



Biochemical parameters in sheep fed diet in presence of mixed *Salix babylonica* extract and exogenous enzyme as feed additives

N RIVERO¹ and A Z M SALEM²

Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca 50200 México

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ABSTRACT

The aim of this study was to evaluate the interaction impacts of *Salix babylonica* L. (SB) extract with exogenous enzyme (EZ) as feed additives on some serum biochemistry parameters in lambs. Suffolk lambs (20), 24±0.3 kg live weight and 6–8 months old were used during 60 days. The lambs were distributed in individual cages of 1.5×1.5 m cages into 4 treatments of 5 lambs each of completely randomized design. All animals were fed a basal diet (BD) of 70% maize silage and 30% commercial concentrate. Treatments were: (i) control; PD without any additives; (ii) EZ; PD plus 10 g of EZ; (iii) SB; BD plus 30 mL of *S. babylonica* (SB) extract; and (iv) EZSB; BD plus 10 g EZ and 30 mL of SB extract. Blood samples were collected on days 0, 15, 30, 45 and 60 and analyzed for total proteins, glucose, creatinine, blood urea N (BUN), alanin aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), calcium, phosphorus and magnesium. Interactions between treatment×time were occurred in creatinine, BUN and magnesium. The highest values of total proteins were obtained with control and SB animals versus EZ or EZSB. The SB treatment tend to increase the levels of creatinine versus other treatments. The ALP level was highest in SB animals. In conclusion, supplementation with *S. babylonica* extract, exogenous enzymes and/or their combination did not cause any major health disorders in lambs within 60 days of experiment.

Key words: Biochemical parameters, Extract, Exogenous enzyme, Lambs

The concept of ‘clean, green and ethical’ animal production has been promoted to address societal demands for agricultural products which are produced with fewer chemical inputs (clean), less negative impacts on the environment (green) and greater care of the animals (ethical), these concepts had modified the livestock production systems (Durmic and Blache 2012). This situation compiled nutritionists to search for natural strategies of low cost and easy to apply; plants and plant extracts are the best solution for alternatives to synthetic drugs based on their potent properties and complex bioactivity (Lu 2011, Durmic and Blache 2012). The use of plants or its extracts in herbivores is restricted by its secondary compounds content (simple phenolic compounds or condensed tannins, saponins or essential oils rich in terpene) as there are inverse relationships between secondary compounds level in forage and palatability, voluntary intake, digestibility and N retention. Although it can cause damage in liver, kidney and digestive tract, these secondary compounds may benefit animal health and production when they are fed in the correct form and dose (Silanikove *et al.* 1996, Acamovic and Brooker 2005, Valizadeh *et al.* 2010, Lu 2011, Vasta and Luciano 2011,

Salem *et al.* 2011b, Durmic and Blache 2012). If the animals were fed some plants containing high levels of antinutritional compounds for extended periods, there would be detrimental effects on animal health and reduced ability to withstand it (Mahgoub *et al.* 2008a, Olafadehan 2011).

The use to exogenous enzymes extracted from ruminal microorganisms in ruminant feeds as by-products, forage, low quality fibrous feeds with a poor nutritive value, reduced the use of antibiotics in the diets and may decrease the pollution of atmosphere because decrease the CH₄ production (Arriola *et al.* 2011, Bedford and Cowieson 2012).

On the other hand, the use of exogenous enzymes has positive relation with palatability, feed intake, rumen microbial N synthesis, digestibility, and improve animal performance as milk production, live weight gain, feed efficiency and immunity (Arriola *et al.* 2011, Gado *et al.* 2011, Salem *et al.* 2011a, Bedford and Cowieson 2012)

It is well known that normal physiological processes are affected long before the death of an organism; hence there is a need to check physiological and biochemical indicators of health. One of the fastest means of ascertaining toxicity of ingested feed in animals is by the assessment of their blood. Blood contains diagnostically relevant parameters which act as a pathological reflector of the status of animals exposed to toxicants (Olafadehan 2011). The objective of

Present Address: ^{1, 2}(asalem70@yahoo.com), Facultad de Veterinaria y Zootecnia.

this study was to evaluate the effects of *Salix babylonica* L. (SB) extract, exogenous enzyme (EZ) and their combination on some serum biochemistry parameters in sheep.

