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EFFECT OF MONENSIN SUPPLEMENTATION ON THE DIGESTION OF MIXED DIET CONTAINING SOYBEAN MEAL AS A PROTEIN SOURCE BY SHEEP

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ABSTRACT

The current study was conducted to evaluate the effect of monensin on the digestibilities of dry matter (DM), organic matter (OM) and crude protein (CP), ruminal fermentation and the degradation kinetics of a basal diet (control) containing soybean meal as a protein source. Three ruminally fistulated male sheep were used in a 3x3 Latin square design experiment to be fed on three rations; 1) control diet, 2) control diet plus monensin at 30 mg/day and 3) control diet plus monensin at 50 mg/day. The results showed that the addition of monensin to the diet had no significant ($P>0.05$) effect on DM, OM and CP digestibilities. When animals were fed monensin diets, the concentration of ammonia-N (NH_3-N) was significantly ($P<0.05$) decreased from 14.6 to 12.15 and 11.99 mg/dl, for control diet and control diet plus monensin at 30 mg/day and control diet plus 50 mg/day, respectively. The molar proportion of propionic acid was significantly ($P<0.05$) increased, while the molar proportion of acetic acid was significantly ($P<0.05$) decreased with the addition of monensin. Butyric acid was not significantly affected with the addition of monensin to the control diet. The effective degradability of DM and CP was significantly ($P<0.05$) decreased with the addition of monensin to the control diet. Microbial nitrogen leaving the rumen was significantly ($P<0.05$) increased with the addition of monensin. These results suggested that the addition of monensin to the diet including soybean meal as a protein source could reduce ruminal protein degradation and enhance the amount of total protein that reaches the post-ruminal tract.

Keywords: *monensin,, digestion, soybean meal, rumen fermentation, degradability, purine derivatives, microbial nitrogen synthesis.*

INTRODUCTION

Monensin was the first ionophore to be shown to have a beneficial influence on ruminant nutrition (Raun *et al.,* 1976). Monensin increases ruminal proportions of propionate and decreases methane production. Of the many benefits attributed to monensin, decreased rumen ammonia production (Chalupa, 1980) and an improved flow of dietary protein to the abomasum (Dinius *et al.,* 1976; Hanson and Klopfenstein, 1979; Poos *et al.* 1979) are believed to be among the most important. Van Nevel and Demeyer, (1977) reported that addition of monensin to rumen fluid *in vitro* apparently inhibited proteolysis, as measured by ammonia production from intact casein. However, it now appears that proteolysis itself might not have been affected, but that the observations were probably due to inhibition of peptides or amino acid catabolism. The most important site of action of ionophores on N metabolism is probably amino acid breakdown. Accumulation of α-amino-N occurred when monensin was added to mixed rumen organisms in vitro (Van Nevel and Demeyer, 1977; Wallace *et al*.

1981; Whetstone *et al*., 1981) and deaminating activity in rumen fluid of cattle decreased when tetronasin was included in the diet (Newbold *et al*., 1990). Recently, El-Waziry and Kamel, (2001) studied the effect of monensin on hay protein digestion in sheep. They found that the monensin had a decreasing effect on the effective degradability of protein and dry matter. Ruminant nutritionists have developed ways for reducing ruminal protein degradation and increasing insoluble protein supplements, which are now commonly applied. However, any good idea can be taken too far, and it is now apparent that the rumen is sometimes starved for amino acid nitrogen. Therefore, additional experiments are needed with ionophores such as monensin to decrease the degradability of highly degradable proteins (such as soybean meal) in the rumen and to increase the passage of amino acids to the small intestine. The main objective of this work is to study the effect of monensin on the degradation of dry matter and crude protein of both the mixed diet and the protein source (soybean meal), digestibility, ruminal fermentation and microbial nitrogen synthesis in the rumen of sheep.

MATERIALS AND METHODS

Animals and diets

Three males Barki×Rahmani crossbred sheep with a mean live weight of 47 kg, (S.D±1.45) fitted with ruminal cannula were used in a 3x3 Latin square design experiment. Animals were offered a control diet (Table 1) at 3% of a live weight. The diets were 1) control diet, 2) control diet plus monensin at 30 mg/day and 3) control diet plus monensin at 50 mg/day. Animals were fed twice daily at 0800 and 1600, and they had free access to fresh water.

Digestibility trials

Digestibility trials (21 days for each treatment) were conducted using three animals for each treatment. Animals for each treatment were separated in individual pens. At the end of the preliminary period (d 15) rams were equipped with bags fitted to the animals with harness for total faecal collection. During the collection period (one week) accurate records were kept for individual feed intake. Total faeces were collected once daily and 10% representative samples were kept in a refrigerator with citric acid until analysis. Nitrogen, DM and OM were analyzed according to the methods of AOAC (1990). NDF was determined according to Goering and Van Soest (1970).

