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# Assessment of probiotics and ruminal juice transplantation therapy in induced lactic acidosis in Baladi sheep

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**ABSTRACT**

This study was conducted to evaluate the therapeutic efficacy of probiotics (Lactobcillus acidophilus, Lactobacillus planterum and Bifidobacterium bifidum) and ruminal juice transplantation in experimental induced lactic acidosis in baladi sheep. Ten rams were used for induction of lactic acidosis in this study in addition ten apparently healthy sheep were used as a control. The sheep with experimental induced acidosis showed inappetance, weakness, depression, tympany, staggering movement and diarrhea. There was significant increase ($p<0.05$) in respiratory rate and pulse rate, while body temperature and ruminal movement were significantly decreased ($p<0.05$). There was significant increase ($p<0.05$) in Hb content, PCV%, RBCs, WBCs, granulocyte, lymphocyte and monocyte in experimental induced acidotic sheep than healthy sheep. Biochemically, there was significant decrease ($p<0.05$) in Na, Cl, Mg, Ca, SOD, catalase, albumin, total protein and vitamin B12; whereas significant increase ($p<0.05$) in K, P, AST, ALT, GGT, ALP, urea, creatinine, MDA, CRP and histamine were detected in experimental induced acidotic sheep. The clinical, hematological, biochemical and ruminal parameters were significantly changed toward the control values after treatment with of probiotics and ruminal juice transplantation from healthy sheep. Therefore, probiotics and ruminal juice transplantation are recommended for treatment of sheep suffering from lactic acidosis.

## 1. INTRODUCTION

The use of antibiotic as feed additive might contribute to an increase of bacterial antibiotic resistance which is a matter of concern when they are used as therapeutic agents, so the European Union has decided to ban the antibiotics as feed additives from 1st January 2006 onwards. This made a way for further alternative agents as growth promoter and antimicrobials. Probiotics are being considered to fill this gap and are already used in preference to antibiotics. Probiotics or direct-fed microbial (DFMs) are live microorganisms that when administered in adequate amounts confer a health benefit to the host (FAO/WHO, 2001). Nutritionists are continually putting their efforts into producing better and more economical feed. Good feed alone will not serve the purpose, but its better utilization is also essential. The term "probiotics" was first introduced in 1953 by Kollath (Hamilton-Miller *et al*., 2003). Probiotic is a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance. (Fuller, 1989). Probiotics have influenced the rumen ecology and thereby nutrient utilization in ruminants and improve colonization of cellulolytic bacteria results in improved digestion process, enhanced nutrient utilization and growth in the small ruminants (Soren *et al*., 2013).

Probiotics appear as promising feed additives, they are of natural origin and generally regarded as safe for animals. Moreover, they may have the potential to improve production performance and health status of small ruminants. Their effects could be related to enhancing nutrient digestibility, stabilizing ruminal ecosystem, stimulating the immune response and increasing milk production (Abd El-Tawab, 2007). Treatment of sheep suffering from lactic acidosis includes single or repeated transfer of ruminal fluid from healthy animal "ruminal juice transplantation", administration of antacids, yeast and chlortetracycline and the intravenous infusion of isotonic sodium chloride and 5% sodium bicarbonate solutions (Braun *et al.,* 1992). Therefore, this study designed to evaluate the usage of probiotics and ruminal juice transplant therapy on hematobiochemical and ruminal juice alterations that associated with experimental induced lactic acidosis in sheep

## 2. MATERIAL AND METHODS

### *2.1. Experimental animals*

Total number of twenty healthy rams from Vet. Teaching farm, Fac. of Vet. Med., Benha University aged from 1-2 years old and weighting 45± 5 kg were used in this study. Ten rams for experimental induction of lactic acidosis and

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ten kept as healthy control .They were kept in clean disinfected pens. 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. All sheep were dewormed with anthelmintic (Ivermectin + Clorsulon 0.2mg Bwt S/C) ®. (R1: Ivomec super ®: Manufactured by Meril Saude Animal Ltd). The animal feed on balanced ration consists of 25%Yellow corn, 10%soya bean meal, 10% sun flower meal, 10% cotton seed meal, 5% germ corn meal, 20% wheat bran, 15% rice bran, 0.3% primex, 1% mix mineral and 2% molasses.

