Bacterial and Biochemical Changes Due to Ruminal Acidosis in Sheep with Trial of Treatment.

El-Bealawy, M.A; Enas, A.H.Farag; Maarouf, A.A., and Mobarak, M.G.

Benha Provincial Laboratory, Animal Health Research Institute, Agriculture Research Center.

Abstract

This study was carried out on 14 Balady sheep (9 suffered from ruminal acidosis and 5 healthy controls). The clinical symptoms were recorded. Blood and serum were obtained for evaluation of R.B.Cs, W.B.Cs, PCV, Hb %, total protein, albumin, globulin, aspartate aminotransferase, alanine aminotrasferase and alkaline phosphatase.

Ruminal juice samples were obtained for determination of pH, protozoal count, total volatile fatty acids. The physical properties of ruminal juice (color, odour and consistency) were observed. Isolation and identification of bacteria which caused the inflammation of the ruminal wall of the acidotic sheep. Treatment was applied for all diseased animals. Affected sheep showed dullness, inappetance, indigestion, diarrhea and unable to move. The laboratory examination revealed a significant decrease of W.B.Cs and significant increase of, PCV, Hb %, AST, ALT and AP. Ruminal juice parameters revealed dramatical; decreased protozoal count, increased total volatile fatty acids and the hydrogen ion concentration changed from neutral to sever acidity .The bacteriological examination of diseased sheep revealed that *Lactobacillus species* and *Clostridium perferinges* were the most predominant pathogens. After treatment, the ruminal flora and blood parameters were returned near to normal as control sheep. So, the trail of treatment is efficient for controlling acidosis in sheep and could be recommended.

Introduction

Ruminal acidosis has been defined as an array of biochemical and physiological stresses caused by rapid production and absorption of ruminal organic acids **(6)**.

Acidosis is anabolic disorder characterized by low hydrogen ion concentration in the rumen **(7)**. Although ruminal acidosis has long term effect in volatile fatty acids absorption in acidotic lambs **(17)** it was considered that the decrease in ciliated protozoa count might be a useful microbial indication of acidotic condition within the rumen **(12)**.

Protozoa may play an important role in preventing the development of acidosis by the control of ruminal fermentation and by engulfing large numbers of bacteria in the rumen which had the same nitrogen source and converting them to amino acids **(18 and 30).** Increased volatile fatty acids (VFA) in the rumen of acidotic animals were due to absence of ciliated protozoa **(24). 13 and 21** reported increased Hb and W.B.Cs in case of lactic acidosis in buffalo calves. Lactic acid is a normal intermediate in ruminal metabolism and may also enter the rumen from exogenous dietary sources as silage. Under normal conditions lactate concentrations in the rumen are low and lactate is quickly metabolized. However under certain conditions particularly those associated with the introduction of high concentrate diets , an unbalanced ruminal fermentation may occur leading to the accumulation of lactic acid (lactic acidosis) **(25)** .

Lactobacillus spp. were the most predominant pathogens in case of acidosis
(8, 15) .The rumen liquor of sheep with acute ruminal acidosis was milky and had a sour smell and with low PH. Also, there was significant increase in transaminase activities, **(1).** Recovery of goat suffered from acidosis with significant improvement to hematological and biochemical values after dosing of sodium bicarbonate and yeast culture were reported by **(4),** while **(11)** noticed that; the biochemical values in addition to VFA and protozoal **count** were not positively affected after feeding rations containing yeast culture and sodium bicarbonate to healthy rams.

The aim of this work is to conduct a trial for treatment of acidosis in sheep after studying the effect of the different changes associated with this disorder.

Materials and methods

Materials:

1- Animals:

This study was carried out on 14 Balady sheep, nine of them were suffered clinically from acidosis by sudden over eating of either maize or wheat and bread.

2- Samples:

Two blood samples were taken from each animal the first with heparin for estimation of blood picture and second one without heparin for separation of clear serum for biochemical analysis.

Ruminal juice samples were taken for physical characters, PH, protozoal count and TVFA determination.

