ISSN 110-2047 ALEX. J. VET. SCIENCE 2010 VOL. 31 NO.1: (53-62)

CLINICAL AND LABORATORY STUDIES ON EXPERIMENTALLY INDUCED ACUTE RUMINAL LACTIC ACIDOSIS IN MALE GOATS.

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ABSTRACT

Acute ruminal acidosis was experimentally induced in six mixed breed of male goats, (15-18 months old and 27-38 kg body weight), by wheat flour, 50 g/kg BW, infused intra-ruminally through a surgically prepared ruminal cannula. Ruminal fluid and blood samples were obtained at 0, 12, 24 and 36 hours to evaluate ruminal fluid contents , serum biochemical changes and complete blood picture. Sever clinical signs of ruminal acidosis were evident by12 hours represented by depression, recumbency, diarrhea, grinding over the teeth, trembling, ruminal stasis, dehydration, polypnea and tachycardia. Sharp drop in ruminal fluid pH with parallel sharp increase in ruminal lactate and glucose levels were recorded. Ruminal protozoa completely disappeared following induction. Significant decrease in venous blood pH and a significant increase of serum levels of lactate, total protein, albumin, Gamma glutamyl transferase (GGT), urea and creatinine were obtained. Significant increase in hematocerit value, total leukocytic count, neutrophil and a significant decrease in lymphocyte were also recorded. Post mortum and histopathological examination were performed to confirm the disease.

Keys: ruminal acidosis, Goat

INTRODUCTION

Acute ruminal acidosis is commonly encountered in goats as a result of accidental access to high amounts of grains containing high carbohydrate content. Ruminal acidosis results from rapid and accelerated fermentation rate in the rumen, resulting in production of large amounts of lactic acid and subsequent non physiological decline in ruminal pH ***(Nagaraja and Titgmeyert, 2007).***

Acute ruminal acidosis in goats is associated with a variety of severe clinical signs including, depression, general weakness, teeth grinding, diarrhea, dehydration and recumbency ***(Braun et al., 1992).***

Acute ruminal acidosis induces severe intra-ruminal biochemical and physiological disturbances, represented by very high amounts of lactic acid content in the ruminal fluid, severe depression in ruminal fluid pH, killing of most protozoal population, shifting in bacterial populations, as well as ruminal wall ulceration ***(Owens et al., 1998).***

Severe systemic reaction usually follows acute and per acute ruminal acidosis including disturbance in acid-base balance, systemic acidosis, dehydration and disturbance in some serum biochemical parameters ***(Brown et al., 1999).*** Death is common sequelae of animals affected with peracute and acute ruminal acidosis ***(Smith, 2002).***

The aim of this study is to study the clinical signs, intra-ruminal changes, systemic biochemical and hematological changes, as well as post mortem and histopathlogical changes associated with acute experimental ruminal acidosis in goats.

MATERIALS AND METHODS

Animals:

Six mixed breed rams, with age ranged from 15-18 months old, and body weight range of (27-38 kg), were used in this study; they were fed a balanced ration composed of berseem and a concentrate mixture. The animals were subjected to thorough clinical examination till all of them proved to be clinically healthy; they were surgically prepared with a ruminl cannula.

Induction of ruminal acidosis:

After obtaining the zero hrs samples, a toxic dose of 50 g/kg BW of corn flour, were dissolved in a suitable amount of water, and infused intra-ruminally through the ruminal cannula.

Sampling:

Blood and ruminal fluid samples were collected at zero hour as a control, then at 12, 24, and 36 hrs post induction to determine the different intended parameters.

Ruminal fluid samples:

Ruminal fluid samples were obtained through the surgically prepared ruminal cannula, using a simple rubber tube (40 cm length and 1 cm in diameter) introduced into the rumen and connected to a 60 ml volume syringe used in suction. The color, odor, and consistency of the collected fluid were examined immediately after sampling, according to ***Rosenbreger et al., (1979).***

Blood samples:

Two blood samples were collected from the jugular vein of each animal; the first one was collected into a heparinized test tube for immediate measuring of venous blood pH and performing a complete blood picture. The second one was collected in a sterilized test tubes and used for separation of the serum and determination of some biochemical changes in the serum.

