Biotechnology in Animal Husbandry 30 (4), p 647-659 , 2014 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 591.132.2:636.3
DOI: 10.2298/BAH1404647Z

CLINICAL, HAEMATOBIOCHEMICAL AND RUMINAL CHANGES DURING THE ONSET AND RECOVERY OF INDUCED LACTIC ACIDOSIS IN SHEEP

M.M. Zein-Eldin, M.M. Ghanem, Y.M. Abd El-Raof, H.M. El-Attar, H.M.El-khaiat

Animal Medicine Department, Faculty of Veterinary Medicine, Benha University.
Corresponding author: E-mail: dr.mohamedmeselhy@yahoo.com.
Original scientific paper

Abstract: A total number of five sheep were used in cross over design with an interval of three weeks for induction of lactic acidosis with sucrose, and treated with sodium bicarbonate as antacid, yeast as probiotics and gentian root powder as medicinal herbs. The acidoteic sheep showed significant ($P<0.05$) decrease in body temp, significant increase in respiratory rate, pulse rate and reduction of ruminal movement with depression, weakness, semisolid feces and stand with their head held lowered. There were significant changes in heamatobiochemical, ruminal parameters, these changes were more obvious at 24 hours after induction of acidosis. The clinical, heamatobiochemical, ruminal parameters of induced lactic acidosis were improved rapidly post-treatment with sodium bicarbonate and yeast, whereas these parameters showed slow improvement post treatment by gentian root powder. It was concluded that treatment of induced lactic acidosis in sheep by sodium bicarbonate and yeast give a good result and improve general health condition of the animal but it's preferable for treatment of lactic acidosis using a combination of both sodium bicarbonate and live yeast as sodium bicarbonate raise the ruminal pH rapidly and yeast stabilizes it. Treatment of lactic acidosis by oral administration of freshly grated gentian root showed slow improvement, so further investigation must be done before using gentian root alone in treating lactic acidosis.

Key words: gentian root, haemato-biochemical, lactic acidosis, sheep, sodium bicarbonate, yeast

Introduction

Acute ruminal acidosis is the most dramatic forms of ruminal microbial fermentative disorders and in some cases is lethal in less than 24 hours. The problem is more common when animals are grouped than when they are separate; probably because the psychology of competition induces them to over - consume

(Radostits et al., 2007). The severity of ruminal acidosis and disease signs vary considerably, depending on the amount and type of carbohydrate-rich feed consumed and the degree of prior ruminal microbial adaption to the carbohydrate substrate *(Gentile et al., 2004).* There are two major phases involved in the etiology of acidosis. The first phase - abrupt increase in the ingestion of readily fermentable carbohydtates accompanied by altered ruminal microbial population profile and subsequent accelerated ruminal fermentation to acids. The second phase - absorption of acids into the blood stream leading to systemic and metabolic acidosis *(Radostits et al., 2007).* Clinical signs of acidosis are manifested by dullness, depression, anorexia, slight dehydration, ruminal stasis and pasty to semi-fluid intermittent diarrhea in sheep. The abdomen was slightly distended and on palpation it was doughy in consistency. Moreover, there was tachycardia and polypnea *(Nikolov, 2003),* while peracute clinical signs which comprised severe dehydration with sunken eyes and the animals had no diarrhea but showed blindness, salivation, grinding of teeth (*Pulina, 2004*). Lactic acidosis was associated with heamatological changes such as significant elevation in erythrocytes, leukocytes and hemoglobin concentration and packed cell volume *(Garry, 2002),* also lactic acidosis associated with biochemical changes such as decreased total protein, hyperglycemia *(Brown et al., 2000***),** hyponatremia, hyperkalemia, hypocalcemia, increase AST, ALT activity *(Jorg and Enemark, 2008),* increase urea nitrogen, creatinine level and serum lactic acid *(Patra et al., 1996).* Treatment of clinical acidosis may be difficult and the chances of success depend on the severity of the case. Sodium bicarbonate is an important buffer of ruminal pH *(Ding and Xu, 2006).* Additives or products as sodium bicarbonate that buffer rumen pH may prevent acidosis and improve the productive performance of feedlot animals that consume high-grain diets *(Wallace and Newbold, 1993).* Addition of yeast culture to the basal diet may alleviate the effect of acidosis that normally resulted in the depression in feed intake as live yeast and other bacterial cell species adhere to feed particles to support ruminal fermentation *(Kawas et al., 2007).* The main modes of action of yeast include supplementation of growth factors to rumen microorganisms; oxygen scavenging that creates more favourable conditions for the anaerobic communities and nutritional competition with autochtonous ruminal species for energy (*James, 2011*). Gentian root infusion, administered orally to sheep at a daily dose of 5 g, before feeding, produced a stimulant effect on secretion of enzymes in the small intestine and used as bitter stomachics (*Wichtl, 2002).* Gentian is stated to possess bitter, gastric stimulant, sialogogue and cholagogue properties. Traditionally, it has been used for anorexia, atonic dyspepsia and gastrointestinal atony. The German Commission approved use for digestive disorders such as loss of appetite, fullness and flatulence *(Schulz et al., 2000).*

This study aimed to follow up the main clinical signs, haematobiochemical changes, and ruminal juice examination associated with induced lactic acidosis in sheep. A further objective was to evaluate the effectiveness of sodium bicarbonate,

yeast and gentian root powder in treatment of such problems to evaluate the best one for veterinary uses.

