An-Najah J. Res: Vol. 4 No. (10) 1996

The Effect of Monensin , Lasalocid and A monensin / lasalocid Rotation of Fungal Population in the Rumen of Concentrate and Roughage Fed Steers.

تأثير أستخدام المضادات الحيوية مفردة او بالتناوب على اعداد الفطريات الناتجه في معد العجول المغذاة على اعلاف مركزة او مالئة

Jamal Abo-Omar*

جمـــال أبوعمـــــر

ABSTRACT

Two studies using three ruminally cannulated beef steers in the first one fed a roughage diet (98% chopped alfalfa at maintenance) and 12 beef steers in the second fed limited ration (85% adlib intake) composed of 90% concentrate diet (80% cracked corn , 10% soybean meal), and 10% chopp-ed alfalfa were allotted to treatment groups receiving a supplement of either monensin / tylan (m27.5 ppm / 11 ppm), lasalocid (33 ppm) or a daily rotation of the two ionophores to examine the effect of the ionophores on rumen population of anaerobic fungi over time and the relationship between fungal numbers and methane production. In the first study , nylon bags containing leaf blades of 5mm length were incubated for 24 hrs. in the rumen. Samples

* **Assistant Professor, Dept. of Aninal Production, A-najah N. University, Nablus.**

were counted on days 0 , 1 , 3 , 5 , 7 , 11 , 14 , 16 , 21 , 22 , 23 , 24 , and 28 . Fungi sporangia on blades were counted microscopically (00x). Study 2 , steers were assigned as above and after methane production was measured using indirect respiration calorimetry , samples of rumen contents were collected by stomach tube pretreatment , and on days 2 , 5 , 16, and 45 after initiation of ionophore treatments . Differences of fungi numbers over time and between treatments were not significant , yet , there was a trend of initial ionophore inhibition on fungi with limited evidence of adaptation over time. There was a positive correlation ($P < 0.05$) between fungi numbers and methane production . These results indicate a fungal/methanogen interaction.

ملخـــص

اشتملت الدراسة على تجربتين اجريب على عجول التسمين ، حيث استخدم في التجربة الاولى ثلاثة عجول غذيت على عليقه ادامه (Maintenence) احتوت على ٩٨٪ قش برسيم . واستخدم في التجربة الثانية ١٢ عجلاً غذيت على علائق محددة (٨٥٪ استهلاك حر Adlib intake) حيث قسمت العجول الى ثلاث مجموعات غذيت على علائق احتوت على الذرة المجروشة وفول الصويا وقش البرسيم بمعدل ٨٥٪ ، ١٠٪ على الترتيب ، وأضيف الى عليقة كل مجموعة احد المضادات الحيوية المستخدمة في التجربه وهي Monensin/tylan ، Lacalocid او اضافة كل من المضادين الحيويين بالتناوب في المجموعة الثالثة ، وذلك لمعرفة تأثير اضافة المضادات الحيوية على تعداد الفطريات في كرش العجول خلال فترة التغذية ، ولمعرفة اثر المضادات الحيوية المستخدمة على كميات غاز الميثان المنتج خلال عملية التخمر .

في التجربة الاولى تم وضع اوراق البرسيم بطول ٥ملم في اكياس نايلون خاصة وتحضينها في الكرش لمدة ٢٤ ساعة، وتمت عملية التحضين في اليوم الذي سبق عملية التحضين ، وفي الايام : ١ ، ٣ ، ٥ ، ٧ ، ١١ ، ١٤ ، ١٦ ، ٢١ ، ٢٢ ، ٢٣ ، ٢٤ ، ٢٨ . بعد تلك العملية ، وقد استخدم المجهر بقوة تكبير ١٠٠ مره في عملية عد الفطريات المتجمعة على الاوراق المحضنة.

وفي التجربة الثانية تم قياس الميثان بواسطة الطريقة غير المباشرة لقياس الطاقة ، وتم اخذ عينات من الكرش مباشرة باستخدام انبوب خاص قبل المعاملة بالمضادات الحيوية ، وفي الايام : ٢ ، ٥ ، ١٦ و ٤٥ من بداية اضافة المضادات الحيوية للعلائق .