Rumen degradability measurement

Two polyester bags (Swiss Nylon Monofilament, Switzerland) of 7 cm × 15 cm and pore size of 45μm were used at each incubation time. The incubation times were 3, 6, 12, 24 and 48 h. Approximately 5 g of mixed diet or soybean meal (ground to 2 mm) were placed in each bags. After removal from the rumen, the bags were rinsed and manipulated in cold water until the water ran clear, then squeezed prior to storing at -20°C. Later, bags

were thawed and washed again in running tap water as described by Kamel *et al*. (1995) to eliminate the microorganism attached to residual sample. Two bags were washed in running water for 15 min. to determine the initial water losses. The kinetics of DM and CP degradation were estimated by the model of Ørskov and McDonald (1979) as described in El-Waziry *et al.* (2000). Ruminal outflow rate were assumed to be 0.05 per h according to Kirkpatrick and Kennelly (1987).

Ruminal fermentation

Rumen contents obtained from fistulated sheep before feeding, 1, 3 and 6 h after feeding were strained through four layers of surgical gauze. Rumen fluid (90 ml) were mixed with 10 ml 50% (v/v) H_2SO_4 and centrifuged at 27,000 x g for 10 min. The supernatant was frozen at -20°C until analysis. Ruminal NH_3-N concentration was determined according to Chaney and Marbach (1962), and volatile fatty acids were determined according to Supelco, Inc. (1975).

Measurement of purine derivatives excretion

Animals were equipped with funnels for urine collection. Twenty-four hours collection of urine was made for one week of each collection period. Urine was collected from three animals into containers which had 75 ml H_2SO4 (1 mol/l) (pH of the final urine < 3). The collected urine was then diluted to a fixed volume of 5 L with water. One subsample was stored at -20 °C for determining of purine derivative (PD) according to the procedure of Chen *et al.* (1990). The supply of microbial N (i.e. entering the small intestine) was then calculated according to Chen *et al.* (1991).

Statistical analysis

Results were analyzed by ANOVA for a Latin square design using the JMP procedure of SAS Institute, (1994).

RESULTS AND DISCUSSION

Digestibility trials

The composition and chemical analysis of the mixed basal diet is shown in Table 1. Sheep fed rations supplemented with monensin had lower ($P<0.05$) dry matter intake (DMI) than that of the control diet by 6% (Table 2). Monensin levels did not express statistical significant differences between the two levels of monensin groups. Gado (1997) reported similar results with goats fed concentrate mixture and berseem hay with the addition of monensin at 20 or 40 mg/day. Joyner *et al.* (1979) found that monensin decreased feed intake by 7% to 11% when lambs were fed monensin diet at levels of 5, 10, 20, or 30 mg/kg diet. Isichei *et al.* (1977) found that daily feed intake was not affected by the addition of monensin to diet. Reduction in feed intake may be due to flavors of ionophore compounds or to that animals regulate intake to maintain body energy balance (Nagaraja, 1995).

Table 1. Formulation and chemical analysis of diet fed to sheep

Ingredients	**%**
Berseem[a] hay	50
Ground corn	30
Soybean meal	12
Molasses	5.0
Lime stone	1.5
Sodium chloride	1.0
Vitamins	0.5
Chemical analysis(%)	
DM	86.53
OM[b]	88.73
CP[b]	14.32
NDF[b]	32.2

[a]***Trifolium alexandrinum***
[b]**Expressed on DM basis**

The addition of monensin did not improve (P>0.05) the apparent digestibility of DM, OM and CP (Table 2). As the results, the digestibilities of DM, OM and CP were 69.6, 73.7 and 76.7 for control diet, 68.8, 72.6 and 76.1 for control diet plus monensin at 30 mg/day and 68.1, 70.30 and 74.3 for control diet plus monensin at 50 mg/day, respectively. Similar results were reported by Gado (1997) for goats fed concentrate mixture and berseem hay with addition of monensin. Laurent *et al.* (1980) found that monensin had no effect on OM and CP digestibility. There was no effect on DM, OM and CP digestibility's in calves fed wheat straw as basal diet with a concentrate mixture when rumensin was added (Singh and Mohini**,** 1999). In most of the earlier studies, no effects on DM and OM digestibility were observed (Dinius *et al.,* 1976; Beever *et al.,* 1987). A decrease in DM digestibility was reported by Lemenager *et al.* (1978), However, Thornton and Owens (1981) reported an increase in DM digestibility. The contradictory of the previous investigations may be due to the changes in fermentation pattern in the rumen and different of the type and pattern feeding.

