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NITROGEN METABOLISM AND RUMEN FERMENTATION OF SHEEP AS AFFECTED BY SALINOMYCIN SUUPPLEMENTATION.

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ABSTRACT

This study was conducted at the Experimental Farm of Animal Production Department, Faculty of Agriculture, Minia University, Egypt, to evaluate the effects of salinomycin as feed additive on animal nutrient digestibilities, feeding values, nitrogen metabolism and rumen fermentation of Ossimi rams. The tested rations (R1, R2 and R3) represented three treatments that contained 0, 12 and 24 ppm. salinomycin, respectively. Results showed that dry matter (DM), organic matter (OM), ether extract (EE) and nitrogen free extract (NFE) digestibilities were significantly increased for animals fed salinomycin in comparison with the control group. Crude protein (CP) digestibility did not significantly increased; the values were 78.64, 80.87 and 81.22 for R1, R2 and R3, respectively. Feeding values as TDN and SV increased gradually for animals fed salinomycin supplemented rations, but the increase was significant only for animals fed R3 compared to the control group. Concerning, total nitrogen excreted, it decreased significantly ($P \geq 0.05$), but fecal and urine nitrogen insignificantly ($P \geq 0.05$) decreased. Nitrogen balance (as g / day or as % digestible nitrogen) significantly increased ($P \leq 0.05$) by increasing salinomycin level, compared to the control group. Ruminal propionic, butyric and isobutyric acids concentrations and acetate/propionate ratio increased significantly ($P \leq 0.05$), while acetic acid concentration significantly ($P \leq 0.05$) decreased for animals fed salinomycin containing rations compared to the control group. Rumen liquor pH was not significantly affected by salinomycin addition to the diets . Sampling time had no significant effects ($P \leq 0.05$) on acetic,

isobutyric and isovaleric, but, significant increase ($P \leq 0.05$) was detected in the case of probionic and significant decrease ($P \leq 0.05$) was noticed for butyric and valeric acids . Significant decrease ($P \leq 0.05$) in ruminal pH from 7.61 to 6.95 and 6.98 at 0, 2 and 4h after feeding, respectively. Blood hemoglobin (Hb g/dl) and packed cell volume (PCV%) increased gradually with salinomycin supplementation than the control.

INTRODUCTION

Ionophores (such as monensin, lasalocid, laidlomycin, salinomycin and narasin) are antimicrobial compounds that are commonly fed to ruminant animals to improve feed efficiency. These antimicrobials specifically target the ruminal bacterial population and alter the microbial ecology of the intestinal microbial consortium, resulting in increased carbon and nitrogen retention by the animal, increasing production efficiency (*Callaway, et. al., 2003)*.

Implementation of comprehensive nutrient management plans on farms may improve efficiency of nutrient utilization, decrease imported nutrients, and nutrient loss to the environment while improving farm profitability (Klausner *et. al.,* 1998; Wang *et. al.*, 2000a, b). The major opportunity to reduce nutrient losses is through animal diet modification (Council for Agricultural Science and Technology, 2002). Absorbed protein (amino acids that are digested and absorbed in the small intestine) that is not synthesized into tissue or milk is excreted in the urine as urea, which is converted to a volatile form (primarily NH_3) and escapes to the environment. Therefore, the goal of the animal nutritionist in developing diets to reduce nutrients in manure is to accurately match dietary amount and sources of protein with animal requirements (Klausner *et. al.,* 1998 and Council for Agricultural Science and Technology, 2002).

Lambs fed on salinomycin gained more weight, feed intake and had greater Longissimus dorsi area than lambs fed on monensin. It appears that salinomycin is a better growth promoter for lambs fed a conventional diet high in concentrates (Salinas-Chavira *et. al.,* 2005). Salinomycin supplementation appeared to be more effective on propionate concentration at the expense of acetic acid (Febel, *et. al.*, 2001). Salinomycin significantly reduced the ammonia concentration of the rumen fluid and microbial N content of the duodenal digesta (Febel, *et. al.*, 2001). Salinomycin inhibited proteolysis and reduced the efficiency of microbial protein synthesis (Febel, *et. al.*, 2001). Because of the complexity and high degree of specificity of ionophore resistance, it appears that ionophores do not contribute to the development of antibiotic resistance to important human drugs. Therefore it appears that ionophores will continue to play a significant role in improving the efficiency of animal production in the future (*Callaway, et. al., 2003).* It is generally recognized that the use of ionophores in ruminants presents no hazard to human health arising from the potential to generate "resistant" foodborne bacteria, this is because ionophores are not used in human therapy due to their narrow therapeutic index, there is no genetic encoded resistance to their biophysical mechanism of action, and there is rapid cell death (Russell and Houlihan, 2003).