بينت النتائج ان اعداد الفطريات لم تتأثر تأثراً ملحوظا على مدار الايام التي استخدمت فيها المضادات الحيوية كما لم يكن لنوع المضاد الحيوي تأثير ملحوظا ايضا على تلك الاعداد ، هذا على الرغم من تدني عدد الفطريات لفترة من الوقت قبل ان تبدأ عملية التأقلم (Adaptation).

كما بينت الدراسة ان هناك ارتباط ايجابي ($P< .05$) بين اعداد الفطريات الموجودة وكمية غاز الميثان المنتج ، وهذا يدل على وجود علاقة بين اعداد الفطر الناتج والبكتيريا المنتجة لغاز الميثان.

Introduction

Rumen anaerobic fungi have been shown to act synergisticly with methanogenic bacteria (Bauchop and Mounfort , 1981 ; Mountfort et. al., 1982) and competitively with cellulolytic bacteria when the bacteria have been inhibited by penicillin or streptomycin (Akin et al., 1983; Windham and Akin , 1984 and Akin , 1987). Monensin and lasalocid are ionophores which are biologically active compounds used in feedlot operations to improve efficiency of feed utilization in growing and finishing cattle . These have been shown to alter bacterial population , selecting for propionate and succinate producers and suppressing formate , H_2, lactate , and butyrate producing bacteria (Chen and Wolin , 1979 ; Dawson and Boling , 1983 ;Bergen and Bates , 1984).

Monensin may also supress fungal and protozoal growth as well (Elliott et al., 1987) . Some of the results of altering the microbial population by ionophore supplementation include changes in volatile fatty acid production in the rumen and a decline in methane production (Schelling ,1984). The decline in methane production has been shown to be a temporary effect suggesting an adaptation by the rumen microflora (Rumpler , et al ., 1986).

One possible explanation for the resumption of methane production in animals fed monensin is a synergistic interaction between fungi and methanogenic bacteria . An increase in fungi number made possible by a change in a niche in the rumen because of ionophore feeding coupled with an adaptation by methanogenic bacteria might result in the rebound of methane production seen in some trials (Rumpler et. al. 1986).

The objective of this study was to examine the effect of monensin and a monensin / lasalocid rotation on fungal numbers , in steers fed two types of diets (total roughage and high concentrate) and to examine the relationship between fungal numbers and measured methane production.

Materials and Methods

Preliminary Study:

Leaf blades from an alfalfa hay bale were hand picked , placed in nylon bags (pore size of 40 um.) and suspended in the rumen of a ruminally cannulated steer for 6, 12 , 18 and 24 hours to determine the optimal incubation period to obtain adequate fungal growth.

Study 1 :

Three ruminally cannulated beef steers were fed a 98% chopped hay diet at maintenance level twice daily at 07:00 a. m. and 4:00 p.m. composition and chemical analysis of the diet are shown in (Table 1) . Each steer was randomly assigned to one of three treatments : 27.5 ppm monensin / 11.0 ppm tylan (M) 33.0 ppm lasalocid (L) , and a continous daily rotation of the two ionophores (M/L) , using these same ionophores and concentrations . Nylon bags (40 um pore size) containing leaf blades (carex spp.) of 5 mm length were incubated in the rumen for 24 hours on days 0 , 1, 3, 5, 7, 11, 14, 16, 21, 23, 24, and 28. Samples removed after 24 hours were rinsed with normal saline , treated with 4% formaldehyde and stored until examination . after staining with lactophenol cotton blue , fungi numbers were obtained by counting sporangia under the light microscope at 100 x mangnigication . Four counts were taken from each bag. Fungi were identified and measurd by size and shape . The occular lens was calibrated using a stage micrometer and standard microscopic procedures.