Table 2. Effect of monensin supplementation on dry matter intake (DMI), and dry matter (DM), organic matter (OM) and crude protein (CP) digestibilities by sheep fed mixed diet containing soybean meal as a protein source (Mean ± SE).

Item	**Monensin mg/head/day**		
	0	**30**	**50**
DMI g/head/day	1339.56±25.21 [b]	1259.11±15.20 [a]	1250.25±20.11 [a]
Digestibilities, (%)			
DM	69.6±1.2	68.8±1.0	68.1±2.1
OM	73.7±1.3	72.6±1.4	70.3±2.1
CP	76.7±1.2	76.1±0.9	74.3±2.5

[a, b] **Means within a row bearing different superscripts differ (P<0.05).**

Degradability in the rumen

Table 3 shows the degradation kinetics of DM and CP of the protein source when it was incubated in the rumen of sheep fed the mixed diet containing test protein. The soluble fractions (*a*) of both DM and CP were significantly (P<0.05) reduced when monensin was added to the control diet. The values of *a* were 21.84 and 18.84% of DM, and 33.27 and 27.27% of CP for control diet and control diet with monensin supplement at 30 mg/day, respectively. Monensin levels did not express statistical significant differences between the two levels of monensin groups (30 mg or 50mg/day) as shown in Table 3. The ruminally degradable fractions (*b*) of DM and CP were significantly (P<0.05) decreased when monensin was added to the control diet (Table 3). Degradation rate (*c*) of DM was not affected, however, it was significantly (P<0.05) decreased in CP when monensin was added to the control diet (Table 3). The effective degradability *(ED*) of DM and CP was significantly (P<0.05) decreased when monensin was added to the control diet. The *ED* values were 62.94, 56.94 and 57.55 of DM, and 71.25, 59.80 and 58.71 of CP for control diet, control diet plus monensin at 30 mg/day and control diet plus monensin at 50 mg/day, respectively, (Table 3).

Table 3. Effect of monensin supplementation on the degradation kinetics and effective degradability (ED) of dry matter (DM) and crude protein (CP) of protein source incubated in the rumen of sheep fed mixed diet containing tested protein (Mean±SE).

Item	Monensin mg/head/day		
	0	30	50
		DM, %	
a[1]	21.84 ± 1.75[b]	18.84 ± 0.85[a]	18.90 ± 0.55 [a]
b[1]	66.41 ± 1.12 [b]	62.22 ± 1.20 [a]	61.65 ± 0.95 [a]
c[1]	0.081 ± 0.001	0.078 ± 0.002	0.075 ± 0.002
ED	62.94 ± 1.00 [b]	56.94 ± 1.01 [a]	57.55 ± 1.25 [a]
		CP, %	
a[1]	33.27 ± 1.25 [b]	27.27 ± 1.10 [a]	26.85 ± 0.85 [a]
b[1]	58.94 ± 1.15 [b]	55.44 ± 0.55 [a]	54.95 ± 1.00 [a]
c[1]	0.091 ± 0.002 [b]	0.071 ± 0.001 [a]	0.069±0.001 [a]
ED	71.25 ± 1.65 [b]	59.80 ± 0.95 [a]	58.71 ± 1.00 [a]

[1] Estimated from the equation of Ørskov and McDonald (1979).
[a,b] Means within a row bearing different superscripts differ (P<0.05).

Table 4 shows the results of degradation kinetics of DM and CP of mixed diet when it was incubated in the rumen. The results revealed that, the values of *a, b* and *c* had similar trends to those of protein source (Table 4). The *ED* values of DM and CP of mixed diet were significantly (P<0.05) decreased with monensin supplementation. There were no statistical significant differences between the two levels of monensin groups (Table 4). Goodrich *et al.* (1984) reported that protein degradability was decreased when rumensin was added to the diet and their results were in agreement with the results of the present study. Zhao *et al.* (1995) reported that the *b*, *c*, and *ED* were decreased when salinomycin was added to goats at 30 mg/kg

DM. Recently, El-Waziry and Kamel (2001) reported that monensin reduced ruminal degradation of hay protein. The earliest evidence that nitrogen metabolism is affected by feeding ionophores came from the report of Dinius *et al.* (1976) who found a decreased ruminal ammonia concentration in cattle fed forage-based diet supplemented with monensin. Ionophores appear to affect ruminal degradation of peptides and deamination of amino acids to a greater extent than affecting proteolysis as such (Van Nevel and Demeyer, 1977; Wallace *et al.* 1981; Whetstone *et al.* 1981; Newbold *et al.* 1990).