Materials and methods

Animals and study design:

Experimental animals:

Five healthy sheep of both sexes, aged from 9-12 months and weighting 30-35 kg were used in this study in a crossover design with an interval of three weeks. They were kept in clean disinfected pens, fed on green fodder and concentrate. All sheep were dewurmed with anthelmentic. They were left for 2 weeks for acclimatization before the beginning of the experiment. During this period they were subjected to a clinical investigation to be ensured healthy and free from any clinical abnormality.

Experimental design:

The first experiment*:*

An average dose of 18 gm/kg b. wt sucrose was estimated to produce the classical clinical picture of the lactic acidosis according to *(Afshin et al., 2011).* All sheep received sucrose after being fasted for 12 h. The sucrose was mixed with 200ml warm tap water, to make a suitable suspension, and was given using stomach tube in a single dose and after the appearance of clinical signs they were treated with oral sodium bicarbonate at a dose of 1g/ Kg. Bwt. at 24,48,72 hours and oral fluid therapy every 12 hours in a form of sacrolyte.

The second experiment:

Lactic acidosis was induced by giving 18 g/kg b. wt of sucrose and treated by 5 g/ head yeast dissolved in 50ml water and was given using stomach tube at 24,48, 72 hours and oral fluid therapy every 12 hours in a form of sacrolyte.

The third experiment:

Lactic acidosis was induced by giving 18 g/kg b. wt of sucrose and treated 5g/ head gentian root dissolved in 50ml water and was given using stomach tube at 24,48,72 hours and oral fluid therapy every 12 hours in a form of sacrolyte. All samples were collected at 0 hr immediately before induction of acidosis, 12hr after induction of acidosis, then treatment begins at 24 hours and samples were taken at 24, 48, 72 and 96 hr after treatment.

2-3- Blood and serum analysis:

Two blood samples were drained from the jugular vein. The first sample was taken with anticoagulant (EDTA) for determination of blood picture using hematology analyzer (RBCs count, Hb content, PCV%, WBCs and differential leucocytic count)**.** The second sample was collected without anticoagulant for biochemical determination of glucose**,** urea nitrogen, creatinine**,** calcium**,** sodium**,**

postassium**,** chloride**,** AST**,** ALT *(Young, 1990),* lactic acid, total protein *(Pagana and Pagana, 2010),* albumin *(Fischbach and Dunning, 2009).* Globulin was determined by the differences between total protein and albumin *(Chernecky and Berger 2008).*

Ruminal juice analysis:

The ruminal juice was collected from all animals by using a simple ordinary stomach tube connecting with a suction plastic syringe 50 ml capacity. These samples were sieved and strained through a 2 folds of sterile gauze and examined immediately to estimate ruminal pH, physical characters *(Radostits et al., 2007*), protozoal activity, motility and numbers (*Abd El-Raof et al., 2007*). Ruminal fluid was preserved for further investigation. Preservation was adopted by the addition of 10% sulphuric acid, then the sample stored at -20◦C till analyzed for lactic acid (*Lorenz et al., 2003*) and rumen ammonia- nitrogen concentration *(Novozamsky et al., 1974*).

Statistical analysis:

The data were statically analysed by two-way analysis of variance (ANOVA) with Dunnet's as a post-hoc test as previously described (*Bailey, 2008*) using SPSS software (Ver. 16). Values (means±S.E.) were considered significantly different from control healthy when $P \leq 0.05$.

Results and Discussion

The clinical examination:

The common clinical signs appeared on the control group were normal appetite, shiny coat, shiny eyes, their tail were fatty and normal defecation in form of small hard pellets. Body temperature, respiratory rate, pulse rate and ruminal movement were within normal range as in (Table 1). Mucous membranes were light rosy red in color. The clinical examination of sheep after induction of lactic acidosis revealed that clinical signs started in sheep within few hours after administration of sucrose the affected sheep showed decrease feed intake, depression, weakness, semisolid feces and stand with their head held lowered. There was increase in pulse, respiratory rates, decrease in ruminal movement and the abdomen was slightly distended. The visible mucous membranes were light rosy red color. At the disease progresses, the classical signs of ruminal acidosis were observed at 12-24 hours after administration of sucrose, the affected sheep appeared dull, inactive and depressed. Pulse and respiratory rate increased while ruminal movements completely absent. Affected sheep showed diarrhea, dyspnea, in coordination and recumbency. Clinical symptoms were returned to the normal after treatment with sodium bicarbonate more rapidly than that treated with yeast and than that treated with gentian root as in (Table 1).