*2.2. Induction of ruminal lactic acidosis:*

For experimental induction of acidosis 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. (2012). Ten sheep received sucrose after being fasted for 12h. The sucrose was mixed with 200 ml warm tap water, to make a suitable suspension, and was given using stomach tube in a single dose. The sheep under experiment was monitored for clinical changes up to the 24 hrs post induction after which blood and ruminal samples were collected for analysis. After induction of acidosis in ten sheep five were treated with probiotic and five sheep were treated with healthy transplanted ruminal juice.

*2.3. Dosing:*

*2.3.1. The first group:*

Five rams with induced lactic acidosis were treated with probiotics (200 CFU/gm) (Lactobcillus acidophilus, Lactobacillus planterum and Bifidobacterium bifidum) powder at a dose 5 gm per head once daily for 5 days. (AVI-5-BAC, origin: Sure Pharmaceutica USA).

*2.3.2. The second group:*

Five rams with induced lactic acidosis were treated with ruminal juice transplantation from healthy sheep at a dose 0.5 liter per head once daily for 5 days.

*2.4. Clinical examination of the animals*

Body temperature (ºC), and respiratory rates, pulse rates, mucous membrane, and ruminal movement of the sheep were examined and recorded following the procedures described by **Kelly (1974)**.

*2.5.Sampling:*

All samples were taken from control healthy sheep and with induced lactic acidosis sheep before treatment and on the 7th day post treatment. Two sets of blood samples were obtained from each sheep.

*2.6. Hematological examination*

Complete blood counts including total erythrocytic count, hemoglobin (Hb) content, PCV%, total leucocytic count and differential leukocytic count (lymphocytes, monocytes, and granulocytes) were done by using Hematology Analyzer (XF9080) according to the method described by Jain (1993).

*2.7. Biochemical examination*

Total protein was determined by colorimetric method by using special kits according to the method that described by Gornall (1949). Albumin was determined by quantitative colorimetric method according to Young (1975). Enzymatic colorimetric test used for the determination of urea in blood serum using of a special kit according to Eisenwiener (1976). Creatinine was determined by colorimetric test by using of the special kits according to Tanganelli *et al*. (1982). Calcium, phosphorus, magnesium, sodium, chloride and potassium levels were determined spectrophotometrically by colorimetric method according to Jansen and Helbing (1991). Kinetic determination of ALT and AST were measured according to klin (1972). Serum GGT activity was determined calometrically according to Beleta and Gella (1990). ALP was determined spectrophotometrically by according to the Rec. GSCC. DGKC (1972 ). SOD was determined according to Nishikimi *et al.* (1972). L- MDA concentration was determined according to Mesbah *et al.* (2004). Catalase was determined according to Aebi (1984). Histamine was determined according to method described by Stites *et al.* (1987). C - reactive protein was determined according to Gerwurz *et al.* (1982). Vitamine B12 was determined according to Segal *et al.* (2004).

*2.8. Ruminal juice examination*

The ruminal juice was collected from healthy and acidotic sheep by using a simple ordinary stomach tube connecting with a suction syringe 50 ml capacity. Each sample (100 ml) was taken from different level of the ruminal contents in a clean dry and sterile flask. These samples were sieved and strained through 2 folds of sterile gauzes and immediately used for estimation of ruminal pH and determine physical characters (color, odor, consistency, and sedimentation activity test), protozoal activity, motility, and numbers. Ruminal fluid was preserved for further investigation as methylene blue reduction test **(Radostits *et al.*, 2007)**.

*2.8. Statistical analysis*

The data were statistically analyzed using one-way analysis of variance **(Bailey, 2008)**. Values were represented as means ± standard error (SE). All differences were considered significantly different when $P< 0.05$.

## 3. RESULTS

*3.1. Clinical examination*

The clinical signs of sheep after experimental induction of lactic acidosis started within few hours after administration of sucrose. The affected sheep showed depression, inappetence, weakness and staggering movement. There was significant increase ($p<0.05$) in respiratory, pulse rates, while the body temperature and ruminal movement were significantly decreased ($p<0.05$). As the disease progressed, the classical signs of ruminal acidosis were observed at 12-24 hours after administration of sucrose, the affected sheep appeared inactive, dull and depressed. Respiratory rate and pulse increased while temperature and ruminal movements completely absent. Affected sheep showed diarrhea, dyspnea and recumbency. Clinical symptoms were changed toward the control values at 7$^{th}$ day post treatment with Probiotics and ruminal juice transplantation (Table1).