Table 4. Effect of monensin supplementation on the degradation kinetics and effective degradability (ED) of dry matter (DM) and crude protein (CP) of mixed diet incubated in the rumen of sheep fed the same diet (Mean±SE).

Item	Monensin mg/head/day		
	0	30	50
		DM, %	
a[1]	22.30 ± 1.45[b]	18.10 ± 0.55[a]	17.90 ± 0.55 [a]
b[1]	54.51 ± 1.22 [b]	49.80 ± 1.21 [a]	48.95 ± 0.95 [a]
c[1]	0.060 ± 0.001	0.052 ± 0.002	0.049 ± 0.002
ED	52.03 ± 1.05 [b]	43.49 ± 1.21 [a]	42.13 ± 1.25 [a]
		CP, %	
a[1]	25.30 ± 1.22 [b]	19.7 ± 1.14 [a]	19.12 ± 0.65 [a]
b[1]	65.10 ± 1.11 [b]	59.7 ± 0.65 [a]	58.0 ±1.30 [a]
c[1]	0.071 ± 0.001 [b]	0.061 ± 0.001 [a]	0.059 ± 0.002 [a]
ED	63.50 ± 1.55 [b]	52.51 ± 1.00 [a]	50.51 ± 1.00 [a]

[1] Estimated from the equation of Ørskov and McDonald (1979).
[a,b] Means within a row bearing different superscripts differ (P<0.05).

Fermentation in the rumen

Ruminal NH_3-N concentrations were significantly (P<0.05) decreased when monensin was supplemented to the control diet (Table 5). The mean values of NH_3-N concentrations of sampling times were 14.6, 12.15 and 11.99 mg/dl for control diet, control diet plus monensin at 30 mg/day and control diet plus monensin at 50 mg/day, respectively**.** Gado (1997) reported that monensin had no effect on NH_3-N concentration. Recently, El-Waziry and Kamel (2001) found slight decreases in NH_3-N concentration with the supplementation of monensin to hay diet. The studies of Dinius *et al.* (1976); Van Nevel and Demeyer, (1977); Wallace *et al.* (1981); Whetstone *et al.* (1981); Newbold *et al.* (1990) have concluded that the lower ammonia concentrations were mainly due to reduced proteolysis, degradation of peptides and deamination of amino acids in the rumen.

Table 5. Effect of monensin supplementation on volatile fatty acids and ammonia nitrogen (NH_3-N) in rumen liquor by sheep fed mixed diet containing soybean meal as a protein source[1] (Mean ± SE).

Item	Monensin mg/head/day		
	0	30	50
NH_3-N mg/dl	14.6±0.85 [b]	12.15± 0.07 [a]	11.99±0.12 [a]
Acetate (A), molar %	64.31±0.13 [b]	58.85±2.19 [a]	58.12±2.15 [a]
Propionate (P), molar %	21.13±3.61 [b]	27.89±0.16 [a]	28.25±1.20 [a]
A to P, ratio	3.04±0.53 [b]	2.11±0.6 [a]	2.06±0.01 [a]
Butyrate, molar %	13.13±1.2	11.30±0.15	11.2±1.2

[1]Values are shown with a mean of sampling times.
[a, b] Means within a row bearing different superscripts differ (P<0.05).

The molar proportion of acetic acid was significantly (P<0.05) decreased when monensin was added to the control diet (Table 5). The supplementation of monensin significantly (P<0.05) increased molar proportion of propionic acid, but it slightly (P>0.05) decreased molar proportion of butyric acid. The ratio of acetate to propionate was significantly (P<0.05) decreased when monensin was added (Table 5). Singh and Mohini (1999) reported that the VFA concentration did not vary among the groups, while there was significant increases in propionate proportion and decreases in the proportion of acetate and butyrate in the rumen of animals fed rumensin. Similar results reported by Gado (1997) and Vuuren et al. (1983) are in agreement with the present study of individual VFA. Ushida *et al.* (1985) reported similar results for propionate and butyrate but there was no effect on acetate with the addition of monensin at 20 mg/kg. Increased propionate production could lead to better feed utilization and improved feed conversion efficiency.