Study 2 :

Twelve beef steers were limit fed (85% ad lib) a 90% concentrate diet consisting of 80% cracked corn, soybean meal (10%) and chopped alfalfa hay (10%) twice daily at 08:00 a.m. and 5:00 p.m. (Table 2). Adapted steers were divided into two replicates of six steers

each. Each steer was randomly assigned to one of the same three treatments liabove. Prior to initiation of ionophore supplementation rumen contents were obtained via stomach tube. Samples were handled as above and stored for later counting .After methane production was measured using indirect respiration calorimetry ; rumen contents were sampled using a stomach tube . This occurred on days 2,5,16, and 45 after ionophore supplementation.

The experimental design used in the study was the complete randomized design with repeated measures for single factor.

Results and Discussion

Preliminary Study :

The nylon bag incubation of alfalf hay leaf blades did not result in adequate fungi numbers to quantify. This might be due to the soluble carbohydrates in alfalfa were too rapidly digested (4-6 hr .) and were unlikely substrate for fungal growth. The results tend to support the findings of others (Bauchop , 1979 ; Windham and Akin , 1984) that concentrate rations are not favorable for fungi growth.

Study 1 :

Three types of fungi were observed and quantified for all three treatments. Type A sporangia were oblong in shape and ranged from 10 - 20 um wide and 30 - 40 um long. Type B sporangia were oval in shape and ranged from 20 - 40- um wide and 40 - 60 um lon. Type C sporangia were darker , oval in shape and ranged from 40 - 80 um wide and 70 - 120 long. Although the fungi numbers were highly variable , there were consistently more fungi counted with the roughage diet . The high variability of the fungi indicate that the quantification techniques used were not sensitive enough to detect and differences over time or between treatments that may have been present.

There was no significant difference in mean fungi numbers over time or between treatments for L,M/L, and M (Table 3). For all three treatments there was an initial decline of fungi numbers on day 1 (23.17 to 14.42) sporangia (S) , per sq.mm.L ; 30. 42 to 16. 42 S, M/l; 30.92 to 19.58 s, M) although they were not statistically significant . The L treatment remained relatively steady throughout the trial falling to allow on day 14(10.58 S) and rising to a high on day 23 (68.08 S). After the initial reduction on day 1 , the M/L treatment rebounded to near normal on day 3 (26.0) and maintained a steady number until dropping to a low of 5.08 S on day 14 which was a monensin day of the rotation then recovered by day 16 (20.0 S) and maintained at this level for remainder of the trial. It is interesting to note that the low days of fungi numbers , although not statistically different , were monensin days of the rotation . Then M treatment declined steadily from the start of the trial until day 7 (30. 92 to 7 . 83 S) then recovered to 18 .75 S on day 11 and fully recovered by day 16 (35.5 S) and maintained a steady level until the end of the trial.

Study 2 :

Three types of fungi, as above , were observed and quantitated for all three treatments. Mean fungi numbers over time and between treatments were not significantly different (Table 4) . The fungi numbers were considerably lower for these receiving the concentrate ration than for the steers of the roughage diet with the highest number for all three treatments of study 2 being 20. 83 S for the M/L group which compares to the approximate base value for the study 1 steers . Initial fungi numbers declined from the start of the ionophore supplementation for the M (1,92 to 0.73 S) and (5.5 to 3.85 S) groups while increasing for the M/L (2.81 to 4.54 S) group. The L treatment chlimbed to a high of 8.11 S by day 5 and then gradually decreased to 6.69 S on day 45. The

M treatment continued to decline to a low of 0.31 S on day 5 and then increased to 6 .79 S on day 16 and reached a high of 9.98 S on day 45. Fugi numbers reached a low (1.35 S)on day 5 for the M/L group and then rose to a high of 20.83 S on day 16. Although decreasing 10 12.02 by day 45 , the numbers were still higher than at the beginning of the trial.

