times/day	2.6 ^c ± .28	1.8 ^d ± .32
Duration of supp. intake, min.	4.0 ± .51	.34 ± .58
Grazing Observation 4 (March 19)		
No. of calves eating supplement	9 ^d	16 ^c
Freq. of supp. intake, times/day	1.3 ^d ± .29	2.6 ^c ± .22
Duration of supp. intake, min.	3.0 ± 1.11	3.4 ± .83

^aA total of 18 calves in each treatment were observed during each grazing observation.

^bMeans are least squares means ± standard error of means.

^{c,d}Means in a row differ significantly ($P < .05$) as determined by Chi-squared.

CHAPTER IV

THE EFFECT OF MONENSIN ON RUMEN TURNOVER RATES, TWENTY-FOUR HOUR VFA PATTERNS, NITROGEN COMPONENTS AND CELLULOSE DISAPPEARANCE

Summary

Two trials were conducted to evaluate the effect of monensin on forage intake, rumen turnover rates, twenty-four hour volatile fatty acid patterns, rumen nitrogen components, in vitro dry matter disappearance and in vivo cellulose disappearance rate. In trial 1 steers were fed ad libitum dry winter range grass and a 30% protein soybean meal supplement with or without 200 mg of monensin per steer daily. In trial 2 steers were fed four levels of monensin with a high concentrate diet to meet NRC requirements for maintenance.

Forage intake of steers fed harvested dry winter range grass was 16% lower ($P < .02$) when monensin was added to the control supplement in trial 1. Liquid turnover rates were 31% ($P < .10$) and 10% ($P < .05$) slower when 200 mg of monensin was fed in trial 1 and trial 2 respectively. Solid rumen turnover rate was 44% ($P < .001$) lower when monensin was fed in trial 1. Liquid rumen volume was reduced ($P < .10$) in trial 1 when monensin was added to the control supplement, but total rumen dry matter was not affected.

Addition of monensin to in vitro fermentation flasks depressed IVDMD of harvested range grass by inocula from both control and monensin adapted steers. Peptide and amino nitrogen levels in rumen fluid of forage fed steers and in vivo cellulose disappearance rate estimated with cotton fiber strips in concentrate fed steers were not affected by monensin feeding.

Introduction

The relationships between rumen volume, fermentation rate, turnover rate, and extent of digestion (Mitchell, 1942) indicate the importance of turnover rate in the economy of feed utilization by the ruminant. The most complete digestion of forages will be obtained with a long retention time in the rumen. This is associated with a large rumen volume and a slow mean fermentation rate (Hungate, 1966). Yet some studies have shown an advantage for the increased turnover when energy intake increases, despite reduced digestibility (Blaxter and Graham, 1956). Increased energy intake simply dilutes the maintenance need to a greater degree. Since the rate of digestion of a particle diminishes with time in the rumen, increased turnover will decrease digestibility but at a decreasing rate. Yet, if conversion of energy to bacterial protein is desired, reduced turnover may prove useful.

The effect of monensin on rumen turnover rate and its relationship with feed intake has not been examined. Decreased rumen turnover rate could partially, if not totally, account for decreased feed intakes observed with high roughage rations (Turner et al., 1977; Raun et al., 1976; Embry and Swan, 1975; Hale et al., 1975; Sherrod et al., 1975; Bolson et al., 1975; Utley et al., 1975 and Lemenager et al., 1977), but

cause and effect are difficult to assess.

The purposes of this study were: 1) to evaluate the effect of monensin on ruminal turnover rate of steers fed low quality harvested dry winter range grass ad libitum or 80% concentrate rations limit-fed, 2) to evaluate the effect of monensin on ruminal nitrogen components and 3) to evaluate the IVDMD of harvested dry winter range grass with inoculum from control and monensin-adapted animals with or without monensin added to the fermentation flask.