Table 1 Clinical parameters (temperature, respiratory rate/ minute, pulse rate/minute and ruminal movement/2 minutes) in experimental induced lactic acidosis sheep and on the 7th day post treatment.

| Parameter | Control (n=10) | Induced acidosis (n=5) | Probiotics treatment(n=5) | Ruminal juice treatment(n=5) |
|---|---|---|---|---|
| Temp. | 39.15±0.08$^{a}$ | 38.55±0.05$^{b}$ | 39.11±0.07$^{a}$ | 39.03±0.06$^{a}$ |
| Resp. rate | 23.75±0.18$^{a}$ | 30.79±0.30$^{b}$ | 23.45±0.14$^{a}$ | 23.85±0.17$^{a}$ |
| Pulse rate | 79.80±0.25$^{a}$ | 92.50±1.65$^{b}$ | 81.60±1.14$^{a}$ | 81.60±1.17$^{a}$ |
| Rum. move. | 2.50±0.17$^{a}$ | 0.35±0.15$^{b}$ | 2.48±0.16$^{a}$ | 2.46±0.14$^{a}$ |

Data are presented as means ± SE with different letters within the same row differed significantly at p<0.05.

### *3.2. Hematological findings*

There was a significant increase (p<0.05) in Hb content, PCV%, RBCs, WBCs, granulocyte, lymphocyte and monocyte in experimental induced acidotic sheep compared to control. The hematological parameters significantly returned toward to the control values at 7th day post treatment with probiotics and ruminal juice transplantation. These results were presented in Table (2).

Table (2): Hematological changes in experimental induced lactic acidosis sheep and on 7th day post treatment with Probiotics and ruminal juice

| Parameter | Control (n=10) | Induced acidosis (n=5) | Probiotics treatment (n=5) | Ruminal juice treatment (n=5) |
|---|---|---|---|---|
| Hb(gm/dl) | 10.98±0.47$^{a}$ | 12.7±0.13$^{b}$ | 10.88±0.43$^{a}$ | 11.26±0.55$^{a}$ |
| PCV% | 31.08±0.58$^{a}$ | 35.70±0.26$^{b}$ | 30.00±0.84$^{a}$ | 30.30±0.99$^{a}$ |
| RBCs($10^{6}$/ul) | 4.78±0.23$^{a}$ | 6.88±0.28$^{b}$ | 4.14±0.27$^{a}$ | 4.34±0.25$^{a}$ |
| WBCs($10^{3}$/ul) | 12.40±0.49$^{a}$ | 19.55±2.94$^{b}$ | 13.75±5.57$^{a}$ | 13.82±2.59$^{a}$ |
| Granulocyte | 2.69±0.39$^{a}$ | 2.95±0.37$^{b}$ | 2.76±0.22$^{a}$ | 2.77±0.14$^{a}$ |
| Lymphocyte | 8.63±0.47$^{c}$ | 18.91±2.68$^{b}$ | 8.94±5.25$^{a}$ | 9.12±2.77$^{a}$ |
| Monocyte | 1.24±0.18$^{a}$ | 4.67±0.35$^{b}$ | 1.73±0.34$^{a}$ | 1.95±0.16$^{a}$ |

Data are presented as means ±SE with different letters within the same row differed significantly at p<0.05.

### *3.3. Biochemical findings:*

There was a significant increase (p<0.05) in AST, ALT, GGt, ALP, urea, creatinine, potassium, phosphorus, MDA, CRP and Histamine. While albumin, total protein, sodium, chloride, magnesium, calcium, SOD, Catalase and vitamin B12 showed significant decrease (p<0.05) in sheep with induced lactic acidosis.

These biochemical changes returned toward the control ranges on the 7th day post treatment with probiotics and ruminal juice transplantation. These results were presented in Table (3, 4, 5).