Methods:

Blood samples with anticoagulant (heparin) were used for determination of total erythrocytic count, total leukocytic count and hemoglobin concentration according to **(14)**. Blood samples without heparin were centrifugated and obtaining of serum for determination of total protein **(29)** albumin **(9)**, AST, ALT and AP **(22)** .These parameters were measured calorimetrally using commercial kets. Ruminal juice samples were collected by stomach tube and suction pump and filtered by goase for determination of PH using PH meter, protozoal count by direct microscopical examination **(2)** and total volatile fatty acids **(23)**.

With strict aseptic conditions, samples of rumen contents from all studied sheep (diseased; before and after treatment and healthy control) were collected by using stomach tube and suction pump and taken to laboratory without delay for bacteriological examination. The samples were thoroughly mixed and 20 ml. were taken from each sample in a sterile McCartney bottles, and centrifuged at 300 rpm for 20 minutes. The sediment of each sample was cultured into nutrient broth and cooked meat broth , incubated aerobically and an aerobically at 37c° for 24-48 hours , then cultured onto nutrient agar , MacConky's agar and blood agar and incubated aerobically and an aerobically at 37 c° for 24 -48- 72 hours. The developed colonies were picked up and subcultured for purification.The pure colonies were identified morphologically by Gram stain and biochemicaly according to **(16 and 20)**.Mixed culture of bacteria which isolated from each diseased animals was subjected to the sensitivity test against 9 different antibiotic discs (Oxoid Standerized discs), using the agar diffusion method of **(16)**.

Treatment:

Treatment of the diseased animals was performed with the aid of **(4 and 28)** as:- enrofloxacin 10mg/kg, sodium bicarbonate 0.5gm/kg body weight

orally and a dose of packing yeast 1 g /kg body weight in addition to freshly ruminal juice from healthy sheep for 5 successive days ..

Statistical analysis of data was performed according to **(26 and 27).**

Results and Discussion

Table (1) showed significant increase in total protein, albumin, globulin, AST, ALT and ALP in sheep suffered from ruminal acidosis in compared with control apparently healthy sheep.

Table (2) showed significant increase in HB%, RBCs, WBCs and PCV in sheep suffered from ruminal acidosis in compared with control apparently healthy sheep.

Table (3) showed significant increase in total volatile fatty acids and acidity of the ruminal fluids, while protozoal count show significant decrease in sheep suffered from ruminal acidosis in compared with control apparently healthy sheep.

The digestion in the rumen supplies the ruminants not only with energy but also with a balance of essential amino acids and most of the vitamin requirement, so dysfunction of that organ caused problems, one of them is ruminal acidosis which caused significant increase in Hb and significant increase in serum transaminases activities (AST, ALT and ALP) which may be due to blood acidosis dehydration and probably to the disturbances occurred in the liver as that obtained by **(1 and 21).**

Protozoa, play an important role in preventing the development of acidosis by the control of bacteria in the rumen, in our study significant decrease in the protozoa on parallel with the results obtained by **(1 and 18)** which attributed this decrease to the great destruction of these protozoa by sever acidity of the rumen ciliated protozoa can help in the absorption of volatile fatty acids from the ruminal wall , so in our study there were significant increase in the total volatile fatty acids which exactly similar to

that obtained by **(24)** who attributed this increase to the absence of ciliated protozoa .

The results of bacteriological examination (Table, 4) showed that ***E.coli*** and ***Clostridium perferinges*** were the most isolated strains from healthy control sheep.Meanwhile ***Lactobacillus spp. ; Clostridium perferinges*** and ***Streptococcus spp.***were the most isolated strains from diseased sheep .Nearly similar results were obtained by **(3, 5, 8, 15 and 19)**.

The increase in these bacteria resulting in dramatic significant increase in ruminal acidosis with clinical symptoms appearance.

The *in - vitro* antibiotic sensitivity test (Table, 5) showed that enrofloxacine was the most effective antibiotic for treatment of diseased sheep. Moreover, the ruminal flora and blood parameters were returned nearly to normal levels after treatment as that of control sheep. So the trail of treatment is efficient for controlling acidosis in sheep and could be recommended.

From this study we can concluded that acidosis affect all organs of the sheep and treatment by sodium bicarbonate, yeast, healthy ruminal juice and antibiotic give best results.

Table (1): Biochemical analysis of acidotic sheep and after treatment. (n=14).