Experimental Procedure

Trial 1

Eight ruminally cannulated mature steers weighing approximately 625 Kg were randomly assigned to two treatments in a cross-over design. A 30% protein soybean meal supplement (Table XI) was fed with or without 200 mg of monensin added per steer per day. Steers were individually housed in slatted floor pens and ad libitum fed low quality dry winter range grass harvested in late December. Grass was chopped to a maximum length of 18 centimeters. Grass intake was recorded daily.

Steers were adapted to their respective supplements for a period of 13 days prior to measuring turnover rate. Liquid and solid rumen turnover rates were estimated by using polyethylene glycol and chromium sesquioxide respectively. On day 13 of each period 50 gm PEG (MW 3000-3500) and 20 gm Cr_2O_3 were mixed and fed at 8:00 a.m. the daily allocation of supplement. A vacuum pump was used to obtain rumen samples at 4, 10, 16 and 22 hours post-supplement feeding. Microbial action was stopped by adding 2½% (v/v) saturated mercuric chloride per 200 ml of rumen contents. Rumen samples were centrifuged at 10,000 g to separate

TABLE XI
INGREDIENT MAKEUP OF PROTEIN SUPPLEMENT IN TRIAL 1

Item	International Reference No.	5 in Supplement
Corn, yellow	4-02-915	22.77
Soybean meal	5-04-604	58.25
Alfalfa hay, grd	1-99-118	10.00
Molasses, cane	4-04-696	5.00
Sodium phosphate, monobasic	6-04-287	2.50
Calcium phosphate, dibasic	6-01-080	.75
Sodium sulfate	6-04-292	.68
Trace mineral mix		.05
Vitamin A palmitate	7-05-143	22,000 IU/kg

liquid and solid fractions. The liquid fraction was analyzed for PEG by the procedure of Smith (1959) and for volatile fatty acids according to Supelco Bulletin 749 (1975) using SP-1200/H₃PO₄ packing. The solid fraction was dried at 100°C for 48-hr, prepared by the procedure of Williams et al. (1962) and analyzed for chromium content by atomic absorption spectroscopy. Peptide nitrogen was determined on the 4 and 22 hour liquid fraction samples by the Lowry procedure (1951). Alpha-amino nitrogen was determined by the ninhydrin procedure outlined by Clark (1964) on 22 hour liquid fraction samples only.

In vitro dry matter disappearance of harvested dry winter range grass was examined using composite inocula from 4 steers adapted to the control supplement and 4 steers adapted to the monensin supplement. Rumen fluid from control or monensin adapted steers was incubated with either 0 or 2 ppm monensin. A two stage Tilley and Terry (1963) in vitro disappearance study was conducted with a 48-hr fermentation and a 24-hr pepsin digestion.

Trial 2

Four ruminally cannulated mature Holstein steers were randomly assigned to four treatments in a Latin square design with 0, 50, 100 and 200 mg of monensin fed per steer daily. A high concentrate diet (Table XII) was limit-fed to meet NRC requirements for maintenance. The ration was fed twice daily at 8:00 a.m. and 4:00 p.m. at a rate of 2.72 kilograms per feeding.

Steers were adapted to their respective supplements for two weeks. On day 9 and 13 of each period, steers were dosed intraruminally with 4.87 gm of CrEDTA prepared by the procedure of Binnerts et al. (1968)

TABLE XII
INGREDIENT MAKEUP OF RATION IN TRIAL 2

Item	International Reference No.	5 in Supplement
Corn, yellow	4-02-935	62.75
Soybean meal	5-04-604	10.00
Alfalfa, dehy	1-00-022	6.00
Molasses, cane	4-04-696	5.00
Cotton seed hulls	1-01-599	14.00
Urea		.10
Ammonium chloride		.50
Limestone		.50
Calcium phosphate, dibasic	6-01-080	.50
Trace mineralized salt		.50
Aurafac - 10		.15

and sampled 4 and 24 hours later. Samples were strained through four layers of cheesecloth, after which the liquid filtrate was centrifuged at 10,000 g and analyzed for chromium content by atomic absorption spectroscopy.