Table 1: Results of clinical examination in sheep with induced lactic acidosis and treated by sodium bicarbonate, yeast and Gentian root:

Parameter	Sodium bicarbonate-treated			Yeast-treated			Gentian root-treated		
	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment
Temp	39.13± 0.03 $^{3\,a}$	38.63± 0.12 $^{1\,a}$	39.13± 0.12 $^{2\,a}$	39.13± 0.03 $^{3\,a}$	38.63± 0.12 $^{2\,a}$	38.93± 0.12 $^{1\,2\,3\,a}$	39.13± 0.03 $^{2\,a}$	38.63± 0.12 $^{1\,a}$	38.9± 0.17 $^{1\,2\,a}$
Pulse rate /min	78.66± 1.2 $^{1\,a}$	102.33± 1.2 $^{3\,a}$	79.33± 1.52 $^{1\,a}$	78.66± 1.2 $^{1\,a}$	102.33± 1.2 $^{3\,a}$	80.35± 1.2 $^{1\,a}$	78.66± 1.2 $^{1\,a}$	102.33± 1.2 $^{3\,a}$	81.33± 1.76 $^{1\,a}$
Resp/min	24.33± 0.33 ^{1a}	38.00± 1.15 ^{3a}	25.66± 0.33 ^{1a}	24.33± 0.33 ^{1a}	38.00± 1.15 ^{3a}	28.66± 0.33 ^{2b}	24.33± 0.33 ^{1a}	38.00± 1.15 ^{3a}	29.66± 0.66 ^{2c}
Rumen mov/2 min	3.00± 0.31 ^{4a}	0.20± 0.20 ^{1a}	2.60± 0.40 ^{4a}	3.00± 0.31 ^{3a}	0.20± 0.20 ^{1a}	2.80± 0.37 ^{3ab}	3.00± 0.31 ^{3a}	0.20± 0.20 ^{1a}	2.80± 0.20 ^{3ab}

Means with different superscript letters in the same raw are significantly different at $P \leq 0.05$.

Hematological examination:

There was a highly significant increase in Hb content, PCV%, and non significant increase in WBCs, lymphocyte, granulocyte and monocyte count while RBCs count was within the normal range, theses changes were more obvious at 24 hours, the hematological picture returned to the normal after treatment with the sodium bicarbonate, treatment with yeast and treatment with gentian root but returned more rapidly after treatment by sodium bicarbonate as in (Table 2).

Table 2. Haematological picture in sheep with induced lactic acidosis and treated by sodium bicarbonate, yeast and Gentian root

Parameter	Sodium bicarbonate-treated			Yeast-treated			Gentian root-treated		
	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment
Hb	10.8± 0.2 ^{1a}	13.36± 0.32 ^{3a}	10.76± 0.46 ^{1a}	10.8± 0.2 ^{1a}	13.36± 0.32 ^{3a}	11.16± 0.58 ^{1ab}	10.8± 0.2 ^{1a}	13.36± 0.32 ^{3a}	11.76± 0.08 ^{2b}
PCV %	29.23± 0.68 ^{1a}	34.66± 0.12 ^{3a}	29.03± 0.88 ^{1a}	29.23± 0.68 ^{1a}	34.66± 0.12 ^{3a}	29.13± 0.74 ^{1a}	29.23± 0.68 ^{1a}	34.66± 0.12 ^{3a}	30.03± 0.17 $^{1\,2b}$
RBCs. Count	11.35± 1.11 ^{1a}	12.36± 1.04 ^{1a}	11.39± 1.20 ^{1a}	11.35± 1.11 ^{1a}	12.36± 1.04 ^{1a}	11.71± 1.08 ^{1a}	11.35± 1.11 ^{1a}	12.36± 1.04 ^{1a}	12.36± 1.15 ^{1b}
WBCs count	8.75± 1.59 ^{1a}	10.94± 1.79 ^{1a}	9.65± 1.24 ^{1a}	8.75± 1.59 ^{1a}	10.94± 1.79 ^{1a}	9.72± 1.39 ^{1a}	8.75± 1.59 ^{1a}	10.94± 1.79 ^{1a}	9.85± 1.56 ^{1a}
Granuolcyte count	3.64± 0.70 ^{1a}	4.61± 0.82 ^{1a}	3.98± 0.62 ^{1a}	3.64± 0.70 ^{1a}	4.61± 0.82 ^{1a}	4.01± 0.67 ^{1a}	3.64± 0.70 ^{1a}	4.61± 0.82 ^{1a}	4.08± 0.72 ^{1a}
Lymphocyt count	4.65± 0.80 ^{1a}	5.76± 0.90 ^{1a}	5.14± 0.58 ^{1a}	4.65± 0.80 ^{1a}	5.76± 0.90 ^{1a}	5.16± 0.67 ^{1a}	4.65± 0.80 ^{1a}	5.76± 0.90 ^{1a}	5.18± 0.80 ^{1a}
Monocyte count	0.44± 0.09 ^{1a}	0.55± 0.08 ^{1a}	0.52± 0.05 ^{1a}	0.44± 0.09 ^{1a}	0.55± 0.08 ^{1a}	0.52± 0.06 ^{1a}	0.44± 0.09 ^{1a}	0.55± 0.08 ^{1a}	0.53± 0.08 ^{1a}

Means with different superscript letters in the same raw are significantly different at $P \leq 0.05$.