MATERIALS AND METHODS

This study was conducted at the experimental farm of the Faculty of Veterinary Medicine and livestock of the Autonomous University of Mexico State. The handling of animals was done according to international bioethical standards and NOM-062 - ZOO-1999.

Animals and treatments: Suffolk lambs (20), 6 to 8-months-old and weighing 24 ± 0.3 kg were housed in individual cages (1.5 m \times 1.5 m) in a completely randomized design for 60 days. After 2 weeks of adaptation to the basal diet (BD) of 70% maize silage and 30% commercial concentrate formulated according to NRC (2007) nutrient requirements; the lambs were weighed and randomly distributed into 4 groups of 5 animals each. The treatments were: (i) Control; BD only without any additive; (ii) EZ; BD plus 10 g of EZ; (iii) SB; BD plus 30 mL of *S. babylonica* extract, and (iv) EZSB; BD plus 10 g EZ and 30 mL of SB extract. The daily dose of SB extract was given orally before the morning feeding; the EZ was mixed with a 200 g of concentrate and the rest to corn silage and offered 1 h before providing the concentrate the rest of the day. The chemical composition of the basal diets is presented in Table 1.

A powdered multi-enzyme commercially available feed additive product produced from *Ruminococcus flavefaciens* by the Academy of Scientific Research and Technology in Egypt (Patent No. 22155, Cairo, Egypt), was used. Prior to this work, the enzyme mixture was assayed for several enzymatic activities, and it was found to contain (per gram of enzyme preparation) 7.1 unit of endoglucanase, 2.3 unit of xylanase, 61.5 unit of α -amylase and 29.2 unit of protease activity.

Preparation of silage: Whole maize plants (at medium stage with about 70% moisture content) were chopped into 1–2 cm lengths using a forage chopper. Chopped maize was immediately filled in a flat 10-ton silo. After 2 months, experimental animals were initiated to be fed the silage as well as the other animals of the experimental farm of the faculty.

Preparation of extract: The *S. babylonica* extract was prepared as per Salem *et al.* (2011b). Briefly, fresh leaves of *S. babylonica* were collected randomly from several young and mature trees (minimum 5 different trees) in autumn and were chopped (1–2 cm) and immediately extracted in the proportion of 1 g leaf per 8 ml of solvent mixture. The mixture of solvents contained 10 ml methanol, 10 ethanol ml and 80 ml distilled water. Leaves were soaked and incubated in solvent in the laboratory at 25–30°C for 48–72 h in closed flasks. After incubation, all flasks were incubated in a water bath at 39°C for 1 h and immediately filtered, and the filtrate collected and stored at 4°C for further use.

Table 1. Chemical composition of the basal diet (commercial concentrate and corn silage) and levels of secondary metabolites (g/kg DM) in the *S. babylonica* extract

	Concentrate	Corn silage	<i>S. babylonica</i> L. extract
<i>Chemical composition</i>			
Dry matter ¹	880	360	
Organic matter	325	684	
Crude protein	157	106	
Ether extract	120	85	
Neutral detergent fiber	160	445	
Acid detergent fiber	28	111	
Lignin	8	18	
<i>Ingredient of concentrate g/kg</i>			
Corn grain flaked		200	
Corn grain cracked		260	
Sorghum grain		154	
Molasses		100	
<i>Dried distillers grains with solubles</i>			
			100
Soya bean meal		96	
Wheat bran		70	
NaCO ₃		10	
Mineral mix ²		10	
<i>Secondary metabolites</i>			
Total phenolics			16.4
Saponins			5.4
Aqueous fraction ³			76.3

