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Influence of *Salix babylonica* extract, exogenous enzyme of xylanase and their combination on blood haematological and biochemical profile in sheep and goats

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ABSTRACT

The study was made to investigate effect of exogenous enzyme of xylanase, *Salix babylonica* extract and their combination on blood haematological and biochemical profile in lambs and goats. Suffolk lambs (4) and Saanen goats (4) were used in a Latin square design (4 animals × 4 treatments in 4 periods) for 15 days of adaptation. Animals were fed the basal diet plus 30 ml of water (control), or plus 30 ml of exogenous enzyme xylanase (EZ), or plus 30 ml of *S. babylonica* extract (SB), or plus 30 ml of xylanase + 30 ml of SB extract (EZSB). The daily dose of treatments was given orally before the morning feeding. Blood samples (5 ml) were collected on day 15 of each period and analyzed for haematological and biochemical profile. Treatments had affected hematocrit in lambs; eosinophils and glucose in goats. Since all the studied blood parameters were within the normal range for healthy goats and there were no signs of disease, it is concluded that xylanase, *S. babylonica* and their combination did not pose any threat to the health of the animals under the conditions of the experiment where both feed additives are innocuous.

Key words: Blood, Exogenous enzyme, Goats, Lambs, *Salix babylonica* extract

The use of feed additives has increased for decreasing the cost of alimentation and enhancing productive parameters (Cedillo *et al.* 2014, Salem *et al.* 2014, Togtokhbayar *et al.* 2015). Exogenous enzyme has positive effects to improve feed utilization and animal performance (Alsersy *et al.* 2015, Rojo *et al.* 2015, Morsy *et al.* 2016) depending on the type of animal and diet (Elghandour *et al.* 2015, Salem *et al.* 2015).

Secondary metabolites presented in *Salix babylonica* extract at low doses and in appropriate mixtures can have beneficial effects on animal nutrition and health (Cedillo *et al.* 2015, Valdes *et al.* 2015). However, some studies indicated that secondary metabolites can cause anaemia by destruction to erythrocytes, decrease the blood cells count, damage liver and kidney, even death if consumed for a considerable time at high doses (Mahgoub *et al.* 2008). In the present experiment, some haematological and biochemical data were used as indication of health status of the experimental animals.

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MATERIALS AND METHODS

Suffolk lambs (4) of 3 to 4-months of age with 24 kg body weight (BW), and Saanen goats (4) of 5 to 8-months of age with 20 kg BW were used. After 2 weeks of adaptation to a basal diet, animals were weighed and randomly distributed into 4 groups of 1 animal (lamb or goat). Animals were housed in metabolic cages in a Latin square design (4 animals, 4 treatments and 4 periods) where the experiment was conducted in 4 periods of 15 days (i.e. total 60 days). Animals were fed the basal diet plus 30 ml of water (control), or plus 30 ml of exogenous enzyme xylanase preparations (EZ), or plus 30 ml of *S. babylonica* extract (SB), or plus 30 ml of xylanase + 30 ml of *S. babylonica* extract (EZSB). The daily dose of treatments was given orally before the morning feeding. The chemical composition of the basal diet is presented in Table 1.

S. babylonica extract was prepared as per Salem *et al.* (2014). Blood samples (5 ml) were collected from each animal in glass plasma tubes with EDTA by jugular vein puncture on day 15 of each period. Samples collected in glasses with EDTA were maintained in cooling temperature and conserved at room temperature until processing after centrifuging at 4,000×g for 10 min where the extracted serum was stored in 1.5 ml eppendorf tubes at –20°C for further biochemical analysis.

Two samples of the diet and SB extract were collected

weekly during the 60 days of the experiment. Samples of diet and extract were pooled and stored at –20°C for further analysis.

