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Annals of Biological Research, 2013, 4 (7):1-8 (http://scholarsresearchlibrary.com/archive.html)



# Effect of using monensin with different source of carbohydrate on brown swiss steers performance

Mah Di Edalati Nasab<sup>\*1</sup>, Hamed Amini Pour<sup>1</sup> and S. Masoud Davoudi<sup>2</sup>

<sup>1</sup>Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran <sup>2</sup>Department of Animal Science, Islamic Azad University of Shahrkord, Shahrkord, Iran

## ABSTRACT

Sixteen male Brown Swiss calve were utilize to study the effects of cereal source (barley and triticale) and monensin on fattening performance using a factorial experiment with four treatments. The number of replicates was four for each treatment and the experiment was last for 90 days. The experimental treatments were: 1) diet containing barley without monensin 2) diet containing barley and monensin 3) diet containing triticale without monensinand 4) diet containing triticale and monensin. The amount of monensin was set to be 30 mgkg<sup>-1</sup> DM. Calf weighing was undertaken monthly, dry matter intake was measured daily. Sampling from rumen fluid was carried out using esophagous tube, and blood sampling was undertaken at the end of each month. The results indicated that average daily gain, feed conversion ratio, final weight and daily feed intake were not significantly affected by cereal source as well as monensin. Blood glucose, BUN and rumen PH were not significantly affected by the experimental treatments. The magnitude of rumen ammonia nitrogen had a non-significant decrease due to using monensin in the diet.

Key words: Monensin, Barley, Triticale, Steer.

## INTRODUCTION

Monensin, a monocarboxylic acid ionophore, is used in feedlot diets to alter ruminal fermentation for improved feed efficiency. Monensin decreases ruminal Proteolysis and thus increases the proportion of dietary protein escaping ruminal digestion [11 and 14].

Monensin increases In Vitro propionate production, without changing total VFA production [2].

An important characteristic of ruminant digestion is the fermentation of ingested carbohydrates to produce volatile fatty acids (VFA) in the rumen. A major factor determining the efficiency with which digestible feed energy is utilized by the ruminant is the relative rates of production of acetate, propionate, and butyrate, the three major VFA.In addition, relative rates of VFA production in the rumen affect the partitioning of nutrients between synthesis of body fat and synthesis of milk fat.The relative rates of VFA production canbe manipulated by changing the amount of roughage included in the diet [1], by changing the rumen fluid dilution rate (2-4), and by using ionophores such as monensin. Monensin increases in vitro propionate production, without changing total VFA production [5], and increases in vivo propionate production as measured by dilution of [1-14C]-propionate (6-8) [22 and 41].

The polyether ionophore antibiotic monensin hashad a profound effect on the cattle feeding industry in the United States. The greatest role of monensin is as

An additive for feedlot cattle to improve feed efficiency. Ionophore antibiotics are characterized by their effectson ruminal fermentation: they increase production of propionate [33 and 34].

Triticale is a hybrid of wheat (Triticum) and rye (Secale) first bred in laboratories during the late 19th century. The grain was originally bred in Scotland and Sweden. Commercially available triticale is almost always a second generation hybrid, i.e., a cross between two kinds of primary (first cross) triticales [9, 35, 36 and 40].

Barley has a very rapid rate of digestion in the rumen. When used as the basal grain in high concentrate feed lot diets, barley may decrease ruminalpH and increase the incidence of acidosis and bloat, compared with corn-based diets [4, 16, 18 and 42].

#### MATERIALS AND METHODS

Sixteen Brown Swiss calve, with initialweights of 170 and 200 kg, were fed sequentially a control and a monensinsupplemented diet. Experiment with four treatments. The number of replicates was four for each treatment and the experiment was last for 90 days. The experimental treatments were: 1) diet containing barley without monensin2) diet containing barley and monensin3) diet containing triticale without monensin and 4) diet containing triticale and monensin. The amount of monensin was set to be 30 mgkg<sup>-1</sup> DM. Experiment. A 2 4 factorial design wasused to test the effects of monensin addition (0 vs.30mg/kg) and TWO grain sources(barley, TRITICALE) onIVDMD.The appropriate diet was fed for at least 21 days before each experimental series. Starting 10 days before an experiment Tal series, the daily ration was divided into 2 equal meals.

Rumen fluid was sampled via the cannula with a stainless-steel probe equipped with a strainer on the distal end and a 50-ml syringe on the proximal end. Samples, each 30 ml, were taken from five locations in the rumen, pooled, and subsampled. At least three such pooled samples were taken, at 30-minute in tervals, starting 6 hours after the PEG infusion began.