Table (3): Biochemical analysis of serum enzymes and metabolites in experimental induced lactic acidosis sheep and at 7th day post treatment.

| Parameter | Control (n=10) | Induced acidosis (n=5) | Probiotics treatment (n=5) | Ruminal juice treatment (n=5) |
|---|---|---|---|---|
| AST(U /l ) | 50.06±2.31$^{a}$ | 90.65±10.08$^{b}$ | 53.22±3.49$^{a}$ | 51.86±11.48$^{a}$ |
| ALT(U /l ) | 20.30±1.40$^{a}$ | 75.59±8.03$^{b}$ | 24.74±1.46$^{a}$ | 22.88±1.32$^{a}$ |
| GGT(U /l ) | 29.07±2.08$^{a}$ | 58.38±5.46$^{b}$ | 31.99±3.97$^{a}$ | 32.03±3.66$^{a}$ |
| ALP(U /l ) | 230.47±4.01$^{a}$ | 358.87±48.10$^{b}$ | 238.33±26.03$^{a}$ | 36.00±24.68$^{a}$ |
| Albumin (g/dl) | 4.11±0.15$^{a}$ | 3.46±0.07$^{b}$ | 4.02±0.03$^{a}$ | 3.95±0.02$^{a}$ |
| Total protein (g /dl) | 7.68±0.12$^{a}$ | 6.67±0.08$^{b}$ | 7.33±0.04$^{a}$ | 7.29±0.14$^{a}$ |
| Creatinine (mg /dl) | 0.25±0.01$^{a}$ | 1.28±0.16$^{b}$ | 0.49±0.02$^{a}$ | 0.52±0.08$^{a}$ |
| Urea (mg /dl ) | 22.15±0.8$^{a}$ | 47.11±1.88$^{b}$ | 24.85±0.49$^{a}$ | 25.01±3.92 |

Data are presented as means±SE with different letters within the same row differed significantly at p<0.05.

Table (4): analysis of serum minerals and electrolytes in sheep experimentally induced with lactic acidosis and at 7th day post treatment.

| Parameter | Control (n=10) | Induced acidosis (n=5) | Probiotics treatment (n=5) | Ruminal juice treatment (n=5) |
|---|---|---|---|---|
| Ca(mg /dl ) | 9.61±0.07$^{a}$ | 9.40±0.24$^{b}$ | 9.53±0.09$^{a}$ | 9.51±0.21$^{a}$ |
| Mg(mg /dl ) | 2.84±0.07$^{a}$ | 1.67±0.09$^{b}$ | 2.53±0.08$^{a}$ | 2.75±0.04$^{a}$ |
| Na(mEq /l ) | 149.92±0.38$^{a}$ | 118.49±2.74$^{b}$ | 147.13±5.02$^{a}$ | 146.56±3.86$^{a}$ |
| K(mEq /l ) | 4.66±0.13$^{a}$ | 6.72±0.19$^{b}$ | 5.43±0.11$^{a}$ | 4.95±0.08$^{a}$ |
| P(mg /dl ) | 5.20±0.06$^{a}$ | 7.33±0.21$^{b}$ | 6.12±0.20$^{a}$ | 5.87±0.13$^{a}$ |
| Cl (mEq /l ) | 99.69±3.82$^{a}$ | 63.04±2.88$^{b}$ | 94.54±4.87$^{a}$ | 93.61±5.03$^{a}$ |

Data are presented as means ±SE with different letters within the same row differed significantly at p<0.05.

Table (5): Biochemical analysis of serum SOD, catalase, MDA, CRP, Histamine and Vitamin B12 in sheep experimentally induced with lactic acidosis and at 7th day post treatment.

