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The Effect of Monensin Supplementation on Ruminal Fermentation Parameters of Sheep

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Research Article

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ABSTRACT

This study was carried out to determine of the effects of monensin on ruminal fermentation parameters in Ghizel sheep. Sixteen male Ghizel sheep were used allocated in completely randomized design. The diet containing of 2.9 ME (Mcal/kg DM) and 150 g/kg DM CP was formulated based on NRC requirement consisting of 400 g kg⁻¹DM alfalfa, 488 g kg⁻¹ DM barley grain, 200 g kg⁻¹ DM soybean meal, 589 g kg⁻¹ DM, corn grain and 20 g kg⁻¹ DM limestone containing predicted metabolizable energy 2.9 Mcal kg⁻¹ DM and containing crude protein 150 g kg⁻¹ DM. The treatments containing different level of ionophores (20 ppm, 25 ppm, 30 ppm and 35 ppm). The experiment period was 21 days. The rumen fluid was collected using stomach tube at 2 and 4 h after morning feeding. The floatation and sedimentation time, methylene blue reduction time, pH, total ammonia nitrogen and total VFA in ruminal samples were determined. The ruminal pH, total VFA, sedimentation and floatation time and methylene reduction time were not significant differences. The effect of monensin on ruminal ammonia concentration was significant difference (P<0.05). The results showed that the monensin had not effect on ruminal parameters except of ruminal ammonia-N resulted low proteolytic bacteria activity.

INTRODUCTION

Ionophores are used widely in fattening animal production. Ionophores biological reactions contain: increasing of energy metabolism efficiency in bacteria and host animal, improvement of nitrogen metabolism of ruminal bacteria and animals and removing or decreasing of digestive disorders in ruminants. Ionophores decrease some bacteria that produce acetate, while the proportion of propionate producer bacteria is increased. Monensin and lasalocid are as ionophores that used extensive in animal nutrition. Ionophores increase propionate production resulting high blood glucose that can be improved energy supply in transit period and early lactation period [1-4].

Ionophores decrease fat metabolism resulting low non estrified fatty acids and low ketone body and increase blood glucose [1]. Ionophores alter gram positive bacterial resulting prevent their vital activity [5]. Gram positive bacteria producing of lactate, acetate, ammonia and methane are sensitive to monensin, whereas gram negative bacteria producing of propionate and succinate have less sensitive to ionophores [5]. This difference is due to variation of between cell wall membrane of gram positive and negative bacteria. Han et al. [6] showed the ionophores increased propionate concentration and decrease acetate: propionate ratio. They reported no effect on total VFA. Domescid and Martin [7] reported that the monensin decreased the ammonia-nitrogen and methan production. They reported increased pH and VFA when ionophores were used in *in vitro* conditions.

Cone et al. [8] did not show any significant differences in fermentation end products in animal fed ionophores compared

to control diet. Gram-negative bacteria are resistance to ionophores, resulting high production succinate that can be altered to propionate. Martin and Macy ^[9] reported that the low methane production in animals fed ionophores can be resulted to incorporation of H₂ in propionate production.

Lana et al. ^[10] indicated low ruminal pH in animal received ionophores resulted due to inhibition of hydrogen concentration that incorporated in propionate production. Ionophores prevent ruminal acidosis due to inhibition of lactic acid producer bacteria. Nagaraja ^[11] showed decreasing of ruminal ammonia-N in animal fed ionophores probably due to low activity of proteolytic bacteria, resulting high escaped protein into small intestine. Ionophores decrease the dry matter intake due to high efficiency of feed intake and improvement of ruminal fermentation and low ruminal passage rate ^[12]. The objective of this study was to determine of effect of monensin on rumen fermentation parameters (floatation and sedimentation time, methylene blue reduction time, pH, total ammonia nitrogen and total VFA) in Ghizel sheep.

MATERIALS AND METHODS

16 Ghizel sheep (45 ± 6.59 kg) are randomly received one of four diets in a completely randomized design. The composition of diet based on NRC (1985)^[13] consisting of 400 g kg-1 DM alfalfa , 488 g kg-1 DM barley grain, 200 g kg-1 DM soybean meal, 589 g kg-1 DM, corn grain and 20 g kg-1 DM limestone containing predicted metabolizable energy 2.9 Mcal kg-1 DM and containing crude protein 150 g kg-1 DM. The treatments containing different level of ionophores (20 ppm, 25 ppm, 30 ppm and 35 ppm). The period of present study was 21 days for each ionophore.

The rumen fluid of each treatment was obtained by stomach tube at 2 and 4 hr after feeding. The effect of treatment was determined on rumen pH, ammonia-N, total volatile fatty acid (VFA) ^[14], methylene blue reduction time and sedimentation and floatation period. The sedimentation and floatation time was determined using filtration of collected rumen liquor from cheese cloth and then collected in experimental tube, as the small particle precipitated, while large particle was suspended in surface of liquor and the spending time was recorded ^[15]. The methylene blue reduction time was measured using combining of 20 ml rumen liquor and 0.3% methylene blue and recording of reduction time.