The samples collected in glasses with EDTA were analyzed for red blood cell count (RBC), the differential white cell count: neutrophils, lymphocytes, basophils, eosinophils (EOS) and monocytes, packed cell volume (PCV), haemoglobin (HMG), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), and plasma protein concentration. Haemoglobin concentration was determined using the cyanmethaemoglobin method. Haematocrit (HMT) was determined by microhematocrit technique. Erythrocyte and total leucocytes and differential leucocyte counts were determined using the haemocytometer method. Total plasma proteins were determined using a refractometer (Archer and Jeffcott 1977). Mean corpuscular volume and MCHC were calculated to diagnose any type of anaemia by taking into account the values of erythrocytes, haemoglobin and haematocrit predetermined (Smith 2007).

$$MCV = \frac{\text{Haematocrit \%}}{\text{Erythrocyte count}} \times 10$$

$$MCHC = \frac{\text{Haemoglobin g/dL}}{\text{Haematocrit \%}} \times 100$$

The serum samples were analyzed also for total protein, glucose, creatinine, urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium, phosphorus and magnesium.

All metabolites were determined by a spectrophotometry analysis using a system analyzer and IL test´s for total protein, glucose, creatinine, urea, ALT, AST, calcium, phosphorus and magnesium.

Samples of diet were analyzed for DM, ash and nitrogen (N), according to AOAC (1997). The neutral detergent fiber (NDF) (Van Soest *et al.* 1991), acid detergent fiber (ADF) and lignin (AOAC 1997) were determined using fiber analyzer unit. The NDF was assayed without use of α-amylase but with sodium sulphite in the NDF. Both NDF and ADF are expressed without residual ash.

Plant secondary metabolites of *S. babylonica* were determined (Salem *et al.* 2014) by using 10 ml of extract and fractionated by funnel separation with a double volume of ethyl acetate to determine total phenolics by drying and to quantify the total phenolics layer in the funnel. After total phenolics separation, a double volume of n-butanol was added to fractionate saponins. The remaining solution was considered to be the aqueous fraction which contains the other secondary metabolites, lectins, polypeptides and starch (Table 1).

Data were analyzed as per SAS (2002) with Latin square design (4 animals, 4 treatments and 4 periods). Significance was declared at a level of $P<0.05$ with Tukey test.

Table 1. Ingredients and chemical composition of basal diet and levels of secondary metabolites in *S. babylonica* extract

Ingredients	*(%)*
Corn stover	35.0
Sorghum	35.5
Soybeans	10.0
Canola	7.0
Wheat bran	10.0
Mineral and vitamin mixture[2]	2.5
Chemical composition (% DM basis)	
Organic matter	92.53
Crude protein	13.2
Neutral detergent fiber	40.6
Acid detergent fiber	14.0
Lignin	2.8
Net metabolizable energy (Mcal/kg)	1.55
Secondary metabolites (%)	
Total phenolics	1.64
Saponins	0.54
Aqueous faction[1]	7.63

[1]Aqueous fraction: lectins, polypeptides, and starch. [2] Mineral and vitamin premix (/kg): Vitamin A (12,000,000 IU), vitamin D3 (2,500,000 IU), vitamin E (15,000 IU), vitamin K (2.0 g), vitamin B_1 (2.25 g), vitamin B_2 (7.5 g), vitamin B_6 (3.5 g), vitamin B_{12} (20 mg), pantotenic acid (12.5 g), folic acid (1.5 g), biotin (125 mg), niacin (45 g), Fe (50 g), Zn (50 g), Mn (110 g), Cu (12 g), I (0.30 g), Se (200 mg), Co (0.20 g).

RESULTS AND DISCUSSION

In lambs, the treatments of control, EZ, SB and EZSB did not affect ($P<0.05$) RBC, HMG, MCV, MCHC, WBC, segmented neutrophils (SGN), lymphocytes (LYM), basophils (BAS), and EOS concentrations. However, SB and EZSB lambs had the highest ($P=0.026$) values of HMT compared with the control and EZ lambs. For the serological parameters, there were no significant statistical differences in all measured parameters (Table 2).

For goats, the results were similar but in their case, there were statistical differences in EOS ($P=0.019$); the highest values were observed in control and EZ goats compared with the other 2 treatments. For the serological analysis, the lowest value of glucose ($P=0.033$) was obtained for the groups EZSB compared with the other 3 groups. However, for the rest of parameters, there were no statistical differences between the 4 treatments (Table 3). In the experiment, both lambs and goats have a similar behaviour in the parameters measured and the animals never showed sign of disease (Tables 2, 3).