| Parameter | Control (n=10) | Induced acidosis (n=5) | Probiotics treatment (n=5) | Ruminal juice treatment (n=5) |
|---|---|---|---|---|
| SOD (U/l) | 3.62±0.22$^{a}$ | 1.32±0.28$^{b}$ | 3.32±0.03$^{a}$ | 3.25±0.45$^{a}$ |
| Catalase(U/l) | 19.46±0.23$^{a}$ | 6.52±0.68$^{b}$ | 17.34±0.25$^{a}$ | 18.16±0.96$^{a}$ |
| MDA(U /l ) | 21.99±1.74$^{a}$ | 66.18±7.49$^{b}$ | 24.16±7.60$^{a}$ | 25.00±2.31$^{a}$ |
| CRP(mg /dl ) | 3.52±0.23$^{a}$ | 15.95±3.10$^{b}$ | 6.28±0.55$^{a}$ | 4.20±0.72$^{a}$ |
| Histamine (U /l) | 7.17±0.47$^{a}$ | 27.23±4.57$^{b}$ | 9.35±1.12$^{a}$ | 7.90±1.06$^{a}$ |
| Vit. B12(Ug /dl) | 2.85±0.04$^{a}$ | 1.18±0.21$^{b}$ | 2.22±0.33$^{a}$ | 2.75±0.16$^{a}$ |

Data are presented as means±SE with different letters within the same row differed significantly at p<0.05.

### *3.4. Ruminal juice parameters*

The color, odor and consistency of ruminal juice were changed in experimental induced lactic acidosis sheep and sedimentation activity time in minutes showed a highly significant increase (p<0.05). The microscopic examination of ruminal juice showed a highly significant decrease (p<0.05) of live protozoa. There was a highly significant decrease (p<0.05) in ruminal pH and Methylene blue reduction test increased in sheep with experimental induced lactic acidosis.

These changes returned toward the control values on the 7th day post treatment with probiotics and ruminal juice transplantation. These results were presented in Table (6, 7).

Table (6): Physical properties of ruminal juice in sheep experimentally induced with lactic acidosis and at 7th day post treatment.

| Parameter | Control (n=10) | Induced acidosis (n=5) | Probiotics treatment (n=5) | Ruminal juice treatment (n=5) |
|---|---|---|---|---|
| Color | Olive green | Yellowish | Olive green | Olive green |
| Consistency | Slightly viscous | Watery | Slightly viscous | Slightly viscous |
| Odor | Aromatic | Soured | Aromatic | Aromatic |
| SAT(minutes) | 6.45±0.15$^{a}$ | 38.75±0.67$^{b}$ | 6.76±0.36$^{a}$ | 6.85±0.37$^{a}$ |

Table (7): Microscopical examination and biochemical analysis of ruminal juice in experimentally induced sheep with lactic acidosis and at 7th day post treatment

| Parameter | Control (n=10) | Induced acidosis (n=5) | Probiotics treatment (n=5) | Ruminal juice treatment (n=5) |
|---|---|---|---|---|
| Protozoal Activity | +++ | + or 0 | +++ | +++ |
| Protozoal ($10^{5}$/ml) | 4.55±0.15$^{a}$ | 1.78±0.14$^{b}$ | 3.35±0.17$^{a}$ | 4.30±0.15$^{a}$ |
| pH | 6.77±0.02$^{a}$ | 5.79±0.07$^{b}$ | 6.65±0.08$^{a}$ | 6.72±0.09$^{a}$ |
| Methylene blue reduction test/ min | 2.99±0.17$^{a}$ | 6.56±0.36$^{b}$ | 4.11±0.25$^{a}$ | 3.22±0.33$^{a}$ |

Values with different superscript letters within the same row differed significantly at p<0.05.

+++ = highly motile and overcrowded. ++ =motile and crowded.
+ = sluggish motile and low number. 0 = no a live protozoa.

## 4. DISCUSSION

Ruminal acidosis is a fermentation disorder in the rumen characterized by a lower than normal ruminal pH, but reflecting an imbalance between microbial production,

microbial utilization, and ruminal absorption of volatile fatty acids (VFA) Castillo et al. (2012).

Most of systemic and clinical changes observed in experimental induced lactic acidosis sheep resulted from the reduction of ruminal pH after the sucrose administration Afshin et al. (2012). The observed clinical alteration were recorded by previous study Plaizier et al. (2008), Commun et al. (2009), Zain El-Din (2013) and Mohamed (2014) that explained that the severity of clinical signs of lactic acidosis varied according to the degree of pH value of ruminal contents and noticed anorexia, dullness, depression, ruminal atony or complete stasis with slight tympany and congested mucous membranes in diseased cases. Most of diseased sheep showed yellowish white soft faeces or even diarrhea. Grinding on teeth was a prominent clinical sign in most severely diseased cases. Conversion of lactic acid to sodium lactate which passed down to the intestinal tract producing an osmotic gradient and draws water into small intestine causing diarrhea (Radostits et al., 2007).The low pH explains the increased respiratory rate with labored respiration by stimulation of respiratory centers and the excessive amount of lactic acid produced increase ruminal osmolarity and water is drawn in from systemic circulation to the rumen causing hemo-concentration and dehydration (Radostits et al., 2007).