The serum biochemical analysis:

There was a highly significant increase in serum levels of glucose, total protein, globulin, potassium, urea nitrogen, creatinine, ALT activity and lactic acid, while albumin level was within the normal range. There was a highly significant decrease in serum levels of sodium, chloride and calcium, while there was a non significant increase in the serum levels of AST activity, theses changes were more obvious at 24 hours. Serum biochemical changes returned to normal after treatment with the sodium bicarbonate, treatment with yeast and treatment with Gentian root but returned more rapidly after treatment by sodium bicarbonate as in (Table, 3).

Table 3. Mean values of selected serum biochemical parameters in sheep with induced lactic acidosis and treated by sodium bicarbonate, yeast and Gentian root:

Parameter	Sodium bicarbonate-treated			Yeast-treated			Gentian root-treated		
	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment	0h	24h Post Induction	72h Post Treatment
Glucose (mg/ dL)	72.49± 1.57 ^{1a}	87.06± 1.22 ^{3a}	70.55± 1.41 ^{1a}	72.49± 1.57 ^{1a}	87.06± 1.22 ^{3a}	71.16± 1.08 ^{1a}	72.49± 1.57 ^{1a}	87.06± 1.22 ^{3a}	74.48± 1.17 ^{2b}
Total protein (gm/dL)	7.05± 0.11 ^{1a}	8.17± 0.11 ^{4a}	7.27± 0.09 $^{1\ 2a}$	7.05± 0.11 ^{1a}	8.17± 0.11 ^{4a}	7.31± 0.15 $^{1\ 2a}$	7.05± 0.11 ^{1a}	8.17± 0.11 ^{4a}	7.44± 0.12 ^{2ab}
Albumin (gm/dL)	3.33± 0.06 ^{1a}	3.11± 0.07 ^{1a}	3.28± 0.03 ^{1a}	3.33± 0.06 ^{1a}	3.11± 0.07 ^{1a}	3.26± 0.08 ^{1a}	3.33± 0.06 ^{1a}	3.11± 0.07 ^{1a}	3.19± 0.05 ^{1a}
Globulin (gm/dL)	3.71± 0.10 ^{1a}	5.06± 0.04 ^{4a}	3.99± 0.15 ^{12a}	3.71± 0.10 ^{1a}	5.06± 0.04 ^{4a}	4.04± 0.13 ^{2a}	3.71± 0.10 ^{1a}	5.06± 0.04 ^{4a}	4.25± 0.15 ^{2ab}
Sodium (mmol/L)	149.47± 1.91 ^{3a}	133.40± 1.73 ^{1a}	147.28± 2.04 ^{3c}	149.47± 1.91 ^{3a}	133.40± 1.73 ^{1a}	146.42± 1.44 $^{3\ 4b}$	149.47± 1.91 ^{3a}	133.40± 1.73 ^{1a}	143.84± 2.05 $^{2\ 3a}$
Chloride (mmol/L)	99.14± 1.42 ^{3a}	88.86± 1.16 ^{1a}	97.50± 1.33 $^{2\ 3b}$	99.14± 1.42 ^{3a}	88.86± 1.16 ^{1a}	96.80± 0.80 $^{2\ 3ab}$	99.14± 1.42 ^{3a}	88.86± 1.16 ^{1a}	95.53± 1.47 $^{2\ 3a}$
Potassium (mmol/L)	4.67± 0.10 ^{1a}	6.24± 0.20 ^{3a}	4.68± 0.16 ^{1a}	4.67± 0.10 ^{1a}	6.24± 0.20 ^{3a}	4.48± 0.10 ^{1a}	4.67± 0.10 ^{1a}	6.24± 0.20 ^{3a}	5.11± 0.12 $^{1\ 2ab}$
Calcium (mg/dL)	10.22± 0.14 ^{4a}	8.21± 0.14 ^{1a}	10.17± 0.14 ^{4ab}	10.22± 0.14 ^{4a}	8.21± 0.14 ^{1a}	10.03± 0.14 ^{3ab}	10.22± 0.14 ^{4a}	8.21± 0.14 ^{1a}	9.66± 0.16 $^{3\ 4a}$
Urea nitrogen (mg/dl)	33.49± 0.76 ^{1a}	44.53± 0.87 ^{4a}	34.44± 0.42 ^{1a}	33.49± 0.76 ^{1a}	44.53± 0.87 ^{4a}	35.37± 0.48 ^{1ab}	33.49± 0.76 ^{1a}	44.53± 0.87 ^{4a}	36.02± 0.71 $^{1\ 2b}$
Creatinine (mg/dl)	1.04± 0.04 ^{1a}	1.47± 0.04 ^{3a}	1.08± 0.04 ^{1a}	1.04± 0.04 ^{1a}	1.47± 0.04 ^{3a}	1.09± 0.06 ^{1a}	1.04± 0.04 ^{1a}	1.47± 0.04 ^{3a}	1.14± 0.04 ^{2a}
AST (I.U/L)	43.55± 2.11 ^{1a}	50.93± 2.19 ^{1a}	43.41± 2.47 ^{1a}	43.55± 2.11 ^{1a}	50.93± 2.19 ^{1a}	44.10± 2.18 ^{1a}	43.55± 2.11 ^{1a}	50.93± 2.19 ^{1a}	45.57± 1.82 $^{1\ 2\ b}$
ALT (I.U/L)	20.26± 1.16 ^{1a}	39.70± 1.45 ^{4a}	21.64± 0.96 ^{1a}	20.26± 1.16 ^{1a}	39.70± 1.45 ^{4a}	20.25± 0.95 ^{1a}	20.26± 1.16 ^{1a}	39.70± 1.45 ^{4a}	24.38± 0.1.21 ^{12b}
Lactic acid mmol/L	1.63± 0.06 ^{1a}	4.77± 0.89 ^{4a}	1.65± 0.04 ^{1a}	1.63± 0.06 ^{1a}	4.77± 0.89 ^{4a}	1.82± 0.07 ^{1a}	1.63± 0.06 ^{1a}	4.77± 0.89 ^{4a}	2.25± 0.14 ^{2b}

Means with different superscript letters in the same raw are significantly different at $P \leq 0.05$.