Yet the initial decline (although not significantly different) for the all treatments , except the M/L group (this due primarily to slight increases of the 2 steers on the L stagger and marginal decreases of the 2 steers on the monensin stagger) suggest a trend of fungal inhibition by ionophore treatment and there is some evidence of adaptation over time . Fungal monensin sensitivity in both studies support the findings of Elliott et al. (1987). There is little reported data on the effects of lasalocid on fungi growth, and it may be that the amounts of lasalocid administered here were inadequate for inhibition of fungi. This may have restricted the effect of the M/L treatment as well.

The correlation between fungi numbers and methane production (as % of gross energy intake) for the individual treatments were significant ($P<0.05$) relating high fungi numbers to high methane production . Spearman rank correlations were for the M treatment r 0.053, L treatment r 0.89, and the M/L teatment r 0.69. Methane production (Table 4) declined on day 2 for all three treatments and then gradually increased untill day 45. After the initial decline on day 2 , methane production for the L treatment rebounded to the control value on day 5 then maintained until day 16 with a slight increase on day 45. The treatment then rose slightly on day 5 while rising sharply on day 16 then dropping slightly on day 45. With methane production continuing to decrease on day 5 , the M/L group then rose sharply on day 16 and fell slightly on day 45. For all three treatments methane production was greater at the end of the trial than at the start with the increase being

marginal for the L and M groups but with the M/L group having a considerabnle increase (3.0% methane to 5.7% methane).

The significant correlation between fungi numbers and methane production indicate a relationship between methanogenic bacteria and fungi as have found (Buachop and Mountfort , 1981 ; Mountfort et al., 1982) . Although the high variability of the fungi counts render these results inconclusive . This, along with the limited evidence of fungi adaptation over time , agrees with the data obtained by Rumpler et al. (1986) and does not disprove the possibility of a synergistic interaction between fungi and methanogenes being resonsilbe for the resumption of methane production.

Application :

Ionophores when used at certain levels in feed-lot operations may increase profits. It is well documented that ionophores have positive effect in increasing rate of gain in feedlot animals. This effect arised through ionophore effects in inhibition of methanogen bacteria and fungi population . Any supresion of both bacteria and fungi counts will improve animals feed efficiency.

Table (1)

Composition and Proximate Analysis of Diet Fed to Cannulated Steers.

Ingredient	%
Chopped Alfalfa Hay	**98.0**
Supplement *	**2.0**
Chemical Analysis : **	
% DM	94.3
% CP	17.9
% NDF	51.9
% ADF	42.6
% Lignin	8.9
% Ash	0.6
% EE	1.65
% NFE	37.01

* Supplement : 85% Soybean meal . 12 % Limestone , 2.86 % Salt, .14 % TMS premix (M 2075).

** DM = Dry matter .
Cp = crude protein
NDF = Neutral detergent fiber
ADF = Acid detergent fiber
EE = Ether extract
NFE = Nitrogen free extract

Table (2)

Composition and Chemical analysis of the Diet Fed to Steers During The Ionophore Treatments

Ingredient	%
Cracked Corn	**80**
ChoAlfalfa Hay	**10**
Supplement *	**10**
Chemical Composition * :	
% CP	14.7
% NEm(Mcal/Kg)	2.2
% NEg(Mcal/Kg)	1.5
% P	0.36
% Ca	0.59
% EE	3.32
% NFE	73.00

* Supplement : 85% Soybean meal . 12 % Limestone , 2.86 % Salt, .14 % TMS premix (M 2075).

** DM = Dry matter .
Cp = Crude protein
NDF = Neutral detergent fiber
ADF = Acid detergent fiber
EE = Ether extract
NFE = Nitrogen free extract

Table (3)
Means of Fungi Numbers for Individual Animals with Standard Errors

Day	Means of four Readings	Standard Error
Steer # Lasalocid		
0	23.17	3.28
1	14.42	1.51
3	38.75	5.91
5	26.67	4.57
7	23.50	4.45
11	21.17	1.45
14	10.58	1.53
16	33.58	5.52
21	39.75	3.86
22	31.33	5.55
23	68.08	10.07
24	48.75	5.83
28	45.25	4.76
Steer # 2 ROTATION		
0	30.42	4.19
1	16.42	2.34
3	27.00	4.51
5	32.67	7.04
7	25.92	5.65
11	47.42	7.60
14	5.08	1.21
16	20.00	2.39