Our results showed that experimental induced lactic acidosis sheep showed relive of signs after treatment with probiotics. These results were in accordance with Vivenk et al. (2014) who recorded that the inclusion of probiotics in ruminant acidosis treatment has improved feed utilization and growth performances, stabilize the rumen ecology and thus nutrient utilization. Its use in livestock feed as a microbial feed additive will improve and maintains the physiological and production status of ruminants for their proper health and wellbeing. In addition, group treatment with ruminal juice transplantation showed improvement of the clinical signs that coincided with Ibrahim (1993) that conducted therapeutic trials for transfer of ruminal juice on six sheep suffering from indigestion. The improvement of these animals could be noticed from the second day of treatment. This was reflected by diminishing the symptoms of indigestion and improvement of the appetite.

Hematological alteration observed in experimental induced lactic acidosis sheep in this study include significant increase RBCs, WBCs, Hb content, PCV%, granulocyte, lymphocyte and monocyte the same result recorded by Soha (2017) and Vieira et al. (2012) that verified the efflux of liquid from intra- and extracellular compartments to the rumen in case of induced lactic acidosis in order to maintain intra rumen balance results in an hematocrit increase. On the other hand, the stress produced by acidosis causes splenic contraction due to the action of epinephrine; hemoconcentration may occur because of the amount of red blood cells released into the peripheral bloodstream and subsequent increase in hematocrit. The leukogram findings are coincided with Garry (2002), Zain El-Din (2013), Soha (2017) and Vieira et al. (2012) that described the mobilization of neutrophils is related to the inflammation of the rumen mucosa caused by the high concentration of lactic acid in the ruminal fluid, which being irritant to epithelium triggers the entire process of ruminitis.

The hemogram and the leukogram findings were altered after usage of probiotics and ruminal juice transplantation which can explained due to correction of ruminal pH, ruminal environment and blood dehydration. Different result was obtained by Zeinab (2011) that exhibit little effects of probiotic on blood and serum parameters on digestive disorder which denote non-harmful effects on the body.

Elevation of AST and ALT activity was previously obtained by Abd El-Samee and Abdou (1997) and Bionaz et al. (2007). Increased activity of ALT reflects hepatocellular damage which may be sublethal degeneration or necrosis, AST activity raised as a result of hepatocellular damage or released from degenerated skeletal muscles (Zain El-Din, 2013). In addition, Xu and Ding, 2011 cited that some hepatic enzymes activities changed in ruminal acidosis because the animal metabolism, environment and pH of the enzymes have changed. Elevated activities of hepatic enzymes were revealed in serum enzyme profiles of acidotic sheep. On contrary, Vieira et al. (2012) recording that the enzymes AST, GGT show no significant variation was observed throughout the experimental period when compared to the initial time.

ALP was elevated as previously recorded by Harmon and Britton (1983) as they found increase in the serum ALP activity of rumen acidosis affected cow that was due to feed intake decrement and urinary excretion of calcium. Although, Antonov et al. (1983) have different result and they believed that the drop of serum alkaline phosphatase activity is chiefly governed by the lowered release in the blood of intestinal alkaline phosphatase as the result of its disturbed synthesis and intestinal absorption.

Experimental induced acidosis sheep showed low level of serum total protein that could be due to the excretion of these parameters in the intestinal lumen with diarrhea (Cao et al., 1987).

The significant elevation in serum urea and creatinine in experimental induced ruminal acidosis is the same as Lal et al. (1992) that attributed these increases to dehydration which results in reduction of effective circulating fluid volume which often also caused alterations in renal function.