The objective was to study the effect of *S. babylonica* extract, exogenous enzyme and their combination on some haematological and serum biochemical blood parameters in growing lambs and goats.The results suggested that treatments did not produced negative effects on parameter measured as indicators for animal health.

For lambs, the values to PCV were modified by treatments where the highest values were in SB and EZSB compared with the other 2 groups; this parameter is a rapid screening technique for seeking anaemia and is used as an indicator of animal hydration. Anaemia occurred due to blood loss, inadequate erythrocyte production and increased

Table 2. Haematological and biochemical profile in lambs consumed *S. babylonica* extract, xylanase enzyme and their combination as feed additives

Parameter	Treatments[1]					
	Control	EZ	SB	EZSB	SEM	P value
RBC ($\times 10^{12}$/l)	9.2	10.4	9.7	10.4	0.68	0.323
PCV (L/l)	0.35[b]	0.36[b]	0.40[a]	0.44[a]	0.023	0.026
HMG (g/l)	117.3	121.0	134.3	132.3	7.91	0.186
MCV (Fl)	37.8	35.3	41.3	38.8	4.45	0.626
MCHC (g/l)	331.5	332.0	331.2	331.5	0.34	0.260
WBC ($\times 10^{9}$/)	11.0	10.0	11.1	12.1	1.06	0.372
SGN ($\times 10^{9}$/l)	3.6	3.7	3.9	3.8	0.45	0.901
LYM ($\times 10^{9}$/l)	7.2	6.2	6.6	8.0	0.80	0.223
BAS ($\times 10^{9}$/l)	0.00	0.02	0.01	0.00	0.022	0.603
EOS ($\times 10^{9}$/)	0.13	0.09	0.16	0.12	0.088	0.897
PSP (g/dl)	65.8	70.0	67.5	67.5	2.13	0.344
Glucose (mmol/l)	3.6	4.4	4.1	4.3	0.28	0.141
Creatinine (mmol/l)	84.1	84.3	87.6	83.7	2.91	0.554
Urea (mmol/l)	6.9	7.9	7.5	7.0	0.77	0.567
ALT (U/l)	15.4	17.3	19.1	18.0	2.11	0.413
AST (U/l)	65.8	67.5	72.8	73.5	4.16	0.266
Phosphorus (mmol/l)	2.5	2.7	2.5	2.7	0.17	0.635
Magnesium (mmol/l)	1.4	1.4	1.2	1.3	0.10	0.267
Calcium (mmol/l)	2.9	2.9	3.0	2.9	0.06	0.802

[1]Animals were fed basal diet plus 30 ml of water (control), or plus 30 ml of xylanase enzyme (EZ), or plus 30 ml of *S. babylonica* extract (SB), or plus 30 ml of xylanase + 30 ml of *S. babylonica* extract (EZSB). RBC, red blood cell; PCV, packed cell volume; HMG, haemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cell; SGN, neutrophils segmented; LYM, lymphocytes; BAS, basophils; EOS, eosinophils; PSP, plasma protein concentration; ALT, alanine aminotransferase; AST, aspartate aminotransferase. Different superscripts following means within the same row indicate differences at P<0.05; SEM, standard error of the mean.

Table 3. Haematological and biochemical profile in goats consumed *S. babylonica* extract, xylanase enzyme and their combination as feed additives

Parameter	Treatments[1]					
	Control	EZ	SB	EZSB	SEM	P value
RBC ($\times 10^{12}$/l)	11.9	11.6	12.0	11.5	0.63	0.849
PCV (L/l)	0.36	0.41	0.37	0.39	0.040	0.619
HMG (g/l)	121.3	135.3	125.0	130.8	12.63	0.709
MCV (Fl)	30.0	34.0	30.8	33.8	4.04	0.688
MCHC (g/l)	332.0	332.5	332.0	331.7	0.44	0.455
WBC ($\times 10^{9}$/l)	12.0	12.2	11.8	12.7	2.06	0.974
SGN ($\times 10^{9}$/l)	5.4	6.0	5.7	5.4	1.27	0.966
LYM ($\times 10^{9}$/l)	6.6	5.6	5.6	6.2	0.93	0.678
BAS ($\times 10^{9}$/l)	0.03	0.05	0.07	0.15	0.114	0.657
EOS ($\times 10^{9}$/l)	0.08[b]	0.29[a]	0.25[a]	0.51[a]	0.091	0.019
PSP (g/dl)	70.3	71.3	71.3	70.5	1.83	0.921
Glucose (mmol/l)	4.1[a]	4.0[a]	4.1[a]	3.1[b]	0.28	0.033
Creatinine (mmol/l)	93.6	84.8	102.5	107.1	6.45	0.051
Urea (mmol/l)	6.7	7.1	8.3	7.5	1.04	0.518
ALT (U/l)	25.1	22.6	23.4	22.0	2.90	0.737
AST (U/l)	72.6	75.1	67.5	70.0	5.63	0.594
Phosphorus (mmol/l)	2.7	2.8	2.8	2.9	0.30	0.895
Magnesium (mmol/l)	1.4	1.4	1.3	1.8	0.22	0.260
Calcium (mmol/l)	2.5	2.3	2.5	2.4	0.32	0.870