Day	Means of four Readings	Standard Error
21	33.50	2.93
22	18.25	3.65
23	28.42	5.37
24	26.67	3.78
28	32.67	3.68
STEER # 3 MONENSIN		
0	30.92	8.12
1	19.58	3.37
3	17.58	4.74
5	11.33	1.54
7	7.83	0.98
11	18.75	2.62
14	17.58	1.84
16	35.83	5.36
21	31.83	4.76
22	20.08	2.49
23	30.83	4.88
24	25.42	2.30
28	28.33	5.62

Table(4)

Means and Standard Errors of Fungi and Methane Production for Steers Fed Lasalocid, Monensin Tylan or Rotation With Standard Errors
(n=4)

Treatment	Pretrt		Day 2		Day 5		Day 16		Day45	
	F*	M	F	M	F	M	F	M	F	M
Lasalocid										
Mean	5.50	4.0	3.85	3.67	8.11	4.25	7.08	3.9	6.69	4.3
SE	5.19	1.7	1.79	1.2	7.63	2.9	3.95	1.3	5.19	1.1
Monensin/ Tylan										
Mean	1.92	2.82	0.73	2.65	0.31	2.7	6.79	4.3	9.98	4.2
SE	1.51	1.1	0.40	0.47	0.16	0.6	5.97	1.4	8.00	0.6
Rotation										
Mean	2.81	4.0	4.54	3.4	1.35	2.65	20.83	6.0	12.02	5.6
SE	2.32	1.2	1.83	0.78	0.66	0.5	12.32	1.7	4.02	0.8

* F=Fungi
M=Methane

Literature Cited

- Akin, D.E. 1987. Association of rumen fungi with various forage grasses. Anim. Feed Sci. Technol. 1`6:273-285.
- Akin , D.E.G.L.R. Gordon and J.P. Hogan . 1983 . Rumen bacteria and fungal degradation of Digitaria perteii grow without sulfur. Apple . Environ . Microbiol , 46:738-748.
- Bauchop, T. 1979. Rumen anaerobic fungi of cattle and sheep . Appl. Environ . Microbiol. 38:148-158.
- Bauchop , T. and D.O. Mountfort. 1981. Cellulose fermentation by a rumen anaerobuic fungi in both the absence and the presence of rumen methanogens. Appl. Environ. Microbiol. 42:1103-1110.
- Bergen W.G. and D.B. Bates. 1984. Ionophores: There effect on production efficiency and mode of action.J.Anim. Sci. 58:1465-1483.
- Chen, M. and M.J. Wolin. 1979. Effects of monensin and lasalocid - sodium on the growth of methanogenic and men saccharolytic bacteria. Appl. Environ . Microbiol.46:160-164.
- Elliott , R., A.J. Ash , F. Calderon - Cortes , B.W. Norton, and Bauchop. 1987. The influence of anaerobic fungi on men volatile fatty acid concentration in vivo.J.Agric. i. Camb. 109:13-17.
- Mountfort , D.O., R.A. Asher, and T. Bauchop. 1982. Fermentation of cellulose to methane and carbon dioxide by a rumen anaerobic fungus in a triculture with Methanobrevibacter sp. Strain RAI and Methanososareina barkeri. Appl. Environ . Microbiol. 44:128-134.

- Rumpler , W.V., D.E. Johnson and D. B. Bates. 1986. The effects of high dietary cation concentration on methanogenesis by steers fed diets with and without ionophores, J.Anim. Sci. 62:1737-1741.
- Schelling , G.T. 1984. Monensin mode of action in the rumen.J. Anim . Sci. 58. 58:1518-1527.
- Windham, W.R. and D.E. Akin. 1984. Rumen fungi and forage ber degradation . Appl. Environ . Microbiol . 48:473-76.