Ruminal juice examination:

Colour, odour and consistency of ruminal juice were changed after induction of lactic acidosis while, sedimentation activity time showed a highly significant increased after induction of lactic acidosis. These changes were more obvious at 24 hours. There was a highly significant decrease in ruminal pH and ammonia level while, there was a highly significant increase in ruminal lactic acid

level after induction of acidosis. Microscopic examination of ruminal juice revealed that presence of few numbers of live protozoa and their number showed a highly significant decrease in sheep after induction of lactic acidosis. These changes returned to normal after treatment with the sodium bicarbonate, treatment with yeast and treatment with gentian root but returned more rapidly after treatment by sodium bicarbonate.

Table 4. Examination of ruminal juice in sheep with induced lactic acidosis and treated by sodium bicarbonate, yeast and Gentian root:

Parameter	Sodium bicarbonate-treated			Yeast-treated			Gentian root-treated		
	0h	**24h Post Induction**	**72h Post Treatment**	**0h**	**24h Post Induction**	**72h Post Treatment**	**0h**	**24h Post Induction**	**72h Post Treatment**
Color	Olive green	yellowish	Olive green	Olive green	yellowish	Olive green	Olive green	yellowish	Olive green
Odor	Aromatic	Soured	Aromatic	Aromatic	Soured	Aromatic	Aromatic	Soured	Aromatic
Consistency	viscous	watery	viscous	viscous	watery	viscous	viscous	watery	viscous
S.A.T	29.66 ± 1.20^{1a}	52.00 ± 1.73^{3a}	31.00 ± 1.15^{1a}	29.66 ± 1.20^{1a}	52.00 ± 1.73^{3a}	33.66 ± 1.45^{12b}	29.66 ± 1.20^{1a}	52.00 ± 1.73^{3a}	36.33 ± 1.45^{2c}
pH	6.83 ± 0.03^{4a}	5.70 ± 0.15^{2a}	6.66 ± 0.08^{4a}	6.83 ± 0.03^{4a}	5.70 ± 0.15^{2a}	6.63 ± 0.03^{4a}	6.83 ± 0.03^{4a}	5.70 ± 0.15^{2a}	6.50 ± 0.11^{3a}
Activity of ruminal protozoa	+++	---	+++	+++	---	+++	+++	---	+++
Protozoal count×10^5/ml	4.16 ± 0.33^{4a}	0.00 ± 0.00^{1a}	3.83 ± 0.16^{34a}	4.16 ± 0.33^{4a}	0.00 ± 0.00^{1a}	4.00 ± 0.28^{4ab}	4.16 ± 0.33^{4a}	0.00 ± 0.00^{1a}	3.66 ± 0.16^{3a}
ammonia	62.93 ± 1.88^{4a}	26.12 ± 1.83^{1a}	58.34 ± 1.58^{4c}	62.93 ± 1.88^{4a}	26.12 ± 1.83^{1a}	57.32± 1.764b	62.93 ± 1.88^{4a}	26.12 ± 1.83^{1a}	50.91± 1.72 3 4a
Lactic acid	0.62 ± 0.02^{1a}	0.97 ± 0.02^{5a}	0.75± 0.012a	0.62 ± 0.02^{1a}	0.97 ± 0.02^{5a}	0.71± 0.012a	0.62 ± 0.02^{1a}	0.97 ± 0.02^{5a}	0.81± 0.023 4a

Means with different superscript letters in the same raw are significantly different at $P\leq 0.05$.

Clinical examination of sheep following oral administration of sucrose in dose of 18 gm/kg bwt according to *(Afshin et al., 2011)* revealed that all animals showed signs of illness within 12-24 h., All these disturbances can be attributed to changes in the pH of the rumen under the effect of excessive lactic acid production, histamine, methanol and its action on the vital organs and nerve centres (*Radostits et al., 2007*). Clinical examination of the sheep after treatment by sodium bicarbonate and yeast revealed that animal began to feed 24h after treatment when pH began to increased, the results were in coincidence with (*Ding and Xu, 2006*)). On the other hand animal begin feeds 96h after treatment by freshly grated gentian root due to its stomachic properties as follows, promotion of saliva secretion, acceleration, inhibition of gastric juice secretion, promotion of viscous liquid secretion, bile secretion and enhancement of stomach motility (*Kohlein, 1991*).

