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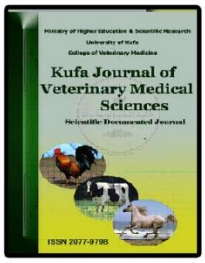

The clinical significance of acute phase proteins and biochemical changes in sheep with acute ruminal acidosis

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Abstract

Acute ruminal acidosis is a common problem in ruminants that feed on high amount of concentrates and has been associated with inflammatory condition of the rumen and metabolic acidosis. The goals of this study were to measure the acute phase proteins (haptoglobin and C-reactive protein concentrations), and biochemical changes and to determine their association with the inflammatory condition and mortality in sheep with acute ruminal acidosis. A total of 25 sheep were included in this study and divided in to two groups [one group suffered from acute ruminal acidosis (n=15) and another control healthy group (n=10)]. Haptoglobin and C- reactive protein (CRP) concentrations were measured by turbidimetric immunoassays. While biochemical parameters were measured using spectrophotometric calorimetric methods. Haptoglobin concentrations were significantly higher in sheep with acute ruminal acidosis compared to control group ($P < 0.05$). However, CRP were not statistically different between both group of sheep ($P > 0.05$) Moreover, serum haptoglobin concentrations were higher in non-survivors sheep with acute ruminal acidosis compared to survivors ($P < 0.05$). The likelihood of mortality was associated with elevated serum haptoglobin, lactate and potassium but also associated with low calcium and chloride concentrations in sheep with acute ruminal acidosis ($P < 0.05$). In conclusion, haptoglobin concentrations were elevated in sheep with acute ruminal acidosis and could be considered more diagnostic and sensitive inflammatory biomarker than CRP in sheep with acute ruminal acidosis. Alterations of biochemical dynamics including lactate, electrolytes and minerals concentrations and energy metabolism could be involved in increased risk of non-survival in sheep with acute lactic acidosis.

Keywords: haptoglobin; CRP; ruminal pH; acute ruminal acidosis; sheep

الدلالات الإكلينيكية للبروتينات الحادة والتغيرات البيو كيميائية في الاغنام ذات حموضة الكرش الحادة

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الخلاصة:
تعتبر حموضة الكرش في الاغنام من المشاكل الخطيرة التي يصحبها تغيرات في البروتينات الحادة و المتغيرات البيو كيميائية في مصل الدم التي تحتاج مزيدا من البحث. لذلك تهدف الدراسة الى قياس تركيزات البروتينات الحادة والمتغيرات البيو كيميائية وعلاقتها بدرجة الالتهاب والوفيات في الاغنام ذات التهاب الكرش الحاد. هذه الدراسة حقلية في الاغنام التي تعانى من حموضة الكرش الحادة وتم تأكيدها عن طريق الاس الهيدروجيني للكرش وعددها (15) ومجموعه اخرى صحية من الاغنام عن طريق الفحص الإكلينيكي وعددها (10). وكانت النتائج وجود زياده في تركيزات الهبتاجلوبين مع عدم حدوث تغير في السي را كتيف بروتين مع حدوث تغيرات بيو كيميائية وارتباطها باحتمالية الوفاه في الاغنام ذات حموضة الكرش الحادة.
الكلمات المفتاحية: هبتاجلوبين , سي را كتيف بروتين, الاس الهيدروجيني للكرش, حموضة الكرش الحادة, الاغنام

Introduction

Acute ruminal acidosis is a common disease of ruminants resulted from ingestion of highly fermentable feed as grain concentrates (Kleen et al., 2003). It has been reported the prevalence of ruminal acidosis is more common in dairy herd and sheep that feed a lot of concentrates to maintain their production and may be appeared in subacute or acute form (Bramley et al., 2008).

Ruminal acidosis usually associated with rapid decrease in ruminal pH due to high production of lactic acid and volatile fatty acids in the rumen (Steele et al., 2009). In addition, it has been documented that ruminal acidosis leads to many pathological disorders such as ruminitis, metabolic acidosis, liver abscessation and pneumonia and associated with high fatality rate (Lean et al., 2000).

Metabolic acidosis resulted from ruminal impaction plays a role in alterations of calcium, phosphorus, electrolytes, acid-base an glucose dynamics in ruminants and also manifested by elevation of inflammatory biomarkers including serum amyloid A and haptoglobin (Danscher et al., 2015; Miller et al., 2010). However, the association of ruminal acidosis in sheep with CRP has been elusive.