#### RESULTS

Data shown that no significant monensin levels and or variety seed source on the feed intake, daily weight grain and feed conversation rate (Table 1).

Significant level	$S^2$	T 4	Т3	T 2	T 1	chart eristic
0/21	0/681	9/39	8/02	9/30	9/56	DM Intake
0/12	0/087	1/56	1/38	1/55	1/48	Weight grain
0/43	0/016	5/95	5/69	5/97	6/35	FCR

Table 1. Feed intake, Daily Weight Grain and Feed Conversation Rate

In the more investigate and research, using Monensin, modified the daily weight grain and feed conversation rate. Effect Monensin on the precede ruminal fermentation caused increase propionate percentage, reduce acetat: propionate rate, reduce proteolysis proteins and deamination Amino acids and reduce production methane. Conclusion exchange overall in proceed fermentation, increase feed efficiency, increase energy and protein remain and finally increase performance.

Van Baale et al (2004) reported that by using two rations on base roughage and grain with monensin or nonmonensin, feed conversation rate and weight grain was significant in calf. Using of levels 0, 11, 22 and 33mg/kg DM ration monensin with meal soybean and or urea in the ration male calf's caused improve daily weight grain and feed conversation rate, but positive effect on feed efficiency and nitrogen with meal soybean was high than urea. They suggested that benefit effects monensin in the beef animals was to be caused improvement energy used. Increase production propionate caused increase product Glucose and finally optimum use of ration protein for weight grain.

Investigate results Goodrich et al (1984) shown that, use of monensin, no affect the daily grain weight, but the feed conversation rate improved 7.5% than control group.

Diverse response to monensin and or seed source may relate to using monensin level, ration composition, variety seed source and or protein.

Barley had high degradability in rumen, but they are limit information about degradability of triticale seed, thus by using nylon bags, estimated Dry matter and protein degradability barley and triticale seeds.

Dry matter and protein degradability barley and triticale seeds shown that, triticale same barley had high degradability in rumen.

## **Rumen Fermentation Parameters**

## Rumen Nitrogen

Use of monensin in different moons caused reduces ruminally nitrogen concentration (Table 2). Reduce is significant and between 3-5mg/dL.

By use of ionophers, can control the protein degrader microorganism and increase inert protein rate to the gut side and also, reduced the protein degradability. Use of monensin in ration, reduce nitrogen concentration in rumen. Main cause reduce of nitrogen and deamination Amino acids, is reduce protein degrader microorganism.

Significant level	T 4	Т3	Т2	T 1	Moons
0/08	10/52	15/52	9/85	14/39	1
0/10	9/65	13/17	10/60	11/82	2
0/13	8/07	11/10	8/92	11/85	3

#### **Table 2. Ruminally Nitrogen Concentration**

#### Rumen pH

pH average of rumen in different treatments has shown in the tables 3 and 4. According to shown tables, there aren't significant different in between treatments.

Van Baale et al (2004) reported that by use of two rations on base rough and grind with or no monensin, increase pH ruminally.

Table 3. pH Average of Rumen in Different Treatments

Significant level	T 4	Т3	T 2	T 1	Moons
0/33	6/00	6/12	6/18	5/95	1
0/41	6/11	6/01	6/16	5/98	2
0/79	6/34	6/44	6/42	6/45	3

#### Table 4. pH of Rumen in Different Treatments

NH3	pН	Effect
		Monensin <sup>n.s</sup>
11/57	6/16	0
10/94	6/20	30
0/8891	0/069	$S^2$
0/29	0/56	Significant level
		Seed <sup>n.s</sup>
12/27	6/91	Barley
10/25	6/17	Triticale
0/8891	0/069	$S^2$
0/11	0/85	Significant level

#### **Blood metabolites**

Glucose: use of monensin with different source of seed source, hasn't significant effects on the blood glucose concentration (table 5).