Cellulose disappearance rate was estimated using unwashed, unsized cotton duck strips weighing approximately 1.5 grams. On days 9 and 13 of each period cotton fiber strips were suspended in the rumen for 24 hours, recovered, washed, dried and weighed to determine cellulose degradation.

Statistical Analysis

In trial 1, liquid and solid rumen turnover rates, liquid and dry matter rumen volumes, VFA's, peptide nitrogen and alpha-amino nitrogen were analyzed as a cross-over design. IVDMD results were analyzed as a factorial arrangement of treatments in a split plot design (Cohran and Cox, 1957). In trial 2, liquid rumen turnover, rumen liquid volume and cellulose disappearance rate were analyzed as a Latin square design.

Results and Discussion

Trial 1

When monensin was fed, average daily forage intakes (Table XIII) were depressed by 15.6% ($P < .02$). These results closely agree with those of cows grazing similar low quality dry winter range grass (Lemenager *et al.*, 1977). A partial explanation for decreased feed intake with monensin feeding may be that rumen digestion, solid turnover and liquid turnover rates were decreased when monensin was fed. Steers fed

TABLE XIII

INTAKE, RUMEN TURNOVER AND RUMEN VOLUME OF STEERS FED HARVESTED
 DRY WINTER RANGE GRASS IN TRIAL 1

Item	Monensin, mg/steer/day		S.E.
	0	200	
Intake, kg	4.60 ^a	3.88 ^b	.21
Liquid turnover, dilution %/hr	6.53 ^c	4.52 ^d	1.07
Solid turnover, dilution %/hr	2.73 ^e	1.54 ^f	.12
Liquid rumen volume, l	236.2 ^c	174.2 ^d	30.3
Dry matter rumen volume, kg	2.50	2.75	.31

a,b Means in a row with different superscripts differ statistically (P < .02).

c,d Means in a row with different superscripts differ statistically (P < .10).

e,f Means in a row with different superscripts differ statistically (P < .001).

monensin had a 30.8% slower liquid rumen turnover rate ($P < .10$) and a 43.6% slower solid rumen turnover rate ($P < .001$) than control steers. Decreased turnover matches the reduction in feed intake with monensin supplementation of high roughage rations (Raun et al., 1976; Embry and Swan, 1976 Hale et al., 1975; Sherrod et al., 1975; Bolsen et al., 1975; Utley et al., 1975 and Lemenager et al., 1977).

Liquid rumen volume of steers fed harvested low quality dry winter range grass was decreased when monensin was fed from 236.2 to 174.2 liters ($P < .10$). The unusually high liquid volume on both treatments may be attributed to incomplete equilibration of markers with rumen contents. When fed, PEG and Cr_2O_3 enters the liquid fraction near the cardia and may exit from the rumen before complete dispersion within the rumen. Consequently, when calculations are based on the amount of marker fed, it will inflate the rumen volumes. Nevertheless, the direction and relative magnitude of rumen volume change appear realistic. Rumen dry matter volume was not changed when monensin was fed, but obtaining a representative sample of solids and liquids is difficult.

Twenty-four hr volatile fatty acid patterns of steers fed harvested dry winter range grass is shown in Table XIV. Acetate was decreased and propionate increased when monensin was fed at the 4, 10, 16, and 22 hour samplings. Butyrate was not affected ($P > .2$) by monensin feeding at any sampling time. Total VFA concentration was lower at the 4 and 16 hour samplings when monensin was fed. These results are not consistent with those obtained by Lemenager et al., (1977) with cows grazing low quality dry winter range grass. In that experiment, the affect of monensin VFA's apparent 4-hr postprandially, had disappeared by 22-hr post-supplement feeding.