Clinical examination:

Animals were subjected to thorough clinical examination through out the experiment and recording of some clinical parameters including rectal temperature, respiratory rate, ruminal sounds, and heart rate at each sampling time according to ***Kelly, (1984)***

Determination of ruminal protozoal activities and counts:

The obtained ruminal fluid was sieved through four layers of gauze, and one drop was poured on a clean slide and protozoal activity was examined directly under the microscope ***(Rosenbreger et al., 1979)***. One ml of the sieved ruminal fluid was fixed and stained with 4 times volume of methyl green formalin saline solution. The count of ruminal protozoa was done using hemo-cytometer slid (***Ogimata and Imai 1981).***

Estimation of ruminal, blood and serum biochemical parameters:

Venous blood and ruminal fluid pH were measured directly after collection using a digital electrical pH meter. Serum and ruminal Lactate were determined after ***Field et al., (1996),*** total serum protein after ***Koller, (1984*)**, serum albumin after ***Gendler, (1984)***, serum AST after ***Murray, (1984b),*** Serum GGT after ***Heersink et al., (1980)***, creatinine after ***Murray, (1984a)***, urea after ***Kaplan, (1984b)***, and ruminal glucose after ***Kaplan, (1984a).***

Estimation of Hematological values:

Total erythrocytic count, hemoglobin, hematocrite and total and differential leukocytic counts were determined according to ***Schalm, (1986).***

Post-mortem and histopathlogical examination:

Post mortem examination was performed on two slaughtered animals, and tissue specimens from the fore stomach (fixed in 10% buffered formalin solution, embedded in paraffin and serial sections of 4-5 micron in thickness then stained with hematoxylin

and eosin) were examined histo-pathologically according to ***Bancroft et al., (1996).***

Statistical analysis:

Statistical analysis was performed using ***SPSS (2001)*** software package, version 13. The values were analyzed by one-way analysis of variance *(ANOVA),* followed by Duncans's square, all results were expressed as means ± SE (standard error).

RESULTS AND DISCUSION

Acute ruminal acidosis was experimentally induced intra-ruminally through a fixed rumen cannula in six mixed breed of male goats, 15-18 months old and 27-38 kg BW. Anorexia is the first sign and is followed by signs of abdominal pain, manifested by grinding over the teeth and arched back ***(fig.1).*** Anorexia and abdominal pain may be attributed to ruminal distention and stasis, as well as to the corrosive inflammatory effects of lactic acid on the ruminal mucosa. These results were coincided with those reported by ***Cao et al., (1987), Patra et al., (1996) and Nour et. al., (1998).***

General weakness, depression, muscular shivering, apathy and tendency for recumbency are recorded ***(fig.2).*** These are usually as a result in absorption of large amounts of ruminal bacterial endotoxins and histamine. Diarrhea was evident by 12 hrs in the form of pasty offensive odor feces**,** followed by signs of dehydration by 24 hrs in the form of loss of skin elasticity, and sunken eyes**.** Withdrawal of plasma fluid into the rumen as a result of increased ruminal fluid osmolarity is the cause of osmotic diarrhea and dehydration *(**Andersen, 2003 and Radostitis et al., 2007)*.

Ruminal motility was completely inhibited by 12 hrs; this may be due to the local effect of volatile fatty acids and lactic acid on reticulo-rumen and as a result of vago-vagal reflex via excitation of the acid-sensitive receptors in the reticulo-rumen ***(Gregory, 1987)***.

Regarding the systemic reaction; the heart and respiration rates were significantly increased at 12 hrs. ***(tab.1),*** these results agrees with ***Cao et al., (1987) and Nour at al. (1998),*** that could be attributed to the compensatory mechanism to correct the systemic acidosis *(**Smith, 2002***). In contrast, significant decreased in body temperature was recorded, this agrees with those reported by ***Haji Hajikolaei et al., (2006)***. Decreased rectal temperature may be attributed to a general weakness and cardiovascular deterioration.