Statistical analysis

Data obtained from *in vivo* study was subjected to ANOVA as a completely randomized design with 4 replicates by the GLM procedure of SAS ^[16], and treatment means were compared by the Duncan test.

$$Y_{ij} = \mu + T_i + e_{ij}$$

Y_{ij} = observation per unit

μ = overall mean effect

T_i = true effect of the *i*th treatment

e_{ij} = error term of the *j*th unit receiving *i*th treatment

Results and Discussion:
The obtained results are shown on Tables 1, 2, 3, 4 and 5. The ruminal pH at 2 and 4 h after feeding were not significant differences due to supplemented monensin. Ives et al. ^[17] showed when ionophores added in the diet ruminal pH was decreased, that is in contrast to our results. These reported that with altering of sampling time from 2 h to 4 h after feeding, ruminal pH was increased that confirm our obtained data. Also, these finding have been reported by Erickson et al. ^[18]. Domescik and Martin ^[7] indicated increased ruminal pH in *in vitro* experiment due to added ionophores. The similar results were confirmed by Baran ^[19]. These finding were in contrast with, compared to obtained data in present experiment (**Table 1**).

Table 1. The effect of monensin on ruminal pH.

Sampling hour	Monensin (ppm)				SEM
	20	25	30	35	
2	5.77	5.72	5.71	5.69	0.05
4	6	5.32	5.85	5.79	0.13

The means within a column without common letter differ (p<0.05).

The effect of monensin on ruminal VFA concentration was not significant difference (**Table 2**). Ives et al. ^[17] showed the ionophore caused high VFA compared to control treatment that is in contrast of our data. These investigators indicated that with changing of sampling time from 2 to 4 h total VFA decreased that is in agreement with our results. Bohnert et al. ^[20] reported numerical increasing VFA concentration with supplemented monensin that is in convenient with the obtained data in current study. These indicated that high VFA when added ionophores is due to increased propionate concentration compared to acetate concentration. The similar results were reported by Cone et al., Domescik and Martin and Han et al. ^[6-8]. These researchers indicated constant ruminal VFA when animal fed by ionophores as in convenient with our results.

Table 2. The effect of monensin on ruminal VFA(mMol/L)

Sampling hour	Monensin (ppm)				SEM
	20	25	30	35	
2	124.75	125.25	126.75	127.5	3.31
4	111.75	113.75	117.5	119.5	4.58

The means within a column without common letter differ ($p < 0.05$).

The ruminal ammonia-N was significantly decreased when the monensin was fed (**Table 3**). Ives et al. ^[17] showed the reduced ammonia-nitrogen when ionophores was fed. Also, they reported that 4 h after feeding the ammonia-N concentration was increased compared to 2 h after feeding. These finding was convenient with our results. The obtained data in present study is in agreement with that were reported by Han et al. ^[6], Lana et al. ^[9] and Domescik and Martin ^[7], who indicated low ammonia-N concentration for animal fed ionophores. Baran ^[19] stated that reduced ammonia-N concentration and deamination of amino acids can be resulted from decreased ruminal proteolytic bacteria population.

Table 3. The effect of monensin on ruminal ammonia-N (mg/L)

Time (after feeding, h)	Monensin (ppm)				SEM
	20	25	30	35	
2	101 ^a	97.75 ^{ab}	91.75 ^{bc}	86.37 ^c	2.85
4	105.5 ^a	100.37 ^{ab}	96.25 ^{bc}	92.37 ^c	2.03

The means within a column without common letter differ ($p < 0.05$).

Bohnert et al. ^[20] showed low ruminal ammonia-N concentration can be related to decreased peptides degradation and reduced amino acids deamination by bacteria. Nagaraja^[11] expressed that ionophores caused decreased ruminal proteolytic bacteria and urease activity. (**Table 4**)

Table 4. The effect of monensin on sedimentation and floatation time (seconds)

Time (after feeding, h)	Monensin (ppm)				SEM
	20	25	30	35	
2	397.5	412.5	427.5	491.25	18.22
4	379	387.5	397.5	412.5	14.08

The means within a column without common letter differ ($p < 0.05$).

The sedimentation and floatation time did not show significant differences due to monensin. These results are in convenient with that reported by Baran ^[19] and Moghadam and Taghizadeh ^[21]. (**Table 5**)

Table 5. The effect of monensin on methylene blue reduction time (seconds)

Time (after feeding, h)	Monensin (ppm)				SEM
	20	25	30	35	
2	221	223.75	228	230.25	3.43
4	205.5	214	216.75	220	5.51

The means within a column without common letter differ ($p < 0.05$).

The methylene blue reduction time (MBRT) was not significantly different between monensin levels. Since, methylene blue reduction time is used as ruminal microbial activity scale and MBRT at 3 minutes introduce as microbial normal activity. So our finding is in agreement with above mentioned hypothesis. These finding is certified ^[21] that reported by Moghadam and Taghizadeh ^[21] and Safaei ^[22].

CONCLUSION

As a whole the monensin can be manipulate rumen ecosystem and decrease ruminal ammonia-N, resulting high ruminal undegradable protein and increasing the ruminal escaped protein.

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