However, effect of salinomycin on ruminal protein metabolism have not been fully investigated. Therefore, this study was carried out to investigate the effects of salinomycin supplementation on feeding values, nitrogen metabolism and rumen fermentation of sheep.

MATERIALS AND METHODS

This study was conducted at the Experimental Farm of Animal Production Department, Faculty of Agriculture, Minia University, Egypt. The main objectives of this study were to evaluate the effects of salinomycin as feed additive on nutrient digestibilities, feeding values, nitrogen metabolism and rumen fermentation of sheep. The tested rations (R1, R2 and R3) represented three treatments that contained 0, 12, and 24 ppm. salinomycin, respectively.

Diet preparation:

The diets used were prepared to contain 75% concentrate feed mixture (CFM) and 25% rice straw (RS) on dry matter bassis (DMB). Salinomycin was added to the concentrate feed mixture at three levels 0, 12 and 24 ppm on DMB from the whole diet (75% CFM +25% RS). Diet chemical composition is presented in Table 1.

Digestibility trials:

Three yearling male Ossimi males of an average 40 kg live body weight were assigned in a Latin square design to determine digestibility coefficients and nitrogen balance of the tested rations. Animals were fed at a rate of 4% of their live body weight (DMB), on diet contained 75% CFM and 25% of rice straw along with 0, 12 and 24 ppm salinomycin. Weighed rations were offered twice daily at 9.0 a.m. and 2.0 p.m. in equal portions, fresh water was available all the time. Each digestibility experiment was continued for 21 days (14 days as preliminary period and 7 days as total collection period of feces, urine and rumen liquor). Feces were weighed daily, mixed thoroughly and 10% representative samples were taken from each animal, dried at 60 °C for 72 hours. Dried feed and fecal samples were ground through 1 mm screen and a sample of 50g / treatment / animal was taken for laboratory analysis. The samples of feed and feces were analyzed for crude protein (CP), crude fiber (CF), ether extract (EE) and ash according to AOAC (1990). Daily acidified urine volume was measured, 10% representative sample was collected and used for urinary-N determination at the end of experiment. Urine-N determination was carried out according to A. O. A. C. (1990) procedure.

Rumen activity:

Rumen liquor samples were collected day by day through the collection period of each experiment at 0, 2 and 4 hrs after the morning meal using stomach tube. Collected rumen fluid was tested immediately for pH using Jenway LTD 3020 pH meter. Few drops of saturated solution of mercuric chloride were added to the rest of filtrate portion to stop the microbial activity, strained through four layers of chesses cloth for each sampling time. Strained samples were frozen storage. VFA's concentrations were estimated using (H.P.L.C).

Statistical Analysis:

Data were subjected to statistical analysis program (SPSS, program 1997), Duncan's multiple range test (1955) was used to detect significant differences among means.

Table 1 : Proximate analysis of concentrate feed mixture (CFM) and rice straw(RS).

Ingredients	Proximate analysis on DM basis						
	DM	OM	CP	CF	EE	NFE	ASH
CFM	89.08	89.08	14.20	13.03	02.09	59.76	10.92
Rice Straw	87.66	83.98	03.11	36.15	02.10	42.62	16.02
75%CFM+25% RS	88.72	87.81	11.43	18.81	02.09	55.48	12.19

Where, DM, OM, CP, CF, EE and NFE are Dry matter, Organic matter, Crude protein, Crude fiber, Ether extract and Nitrogen free extract, respectively (CFM) composed of 30% wheat middling, 20% undecorticated cotton seed cake, 22% wheat bran, 12% yellow corn, 9% rice germ, 4% molasses, 2% limestone and 1% common salt.