Acute ruminal acidosis was confirmed by some laboratory tests; The initial mean value of ruminal pH was 6.10±0.13, then it significantly decreased gradually reaching to its lowest value (4.23±0.07) at 24 hrs ***(Tab. 2)***. Ruminal pH is indicative of severe ruminal acidosis, and it is considered an acute form of the disease according to ***Nagaraja and Titgemeyert, (2007)***, who reported that a ruminal pH below 5.0, approaching 4.5 or lower, is considered acute acidosis. The severe reduction in ruminal pH is accepted to be a result of the huge amounts of lactic acid produced intra-ruminally which increased dramatically from 3.25±0.12 mmol/l at zero hrs, reaching to its maximum concentration 27.85 ±2.41 mmol/l at 24hrs, ***(Tab. 2).*** The production of lactate are due to rapid growth of lactate producing bacteria; *Streptococcus. bovis* and *lactobacillus*, that are an acid resistant bacteria, capable on producing large amounts of lactate when a carbohydrate substrate is available. In the same time, the production of lactate is aggravated by the death of ruminal protozoa and lactate utilizing bacteria which are acid sensitive organisms ***(Asanuma and Hino, 2002 and Kamra, 2005).***

The significant decrease of venous blood pH was obtained from 7.49±0.01at zero hrs reaching to 7.29±0.02 at 36 hrs ***(Tab. 3)***, this indicates a systemic acid-base

disturbance, and it shift towards systemic acidosis. The cause of such decline is probably to the absorption of ruminal lactate into systemic circulation, this results are supported by the parallel increase in serum lactate levels from 2.58±0.05 mmol/L at zero hrs reaching to 4.21±0.26 mmol/L 36 hrs ***(Tab. 3)***. This result is in agreement with previous work performed by ***Cao et al., (1987), Patra et al., (1996) and Haji Hajikolaei et al., (2006)***.

The significant increase in ruminal glucose concentration ***(Tab. 2)*** is a result of increased substrate availability of carbohydrate substance of the wheat flour into the the amylolytic ruminal bacteria giving rise to glucose as one of the end products of bacterial digestion **(*Nour at al., 1998*).** The highly active and crowded ruminal protozoa at zero hrs ***(fig.3)*** which counted (894.85±92.28×10^3/ml ruminal fluid) was dramatically affected by acute ruminal acidosis, as indicated by its complete absence by 12hrs ***(fig. 4)***. This result is in agreement with ***Nour et al., (1998)***.

Ruminal fluid was having olive green color at zero time ***(fig. 5),*** it changed to milky white or whitish yellow in the following sampling times ***(fig. 6)*** **,** this is probably due to the nature of the flour used which have normally the white color, besides the large amounts of lactate produced that aids in increasing the white color. The normal aromatic odor turned into a strong repugnant sour odor, the viscous nature of ruminal fluid at zero hrs, changed to pasty, highly viscous in consistency, this is related to the nature of flour in absorbing water. This result agrees with recorded by ***Rosenberger (1979) and Braun et al., (1992)***.

Significant increase in total serum protein and albumin level at 12 hrs were obtained ***(Tab.3)***, this may be attributed to plasma fluid losses and subsequent hemo-concentrtion ***(Cao et al., 1987 and Patra et. al., 1996).***

Non-significant increased of AST was recorded in all blood serum samples, however, γ GGT was significantly increased from 26.32 ±1.03 u/l at zero hrs to 39.73±3.17 at 36 hrs post induction ***(Tab. 3).*** These results indicate some degree of hepato-biliary injury ***(Smith, 2002 and Andersen 2003)***. This result was supported by congested liver and enlarged gall bladder during post-mortem examination ***(fig. 9).***

Serum urea level was increased significantly in all samples post induction, while creatinine was increased significantly at 24 and 36 hrs (***Tab. 3)*****,** these values may be attributed to hemo-concentration and/or renal dysfunction. This coincided with those reported by ***Patra et. al., (1996), Nikolov, (1998) and Brown et al., (1999)***.