Haptoglobin is a sensitive inflammatory biomarker in sheep and protect against free hemoglobin and myoglobin in addition to it is considered more specific and less likely to give false positive or/and negative results than other hematological examination (Skinner and Robert, 1994).

C-reactive protein is acute phase reactant protein that is produced by hepatocytes and has been associated with inflammation and cellular injury and rise rapidly within the first 6 to 8 hours and peak at levels of up to 350-400 mg/L after 48 hours (Vermeire et al., 2005).

CRP binds to phosphocholine on the surface of damaged cells, as well as to polysaccharides and peptosaccharides expressed on bacteria, parasites and fungi. This binding potentiates the classical complement cascade of the immune system and modulates the activity of phagocytic cells, supporting the role of CRP in the opsonization (i.e. the process by which a pathogen is marked for ingestion and destruction by a phagocyte) of infectious agents and dead or dying cells. When the inflammation or tissue destruction is

resolved, CRP levels fall, making it a useful marker for monitoring disease activity (Vermeire et al., 2005).

It was documented that ruminal acidosis was associated with inflammatory condition of the rumen in ruminant (Lean et al., 2000). This open a question whether CRP is a diagnostic inflammatory biomarker for sheep with ruminal acidosis beside other hypothesizes that mentioned that haptoglobin and serum amyloid A concentrations are the most diagnostic inflammatory biomarkers in sheep (Gonzalez et al., 2010).

The aims of this study were to measure haptoglobin and CRP concentrations in sheep with ruminal acidosis and to investigate their diagnostic values in sheep with acute ruminal acidosis. We hypothesized that elevated haptoglobin concentrations will be associated with acute ruminal acidosis and will be considered more diagnostic inflammatory biomarker and predictor of mortality than CRP

Materials and Methods

Animals' criteria

A completely randomized field study of a total of 25 sheep with different age ranged between 3-5 years old and sex were included [one group with ruminal acidosis (n=15) and another control healthy group (n=10). The body weight of the used sheep in the experiment ranged from 40-45 kg. The diagnosis of sheep with acute ruminal acidosis was based on case history of accidental eating of large amount of grains, clinical signs (e.g. dehydration, sub-normal body temperature, decreased pulse rate and ruminal motility) and assessment of ruminal pH while healthy control group was confirmed by physical examination. Ruminal juice was collected by small ruminant stomach tube to measure ruminal pH (Gonzalez et al., 2010). Survivors (n=9) were these sheep that treated from acute ruminal acidosis, while non-survivors (n=6) were these sheep that died due to poor prognosis (subnormal body temperature and severe dehydration).

Sampling

Blood was collected from jugular vein in serum clot tubes. Serum clot tubes were centrifuged at 4,000 rpm for 15 minutes. Serum samples were stored at -20 °C until analysis. Rumen liquor samples 10 ml were collected by small ruminant stomach tube with a suction plastic syringe to determine ruminal pH to confirm ruminal acidosis.

Physical examination

Heart and respiratory rates in addition to ruminal movements, body temperature were examined in both groups (Sherman and Robinson, 1983). Capillary refilling time was assessed by gum pressure by tip of the finger to determine degree of dehydration in sheep with acute ruminal acidosis.

Ruminal pH

Ruminal pH was determined by pH meter to confirm diagnosis of acute ruminal acidosis in sheep (Gonzalez et al., 2010).

Measured Parameters

Haptoglobin concentrations were measured in serum by immune turbidimetry method (Spectrum dignostics; Egypt), with detection range 10-5000 mg/L. Serum C-reactive protein concentrations were measured by tubidimetric assay (Spectrum dignostics; Egypt) according to method described by (Ritchie, 1967). The inter and intra-coefficient assay of variation for CRP were 2.5% and 4%, respectively (Vojtic and Krajnc, 2000). While serum lactic acid, calcium, phosphorus, glucose, and electrolytes concentrations were measured calorimetrically according to method described by (Tietz, 1999; Thomas, 1998; Young, 1990).

