Research Article

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Effect of Addition of Different Concentrations of Bentonite to the Ration on Concentration of Blood Minerals and Ruminal Fluid Traits of Awassi Lambs

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Abstract

The study was conducted on know the effect of different levels of local Bentonite in a percent of 0, 1 and 3% to the ration of Awassi lambs on concentration of minerals and Ammonia concentration and the numbers of microflora of ruminal fluid. Fifteen Awassi lambs were used in this study, aged between 4-6 months with mean body weight of 27.57 ± 0.71 kg. The animals were an individual pens in a space of 1.5 × 2 m. The animals were divided randomly into three equal groups (5 lambs/ groups). The 1st group regarded as a control group, While the 2nd and 3rd group adding to its concentrated ration the local Bentonite in a percentage of 1 and 3% respectively. The animals were fed on a concentrated ration 3% of the body weight and the rough ration (alfalfa hay) were given ad libtium. The results of experiment showed there were a significant increase ($P<0.05$) in concentration of Ammonia nitrogen of ruminal fluid in the control and 3rd group (3% Bentonite), before morning fed. While there were significantly ($P>0.05$) decrease in ruminal fluids of animals fed at the 2nd and 3rd group ration (1, 3% Bentonite) after two hours of morning fed as compared with the control group. The results also showed after four hours of morning feeding there was no significant difference between different groups in concentration of Ammonia in ruminal fluid. There was a significant decrease ($P>0.05$) in sodium salts in blood of lambs fed on 2nd and 3rd ration as compared with control ration. The results showed that addition of Bentonite at different levels (0, 1 and 3) % on lamb ration have no effect on concentration of blood Ca, Ma, K and Al. It has been observed that there was a significant increase ($P<0.01$) in the numbers of ruminal fluid microflora after addition of 3% Bentonite as compared with control and 1% Bentonite. It was concluded from this study that addition of Bentonite to the lamb ration have a beneficial effect on blood sodium, ruminal fluid Ammonia and the numbers of ruminal microflora.

Keywords: **Bentonite salts, Ammonia nitrogen, Microflora, Blood minerals, Awassi lambs.**

تأثير إضافة تراكيز مختلفة من البنتونايت إلى العلائق في تركيز المعادن في الدم وصفات سائل الكرش في الحملان العواسية

الخلاصة

أجريت هذه التجربة معرفة تأثير إضافة مستويات مختلفة من البنتونايت المحلي بنسبة 0، 1 و3% إلى علائق الحملان العواسية في تركيز بعض أملاح الدم وتركيز نتروجين الأمونيا وأعداد الأحياء المجهرية في سائل الكرش. استخدم 15 حملاً عواسياً بعمر 4- 6 شهر وبمعدل وزن 27.57 ± 0.71 كغم، وضعت الحيوانات داخل حضائر فردية بأبعاد 1.5 × 2م، ووزعت بشكل عشوائي إلى ثلاث مجاميع متساوية (5 حمل/ مجموعة) عدت المجموعة الأولى مجموعة سيطرة، أما الثانية والثالثة فأضيف إلى علائقها المركزة البنتونايت المحلي بنسبة 1 و3% على التوالي. تم تقديم العلف المركز بنسبة 3% من وزن الجسم أما العلف الخشن (دريس الجت) فقدم بصورة حرة. تشير نتائج التجربة إلى وجود تفوق معنوي ($P<0.05$) لعليقة السيطرة والعليقة الثالثة (0 و3% بنتونايت) في تركيز نتروجين الأمونيا لسائل كرش الحيوانات قبل التغذية الصباحية في حين انخفض التركيز في سائل كرش الحيوانات التي تناولت العليقة الثانية والثالثة (1 و3% بنتونايت) على مستوى معنوي ($P<0.05$) مقارنة مع عليقة السيطرة بعد ساعتين من التغذية الصباحية. وبعد أربع ساعات من التغذية الصباحية أظهرت النتائج غياب الفروقات المعنوية بين العلائق الثلاثة التجريبية في تركيز أمونيا سائل الكرش. كما تبين حصول انخفاض معنوي ($P>0.05$) في تركيز أملاح الصوديوم في دم الحملان المغذاة على العليقتين الثانية والثالثة مقارنة بعليقة السيطرة، لم يؤثر إضافة البنتونايت بمستويات مختلفة 0، 1 و3% إلى علائق الحملان في تركيز أملاح الكالسيوم والبوتاسيوم والمغنسيوم والألمنيوم في الدم إضافة إلى وجود تأثير عالي المعنوية ($P<0.01$) في أعداد هدبيات سائل الكرش عند إضافة 3% بنتونايت مقارنة بمعاملة السيطرة و1% بنتونايت. نستنتج من الدراسة ان إضافة بنتونايت إلى علائق الحملان أثر على الصوديوم في الدم، وتركيز الأمونيا في سائل الكرش والأحياء المجهرية.