Increased PCV following ruminal acidosis from 25.67±1.40% at zero hrs to 32.00 ±2.06 % after 12 hr**s *(Tab.4)***, This is due to withdrawal of plasma fluids into the rumen as a result of increased osmolarity of its contents, leading to dehydration and hemo-concentration ***(Radostitis et al., 2007)***. Our results were in agreement with that obtained by ***Cao et al., (1987)*** and ***Nour et al., (1998)***.

Total WBCs count increased significantly in all blood samples from 12.12±0.44 10^3/µl at zero hrs, reaching to its highest value (17.43± 0.41 10^3/µl) at 24 hrs. WBCs differential count showed a stress response indicated by the significant increase of neutrophil % with parallel significant decrease of lymphocyte % following induction **(*Tab.4)*,**these leukogram change is probably to counteract the severe inflammatory reactions encountered in the ruminal wall as a result of increased ruminal acidity and osmolarity. These results are in agreement with **Cao *et al., (1987) and Nour et al., (1998)***.

Post mortem findings observed in this study were, easily detached ruminal papillae, patchy areas of sloughed ruminal papillae ***(fig.7)***, hemorrhages in different areas of internal ruminal and reticular wall ***(fig.8),*** and some times associated with ruminal wall ulceration, these changes are a result of the highly acidic ruminal PH, and the corrosive effects of lactate. Liver showed different degrees of congestion and gall bladder was enlarged ***(fig.9)***,

these changes probably are a result of the systemic inflammatory responses as a result of systemic endotoxicosis and histaminosis ***(Anderson, 2003)***

The histopathological findings were evident in the fore-stomach in the form of sloughing of mucosal layer covering the rumen, with loss of the keratin layer covering the ruminal papillae in different areas ***(fig.10)***, besides marked sub-mucosal infiltration with leukocytes and sub-mucosal hemorrhages ***(fig.11)***.The pervious results are in agreement with ***Vestweber and Leipold,(1974) and Nour et.al,(1998).***

In conclusion, 50 g wheat flour/kg BW has induced a severe form of experimental induction of acute ruminal acidosis in goat as indicated by the rapid onset, severe clinical signs and the significant changes in the ruminal fluid constituents, blood biochemical and hematological parameters, In addition to post-mortem and histo-pathological findings.

Table (1) Clinical parameters changes in male goats after induced ruminal acidosis

parameter	Time (hour)			
	Zero	12	24	36
Respiratory rate(cpm)	$23.33^{b} \pm 1.1$	$31.33^{ab} \pm 3.63$	$37.67^{a} \pm 4.51$	$33.33^{ab} \pm 4.21$
Rectal temperature ^{o}C	$39.58^{a} \pm 0.06$	$39.33^{a} \pm 0.09$	$38.53^{b} \pm 0.3$	$38.2^{b} \pm 0.03$
Heart rate (bpm)	$93.33^{b} \pm 6.25$	$138.67^{a} \pm 3.04$	$121.67^{a} \pm 8.1$	$124.00^{a} \pm 9.06$

^{abcd}Means with in the same row carry different superscripts are significantly different ($p< 0.05$

Table (2) Ruminal biochemical changes in goats after induced ruminal acidosis

parameter	Time (hour)			
	Zero	12	24	36
Ruminal pH	$6.10^{a} \pm 0.13$	$4.27^{b} \pm 0.06$	$4.23^{b} \pm 0.07$	$4.34^{b} \pm 0.04$
Ruminal lactate (mmol/l)	$3.25^{b} \pm 0.12$	$24.93^{a} \pm 2.67$	$27.85^{a} \pm 2.41$	$24.40^{a} \pm 2.67$
Ruminal glucose mg/dl	$48^{c} \pm 5.33$	$123.00^{a} \pm 9.43$	$88.17^{b} \pm 6.57$	$96.00^{b} \pm 3.31$

^{abcd}Means with in the same row carry different superscripts are significantly different ($p< 0.05$