[1]Animals were fed the basal diet plus 30 ml of water (control), or plus 30 ml of xylanase enzyme (EZ), or plus 30 ml of *S. babylonica* extract (SB), or plus 30 ml of xylanase + 30 ml of *S. babylonica* extract (EZSB). RBC, red blood cell; PCV, packed cell volume; HMG, haemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cell; SGN, neutrophils segmented; LYM, lymphocytes; BAS, basophils; EOS, eosinophils; PSP, plasma protein concentration; ALT, alanine aminotransferase; AST, aspartate aminotransferase. Different superscripts following means within the same row indicate differences at P<0.05; SEM, standard error of the mean.

erythrocyte destruction or haemolysis (Archer and Jeffcott 1977, Smith 2007). In the present study, the modified PCV values by treatments can not be considered as an indicator of anaemia or dehydration occurrence, because these values were within reference values for sheep (0.27 – 0.45 l/l, Archer and Jeffcott 1977, Smith 2007).

Mahgoub *et al*. (2008) reported decreased PCV values in sheep after feeding with non-conventional feeds containing phenols and condensed tannins, but this decrease was within normal range of healthy sheep and can not be considered as an indicator of injury or disease (Smith 2007). In contrast to that study, we did not observe any decease in lymphocytes and EOS numbers. Parallel results were obtained by Olafadehan (2011) who reported low PCV in goats fed with tannins-rich forage.

Haemoglobin, MCV and MCHC concentrations were in normal ranges, which suggested the absence of microcytic hypochromic anaemia occasioned by iron deficiency and improper utilization for the formation of haemoglobin; the values of blood indices (MCV=28 - 40 FL and MCHC=310 – 340 g/l) confirmed that the animals were not having anaemia (Smith 2007, Olafadehan 2011).

Phosphorus and magnesium levels increased; as the increases were in all treatments including control group, which can be due to supra-nutritional levels where its levels in serum are influenced by food supply (Smith 2007). Mahgoub *et al*. (2008) observed the same increase in both minerals, but they did not explain the reasons.

In goats, there were statistical differences for EOS in the haematology analysis and glucose in serum biochemical analysis. Eosinophils are produced in bone marrow and are more important in regulating inflammatory of allergic reactions, but principally controlling parasitic infection (Smith 2007). In the present study, the levels of EOS were in normal range (0.05 – 0.65 $\times 10^{9}$/l) (Archer and Jeffcott

1977, Smith 2007) for all treatments. Moreover, the current study demonstrated that use of feed additives as EZ or/and SB in animal nutrition have positive effects on levels of blood EOS because the levels of this parameter were lower in the control group in contrast to the other 3 groups that consumed EZ or/and SB treatments.

In other experiments, on lambs grazed willow (*Salix* spp.), fodders blocks had increased levels of EOS,WBC and lymphocytes, suggesting that in addition to the benefit of rumen escape protein, feeding condensed tannin - containing forages may stimulate cell-mediated immune response (Ramirez-Restrepo *et al.* 2010). Moreover, it is well known that secondary metabolites such as saponins and phenolic compounds can modulate the immune system and EOS level, which has an important role in innate immune response. The results of EOS treatment were different to those obtained by Mahgoub *et al*. (2008) and Olafadehan (2011) who reported lower WBC, lymphocytes and monocytes but not EOS in sheep and goats fed with tannins-rich non-conventional feeds and tannins-rich forage, respectively; but these parameters in both experiments were within the normal range, and confirmed that the concentration of tannins in these experiments were lower than the level that could induce toxicity or ill health to the animals (Mahgoub *et al.* 2008, Olafadehan 2011).