Statistical analysis

Data normality was examined by Shapiro-Wilk statistic and was normally distributed. Values are expressed as mean with standard error (SE). Comparisons

between two groups of sheep were carried out by t-tests include comparison between healthy sheep and another group of acute ruminal acidosis and comparison between survivors and non-survivors. Pearson correlation (*r*) was used to determine association of the measured variables. Cut-off values of measured parameters were determined based on 95% CI from healthy sheep. Univariate logistic regression and odds ratios (OR) with 95% confidence interval (CI) for mortality were calculated. The Hosmer-Lemeshow goodness-of-fit test indicated that the data fitted the model (P = 0.65). Data was analyzed with SPSS and prism 6 software. Significance was set at P<0.05.

Results

The recorded clinical signs of sheep with acute lactic acidosis.

Heart rate and capillary refilling time were significantly higher in sheep with ruminal acidosis than healthy sheep (P < 0.05), but body temperature and ruminal motility were significantly lower in sheep with ruminal acidosis compared to healthy ones (P<0.05; **Table 1**).

Table 1. The recorded clinical signs of sheep with acute acidosis.

Variables	Healthy sheep (n=10)	Sheep with ruminal acidosis (n=15)
Ruminal pH	6.62±0.12	5.24±0.45*
Heart rate/min	68.2±0.3	80.2±1.05*
Respiratory rate/min	25.4±0.06	24.2±2.3
Ruminal motility/2 min	3.5±0.2	1.2±0.1*
Temperature	39.2±0.7	38.1±0.5*
Capillary refilling time/S	1.2±0.1	3.2±0.1*

*P<0.05-min; minute-s; second- n; number of sheep

Serum haptoglobin, CRP and bio-chemical parameters of healthy and lactic acidosis group of sheep.

Serum haptoglobin concentrations were significantly higher in sheep with acute ruminal acidosis (791.1±2.4 mg/L) than control group (30.5±4.3 mg/L). Serum C-reactive protein concentrations were not significantly different in sheep with ruminal lactic acidosis (9.2±1.2 mg/L) compared to healthy ones (7.8±0.6 mg/L) (P >0.05) as presented in **Table 2**.

Ruminal pH was significantly lower in sheep with ruminal acidosis compared to control (P <0.05). Lactic acid concentrations were significantly higher in group of sheep with ruminal acidosis compared to healthy ones (P< 0.05). In group of sheep with ruminal lactic acidosis, sodium and chloride concentrations were significantly lower compared to healthy ones (P < 0.05). Potassium concentrations were significantly higher in sheep with ruminal acidosis compared to healthy sheep (P < 0.05; **Table 2**). Serum calcium and glucose concentrations were significantly lower in sheep with ruminal acidosis than healthy control ones (P < 0.05), while phosphorus concentrations were significantly higher in sheep with ruminal acidosis compared to control group (P < 0.05; **Table 2**).

Table 2. Bio-chemical parameters of healthy and lactic acidosis group of sheep.

Variables	Healthy sheep (n=10)	Sheep with ruminal acidosis (n=15)
Haptoglobin (mg/L)	30.5±4.3	791.1±2.4**
CRP (mg/L)	7.8±0.6	9.2±1.2
Lactic acid (mmol/L)	2.01±0.06	5.3±1.2*
Sodium (mEq/L)	148.7 ±0.4	134.2± 0.2*
Potassium (mEq/L)	4.3 ±1.2	5.9 ±0.5*
Chloride (mEq/L)	100.4 ±0.05	90.2± 0.1*
Calcium (mg/dL)	11.3±1.5	10.2±0.6*
Phosphorus (mg/dL)	4.8±0.1	6.2±0.03*
Glucose (mg/dL)	85.4±0.8	75.2±1.1*
Albumin (mg/L)	2.5±0.3	3.6±0.7*

n-number of sheep; *P <0.05

Haptoglobin and CRP and biochemical parameters in survivors and non-survivors' sheep with acute ruminal acidosis

Serum haptoglobin concentrations were significantly higher in non-survivors compared with survivors (P <0.05). CRP protein concentrations were not significantly different in non-survivors sheep with acute ruminal acidosis compared to survivors (P < 0.05). Serum calcium and glucose concentrations were significantly lower in non-survivors than survivors. However, serum phosphorus concentrations were not significant between both groups (P> 0.05) as shown in **Table 3.** Chloride concentrations were significantly lower in non-surviving sheep with acute ruminal acidosis compared with surviving ones (P<0.05). Potassium concentrations were higher in non-survivors than survivors sheep with acute ruminal acidosis (P<0.05). However, sodium concentrations were not significant between surviving and non-surviving sheep with acute ruminal acidosis (P > 0.05).