Group / parameter	CONTROL Sheep	DISEASED Sheep	TREATED sheep	LSD P≤0.05
Total protein(g/100ml)	7.778 ^{b}± 0.130	8.310^{a}± 0.141	7.122 ^{b}± 0.136	0.6556*
Albumin(g/100ml)	4.255 ^{b}± 0.104	4.801^{a}± 0.151	4.178 ^{a}± 0.118	0.0667 (NS)
Globulin(g/100ml)	3.523 ^{a}± 0.107	3.509^{a}± 0.131	2.944^{b}± 0.050	0.4444*
AST (ug/dl)	38.522 ^{b}± 0.594	45.711^{a}± 1.238	39.278 ^{b}± 0.764	6.4333*
ALT(ug/dl)	21.756 ^{b}± 0.467	28.544 ^{a}±03.5	21.789 ^{b}± 0.711	6.7556*
AP(ug/dl)	12.344 ^{b}± 0.297	16.578 ^{a}± 0.565	12.533 ^{b}± 0.539	4.0444*

(*): Significance at P≤0.05
LSD: Least significance difference among means at P≤0

Table (2): Some blood parameters of acidotic sheep and after treatment (n=14).

Group / Parameter	CONTROL sheep	DISEASED sheep	TREATED sheep	LSD at P≤0.05
Hb % (g/dl)	12.967 ^{b}± 0.274	13. 970^{a}± 0.242	13.767 ^{b}± 0.23	2.2111*
RBCs (cell×10^{6})	12.478 ^{a}± 0.234	14.011 ^{b}± 0.231	13.97 ^{a}± 0.132	0.1667(NS)
WBCs (cell×10^{6})	8.878^{b}± 0.134	10.089 ^{a}± 0.330	8.867 ^{b}± 0.091	1.2111*
PCV%	35.133 ^{b}± 0.38	40.244 ^{a}± 0.463	34.700^{b}± 0.416	5.1111*

Table (3): Ruminal juice of acidotic sheep and after treated (n =14).

Group / Parameter		CONTROL sheep	DISEASED sheep	TREATED sheep	LSD at P≤0.05
Physical Propert-ies	color	Gray	Greenish brown	Greenish white	
	odor	Aromatic	offensive	Slightly aromatic	
	consistency	Viscus	watery	slimy	
PH		7.033 [a] ±0.075	5.800 [b] ±0.107	7.450 [b] ±0.135	1.2333*
Protozoal count (count×10^3)		325.890 [a] ±21.861	53.444 [c] ±7.018	160.333 [b] ±33.194	106.8889*
Total volatile fatty Acida (mg/dl)		79.667 [c] ±0.631	90.244 [a] ±0.889	85.478 [b] ±1.004	4.7667*

(*): Significance at P≤0.05
LSD: Least significance difference among means at P≤0.05

Table (4): Bacterial isolates from rumen of apparently healthy (control) and diseased sheep (Before and after treatment).

Type of bacterial isolates	Healthy control sheep (n=5)		Diseased sheep			
			Before treatment		After treatment	
	No.	% *	No.	% *	No.	% *
E.coli	3	20.0	2	6.1	1	9.1
Enterobacter aerogenes	1	6.7	0	0.0	0	0.0
Pseudomoneus aerogenosa	0	0.0	1	3.0	1	9.1
Cetrobacter freudis	2	13.3	1	3.0	0	0.0
Klebsilla pneumonae	1	6.7	0	0.0	0	0.0
Streptococcus spp.	2	13.3	7	21.2	2	18.2
Lactobacillus spp.	2	13.3	9	27.3	3	27.3
Staphylococcus epidermidis	1	6.7	5	15.2	1	9.1
Clostridiumperferinges	3	20.0	8	24.2	3	27.3
Total	15	100.	33	100.0	11	100.0

- Percentage in relation to total number of isolates in each group (15 for control; 33 for diseased before treatment and 11 after treatment).

Table (5): The in-vitro antibiotic sensitivity for whole mixed cultures of bacteria isolated from diseased sheep.