RESULTS AND DISCUSSION

Digestibility coefficients and feeding values:

Digestibility coefficients of nutrients and feeding values as total digestion nutrients (TDN) and starch value (SV) are presented in Table 2. The results show that DM and OM digestibilities significantly increased ($P \leq 0.05$) for animals fed salinomycin, especially with the higher level (R3) compared to the control animals. For CP digestibility, non significant effect was observed, but the values tended to increase by 2.84 and 3.28% for R2 and R3, respectively compared to the control value. But, EE and NFE digestibilities increased significantly ($P \leq 0.05$) for R2 and R3, compared to the control (R1). Feeding values as TDN and SV increased gradually for animals fed salinomycin supplemented rations, but the increase was significant only for animals fed R3 compared to the control group. The values of TDN were 57.28, 61.07 and 62.31 and those of SV were 56.36, 60.09 and 61.33 for R1, R2 and R3, respectively. Hristov, *et. al.*, (2000) reported that, apparent digestibilities of DM, neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude protein were unaffected ($P > 0.05$) by salinomycin supplementation to the steers diet. The present results are in contrast with Merchen and Berger (1985) and Richter and Flachowsky (1991). They reported that salinomycin supplementation to the ruminants diet insignificantly affected the apparent digestibility of organic matter or other nutrients.

Table 2 : Nutrient digestibility coefficients and feeding values (DM basis) of the tested rations (R1, R2 and R3).

Item	Rations			SE
	R1	R2	R3	
Digestibility coefficients %				
DM	62.46^{a}	66.88^{ab}	67.96^{b}	2.10
OM	63.67^{a}	67.75^{ab}	69.17^{b}	2.01
CP	78.64	80.87	81.22	1.12
CF	55.13	53.52	55.55	2.93
EE	51.55^{a}	60.42^{b}	60.19^{b}	2.59
NFE	64.00^{a}	70.15^{b}	71.65^{b}	1.87
Feeding values:				
TDN %	57.28^{a}	61.07^{ab}	62.31^{b}	2.27
SV %	56.36^{a}	60.09^{ab}	61.33^{b}	1.83

±SE: Plus or Minus Standard error.

a and b: Averages in the same raw with different superscripts are different ($P \leq 0.05$).

Nitrogen metabolism:

Total nitrogen excreted was significantly decreased ($P \geq 0.05$), but fecal and urinary nitrogen were insignificantly decreased ($P \geq 0.05$) with salinomycin addition to the diet compared to the control (Table 3). Nitrogen balance (as g / day or as % of digestible nitrogen) was significantly increased ($P \leq 0.05$), compared to the control group (Table 3). Also, between the two levels of salinomycin in the diet the higher level showed the better response for nitrogen balance. Daily urinary N output was reduced by lasalocid supplementation. Yang, *et. al.*, (2003) stated that the proportion of urinary N relative to digested N tended to decrease, and the retained N as a proportion of digested N tended to increase with ionophore supplementation. Salinomycin significantly lowered microbial N content of the duodenal digesta, inhibited proteolysis and reduced the efficiency of microbial protein synthesis (Febel *et. al.,* 2001). They suggested that the effect of salinomycin on ruminal N metabolism was independent of the composition of substrate. Unlike salinomycin tended to increase proteolysis in the rumen and did not inhibit protein synthesis. The obtained data are in agreement with those reported by Luis, *et. al.,* (2003), who indicated that monensin in the diets of ruminants may decrease protein degradation in the rumen and may increase feed protein utilization by an average of 3.5 percentage units. These changes would have an effect in reducing N losses and decreasing fecal N and the amount of protein that must be fed to meet animal requirements.

Table 3 : Nitrogen metabolism as affected by salinomycin tested rations.

Item	Rations			±SE
	R1	R2	R3	
Fecal nitrogen g/day	04.84	04.34	04.26	0.19
Urine nitrogen g/day	16.31	15.74	15.23	0.37
Total nitrogen excreted g/day	21.16[a]	20.07[b]	19.49[c]	0.21
Digestible nitrogen (DN) g/day	17.83	18.33	18.41	0.18
Nitrogen balance g/day	01.52[a]	02.60[b]	03.18[c]	0.24
Nitrogen balance as % of DN	08.53[a]	14.17[b]	17.24[c]	1.69

±SE: Plus or Minus Standard error.
A, b and c: Averages in the same raw with different superscripts are different ($P \leq 0.05$).