Induced ruminal acidosis led to change in blood constituents due to systemic dehydration and degree of heamo concentration (*Radostits et al., 2007*). The haematological picture returned to the normal after treatment with the sodium bicarbonate, yeast and freshly grated gentian root but returned more rapidly after treatment by sodium bicarbonate due to correction of dehydration. The highly significant increase in serum levels of glucose after induction of lactic acidosis may be due to the fact that the absorbed lactic acid is used for the process of gluconeogenesis (*Garry, 2002*), while the significant increase in serum total protein and globulin at 24h after induction of lactic acidosis may be attributed to dehydration due to passage of water from the intravascular compartment into the rumen (*Brown et al., 2000*) and production of immunoglobulins (*Lomborg et al., 2008*) respectively. Decrease in serum sodium and chloride accompanied with ruminal lactic acidosis may be due to the shift of these electrolytes by osmolarity from the blood to hypertonic rumen or due to their losses (Na^+ and Cl^-) due to diarrhea (*Jorg and Enemark, 2008*). He also added that hyperkalemia may be attributed to heamoconcentration which occurred to the constituent of the blood due to dehydration, while hypocalcemia may be due to a temporary malabsorption of calcium due to damaged mucosa of intestine (*Radostits et al., 2007*). The significant increase in serum urea and creatinine are an index of decreased glomerular filtration rate in acidotic sheep, theses due to renal damage or reduction in effective renal flow and fall in arterial blood pressure which results in subnormal renal function as recorded by *(Lal et al., 1992*). Increased activity of ALT reflects hepatocellular damage which may be sublethal degeneration or necrosis, whereas non-significant rise in AST may be due to hepatocellular damage or released from degenerated skeletal muscles *(Kromer and Hoffman, 1997).* The concentration of lactic acid in serum and rumen was found to be directly related to each other, the excessive production of lactic acid in the rumen, less rapid metabolism and clearance causing its gradual accumulation and reach its peak level in the blood (*Ivany et al., 2002*). After treatment with alkalizing buffer sodium bicarbonate, lactic acid decreased significantly and more rapid than in animals treated with yeast and freshly grated gentian root. The changes in physical properties of ruminal juice and the prolonged period which was taken for complete the sedimentation activity test were attributed by (*Garry, 2002*) to poor microbial fermentation in the rumen. Physical properties of ruminal juice after treatment was improved and the SAT test take shorter time than before treatment this may attributed to decrease the level of lactic acid by alkalizing agent "sodium bicarbonate" and by activation of lactic acid utilizing bacteria leading the pH to increased and refreshment of microflora by yeast and gentian root (*Giger- Reverdin et al., 2004*). Fall in rumen pH is associated with increased production of lactic acid in the rumen due to increase the fermentation of starch by amylolytic bacteria in the rumino-reticular compartment (*Ding et al., 1997*). Also (*Owens et al., 1998*) recorded that pH drops because of the high rates of production and accumulation of TVFAs and lactic acid. When the

rumen pH is low, microbial diversity is reduced, as protozoa numbers may sharply decline and the bacterial population is altered (32). These indicate the inverse relationship between lactic acid concentration and PH as recorded by (*Martin et al., 2006*). The significant decreased of ruminal level of ammonia in sheep after induction of lactic acidosis were attributed by (*Henning et al., 2010*) due to death of microflora and microfuna in the rumen. Ruminal pH returned to the normal after treatment as sodium bicarbonate is considered alkalinizing agent or its neutralizing effect and yeast was efficient at stabilizing ruminal pH (James, 2011). Live yeast was efficient at stabilizing ruminal pH by stimulating ciliated protozoa, which are known to rapidly engulf starch granules and compete effectively with amylolytic bacteria for substrate *(Bach et al., 2007*). Moreover, ciliated protozoa are also able to take up some of the lactic acid and thus may prevent its accumulation in the rumen. Therefore, an increase in viable microbial cell numbers in the rumen promoted by live yeast supplementation may minimize the increase in ruminal concentrations of volatile fatty acids thereby avoiding a decrease in ruminal pH (*Giger- Reverdin et al., 2004*). Regarding lactic acid is decreased after treatment than in case of acidotic sheep due to the elevating effect of yeast on ruminal pH and lactic acid may be reduced due to reduced lactate concentrations in the rumen (*Williams and Coleman, 1997*), through the increase of activity of lactate-utilizing bacteria such and/or the decrease of activity of lactate producing bacteria (*Martin and Nisbet 1992*). Ammonia concentration was increased after treatment with sodium bicarbonate and yeast, in a study with adult ruminants, a similar effect on ammonia concentration occurred with daily yeast feeding (*Kumar et al., 1994*). They also suggested that some changes in the nitrogen metabolism of rumen microorganisms in the presence of yeast. Death of microflora may be due to decrease of ruminal pH and increase level of lactic acid as microflora accustoms the life in neutral media 6.2-7.2 (Steen, 2001). Microbial population was increased after treatment in all groups these results were similar to that obtained by (Chaucheyras- Durand *et al.,* 2008) and this may be due to increase of pH and decrease lactic acid, restore the normal ruminal function and the stomachic effect of gentian root (*Wichtl, 2002*).