TABLE XIV

24-HOUR VOLATILE FATTY ACID PATTERN OF STEER FED HARVESTED
 DRY WINTER RANGE GRASS IN TRIAL 1

Item	Monensin, mg/steer/day		S.E.
	0	200	
4 hr sampling			
Acetate, molar %	68.50 ^a	63.40 ^b	1.52
Propionate, molar %	25.56 ^b	30.48 ^a	1.79
Butyrate, molar %	5.95	6.12	.75
Total, mM/l	41.54	44.95	2.80
10 hr sampling			
Acetate	73.00 ^c	64.14 ^d	.81
Propionate, molar %	20.20 ^c	29.27 ^d	.80
Butyrate, molar %	6.80	6.59 ^f	.23
Total, m/Ml	46.45 ^e	33.05 ^f	3.55
16 hr sampling			
Acetate, molar %	76.09 ^c	63.89 ^d	1.53
Propionate, molar %	17.96 ^d	30.02 ^c	1.40
Butyrate, molar %	5.96	6.08	.35
Total, mM/l	45.71	41.74	4.35
22 hr sampling			
Acetate, molar %	72.56 ^e	66.54 ^f	1.70
Propionate, molar %	21.10 ^e	27.15 ^f	1.61
Butyrate, molar %	6.33	6.31 ^b	.50
Total, mM/l	45.78 ^a	35.63 ^b	3.75

^{a,b} Means in a row with different superscripts differ significantly (P < .10).

^{c,d} Means in a row with different superscripts differ significantly (P < .001).

^{e,f} Means in a row with different superscripts differ significantly (P < .05).

Nitrogen components of liquid rumen contents from steers fed harvested dry winter range grass are shown in Table XV. Peptide nitrogen was higher at the 22 hour than the 4 hour sampling. Alpha-amino nitrogen was not affected at the 22 hour sampling. These data suggest that monensin did not cause free amino acid or peptide nitrogen accumulation in the rumen of cattle fed low quality dry winter range grass.

Addition of monensin to in vitro vessels depressed IVDMD of harvested dry winter range grass with rumen fluid from both control and monensin adapted steers (Table XVI). Dinius et al. (1976) observed a similar depression of cellulose disappearance in vitro with inocula from animals unadapted to monensin. Monensin adapted rumen fluid produced lower IVDMD ($P < .05$) than the control without monensin addition, however IVDMD from control or monensin adapted inocula did not differ statistically when 2 ppm monensin was added to the fermentation.

Trial 2

In vivo cellulose disappearance and liquid rumen turnover of steers limit fed a high concentrate ration are shown in Table XVII. In vivo cellulose disappearance rate was not significantly altered when monensin was added to the control supplement, however, it appears that 50 and 100 mg of monensin may depress cellulose digestibility. These results concur with those reported by Dinius et al. (1976). Turnover rates of rumen liquid tended to be slower in the 50, 100 and 200 mg of monensin treatments than the control, with an apparent linear effect ($P < .05$) as monensin level increased. These results agree with results obtained from feeding the low quality dry winter range grass diet in trial 1.

Several theories exist that may explain the relationship between

TABLE XV
 LIQUID RUMEN NITROGEN COMPONENTS OF STEERS FED HARVESTED
 DRY WINTER RANGE GRASS IN TRIAL 1

Item	Monensin, mg/steer/day		S.E.
	0	200	
4 hr sampling ^a			
Peptide nitrogen, mg/ml	2.72	3.04	.25
22 hr sampling ^b			
Peptide nitrogen, mg/ml	4.07	3.73	.56
α -Amino nitrogen, mM	4.35	4.50	.46

^a Sampled 4 hr post-supplement feeding.

^b Sampled 22 hr post-supplement feeding.

TABLE XVI
HARVESTED DRY WINTER RANGE GRASS IVDMD

Rumen Fluid Source ^a	Monensin Added to Fermentation ^b		S.E. ^c
	0	200	
Control	28.1 ^{dh}	24.7 ^e	
Monensin	25.8 ^{fi}	24.8 ^g	.79
S.E. ^j	.47		

^aSteers adapted to control or 200 mg of monensin and harvested dry winter range grass.