Introduction

The most important difficulties that faces the ruminants farmer in dry seasons was the availability of food and its high cost which makes feeding of the animal is so difficult (1, 2). Ruminant animals having the ability to respond to different food additives the leads to improvement of animal performance through the improvement of efficiency of different food elements and prevents the danger resulted from affection with metabolic diseases (3,4). The food additives may be nutritional like addition of animal fats (5) or non-nutritional additives like Bentonite salts which in organic materials gaseous clays originated from valconies and having a great ability of increase absorption and ions exchange (6). Bentonite clay have a white green light colour or blue, when exposed to the air or sun light becomes deep blue or red or light brown (7). The Bentonite composed of huge numbers of elements including; mainly Montmorillonite and little amount of illite, kaolin, cristobalite, Aluminum silicate and basic elements which constitute the main elements that form Bentonite (8).

Bentonite characterized by several traits especially the adsorption which is beneficial for control the release of ammonium ions in the rumen in a certain concentration that required by the body lead to increase of efficiency of the usefulness of nitrogen especially that greatly analysis in the rumen. The addition of Bentonite to ration also improve rumen fermentation (9). (10) observed that addition of Bentonite sodium or Bentonite calcium in a percent of 2% for cattle nutrition leads to increase Ammonia nitrogen in ruminal fluid. Also the addition of Bentonite have an effect on the numbers of ruminal fluid microflora. (11) reported that addition of Bentonite to the ration of calves in a percent of 1.5% leads to increase the numbers of microflora of ruminal fluid as compared with control treatment. The addition of Bentonite to the ruminants ration have an effect on the concentration of blood minerals which presents in blood plasma like K, Ca, Na, Fe, Cl and Mg. These minerals are chemical compounds changed into charged ions positive or negative after their solution in water. It is very important to keep the body in a good health and different functions which includes; anabolism of bone, synthesis of hormones and control of cardiac pulse, muscle contractions and keeping the blood pressure and pressure of fluid in the body. The aim of this study was to know the effect of different levels of Bentonite (local) in 0, 1 and 3% to the ration and their effect on concentration of blood minerals, Ammonia nitrogen and the numbers of ruminal fluid microflora in Awassi lambs.

Materials and Methods

The study was conducted on animal farm which belonged to the College of Agricultural Engineer Sciences, University of Baghdad/ Abu-Graib, during the period of 60 days extends from May 3, 2019 to July 2, 2019 with a training before the study for 14 days (preliminary period). The Iraqi local Bentonite have been taken from the local market and prepared for its mixing with the rations. It washed with an enough amount of tape water and mixed very well then leave it for 24 hours. The water has been removed a blastic narrow tube and then the Bentonite distributed in shallow instrument and exposed to sunlight for dryness. The Bentonite was grinding to get the powder of Bentonite. As showed in the Table-1 the Bentonite chemical constituent.

During preparing the rations, three different levels of Bentonite has been added 0. 1 and 3% to the concentrated ration to study the effect of these additives on concentration of Ammonia nitrogen of ruminal fluid and the total numbers of ruminal fluid microflora. The concentration of blood plasma minerals were measured including: Sodium, calcium, potassium, magnesium and aluminum.

Fifteen Awassi lambs were used in this study, aged between 4-6 months with a mean of body weight of 27.57 ± 0.71 kg. The animals were put in an individual pens in a space of 1.5×2 m. The animals were divided randomly into three equal groups (5 lambs/ group). The 1st group regarded as a control group. While the 2nd and 3rd group adding to its concentrated ration the local Bentonite in a percent of 1 and 3% respectively. The animals were fed on a concentrated ration 3% of the body weight and the rough ration (alfalfa hay) were given ad libtium.