Rumen activity:

The effect of treatments on VFA's concentration and pH in rumen liquor is presented in Table 4. Both of propionic, butyric acids concentrations increased significantly ($P \leq 0.05$) for animals fed salinomycin containing rations compared to the control group. In contrast, acetic acid concentration and acetate/propionate ratio were significantly ($P \leq 0.05$) decreased in both rations containing salinomycin compared to the control diet. Supplementation of salinomycin reduced molar concentration of acetate and acetate / propionate ratio and increased propionate in rumen liquor, ((Merchen and Berger, 1985, Olumeyan *et. al.,* 1986, Bagley, *et. al.*, 1988 and Tadahisa, *et. al.,* 2006). Salinomycin had no significant effect on ruminal pH, with slight increase with R2 and R3 diets compared to the control (7.19 and 7.29 for R2 and R3 *vs.* 7.08 for control, respectively). The present results are in agreement with those obtained by Hristov *et. al.,* (2000), who reported that ruminal pH was unaffected by salinomycin addition to the diet. These data suggested that the inclusion of salinomycin in the diet increase ruminal pH in sheep fed ionophores (Clayton *et. al.*, 1999 and Ives *et. al.*, 2002).

Table 4 : Effects of salinomycin treatments on VFA's concentrations and pH in rumen liquor of sheep.

Item	Treatments			±SE
	R1	R2	R3	
Acetic	50.21[a]	48.01[b]	47.10[b]	1.00
Propionic	28.29[a]	29.51[b]	31.02[b]	0.30
Isobutyric	01.72	01.71	01.81	1.00
Butyric	18.58[a]	20.51[b]	19.10[ab]	0.15
Isovaleric	00.46	00.56	00.32	0.25
Valeric	00.73	00.70	00.65	0.73
Acetic/Propionic ratio	01.78[a]	01.63[b]	01.54[b]	0.23
Rumen liquid pH	07.08	07.19	07.29	0.17

±SE: Plus or Minus Standard error.

a and b: Averages in the same raw with different superscripts are different ($P \leq 0.05$).

Sampling time (0, 2 and 4h after feeding) had, no significant effects ($P \leq 0.05$) on acetic, isovaleric, valeric and acetic / propionic ratio (Table 5). Significant increase ($P \leq 0.05$) was detected in the case of propionic and significant decrease ($P \leq 0.05$) was noticed for butyric acid as the times of sampling was increased. Significant decrease ($P \leq 0.05$) was detected in ruminal pH from 7.61 to 6.95 and 6.98 at 0, 2 and 4h after feeding, respectively (Table 5).

Table 5 : Effects of sampling time on VFA's concentrations and pH in rumen liquor of sheep.

Item	Time of post-feeding			SE
	0h	2h	4h	
Acetic	47.69	48.30	49.33	0.62
Propionic	27.87[a]	30.19[ab]	30.77[b]	0.81
Isobutyric	01.00	01.38	01.85	0.34
Butyric	21.63[a]	19.31[ab]	17.25[b]	0.86
Isovaleric	00.45	00.48	00.41	0.14
Valeric	01.35	01.34	00.39	0.15
Acetic/Propionic ratio	01.73	01.61	01.62	0.05
Rumen liquar pH	07.61[a]	06.95[b]	06.98[b]	0.09

±SE: Plus or Minus Standard error.

a and b: Averages in the same raw with different superscripts are significantly different ($P \leq 0.05$).

Hematological parameters:

The results of hematological parameters such as hemoglobin concentration (Hb g/dl) and packed cell volume (PCV%) are presented in Figure 1. Salinomycin supplemented diets recorded the highest values of Hb and PCV% compared to the control diet. The values of Hb and PCV% were (10.3 and 28.7), (11.5 and 29.0) and (12.7 and 31.7), for animals fed on R1, R2 and R3, respectively. The increase of Hb and PCV for animals fed salinomycin could be attributed to the effect of such ionophore on rumen fermentation which may account for the enhanced performance (Soliman *et. al.,* 2002).