Kliničke, hematobiohemijske i promene u rumenu kod nastanka i oporavak od indukovane mlečne acidoze kod ovaca

M.M. Zein-Eldin, M.M. Ghanem, Y.M. Abd El-Raof, H.M. El-Attar, H.M.El-khaiat

Rezime

Ukupno pet ovaca su korišćene u ogledu sa intervalom od tri nedelje za indukciju mlečne acidoze sa saharozom, i terapijom sa natrijum bikarbonatom kao antacidom, kvasacom kao probiotikom i prahom korena lincure kao lekovitog bilja. Acidotične ovce su pokazala značajno ($P<0,05$) smanjenje telesne temperature, značajno povećanje disanja, pulsa i smanjenje kretanja rumena sa depresijom, slabošću, polučvrsti izmet i držanjem oborene glave. Bilo je značajne promene u hematobiohemijskim parametrima, parametarima buraga, ove promene su bile očiglednije 24 sati po indukciji acidoze. Klinički, hematobiohemijski i parametari buraga kod indukovane mlečne acidoze su poboljšani ubrzo nakon tretmana sa natrijum-bikarbonatom i kvascom. Isti parametri su pokazali spor napredak makon tretmana sa korenom lincure u prahu. Zaključeno je da tretman indukovane mlečne acidoze ovaca sa natrijum-bikarbonatom i kvascem daju dobar rezultat i poboljšavaju opšte zdravstveno stanje životinje, ali poželjno je za lečenje mlečne acidoze koristiti kombinaciju natrijum-bikarbonata i kvasca, pošto natrijum-bikarbonat utiče na brzo povećanje pH buraga a kvasac ga stabilizuje. Tretman mlečne acidoze oralnom primenom sveže rendanog korena lincure pokazao je spor napredak, tako da dalje istraživanje mora da se uradi pre upotrebe ovog korena u lečenju mlečne acidoze.

References

ABD EL-RAOF, Y.M., GHANEM, M.M., GALBAT, S., 2007: Cryopreservation of rumen protozoa using three different cryoprotectant methods in sheep. The Second Scientific Conference, Fac. Vet. Med., Benha University - Ras Sedr 25-28 January, Pp. 314-332.

AFSHIN JAFARI-DEHKORDI; MOHAMMAD RAHIM HAJI-HAJIKOLAEI, ZAHRA KARIMI-DEHKORDI, 2011: ECG Changes in Acute Experimental Ruminal Lactic Acidosis in Sheep. veterinary reaserch forum. 2, (3): 203-208.

BACH, A., IGLESIAS, C., DEVANT, M., 2007: Daily rumen pH pattern of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation. Anim. Feed Sci. Technol. 136: 156–163.

BAILEY, R.A., 2008: Design of Comparative Experiments. Cambridge Univ. Press.Pp.126-128.

BROWN, M.S., KREHBIEL, C.R., GALYEAN, M.L., REMMENGA, M.D., PETERS, J.P., HIBBARD, B., ROBINSON, J., MOSELEY, W.M., 2000: Evaluation of models of acute and subacute acidosis on dry matter intake, ruminal fermentation blood chemistry, and endocrine profiles of beef steers. J. Anim. Sci. 78: 55-68.

CHAUCHEYRAS- DURAND, F., WALKER, N.D., BACH, A., 2008: Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. Animal Feed Science and Technology 145: 5–26.
CHERNECKY, C.C., BERGER, B.J., 2008: Laboratory Tests and Diagnostic Procedures, 5th ed. St. Louis: Saunders.
DING, Z., XU, Y., 2006: A model for exploring lactic acidosis: 1. Model description. Belg. J. Zool. 136 (2): 117-124.
DING, Z., ROWE J.B., GODWIN, I.R., Y. XU, 1997: The buffering capacity of caecal digesta exceeds that of rumen digesta from sheep fed pasture or roughage diets. Aust. J. Agr. Res., 48: 723-728.
FISCHBACH, F.T., DUNNING, M.B., 2009: Manual of Laboratory and Diagnostic Tests, 8th ed. Philadelphia: Lippincott Williams and Wilkins.
GARRY, F.B., 2002: Indigestion in ruminants. In: Large animal internal medicine (Smith B.P., ed). Mosby-Year Book, Mosby, St Louis, MO, USA. pp. 722-747.
GENTILE, A., SCONZA, S., LORENZ, I., 2004: D-Lactic Acidosis in Calves as a Consequence of Experimentally Induced Ruminal Acidosis. Journal of Veterinary Medicine Series A. (51): 64-70.
GIGER- REVERDIN, S., SAUVANT, D., TESSIER, J., BERTIN, G., MORAND-FEHR, P., 2004: Effect of live yeast culture supplementation on rumen fermentation in dairy lactating goats. J. Animal Sci. 34 (1): 59- 61.
HENNING, P.H., HORN, C.H., STEYN, D.G., MEISSNER, H.H., HAGG, F.M., 2010: The potential of Megasphaera elsdenii isolates to control ruminal acidosis. Animal Feed Science and Technology 157: 13–19.
IVANY, J.M., RINGS, D.M., ANDERSON, D.E., 2002: Reticuloruminal disturbances in the bovine. The Bovine Practitioner 36: 56-64.
JAMES S., 2011: Effects of Saccharomyces cerevisiae supplementation on ruminal parameters, nutrient digestibility and growth in sheep: A meta-analysis. Small Ruminant Research 10: 19– 29.
JORG, M.D., ENEMARK, 2008: The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. The Veterinary Journal 176: 32–43.
KAWAS, J.R., GARCIA-CASTILLO, R., FIMBRES-DURAZO, H., GARZA-CAZARES, F., HERNANDEZ-VIDAL, J.F., OLIVARES-SAENZ, E., 2007: Effects of sodium bicarbonate and yeast on nutrient intake, digestibility, and ruminal fermentation of light-weight lambs fed finishing diets. Small Ruminant Research (67)149–156.
KOHLEIN, F., 1991: Gentians. Timber Press, Portland, pp 25–27.
KROMER, J.W., HOFFMAN, W.E., 1997: Clinical enzymology. In clinical Biochemistry of domestic animals. Ed. Kaneko, J.J.; Harvey, J.W. and Bruss, M.L. San Diegioi Academic press.
KUMAR, U., SAREEN, V.K., SINGH, S., 1994: Effect of Saccharomyces cerevisiae yeast culture supplement on ruminal metabolism in buffalo calves given a high concentrate diet. Anim. Prod. 59: 209–215.