Ruminal fluid was collected by stomach tube connected with manual pump. Blood samples were collected via jugular vein puncture and put in 10 ml tube then leave it to clot and centrifuged with 3000 r. minute for 10 minute then the serum drawn off for measurements of serum mineral measurement the concentration of Ammonia

nitrogen (mg/ 100 ml):

10 ml of ruminal fluid has been taken after stored and put it 50 ml plastic tube tightly closed and adding to it 5 ml of HCl 0.1% and keep it in a freeze °C till chemical analysis according to the following steps:

1. The samples were thawed and centrifuge for 3000 r. min. for 25 min. in order to remove unwanted and precipitate substance and get a yellow transparent coloured fluid.
2. 0.5 ml of ruminal fluid has been taken and adding to it 0.5 g from magnesium oxide and calcium chloride then put the mixture in a microkeldal system and then measured the volume of collected fluid.
3. Titration of collected solution in recipient flask with 0.05% HCl with mixing until change of the colour of methyl red stain to purple colour and then measured the concentration of Ammonia nitrogen in the ruminal fluid according to the method of (12) with the equation:

$$\text{NH3-N(mg/ 100ml)} = \frac{\text{The volume of titrated acid- Blank} \times 0.05 \times 0.014}{\text{The volume of the sample}} \times 100$$

The total microflora count: one ml from saved ruminal fluid has been taken and put it in 10 ml plastic tube then added 8 ml from distilled water then transported directly to the lab. The measurement of the numbers of microflora has been done according to the method of (13), the use of light microscope. 1 ml of ruminal fluid diluted to 10^6 put it on slide in an area of one cm^3. The mean of 5 reading were taken and multiplied with dilution then the figures changed logarithmic numbers.

Measurement of blood minerals: The serum samples were transported with cool box directly to the lab for analysis of Ca, K, Na, Mg and Al according to the method of in the biological lab/ College of Science, University of Baghdad using atomic absorption spectrophotometer.

Statistical analysis of data were done according to SAS (14) and Duncan multiple range test (15) using level significant.

Results and discussion

Concentration of Ammonia nitrogen (mg/ 100 ml): Table-4 showed the effect of addition of different levels of Bentonite 0, 1 and 3% on the rations of Awassi lambs on the concentration of Ammonia nitrogen of ruminal fluid. The results showed that there was a significant difference ($P<0.05$) between control and third group ration as compared with the 2nd group in the concentration of Ammonia nitrogen before given the morning food when it reaches 9.80, 5.30 and 11.37 mg/ ml respectively. The results also showed that there was a significant difference ($P<0.05$) between different treatments after 2 hours from morning food when it reaches 59.25, 32. 80 and 43.47 mg/ 100 ml respectively. These results were in agreement with (9) after addition of 2% of Bentonite calcium or 2% of Bentonite sodium to the ration of Holstein bulls that leads an increase in Ammonia nitrogen in control group.

Also the results of this experiment agreed with the results of (16) after addition of Bentonite in 2.5 and 5% to the rations of Angora goats aged 6 months that leads an increase in Ammonia nitrogen. While this experiment disagreed with the results of (17) after addition of Bentonite to rations of Karadi lambs in a dose of 20 g/ lamb/ day; that leads to a significant decrease in Ammonia nitrogen of ruminal fluid as compared with control one. These might be due to increase adsorption of gases by Bentonite as compared with the control group (9). In the same table the results showed no significant difference between different three rations in concentration of Ammonia nitrogen of ruminal fluid after 4 hours from morning nutrition when it reaches 6.33, 6.75 and 5.57 mg/ 100 ml respectively. These results were disagreed with (18) after addition of Bentonite 1% in the cow rations that leads to a decrease in Ammonia nitrogen. It also the results of this study were disagreed with the results of (19) after addition of Bentonite 4% to the rations of Burqi lambs, that leads to a decrease in Ammonia nitrogen of ruminal fluid as compared with the control treatment.

Total numbers of ruminal fluid microflora: Table- 5 showed the effect of addition of different levels of Bentonite of 0, 1 and 3% on the numbers of ruminal fluid microflora in Awassi lambs. The results showed a significant increase in the numbers of ruminal microflora in the 3rd ration ($P<0.01$) when it reaches 34.20 as compared with the 2nd and 3rd rations when it reaches 18.06 and 23.00 cells × 10^5/ ml. It is also showed that the superiority of the 2nd treatment when it reaches

23.00 and 18.06 cells × 10^5/ ml for the 2nd and 1st treatment respectively.

These results were in agreement with the (10) after addition of Bentonite 1.5% to the calves rations leads to increase in microflora of ruminal fluid as compared with control ration. While this study was disagreed with (20) who found that addition of Bentonite to lamb ration also this study disagreed with the (21) who observed a decrease in the numbers of ruminal microflora after addition of Bentonite to the lambs rations.

Concentration of blood serum minerals: Table-6 showed the effect of addition of different levels of Bentonite (0, 1 and 3)% on concentration of blood serum minerals in Awassi lambs.