Antibiotic	Sensitivity		Resistivity	
	No.	%	No.	%
Amoxycilline	3	33.3	6	66.7
Chloromphenicol	5	55.6	4	44.4
Colistine sulphate	4	44.4	5	55.6
Enrofloxacine	7	77.8	2	22.2
Gentamycine	7	77.8	2	22.2
Penicilline	3	33.3	6	66.7
Neomycin	5	55.6	4	44.4
Oxytetracycline	2	22.2	7	77.8
streptomycine	2	22.2	7	77.8

References

1-Abd El – Samee, A.A. and Abdou, T.A. (1997): Investigation on the influence of rumen acidosis on blood chemistry and some rumen liquor parameters in goats. J. Egypt. Vet .Med. Ass. 57, No. 1: 509-522.

2-Abou – El- Naga, M.M. (1967): Some metabolic studies on rumen microorganisms. Ph. D. Thesis Alex. Univ. Faculty of agriculture, (Animal Pro. Dept.) .

3-Aly, S.M.; Khalil, N., GH. and Amer, A.A. (1991): Aerobic bacterial flora from ruminal juice of experimentally impacted sheep. Assiut Vet. Med.J.Vol.24, No.48: 131-136.

4-Aslan, V.; Thamsborg, S.M.; Jorgensen, R.J. and Basse, A. (1995): Induced acute ruminal acidosis in goats treated with yeast (*Saccaromces cerevisiae*) and bicarbonate. Acta.Vet. Scand.; 36(1):65-77.

5-Braun, U., Rihs, T.and Scher, U. (1992): Ruminal lactic acidosis in sheep and goats. Vet. Rec., 130(16): 343-349.

6-Britton, R.A. and Stock, R.A. (1987): Acidosis rate of starch digestion and intake. In: F.N.Owens (Ed.). Symposium Proceeding: Feed intake by beef cattle. Publ.MP 121. p 125. Oklahoma State Univ., Stillwater.

7-Cooper, R., and Klopfenstein, T., (1996): Effects of Rumensin and feed intake variation on ruminal pH. Update on Rumensin / Tylan / Micotyl for the Professional Feedlot Consultant. Elanco Animal Health, Greenfield,IN.

8-Counotte, G.A.M.and Prins, R.A. (1978): Regulation of rumen lactic acid in nutritional disorders of ruminants. Vet. Sci.Commun. 3: 277-303.

9-Drupt, F. (1974): Colorimetric determination of serum albumin using bromocresol green .Pharm. Biol., 2: 77.

10-El- Bealawy, M.A. and Maarouf, A.A. (2002): Some causes of lamb mortalities. Benha Vet. Med. J., 13 (2): 71-86.

11-Galip, N. (2006): Effect of supplemental yeast culture and sodium bicarbonate on ruminal fermentation and blood variable in rams. J. Anim. Physiol. Nutr. (Berl) Dec; 90 (11-12):446-52.

12-Goad, D.W.; Goad, C.L., and Nagaraja, T.G., (1998): Ruminal microbial and fermentative changes associated with experimental induced subacute acidosis in steers. J. Anim. Sci.1998.76: 234-241.

13-Halimi, M.; Ahmed, S.I. and Roi, M.T. (1991): Studies on experimental ruminal acidosis in calves fed with Ragi Slurry. M. J. Agri. Sci. 25, I: 86-88.

14-Jain, O.W. (1986): (Schalm's Veterinary Haematology) th Ed., Lea and Febiger, Philadelphia, USA.

15-James, C.N. and John, R.W. (1988): Effects of ionophores monensin and tetronasin on simulated development of ruminal lactic acidosis in vitro. Appl.and Environ. J. Microbiol., 54(12): 2981-2985.

16-Koneman, E.W.; Allen, S.D.; Janda, W.M.; Schrechtenberger, P.C. and Winner Jr., W.C. (1997): Colour Atlas and Text book of Diagnostic Microbiology. 4th Ed. J.B.Lippincottco., U.S.A.

17-Krehbiel , C.R. ; Britton , R.A. ; Harmon , D.L.; Wester ,T.J. and Stock, R.A. (1995): The effects of ruminal acidosis on volatile fatty acids absorption and plasma activities of pancreatic enzymes in lambs.J. Anim. Sci., 73: 3111- 3121.

18-Ludovic, B.; Cecile, M.; Frederique, C.D. and Brigitte, M.D. (2004): (Protozoa in valved in butyric rather than lactic fermentation pattern during lactic acidosis in sheep reprted). Nutr. Dev. 44: 195-206.