Table (3) Blood and serum biochemical changes in goats after induced ruminal acidosis

parameter	Time (hour)			
	Zero	12	24	36
Blood pH	$7.49^{a} \pm 0.01$	$7.41^{ab} \pm 0.04$	$7.36^{bc} \pm 0.03$	$7.29^{c} \pm 0.02$
Serum Lactate (mmol/l)	$2.58^{c} \pm 0.05$	$3.36^{b} \pm 0.27$	$3.92^{ab} \pm 0.21$	$4.21^{a} \pm 0.26$
Serum total protein (g/dl)	$7.12^{b} \pm 0.14$	$8.24^{a} \pm 0.04$	$7.64^{b} \pm 0.24$	$7.46^{b} \pm 0.23$
Serum albumin (g/dl)	$3.04^{c} \pm 0.06$	$3.81^{a} \pm 0.06$	$3.29^{b} \pm 0.08$	$3.22^{bc} \pm 0.05$
Serum AST IU/L	$55.67^{a} \pm 5.60$	$60.50^{a} \pm 6.58$	$64.17^{a} \pm 8.31$	$70.17^{a} \pm 8.19$
Serum GGT IU/L	$26.32^{b} \pm 1.03$	$34.20^{a} \pm 3.05$	$37.04^{a} \pm 2.80$	$39.73^{a} \pm 3.17$
Serum urea mg/dl	$32.17^{b} \pm 3.03$	$46.67^{a} \pm 6.05$	$44.67^{a} \pm 4.89$	$44.83^{a} \pm 4.33$
serum creatinine mg/dl	$0.78^{b} \pm 0.01$	$0.98^{ab} \pm 0.04$	$1.06^{a} \pm 0.11$	$1.10^{a} \pm 0.08$

[abcd]Means with in the same row carry different superscripts are significantly different ($p< 0.05$

Table (4) Hematological changes in goats after induced ruminal acidosis

parameter	Time (hour)			
	Zero	12	24	36
RBC (10^{6}/µl)	$13.17^{b} \pm 0.81$	$16.58^{a} \pm 0.91$	$15.40^{ab} \pm 0.95$	$14.77^{ab} \pm 1.10$
Hb g/dl	$10.30^{b} \pm 0.37$	$11.73^{a} \pm 0.49$	$11.07^{ab} \pm 0.32$	$11.23^{ab} \pm 0.35$
PCV %	$25.67^{b} \pm 1.40$	$32.00^{a} \pm 2.06$	$29.83^{ab} \pm 1.1$	$30.00^{ab} \pm 0.93$
WBC (10^{3}/ µl)	$12.12^{c} \pm 0.44$	16.52^{ab} 0.34	$17.43^{a} \pm 0.41$	$15.48^{b} \pm 0.54$
Neutrophil (%)	$29.50^{d} \pm 1.64$	$46.67^{c} \pm 2.17$	$65.17^{a} \pm 2.90$	$53.83^{b} \pm 2.41$
Lymphocyte (%)	$64.83^{a} \pm 1.68$	$45.50^{b} \pm 2.37$	$29.33^{d} \pm 2.21$	$36.50^{c} \pm 2.17$
Monocyte (%)	$3.83^{a} \pm 0.40$	$4.00^{a} \pm 0.44$	$3.17^{a} \pm 0.40$	$4.33^{a} \pm 0.49$
Eosinophil (%)	$2.50^{a} \pm 0.42$	$2.67^{a} \pm 0.55$	$1.50^{a} \pm 0.50$	$3.17^{a} \pm 0.70$
Basophil (%)	$0.33^{a} \pm 0.21$	$1.00^{a} \pm 0.36$	$0.83^{a} \pm 0.30$	$1.33^{a} \pm 0.42$

[abcd]Means with in the same row carry different superscripts are significantly different ($p< 0.05$

Fig.1; Arched back position.

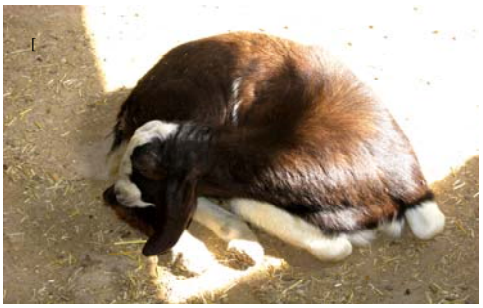

Fig. 2; Depression and recumbency.