The results showed no significant difference in concentration of serum Ca when its values 50.56, 43.76 and 48.16 µgm/ ml, serum K 1000, 754.33 and 879.33 µgm/ ml, serum Mg 20.80, 19.10 and 20. 93 µgm/ ml and serum Al 6.53, 5.33 and 8.76 µgm/ ml. While the values of serum Na showed a significant decrease ($P<0.05$) in the 2nd and 3rd groups at a different levels of Bentonite treated lambs.

These results are in agreement with that found by (22) in a study on dairy cattle when the results showed no significant difference between treatments in blood minerals.

The results also agreed with (23) after addition of Bentonite sodium to the rations of Holstein cows in a value of 150 g/ day/ cow when there is no significant effect on blood Ca. The effects of Bentonite on blood minerals might be due to the ion exchange resulted from its addition (6).

Conclusion

It can be concluded that addition of Bentonite to the ration of Awassi lambs have a beneficial effect on ruminal fluid microflora, Ammonia nitrogen and on blood mineral levels.

Table (1) Chemical analysis of Bentonite

Minerals	Concentration ppm
Al	21
Mg	370
Na	4411
Ca	570
K	295

Table (2) The composition of concentration ration%

No.	Composition	Ration 1	Ration 2	Ration 3
1	Barley	40	40	39
2	Yellow corn	10	9	8
3	Soybean earned it	15	15	15
4	Wheat bran	33	33	33
5	Salts and minerals	2	2	2
6	Bentonite	0	1	3

Table (3) Chemical structures of experimental rations and alfalfa hay according to the dry matter%

Traits / Ration Subs.	Dray matter	Organic matter	Ash	Crude protein	Crude fiber	Ether extract	Nitrogen free extraction*	Metabolizable energy (mjol/ kg dry matter)**
Concentration ration T1	91.19	86.35	10.3	14.8	8.2	5.8	60.9	12.51
Concentration ration T2	91.45	86.64	10.3	14.3	8.2	5.8	61.4	12.52
Concentration ration T3	90.88	85.85	10.1	14.75	8.4	8.6	61.15	12.48
Alfalfa hay	93.96	80.73	13.23	12.12	21.5	1.54	51.61	10.23

*Non protein nitrogen: NFE= 100- (CF + EE + Ash + Cp)

**Metabolizable Energy (Megajol/ Kg dry matter). ME= 0.012 × Cp + 0.031 × EE + 0.005 × CF + 0.014 × NFE (11)

T_1= Control ration.

T_2= Control ration + 1% Bentonite.

T_3= Control ration + 3% Bentonite

Table (4) Effect of different levels of Bentonite 0, 1 and 3% on concentration of Ammonia nitrogen of the ruminal fluids before and after 2 and 4 hour from morning feeding (mg/ 100 ml)

Mean ± SE for NH3-N			
Rations	**Before morning fed**	**After 2 hours**	**After 4 hours**
T_1	9.80 ± 3.82^a	59.25 ± 11.95^a	6.33 ± 0.84
T_2	5.30 ± 1.27^b	32.80 ± 7.23^c	6.75 ± 1.95
T_3	11.37 ± 2.47^a	43.47 ± 6.56^b	5.57 ± 1.66
Significant levels	*	*	N.S.

T_1= Control ration.
T_2= Control ration + 1% Bentonite.
T_3= Control ration + 3% Bentonite.
*=Significant difference at 5% level.
N.S= No significant difference.

Table (5) Effect of addition of different levels of Bentonite on the numbers of ruminal microflora

Rations	Number of microflora (Mean ± SE)
T_1	18.06 ± 0.43^c
T_2	23.00 ± 0.52^b
T_3	34.20 ± 0.94^a
Significant levels	**

T_1= Control ration.
T_2= Control ration + 1% Bentonite.
T_3= Control ration + 3% Bentonite.
**=Significant difference at 1% level.

Table (6) Effect of different levels of Bentonite on the concentration of blood minerals (µg/ ml) (Mean ± SE)

Rations	Ca	K	Mg	Na	Al
T_1	50.86 ± 2.81	1000 ± 25	20.80 ± 0.49	2235 ± 78.35^a	6.53 ± 0.08
T_2	43.76 ± 3.24	754.33 ± 24.79	19.10 ± 1.38	1679.67 ± 214.30^c	5.33 ± 0.37
T_3	48.16 ± 2.82	879.33 ± 35.50	20.93 ± 1.16	1981.33 ± 195.59^b	8.76 ± 1.72
Significant levels	N.S.	N.S.	N.S.	*	N.S.

T_1= Control ration.
T_2= Control ration + 1% Bentonite.
T_3= Control ration + 3% Bentonite.
*=Significant difference at 5% level.
N.S= No significant difference.

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