Table 3. Serum biochemical parameters in non-surviving and surviving sheep with acute ruminal acidosis.

Variables	Non-survivors (n=6)	Survivors (n=9)
Haptoglobin (mg/L)	850.3± 3.2 **	728.4± 0.5
CRP (mg/L)	9.1±2.1**	9.4±0.8
Lactate (mmol/L)	5.1±0.1**	3.2±0.1
Calcium (mg/dL)	10.1±0.4*	11.4±1.5
Phosphorus (mg/dL)	5.8±0.7	6.1±0.8
Glucose (mg/dL)	70.6±1.4*	88±0.3
Sodium (mEq/L)	137 ±0.7	139± 0.1
Potassium (mEq/L)	5.4 ±0.2*	3.5 ±0.05
Chloride (mEq/L)	87.2 ±0.6*	95.4± 0.3

n-number of sheep ;*P <0.05, ** P<0.01

The association of CRP , haptoglobin concentrations and biochemical parameters with likelihood of mortality in sheep with acute ruminal acidosis

Sheep with serum concentrations of haptoglobin > 45 mg/L were more likely to die 3.2 times more than sheep with normal concentrations (95% CI=1.3-20.4; P= 0.01). Sheep with CRP concentrations > 10.2 mg/L were not associated with likelihood of non-survival (P=0.02). The likelihood of mortality was associated with sheep with high blood lactate concentrations (OR=4.8; 95% CI=1.5-10.8; P=0.01). Sheep with low concentrations of calcium and glucose were more likely to die [(OR=1.6; 95% CI=1.1-4.6; P=0.03) (OR=3.1; 95% CI=1.2-14.5; P=0.03)], respectively (**Table 4**). The likelihood of non-survival was associated with low concentrations of chloride and also was associated with higher concentrations of potassium (P < 0.05; **Table 4**).

Table 4. Univariate logistic regression in sheep with ruminal acidosis

Variables	**Range**	**OR**	**95% CI**	**P value**
CRP (mg/L)	5.8-10.2	Referent		
	>10.2	2.4*	1.04-6.4	0.02
Haptoglobin (mg/L)	30.2-45			
	> 45	3.2	1.3-20.4	0.01
Lactate (mmol/L)	1.1-3.4	Referent		
	>3.4	4.8*	1.5-10.8	0.01
Calcium (mg/dL)	11.2-12.4	Referent		
	<11.2	1.6*	1.1-4.6	0.03
Phosphorus (mg/dL)	4.2-6.5	Referent		
	> 6.5	0.8	0.5-3.4	0.4
Glucose (mg/dL)	51.8-83.6	Referent		
	<51.8	3.1*	1.2-14.5	0.03
Sodium (mEq/L)	139-148	Referent		
	< 139	0.74	0.62-3.45	0.2
Potassium (mEq/L)	2.7-3.6			
	> 3.6	2.4*	1.4-10.5	0.03
Chloride (mEq/L)	95.2-98.1	Referent		
	< 95.2	1.7*	1.14-5.6	0.04

CRP-creative protein; OR-odds ratios; CI-confidence interval; * P<0.05

Association of blood lactate with CRP, calcium, phosphorus, glucose and electrolytes concentrations in sheep with ruminal lactic acidosis

Serum haptoglobin concentrations were inversely associated with lactate (r=-0.48; P< 0.01). Serum calcium and glucose concentrations were negatively associated with blood lactate (r=-0.42; P=0.02; r=-0.52; P=0.01), respectively. Serum phosphorus concentrations were positively correlated with lactate (r=0.45; P=0.03). Serum potassium concentrations were positively associated with lactic acid concentrations (r= 0.40; P<0.05).

Discussion

In the present study, ruminal pH was reduced in sheep with acute ruminal acidosis and associated with alterations in clinical and biochemical changes in electrolytes, mineral and energy dynamics and was inversely correlated with elevated haptoglobin concentrations.