Significant level	Т4	Т3	T 2	T 1	Moons
0/11	66/25	75/00	74/25	61/05	1
0/24	49/25	49/00	47/25	42/00	2
0/09	74/25	66/5	61/00	58/25	3
Significant level	T 4	Т3	T 2	T 1	Moons
0/07	19/75	21/25	18/75	18/5	1
0/13	11/5	10/75	13/00	11/5	2
0/09	12/25	16/5	15/00	14/75	3
	S	EM = 4/90	)		

**Table 5. Blood Glucose Concentration** 

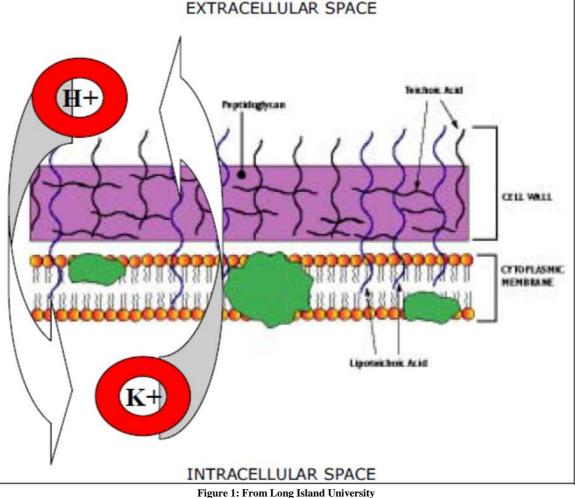
Main substrate for make glucose is, acids obtain of fermentation, carbon structure of amino acids deamination and glycerol.

Maas et al (2001), experiment the season effect and monensin on the alimentary characteristics of rough autumn and spring fed in sheep's and suggested that use of monensin, increase the plasma glucose levels. They reported that the blood glucose increase due to exchange in ruminal fermentation process and increase propionate molar rate than other acids.

#### DISCUSSION

#### Antimicrobial mode of action of the polyetherIonophores

Comprehensive descriptions of the mode of antimicrobial action of the ionophores have been presented [3, 12, 13, 27 and 32], but only a brief synopsis of the key features of the mode of action is presented here. The term 'polyether' refers to the unusual structural feature whereby there are a considerable number of heterocyclic tetrahydro-pyrans and-furans. While the backbone of the ionophores provides an alkyl-rich, lipid-soluble exterior, the ether, carboxyl, hydroxyl and carbonyloxygens are oriented internally forming a cage of potential ligands binding entrapped cations. Monensin is effectively cyclized by head-to-tail hydrogen bonding between the carboxyl group at the head and one or two hydroxyl groups at the tail. The result is a mobile cation Carrier (aptly termed an ionophore from the Greek 'ion bearing' as recommended by Pressman, (1976) that readily traverses the thick but porous peptidoglycan cell wall of Gram positive organisms, and is able to transport cations across the bilaminar lipid cytoplasmic membrane, much like a Trojan horse, though smaller, as illustrated in Figure 1.



at http://www.liu.edu/cwis/bklyn/acadres/facdev/FacultyProjects/WebClass/micro-web/htmlfiles/ ChapterA-4.html

Selectivity of cation binding is a distinguishing feature of each polyether ionophore, and relates to each compound's characteristic dimensions and electromechanical properties [6, 25 and 31].

Monensin is a monovalent polyether with the following selectivity: Na>K>Rb>Li>Cs. Affinity for Na+ is approximately tenfold that for K+. By contrast, lasalocid is a divalent polyether, with a monovalent selectivity series Cs>Rb~K>Na>Li and divalent series Ba>Sr>Ca>Mg. In terms of relative potency, monensin has a 31-fold greater

affinity for Na+ than lasalocid, while by contrast lasalocid has a 10,000 fold greater affinity for Ca++ than monensin [8 and 23].

Because intracellular [K+] far exceeds [Na+], monensin exposure rapidly leads to K+ efflux from cells matched by rapid H+ influx. The disturbance of ionic equilibrium and pH activates a variety of homeostatic mechanisms that are active ATP-consuming processes which exhaust cellular energy supplies and ultimately lead to cell death.

## **RUMINANT BENEFITS**

### Key findings

Ionophores modify the microbial population of the rumen and hindgut leading tochanges in diet fermentation patterns allowing increased energy, protein and lipidavailability through:

- Increased production of propionic acid which allows increased synthesisof glucose and consumes hydrogen otherwise directed to methaneproduction

- decreased lactic acid fermentation allowing highly fermentable diets to be fed safely
- decreased protein degradation of protein in the rumen and higher flow to the small intestine
- improved nutrient disposition results in:
- increased retention of nitrogen (N
- increased retention of phosphorus (P)
- decreased dietary requirements for P
- increased retention of energy
- reduced production and emission of methane
- favorable patterns of fermentation underpin all major uses including:
- improved feed conversion efficiency (FCE) and daily gain in confined cattle production
- improved daily gain in grazing cattle
- decreased incidence and severity of bloat in both feedlot and grazing animals
- prevention of clinical and subclinical ketosis in dairy cattle
- potential for increased milk production in dairy cattle
- decreased incidence of acute pneumonia caused by toxic fermentation of lush pasture
- control of coccidiosis in cattle and sheep
- Prevention of abortion in ewes caused by toxoplasmosis.