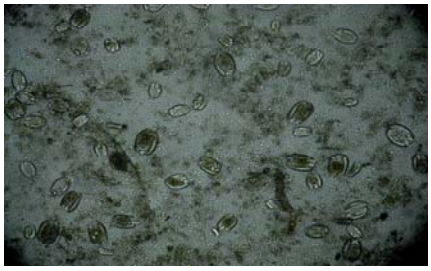

Fig.3; Ruminal protozoa at zero hour hour

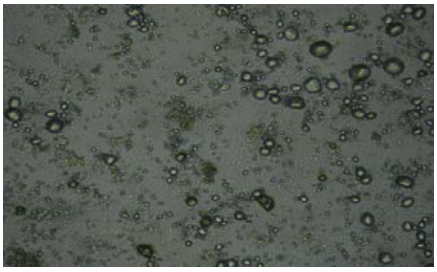

Fig.4; Ruminal protozoa at 12

Fig.5; Ruminal fluid at zero hrs

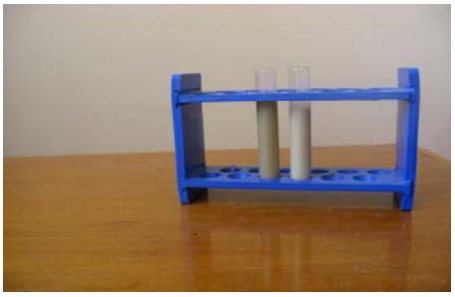

Fig.6; Ruminal fluid at 12 hrs.

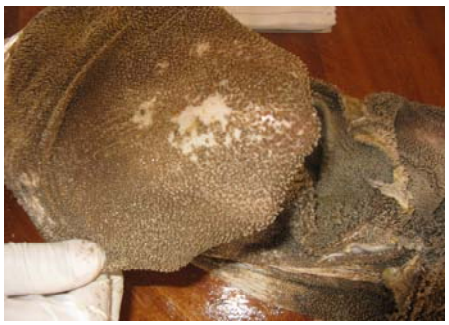

Fig. (7) Patchy areas of sloughed internal ruminal papillae.

Fig. (8) Hemorrhagic areas of ruminal wall.

Fig. (9) Liver showing congestion, and enlargement of gallbladder.

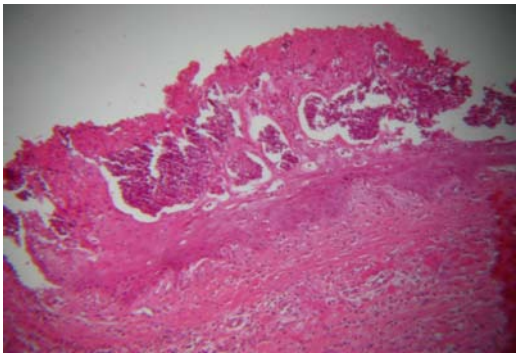

Fig. (10) Rumen , showing, Sloughing of ruminal mucosa and loss of keratin layer.

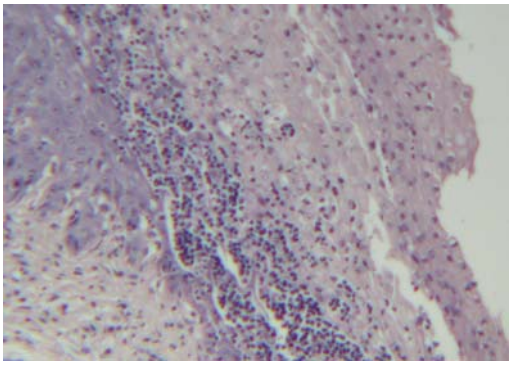

Fig. (11) Rumen, showing marked sub mucosal leukocytic infilteration.

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Received: August 21, 2010.
Accepted for Publ. : August 29, 2010.