The levels of glucose were modified by the treatments, where the lower value was recorded for EZSB treatment and similar in control, EZ and EZSB treatments; however, the levels were in normal range (2.66 – 2.49 mmol/l) (Archer and Jeffcott 1977, Smith 2007). Mahgoub *et al*. (2008) indicated that the decrease in glucose level can be due to feed consumption with reduced nutritive value. In previous studies, it was mentioned that depressed serum glucose is not due to tannic acid intoxication, but that the dietary energy was sufficiently utilized for growth and the animals were not surviving at the expense of body tissues (Olafadehan 2011).

Mean corpuscular volumes reflect the mean erythrocyte size. In goats in this study, it increased; this increase is a common problem by inadequate spinning of blood which causes a spurious of MCV by trapped plasma (Smith 2007). For this reason, all treatments had higher values than normal range (16 – 25 FL) (Archer and Jeffcott 1977). In a study in sheep, MCV values were lower than normal range, but not due to the treatments (Mahgoub *et al.* 2008). With respect to the other parameters evaluated, the treatments did not produce any negative effect and it was in normal range for both lambs and goats. Anaemia was rejected because RBC, HMG, MCV and MCHC were within normal range (Smith 2007, Mahgoub *et al.* 2008, Olafadehan 2011). The present result of no impact of studied treatments on blood parameters, is an important finding in those lambs and goats that consume extract of *S. babylonica* because they contain secondary metabolites, which could be expected to have a detrimental effect on cellular components and protein in blood mainly by the presence of tannins and saponins (haemolytic activity when it was used in high concentration). These results are similar to those obtained by Ramirez-Restrepo (2010) who did not observe consistent effects of the grazing willow (*Salix* spp.) fodder blocks on RBC, HMG, MCV and MCHC concentrations.

The biochemical analysis indicated that treatments did not produce any negative effect on kidney, liver or animal health. The enzymes ALT and AST are used as indicators for domestic animal hepatic damage; and both were within the normal ranges for sheep and goats suggesting that liver was not affected by the treatment (Mahgoub *et al.* 2008, Olafadehan 2011).

Damage to the kidneys was implicated for renal failure and changes in serum blood urea-N, creatinine, uric acid and mineral concentrations. Increased levels of these metabolites above the normal values suggested a necrotic damage to the kidney; and this was not observed in the current experiment.

In the current experiment, animals particularly those who consumed *S. babylonica* extract containing secondary metabolites, did not show clinical signs of ill health or signs of tannin toxicity such as brisket edema, diarrhea, constipation, anorexia, or hard pelleted faeces coated with blood and mucous (Olafadehan 2011). In previous studies, it was observed that tannins which can be consumed by goats without signs of toxic effect may be toxic for cattle and sheep as the secretion of proline rich proteins is higher during eating in goats than that in sheep. The parotid saliva of goats is relatively rich in proline (6.5%), glutamine (16.5%) and glycine (6.1%), which have high tannin-binding capacity, by enhancing the affinity of proteins to tannins. Moreover, the ability of goats to consume large amounts of tannin-rich fodder without exhibiting toxic effects was found related to their ability to avoid consuming browses in amounts exceeding their capacity to detoxify and their enhanced capacity in comparison with other ruminant species to detoxify tannins (Olafadehan 2011). In this study, no sign of toxic effects in lambs or goats was observed, which confirmed that EZ and SB treatments are innocuous (Rivero *et al.* 2012) in both species when used as feed additives. These results are different to other studies by the type of secondary compounds.

The absence of clinical signs of ill health, tannin toxicity symptoms and the findings of all haematological and serum metabolites within the established ranges for healthy lambs and goats, suggested that EZ, EZSB treatments and their combination were well tolerate innocuous in lambs and goats. Both of *S. babylonica* and exogenous enzyme preparations can be used effectively at the rate of 30 ml of exogenous enzyme preparations and 30 ml of *S. babylonica* extract/head/day.

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