Muir and Barreto (1979) evaluated the sensitivity of *Streptococcus bovis*(ATCC 15351) to avariety of antibiotics, observing that relative to the highly active antibiotic thiopeptin assigned a reference activity of 100 per cent, the rank order of activity of the polyether ionophores wassalinomycin (21 per cent) >lasalocid (11 per cent) >monensin (5 per cent). Members of the ruminal ciliated protozoa fauna vary in their susceptibility to theionophores (Poos et al, 1979), with entodiniomorphs (*Entodinium, Diplodinium* and *Ophryoscolex*) susceptible andholotrichid ciliates (such as *Dasytricha, Isotricha* and *Charonina*) generally resistant. Monensin is fungistatic at low concentration (1µg/mL), becoming fungicidal at high concentration (16µg/mL) [21 and 26].

#### Effects of Ionophores on rumen VFA production

Richardson et al (1974, 1976) and Raun et al (1976) undertook a number of studies both in vitro and *In vivo* to assess the effect of monensin on rumen production of VFA and the proportions of the main acids, acetic, propionic and butyric. Many investigations have confirmed the characteristic shifts in VFA production induced bythe use of monensin [5 and 20] and shown that similar VFA patterns are associated with the use of salinomycin[19, 30 and 44] and lasalocid [28, 43 and 45].

Compound	Propionate		Acetate:Pr	opionate	Butyrate	
	<sup>1</sup> EC <sub>25</sub> (µg/ml)	<sup>2</sup> † <sub>max</sub> (%)	<sup>3</sup> IC <sub>25</sub> (µg/ml)	<sup>4</sup> ↓ <sub>max</sub> (%)	<sup>5</sup> IC <sub>25</sub> (µg/ml)	<sup>6</sup> ↓max (%)
Lasalocid	3.27	37	8.98	30	0.94	42
Monensin	14.42	25	>24.00	22	3.31	38
Narasin	2.44	32	>24.00	25	0.41	44
Salinomycin	2.21	36	7.92	28	0.35	43
Monensin + Tylosin <sup>7</sup>	-	8	-	6	>24.00	16

#### Table 6. VFA Concentrations

In general, in a broad array of investigations of both high carbohydrate and high fiber diets, total production of VFA was little affected, but significant and reproducible shifts in the relative proportions of individual VFAs were observed. While acetic and butyric acid concentrations fell, propionic acid increased significantly. Furthermore, as demonstrated in the study of feedlot cattle, the response to monensin was maintained for the entire 148-day feeding

period.Nagaraja et al (1987) examined the ability of the ionophores to influence VFA production by incubating a ruminal fluid inoculum obtained from a steer fed an alfalfa hay and grain diet with a carbohydrate mixture and graded doses of each of the ionophores. The results of this study are presented in Table 6.

Nagaraia et al 1987

 Antimicrobial concentration required to increase propionate by 25 per cent above the control 2Maximum observed increase in propionate concentration
Antimicrobial concentration required to reduce acetate:propionate by 25 per cent of the control 4Maximum observed reduction in acetate:propionate
Antimicrobial concentrations required to inhibit butyrate by 25 per cent of the control 6Maximum observed inhibition of butyrate concentration

7Monensin and tylosin mixed at a ratio of 3:1 (w:w)

Studies by van Maanen et al (1978) corroborated the VFA results of Richardson et al (1976) for both high roughage and high grain diets. In addition, however, van Maanen and coworkers evaluated the rumen production kinetics of propionate and found that, for roughage and grain diets, propionate production increased above control levels by 49.4 per cent and 76.3 per cent respectively. These increases should be compared with observed molar percentage increases of propionate of 15.1 per cent and 24.6 per cent respectively. The notable difference between production rate and molar percentage underlines the importance of appropriate interpretation of VFA percentage which may be an unreliable predictor of VFA production rate. Similar observations of the discongruity of production and proportional concentrations were made by Prange et al (1978) and Rogers and Davis (1982) and echoed the prior warnings of Leng and co-workers.