Purine derivatives excretion

The urinary purine derivatives measured as uric acid, allantoin and xanthin+hypoxanthin are shown in Table 6. There were significant (P<0.05) increases in the purine derivatives in the urine when monensin was added to the diet and hence, there was a significant increase (P<0.05) in the calculated flow of microbial nitrogen from the rumen (Table 6). The present results are in agreement with the results of El-Waziry and Kamel (2001), who found an increase in the microbial nitrogen leaving the rumen when monensin was added to hay diet. Yang and Russell (1993) reported that monensin increased amino acid nitrogen passage from the rumen, and the quantity passing from the rumen was dependent on the protein source. The greatest response to ionophores such as monensin would be expected when dietary protein is not excessive and is supplied in a soluble form likely to be fermented rapidly in the rumen (Hanson and Klopfenstein, 1979).

Table 6. Effect of monensin supplementation on urinary purine derivatives and microbial nitrogen leaving the rumen by sheep fed mixed diet containing soybean meal as a protein source (Mean ± SE).

Item	Monensin mg/head/day		
	0	30	50
Allantoin, mmol/d	15.42±0.84 [b]	17.73± 0.11 [a]	17.81±0.14 [a]
Uric acid, mmol/d	2.01±0.11[b]	2.33±1.99 [a]	2.39±1.95 [a]
Xanthin+Hypoxanthin, mmol/d	2.61±3.58 [b]	3.02±0.16 [a]	3.17±0.20 [a]
Total, mmol/d	20.04±0.51 [b]	23.08±0.22 [a]	23.30±0.01 [a]
Microbial nitrogen leaving the rumen[1], g/d	17.16±1.00 [b]	19.76±0.14 [a]	19.95±0.18 [a]

[1]Values were calculated from urinary purine derivatives, (Chen *et al.* 1991).
[a, b] Means within a row bearing different superscripts differ (P<0.05).

In conclusion, the results suggested that the addition of monensin to the diet including rapidly degradable protein could reduce ruminal protein degradation and enhance total amount of protein that reaches the post-ruminal tract. In addition, the supplementation of monensin increased the production of propionate and this increment could lead to better feed utilization and improved feed conversion efficiency. These results suggested that increasing the addition of monensin levels in the daily ration for sheep from 30 to 50 mg/head/day did not show any significant beneficial effects on the metabolism of the animals, and it is recommend to use the level of 30 mg/head/day.

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تأثيراضافة المونسين (Monensin) علي هضم العليقة المحتوية علي كسب فول الصويا كمصدر بروتيني بواسطة الأغنام

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تهدف هذه الدراسة إلى تقييم تأثير المونسين (Monensin) علي معاملات هضم المادة الجافة والمادة العضوية والبروتين الخام وتخمرات الكرش وكذلك لمعرفة درجة تحلل المادة الجافة والبروتين للعليقة وكذلك مصدر البروتين في الكرش. استخدم ثلاثة ذكور من الأغنام مزودة بكانيولات مستديمة بالكرش ومغذاة علي (١) عليقة مقارنة تحتوي علي كسب فول الصويا كمصدر بروتيني (٢) العليقة المقارنة مضاف إليها المونسين بمعدل ٣٠ مجم / يوم (٣) العليقة المقارنة مضاف إليها المونسين بمعدل ٥٠ مجم / يوم وصممت التجربة باستخدام المربع اللاتيني ٣ X ٣. وكانت النتائج كما يلي:-

إضافة المونسين لم تؤثر علي معاملات هضم المادة الجافة والمادة العضوية والبروتين الخام وعندما غذيت الحيوانات علي العلائق المحتوية علي المونسين حدث انخفاض معنوي في تركيز امونيا الكرش من ١٤,٦ إلى ١٢,٥ و١١,٩٩ مجم/١٠٠ مل سائل كرش لكل من عليقة المقارنة وعليقة المقارنة مضاف إليها المونسين بمعدل ٣٠ مجم / يوم و عليقة المقارنة مضاف إليها المونسين بمعدل ٥٠ مجم / يوم علي الترتيب أما في حالة الأحماض الدهنية الطيارة فوجد أن هناك زيادة معنوية في تركيز حامض البروبيونيك وانخفاض معنوي في تركيز حامض الخليك ولم يتأثر حامض البيوتريك بإضافة المونسين. و أدت إضافة المونسين إلى حدوث انخفاض معنوي واضح في معدل تحلل المادة الجافة والبروتين للعليقة في الكرش وكذلك مصدر البروتين. وكان تأثير إضافة المونسين معنويا علي النتروجين الميكروبي المخلق والمار من الكرش و المقدر بواسطة مشتقات البيورين في البول. وخلاصة القول إن إضافة المونسين قد يؤدي إلى خفض تحلل بروتين كسب فول الصويا وبروتين العليقة المحتوية علي كسب فول الصويا وكذلك زيادة كمية البروتين الكلي المار الي الأمعاء.