19-Nassar, M.S.; Ali, M.A.; Mahmoud, A.A.and Hassan, I.M. (1986): Effect of green fodder and dry concentrate ration on rumen microorganisms in goat. Assiut Vet. Med., J. 15: 30: 82-89.

20-Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donelly, W.J.C. and Leonard, F.C. (2002): Veterinary Microbiology and Microbial Diseases. 1st. Iowa State University Press Black well Science.

21-Randhawa , S.S. ; Ahuja , A.K. and Ranshawa , G.S.(1993) : Effect of acute lactic acidosis and acid – base . States and blood gas dynamics in buffalo calves (Bubalus bubalis).

22-Reitman, S.and Frankel, S. (1957): A colorimetric method for determination of serum glutamic pyruvic transaminase and glutamic oxalo acetic transaminase activity. Am. J. Clin Path., 28:56.

23-Salem, O.A.I. (1980): (Studies with different short time alkali treated roughages in metabolism trails with sheep reference to detailed nutrients, ruminal activity and animal behavior). Ph.D.Thesis CairoUniv.Fac. Agric.

24-Santra, A. and Karim, S.A. (2002): Influence of ciliated protozoa on biochemical changes and hydrolytic enzymes profile in the rumen ecosystem. J. Appl. Microbiol.; 92(5); 801-11.

25-Slyter, L. (1976): Influence of acidosis on rumen function. J. Animal Sci. , 43 : 910.

26-Snedecor, G.W. and Cochran, W.G. (1980): Statistical methods. Iowa State University Press, Ames, P. 43.

27-SPSS for windows, Version: 11 (19 September, 2001) .Copyright SPSS Inc. 1989 - 2001. All rights reserved.

28-U.S Grains Council (2008).

29-Weichselbaum, P.F. (1946): An accurate and rapid method for determination of protein in Small amount of blood serum and plasma Am. J. Clin. Path. Tech. Sect.:10-40.

30-Williams, A.G.and Coleman, G.S. (1997): (The Rumen Protozoa). In: Hobson P.N. and C.S.Stwart (Eds), New York, PP.73-139.

التغيرات البكتيرية والبيوكيميائية الناتجة عن حموضة الكرش في الأغنام مع محاولة العلاج.

معروف عابدين البعلاوي ، إيناس عبد الرحمن حسن فراج،أحمد عفيفي عبد الغفار و محمد جمال مبارك.
معهد بحوث صحة الحيوان بالدقى

الملخص العربي

هذه الدراسة تمت علي أربعة عشر من الأغنام البلدي ، وكان تسعة منهم يعانون من حموضة الكرش وخمسة أصحاء ظاهريا . وقد تم تسجيل الأعراض المرضية علي الحالات التي تعاني من الحموضة وتم أخذ عينات دم وتم فصد جزء منها وذلك لقياس بعض وظائف الكبد وكذلك عد كرات الدم البيضاء والحمراء وقياس نسبة الهيموجلوبين في الجزء الأخر من الدم لجميع الحيوانات . كما تم أخذ عينات من الكرش من جميع الحيوانات وذلك لتسجيل الصفات الطبيعية وقياس الأس الهيدروجيني وعد الأوليات وتعيين الدهون الكلية العطرية. كما تم اخذ عينات من الكرش والمستقيم لعزل البكتريا المسببة لحموضة الكرش . بعد ذلك تم علاج الحيوانات المريضة بمضادات الحموضة وإضافة عصارة كرش من حيوانات سليمة وإعطاء مضادات حيوية للبكتيريا المعزولة . وقد أظهرت النتائج المعملية زيادة معنوي في عدد كرات الدم البيضاء وزيادة معنوية في الهيموجلوبين وPCV كما وجدت زيادة معنوية في وظائف الكبد . وقد اظهر العزل البكتيري وجود ميكروب الكلوستريديوم بيرفرنجيز . أظهرت نتائج العلاج سالفة الذكر عن عوده جميع النتائج الي وضعها الطبيعي . من هذه النتائج نستخلص أن علاج حالات الحموضة في كرش الأغنام يتم بنجاح إذا ما تم بطريقة صحيحة و منها نقل عصارات كرش من حيوانات سليمة إلي هذه الحيوانات بالإضافة إلى مواد قاعدية ولمده خمسه أيام .