Richardson et al (1976) noted that in changing the molar proportions of the rumen VFA in favor of propionate, monensin theoretically increases the efficiency of conversion of feed energy to energy in the VFA end products which are available for absorption. In changing molar proportions from 60:30:10 (acetic:propionic:butyric) to 52:40:8 gross energy savings of 5.6 per cent were calculated. On the basis of fermentation balance equations, it has been predicted that propionic acid production increases should be associated with reductions in methane production. Indeed reductions in methane production of 4-31 per cent have been described by Schelling (1984).

Rowe et al (1981) examined rumen fermentation in sheep and found increased propionate production accompanied reductions in methane of 37.5 per cent [17 and 24].

#### **Digestion in the rumen**

While monensin in vitro decreased digestion of organic matter, protein and cellulose, butusually not starch [37], *In vivo*ruminal digestion of organic matter and cellulose is not normally decreased by monensin, possibly because of an increased retention time for solids and liquids in the rumen [7].

Zinn and Borques (1993) studied the effect of monensin on utilisation by feedlot steers of a fat-supplemented, highenergydiet. While ruminal organic matter digestibility was decreased, postruminal digestion was increased which may more than compensate as assimilation of nutrients from the small intestine may be superior. It is clear that many factors must affect digestibility, especially the Chemical and physical properties of the different fiber sources as well as total quality and quantity of the diet.

#### **Reductions in methane production**

The characteristic and consistent increase in propionate production in response to monensin is accompanied by a reduction in methane production [38]consistent with the diversion of hydrogen (H2) from methane synthesis to the production of propionate [1 and 29].

Henderson et al, (1981) in a study of the effect of monensin on pure and mixed cultures of rumen bacteria found that the ruminococci and *Butyrivibriofibrisolvens*, both significant rumen acetate and H2 producers, were inhibited by monensin, which would lead to decreased availability of H2 for methane production by methanogenic bacteria. Wedegaertner and Johnson (1983) studied the effect of monensin on the partition of energy by growing-finishing steers fed a basal corn grain, corn-silage diet. Methane production was significantly reduced when monensin was included in the diet. It was reduced by 26.6 per cent compared with control animals, a reduction that was similar to the 16–24 per cent reductions reported by Thornton and Owens (1981) in steers on 20–70 per cent roughage diets, and the 31 per cent reduction noted by Joyner et al, (1979) in a study of lambs consuming chopped hay and corn mixed with 20 ppm monensin.

Singh and Mohini (1999) examined the effects of monensin-induced manipulation of rumen fermentation in crossbred calves offered a low quality diet of wheat straw or rice straw. Groups of calves were fed differing proportions of straw and concentrate mix (peanut meal, corn and wheat bran) offered for twenty days at which time rumen fluid was sampled and production of methane determined. While total gas production by rumen fluid samples Incubated for 24 hours remained relatively constant at about 110 l/kg digestible dry matter (DDM), irrespective of source of straw, proportion of straw and concentrate or presence of monensin, significant changes in methane production were observed. With both types of straw, as the proportion of straw was reduced the production of methane was also reduced. While monensin led to a fall in methane production in the rice straw diet of around 25 per cent at all straw:concentrate ratios, with wheat straw, increasing quantities of concentrate were associated with increasing reductions in methane production in the monensin group. The trends in methane production found in this study are consistent with Those previously found by Blaxter and Wainman (1964) and Ørskov et al, (1968).

#### REFERENCES

- [1] Adams DC, Galyean ML, Kiesling HE, Wallace JD and Finkner MD. 1981. J AnimSci 53: 780–789.
- [2] Ammerman, C. B., C. F. Chicco, P. E. Loggins and L. R. Arlington. 1972. J. Anita. Sci. 34:122.
- [3] Armentano LE and Young JW. 1983. Journal of Nutrition 113: 6, 1265–1277.
- [4] Bergen WG and Bates DB .1984. J AnimSci; 58 (6):1465-83.
- [5] Blaxter KL and Wainman FW .1964. J AgrSci (Camb) 63: 113-128
- [6] Duff GC, Galyean ML and Branine ME .1990b. J AnimSci 68 (Suppl 1): 518.
- [7] Duff GC, Galyean ML and Estell RE .1990a. J AnimSci 68 (Suppl 1): 518.
- [8] Elsasser TH. .1984. J AnimSci 59 (3): 845–53.
- [9] Fontenot JP, Webb KE and Lucas DM .1980. J AnimSci 51 (Suppl 1): 360.
- [10] Goodrich, R. D., J. E. Garrett., D. R. Gast., M. A. Kirick., D. A. Larson and J. C. Meiske. **1984**. *J. Anim Sci.* 58: 1484 1498.
- [11] Haney ME and Hoehn MM .1967. Antimicrobial Agents and Chemotherapy 1967: 349–352.
- [12] Harmon, D. L., K. K. Kreikemeier., and K. L. Gross. 1993. J. Anim Sci. 71: 218 225.
- [13] Joyner AE, Brown L J, Fogg TJ and Rossi RT .1979. Journal of Animal Science 48: 5, 1065–1069.
- [14] LOUIS E. ARMENTANO2 AND JERRY W. YOUNG3.**1983**. Production and Metabolism of Volatile Fatty Acids, Glucose and CO2 in Steers and the Effects of Monensin on Volatile Fatty Acid Kinetics, *Nutritional*
- Physiology Section, Department of AnimalScience, Iowa State University, Ames, Â;A50011.