^bZero or 2 ppm monensin added to in vitro vessel.

^cStandard error of mean for means in a row.

^{d,e}Means in a row with different superscripts differ statistically (P < .05).

^{f,g}Means in a row with different superscripts differ statistically (P < .10).

^{h,i}Means in a column with different superscripts differ statistically (P < .05).

^jStandard error of means for means in a column.

TABLE XVII
 CELLULOSE DISAPPEARANCE AND LIQUID RUMEN TURNOVER OF STEERS
 LIMIT FED A HIGH CONCENTRATE RATION IN TRIAL 2

Item	Monensin, mg/steer/day				S.E.
	0	20	100	200	
Cellulose disappearance rate, %	18.8	15.9	13.6	19.1	2.47
Liquid turnover, dilution, %/hr	5.33 ^a	4.82 ^{a,b}	4.78 ^{a,b}	4.16 ^b	.24
Rumen liquid volume, l	53.0	67.8	58.0	65.9	5.97

^{a,b} Means in a row with different superscripts differ statistically (P < .05).

reduced forage intake and rumen turnover rate when monensin is fed. First, rumen turnover rate may be decreased because intake is decreased (Balch and Campling, 1965). Rumen turnover rate was slower in trial 1, with steers ad libitum fed harvested low quality range grass diet. This may be the result of reduced intake. But in trial 2, with steers limited a high concentrate diet intake of feed was maintained while turnover rate declined. This suggests that monensin depressed rumen turnover rate independent of its effect on intake. Consequently, the depression in rumen turnover appears independent, and therefore probably the cause and not simply a result, of reduced forage intake.

Based on research findings to date, it seems that monensin decreases intake of low quality forages as well as rumen turnover rate. One explanation for reduced forage intake is decreased rate, but not necessarily extent, of ruminal digestion. Decreasing the rate of digestion of particulate matter in the rumen would prolong rumen retention and slow rumen turnover. Reduced rumen turnover would decrease feed intake if bulk fill limits intake. The decreased energy intake of monensin fed cattle may not reduce performance however, due to compensating factors. These include: 1) increased propionate production, 2) decreased methane production, 3) decreased heat loss, 4) decreased energy expenditure for grazing and 5) decreased metabolic fecal energy loss.

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APPENDIX

TABLES

TABLE XVIII
 ANALYSIS OF VARIANCE FOR COW WEIGHT CHANGE AND FORAGE
 INTAKE (CHAPTER III, TRIAL 1)

Source of Variation	df	Mean Squares	
		Weight Change	Intake
Squares	22	783.9684*	1.7281*
Cows within square	46	125.5749	1.3285*
Period	2	1542.8599*	57.3943*
Period x square	44	652.8801*	.7861
Unadjusted direct effects	2	1124.7874*	63.6768*
Treatment x square	44	485.5198	.4801
Adjusted direct effects	(2)	1101.3935*	45.5805*
Carry-over effects	2	59.3925	.9048
Error	40**	335.9248	.5830

*(P < .05).

**Four missing observations were estimated, therefore, 4 degrees of freedom were subtracted from the error degrees of freedom (44-4).

TABLE XIX

ANALYSIS OF VARIANCE FOR RUMEN VOLUME, RUMEN TURNOVER, NITROGEN
COMPONENTS AND FORAGE INTAKE (CHAPTER IV, TRIAL 1)

Source of Variation	df	Mean Squares		
		Dry Matter Volume	Solid Turnover	Intake
Columns	7	1287.7941**	.6996**	6.7529**
Rows	1	8.7844	4.4310**	67.4041**
Treatment	1	260.1765	5.6882**	10.1124**
Error	6	375.9189	4.0544	.8328

* (P < .05).

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