We have shown that pulse rate was significantly higher in sheep with ruminal lactic acidosis compared to healthy ones. However, body temperature and capillary refilling time were significantly lower in sheep with lactic acidosis. Our result was in the same line with another study in sheep (Mohamed Nour et al., 2016). The increase in pulse rate could be due to inflammatory condition of the rumen, while dehydration resulted from lactic acidosis could be a reason for low body temperature and elevated capillary refilling time than normal (Mohamed Nour et al., 2016; Patra et al., 2016).

Interestingly, haptoglobin concentrations were inversely correlated with ruminal pH and lactate in sheep with ruminal acidosis. It has been reported that ruminal acidosis is associated with metabolic lactic acidosis that in turn leads to tissue hypoperfusion and cellular injury (Delesalle et al., 2007). Therefore, it is reasonable to assume that ruminal injury resulted from parakeratosis or ruminitis in sheep with ruminal acidosis could be a major contributory factor in the pathogenesis of haptoglobin concentrations in circulation of sheep with acute ruminal acidosis. Additionally, elevated haptoglobin concentrations were associated with mortality in sheep with acute ruminal acidosis suggesting that sever metabolic complications from acute ruminal acidosis.

Serum CRP was not significantly different between groups of sheep and not associated with likelihood of non-survival suggesting that it is not inflammatory biomarker in sheep with acute ruminal acidosis. However, it has been reported CRP concentrations are increased in sheep with pneumonia (Haligur and Ozmen, 2011).

Serum lactic acid concentrations were significantly higher in sheep with ruminal acidosis compared with healthy ones and associated with mortality. Similar result was observed by different study in sheep (Minuti et al., 2014). Potential explanation for elevated lactic acid concentrations in sheep with ruminal acidosis is ingestion of concentrates that undergoes excessive fermentation in the rumen leading to lactic acid production and tissue hypoperfusion which could be a potential explanation for its association with non-survival in sheep (Lettat et al., 2010).

Sodium and chloride concentrations were significantly lower in sheep with acute lactic acidosis compared to control group and also associated with the likelihood of non-survival. Moreover, we documented that serum potassium concentrations were significantly higher in sheep with acute ruminal lactic acidosis compared to healthy one. Potential explanation include dehydration that resulted from lactic acidosis could stimulate chloride loss (Enemark, 2008). In addition, hyperkalemia could be attributed to extracellular shift due to hemoconcentration (Enemark, 2008).

Serum calcium concentrations were significantly lower in sheep with ruminal lactic acidosis. Furthermore, we documented that serum calcium concentrations were inversely associated with lactic acid concentrations and also associated with mortality. The decreased serum concentrations of calcium could be attributed to binding of excessive lactate concentrations with ionized calcium forming calcium lactate resulting in low calcium concentrations (Enemark, 2008). Therefore, we speculate lactic acidosis could directly decrease ruminal motility through local inflammatory condition of the rumen and

indirectly through hypocalcaemia as calcium is essential for contraction of the smooth muscles of the rumen (Daniel, 1983).

Interestingly, serum phosphorus concentrations were higher in sheep with ruminal acidosis. In addition, serum phosphorus was positively correlated with lactic acid concentrations in sheep with lactic acidosis. Similar result was reported in deer with ruminal acidosis (Sahinduran, 2016). The increase in serum phosphorus concentrations could be due to intracellular shift of phosphorus to extracellular space as a result of cellular injury by lactic acidosis (O'Connor et al., 1977).

Our result showed that serum glucose concentrations were significantly lower in sheep with acute ruminal acidosis compared to healthy ones. Moreover, we reported that serum glucose concentrations were negatively associated with lactic acid concentrations and non-survival. Our result was agreed with a recent study in sheep (Minuti et al., 2014). It has been reported that lactic acidosis and decreased vitamin B12 synthesis in the rumen play essential role in conversion of propionic acid which in turn led to decreased glucose concentrations (Odongo et al., 2009).

Conclusions

In conclusion, haptoglobin concentrations are more diagnostics inflammatory biomarker and predictive of mortality than CRP in sheep with acute ruminal acidosis. Hyperlactatemia resulted from acute ruminal acidosis could be associated with many metabolic disorders including hypocalcemia, hyperphosphatemia, hyponatremia, hyperkalemia, hypochloremia, and hypoglycemia that could be a reason for increased risk of mortality in sheep with acute lactic acidosis that will require rapid correction and treatment.

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Conflict of interest

The authors declare that no conflict of interest

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