[15] Maas, J. A., G. F. Wilson., S. N. Mccutcheon., G. A. Lynch., D. L. Burnham., and J. France. **2001**. *J. Anim Sci.* 79: 1052 – 1058.

- [16] Muir LA and Barreto A .1979. J AnimSci 48: 468–473.
- [17] Nagaraja TG, Taylor MB, Harmon DL and Boyer JE .1987. J AnimSci 65: 1064–1076.
- [18] Ørskov ER .1975. World Review of Nutritionand Dietetics 22: 152-182.
- [19] Ørskov ER, Flatt WP and Moe PW .1968. J Dairy Sci 51: 1429–1435.
- [20] Owens FN, Secrist DS, Hill WJ and Gill DR .1998. J AnimSci 76: 275–286.
- [21] Pressman BC .1976. Ann Rev Biochem; 45: 501–530.
- [22] Pressman BC and Fahim M .1982. AnnRev PharmacolToxicol; 22: 465-490.
- [23] Phillips MW and Gordon GLR .1992. LettApplMicrobiol 15: 116–119.
- [24] Poos MI, Hanson TL and Klopfenstein TJ .1979. Journal of Animal Science 48: 1516–1524.
- [25] Prange RW, Davis CL and Clarke JH .1978. Journal of Animal Science 46: 1120–1124.
- [26] Russell JB .1987. J AnimSci; 64: 1519–1525.
- [27] Russell JB and Strobel HJ .1988. J AnimSci 66 (2): 552–558.
- [28] Russell JB and Strobel HJ .1989. Appl Environ Microbiol; 55: 1-6.
- [29] Richardson LF, Raun AP, Potter EL, Cooley CO and Rathmacher RP .1974. J AnimSci 39: 250.
- [30] Richardson LF, Raun AP, Potter EL, Cooley CO and Rathmacher RP .1976. J AnimSci 43: 657-664.
- [31] Ronne H and Jensen JCE .**1992**. *Veterinary Record* 131: 239–240.
- [32] Rogers JA and Davis CL .1982. J. Dairy Sci. 65:944-952.
- [33] Shell LA, Hale WH, Theurer CB and Swingle RS .1983. J AnimSci 57: 178–185.
- [34] Spears JW and Harvey RW .1984. J Anim Sci. 58 (2):460-464.
- [35] Schelling GT .1984. Monensin mode of action in the rumen. J Anim. Sci. 61:1518–1527.
- [36] Singh GP and Mohini M .1999. Asian-Australasian Journal of Animal Sciences 12: 8, 1215–1221.
- [37] Surber, L. M., and J. G. Bowman. 1998. J.Anim. Sci. 76: 1945-1954.
- [38] Tartakoff AM .**1983**. Perturbation of vesicular traffic with the carboxylic ionophoremonensin. Cell; 32 (4):1026–8.
- [39] Thornton JH and Owens FN .1981. Journal of Animal Science 52: 3, 628–634.
- [40] Van Baale, M. J., J. MI Sargeant, D. P. Gnad, B. M. Debey, K. F. Lechtenbery, and T. G. Nagaraja. **2004**. *Applied and Environmental Microbiology*, P. 5336 5342.

[41] Van Maanen RW, Herbein JH, McGillard AD and Young JW .1978. Journal of Nutrition 108: 1002–1007.

- [42] Van Nevel CJ and Demeyer DI .1977. Applied and Environmental Microbiology 34: 251–257.
- [43] Wallace RJ, Czerkawski JW and Breckenridge G .1981. Br J Nutr 114: 101–105.
- [44] Wedegaertner TC and Johnson DE .1983. Journal of Animal Science 57: 1, 168–177.
- [45] Zinn RA .1993. J AnimSci71: 3–10.