LAL, S.B., DWIVEDI, S.K., SHARMA, M.C., SWARUP, D., 1992: Biopathological studies in experimentally induced ruminal acidosis in goats. Indian J. Anim. Sci. 62: 200-204.
LOMBORG, S.R., NIELSEN, L.R., HEEGAAR, P.M.H., JACOBSEN, S., 2008: Acute phase proteins in cattle after exposure to complex stress. Vet. Res. Commun. 32: 575-582.
LORENZ, I., HARTMANN, I., GENTILE, A., 2003: Determination of d-lactate in calf serum samples – an automated enzymatic assay. Comp. Clin. Pathol. 12: 169–171.
MARTIN, C., BROSSARD, L., DOREAU, M., 2006: Mechanisms of appearance of ruminal acidosis and consequences on physiopathology and performances. INRA Prod. Anim. 19: 93–108. (In French, with English abstract).
MARTIN, S.C., NISBET, D. J., 1992: Effect of direct-fed microbials on rumen microbial fermentation. J. Dairy Sci. 75: 1736–1744.
NIKOLOV, Y., 2003: Biochemical alterations in rumen liquor, blood, cerebrospinal fluid and urine in experimental acute ruminal lactic acidosis in sheep. Indian Vet. J., 80: 36-39.
NOVOZAMSKY, R.E., SCHONWENBURG, J., WALLING, I., 1974: Nitrogen determination in plant material by means of indophenol blue method. Neth. J. Agric. Sci. 22: 3–5.
OWENS, F.N., SECRIST, D.S., HILL, W.J., GILL, D.R., 1998: Acidosis in cattle: a review. J. Anim. Sci. 76: 275-286.
PAGANA, K.D., PAGANA, T.J., 2010: Mosby's Manual of Diagnostic and Laboratory Tests 4th ed. St. Louis: Mosby Elsevier.
PATRA, R.C., LAL, S.B., SWARUP, D., 1996: Biochemical profile of rumen liquor, blood and urine in experimental acidosis in sheep. Small Ruminant Research. 19 (2):177-180.
PULINA, G., 2004: Dairy sheep nutrition. CABI Publishing, Wallingford, UK.
RADOSTITS, O.M., GAY, C.C., HINCHCLIFF, K.W., CONSTABLE, P.D., 2007: Veterinary Medicine A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10 Ed. B. Saunders, London, New York, Philadelphia, Sydney and Toronto.
SCHULZ, V., HANSEL, R., TYLER, V., 2000 : Rational Phyto therapy. A Physicians' Guide to Herbal Medicine, 4th Ed. Berlin: Springer-Verlag.
SMITH, B.P., 1996: Large animal internal medicine, A text book of diseases of horse, cattle, sheep and goats. 2nd Ed., Mosby, st. Louis, Baltimor Madried Mexicocity sigpore Sydney Tokyo Toronto Wiesbaden, pp. 1513- 1518.
STEEN, A., 2001: Field study of dairy cows with reduced appetite in early lactation: Clinical examination, blood and rumen fluid analysis. Acta. Vet.Scand. 42 (2): 219-228.
WALLACE, R.J., NEWBOLD, C. J., 1993: Rumen fermentation and its manipulation: the development of yeast culture as feed additives. In: Lyons, T.P.

(Ed.), Biotechnology in the Feed Industry. Alltech Technical Publications, Nicholasville, KY, p. 173.
WICHTL, M., 2002: Teedrogen und Phytotherapeutika. 4. Aufl.; Stuttgart; Wissenschaftliche Verlagsgesellschaft mbH.
WILLIAMS, A.G., COLEMAN, G.S., 1997: The rumen protozoa. In: Hobson, P.N., Stewart, C.S. (Eds.), The Rumen Microbial Ecosystem, second ed. Chapman & Hall, London, UK, pp. 73–139.
YOUNG, D.S., 1990: Effect of drugs on clinical Laboratory tests, 3rd Edition. AACC Press, Washington, D.C. Pp. 3122-3131.

Received 15 October 2014; accepted for publication 12 November 2014