Hypocalcemia, hypomagnesemia, hyponatremia, hyperkalemia and hyperphosphatemia were previously reported by Furll (1994) and Ahmed et al. (2017). Low level of serum sodium and chloride may be due to the shift of these electrolytes by osmolarity from the blood to hyper tonic rumen (due to high lactic acid increase hypertonicity in rumen) or due to their losses (Cl and Na) in lactic acidosis associated with diarrhea (Jorg and Enemark, 2008). Hyperkalemia may be due to haemoconcentration and dehydration. The decrease in calcium level might be attributed to a temporary malabsorption due damaged mucosa of intestine (Radostits et al., 2007).

Regarding to serum SOD and Catalase reduction in their level were noticed in experimental induced lactic acidosis that previously obtained by Dimri et al. (2010) and Zain El-Din (2013). While serum MDA was increased in experimental induced acidotic sheep these changes were coincided with Deger et al. (2008) and Zain El-Din (2013). The higher MDA concentration in plasma of sheep suffering from ruminal acidosis suggests increased production of lipid peroxidation in the liver, and indirectly pointed to enhanced free radical generation Maffei Facino et al. (1993).

It was documented that ruminal acidosis was associated with inflammatory condition of the rumen in ruminant. 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 concentration are the most diagnostic inflammatory biomarkers in sheep (Gonzalez et al., 2010). Significant increased serum CRP in sheep with experimental induced acidosis was also observed by Danscher et al. (2015).

Rumen pH depression triggers the release of vasoactive substances, such as histamine and LPS, which damage the capillaries of the lamellae in the foot, thus causing hemorrhage, inflammation, and lameness (Nocek et al.2000). This explain the obtained result that show increase level histamine in sheep with experimental induced acidosis as recorded Aschenbach et al. (2000).

Interestingly, ruminants utilize Co contained within pasture for the synthesis of vitamin B12 by ruminal bacteria. This is then bound with vitamin B12 binding proteins (commonly known as R-proteins or R-binder) and is eventually absorbed from the small intestine (Guèant and Nicolas 1990). The significant reduction in serum levels of Vitamin B12 in sheep with experimental induced lactic acidosis could be due to acidic ruminal pH that has harmful effect on production and multiplication of beneficial bacteria that produce vitamin B 12.Our result exhibited that after treatment of the experimental induced lactic acidotic sheep with probiotic and ruminal transplantation there was improvement of all biochemical parameters. This is because the effect of probiotics in improvement of production by their direct nutritional effect as bioregulators of the intestinal microflora and reinforcing the host's natural defenses (Guillot 1998). In addition, they influenced the rumen ecology and thereby nutrient utilization in ruminants. Improvement in colonization of cellulolytic bacteria results in improved digestion process, enhanced nutrient utilization (Soren et al. 2013).

This study detected changes in the physical properties of ruminal fluid in experimental induced lactic acidotic sheep include milky gray color, sour odor, watery consistency and prolonged SAT. While, microscopic examination of ruminal fluid revealed reduction in protozoal count and protozoal activity with reduction in ruminal juice pH and prolonged Methylene blue reduction test. These changes also observed by Salem (2006), Karapinar et al. (2008) and Khan et al. (2013). Milky–grey color of ruminal juice arisen from sucrose in induced cases and the watery consistency and sour odor was due to excessive production of lactic acid (Radostits et al., 2007). The decreased rumen pH in the sheep with induced ruminal acidosis was due to increase production of lactic acid. Reduced number and activity of ruminal microflora could be due to decrease of ruminal pH and increase level of lactic acid as the microflora needs neutral media 6.2-7.2 (Steen 2001). After treatment with probiotics, the characters of ruminal fluid returned to olive green color, aromatic odor, slightly viscous consistency and the SAT time was shorter than before treatment. These results agreed with Braun et al. (1992) and Mohamed (2014). Thus, they have a beneficial effect on the ruminal ecosystem and the digestive process in the rumen Zeinab (2011).

Ruminal juice transplantation help relive the changes in the characters of ruminal fluid of experimental induced lactic acidosis as it improvement in ruminal protozoa induced marked changes in the concentration of the constituents of the rumen cause marked increase in rumen pH (Ibrahim, 1993), improve digestibility and refaunation of ruminal protozoa (Galbt, 2007).

## 5. CONCULSION

According to the obtained results of this work, the use of probiotics and ruminal juice transplantation therapy has beneficial effects on hematobiochemical alterations that associated with induced lactic acidosis in Baladi sheep.

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