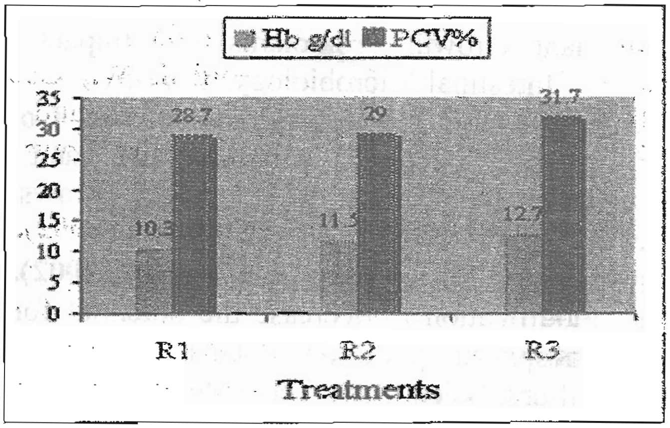

Figure 1: Effects of salinomycin level on hemoglobin concentration (Hb) and packed cell volume (PCV%) of Ossimi rams.

CONCLUSSION

Supplementation of salinomycin to sheep diet alter ruminal fermentation primarily by changing ruminal microbial populations, that increased ruminal pH, increased propionate concentration at the expense of acetic acid. Salinomycin leads to increase N retention at the expense of N excreted as noticed from the obtained results of the present study.

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تأثير إضافة السالينوميسين إلى العليقة على القيمة الغذائية، التمثيل الغذائى للنيتروجين والنشاط الميكروبى بالكرش فى الأغنام.

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أجريت هذه الدراسة فى مزرعة الإنتاج الحيوانى بكلية الزراعة جامعة المنيا لتقدير تأثير إضافة السالينوميسين كمنشط نمو إلى علائق ذكور الأغنام الأوسيمى الناضجة على معاملات الهضم للمركبات الغذائية ، القيمة الغذائية ، التمثيل الغذائى للنيتروجين وكذلك النشاط الميكروبى. أضيف السالينوميسين إلى العليقة التى تكونت من (٧٥% مخلوط علف مركز + ٢٥% قش أرز)على أساس المادة الجافة للحصول على أربعة معدلات من السالينوميسين بالعليقة الكلية (المأخوذ الكلى من المادة الجافة) وهى:-

(R1) عليقة مقارنة خالية من السالينوميسين.

(R2) عليقة مقارنة + ١٢ جزء/المليون سالينوميسين.

(R3) عليقة مقارنة + ٢٤ جزء/المليون سالينوميسين.

وكان من أهم النتائج المتحصل عليها مايلى:

أدت إضافة السالينوميسين إلى زيادة معنوية عند مستوى ٠,٠٥% فى معاملات هضم كلا من المادة الجافة والمادة العضوية والدهن الخام وكذلك المستخلص الخالى من الأزوت بينما لم يتأثر معامل الهضم لكلا من البروتين الخام والألياف الخام بإضافة السالينوميسين عن المجموعة المقارنة ، بينما حدثت زيادة معنوية عند مستوى ٠,٠٥% فى كلا من مجموع المركبات الغذائية المهضومة وكذلك معادل النشا وكانت هذه الزيادة تدريجية مع زيادة السالينوميسين بالعليقة.

حدث انخفاض تدريجى غير معنوى عند ٠,٠٥% مع زيادة السالينوميسين بالعليقة فى كلا من أزوت الروث والبول ، وانخفاض معنوى عند ٠,٠٥% فى الأزوت الكلى الخارج ، وارتفاع المحتجز من الأزوت معنويا عند مستوى ٠,٠٥% مع إضافة السالينوميسين وكانت الزياة تدريجية مع زيادة السالينوميسين بالعليقة بالمقارنة بالمجموعة المقارنة.

زيادة كلا من حمض البروبينيك والبيتريك معنويا عند ٠,٠٥% مع انخفاض كلا من الخليك ونسبة الخليك/البروبينيك معنويا عند ٠,٠٥% فى الحيوانات التى تغذت على السالينوميسين بالمقارنة بالمجموعة المقارنة ، فى حين أن الإرتفاع الحادث فى قيمة ال pH عند المستويات المختلفة من السالينوميسين لم يكن معنويا عند ٠,٠٥% عن المجموعة المقارنة.

أظهرت نتائج كلا من الهيموجلوبين ونسبة المكونات الخلوية بالدم ارتفاع تدريجى مع زيادة السالينوميسين بالعليقة بالمقارنة بالمجموعة المقارنة.