¹DM expressed as g/kg fresh silage. ²Mineral mixture: Ca, 190 g/d; P, 115 g/d; Mg, 63 g/d; Cl, 167 g/d; K, 380 g/d; Na, 70 g/d; S, 53 g/d; Co, 3.3 mg/d; Cu, 197 mg/d; Fe, 360 mg/d; Mn, 900 mg/d; Se, 2 mg/d; Zn, 810 mg/d; vit A, 940(1000 IU/d); vit D, 165 (1000 IU/d); vit E, 374 (1000 IU/d). ³ The aqueous fraction contains lectins, polypeptides and starch (Cowan 1999).

Sampling and measurements: Peripheral blood (5 mL) was collected from each lamb by jugular venipuncture in red vacutainer tubes on days 0, 15, 30, 45 and 60 of the experiment. The samples were conserved at room temperature until processing, after which it was centrifuged at 1500 rpm for 10 min for serum extraction which was stored in 1.5 ml eppendorf tubes at –20°C according to groups for further biochemical analysis.

Samples of both concentrate and silage were also collected during the experimental period and stored at –20°C for later chemical analysis.

Two samples of the concentrate, silage and SB extract were collected weekly during the 60 days of experiment. Each sample type (i.e., concentrate, silage or extract) were pooled and stored for further analysis.

Sample analysis: The choice of biochemical measurements is that conventionally used for diagnosing human and domestic animal hepatic and kidney damage, and general metabolic disorders (Silanikove *et al.* 1996). The serum samples were analyzed using specific kits IL for total proteins (#0018481300), glucose (#0018480000), creatinine (#0018480900), blood urea N (BUN;#0018480400), alanine aminotransferase or glutamic pyruvate transaminase (ALT/GPT; #0018480700), aspartate

aminotransferase or glutamic oxalacetic transaminase (AST/GOT) (#0018480800), alkaline phosphatase (ALP) (#0018480600), calcium (#0018258840), phosphorus (#0018481900) and magnesium (cat. #0018481600).

All metabolites were determined using spectrophotometry analysis using a chemistry system analyzer. Samples of concentrate and silage were analyzed for DM (#934.01), ash (#942.05), N (#954.01), and ether extract (EE; #920.39) according to AOAC (1997). The neutral detergent fiber (NDF; Van Soest *et al.* 1991), acid detergent fiber (ADF) and lignin (AOAC 1997, #973.18) were analyzed using fibre analyser 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 extract were determined using 10 ml of extract liquor and fractionated by funnel separation with a double volume of ethyl acetate (99.7/100, analytical grade) 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 (99.9/100, analytical grade) was added to fractionate saponins (Ahmed *et al.* 1990). The remaining solution was considered to be the aqueous fraction which contains (Table 1) the other secondary metabolites lectins, polypeptides and starch (Cowan 1999).

Endoglucanase activity was assayed by liberating glucose from carboxymethyl cellulose, which was determined calorimetrically using alkaline copper reagent as described by Robyt and Whelan (1972). One unit of endoglucanase catalyzes the liberation of 1 mmol of glucose per min from sodium carboxymethyl cellulose at 40°C and pH 4.5. Furthermore, α -amylase was assayed by its ability to produce reducing groups from starch, which were measured by the reduction of 3,5-dinitrosalicylic acid (Bernfeld 1955). One unit of α -amylase catalyzes the liberation of 1 mmol of reducing groups per min from soluble starch at 25°C and pH 6.0, calculated as maltose equivalents. Protease activity was determined by the hydrolysis of dimethyl casein (DMC) and the liberated

Table 2. Serum biochemistry parameters in growing lambs treated with *S. babylonica* L. (SB), exogenous enzyme preparation (EZ) and their mixture (EZSB) sampled the days 0, 15, 40, 45 and 60 of the experiment

Treatment	Time	Protein g/L	Glucose mmol/L	Creatinine μ mol/L	BUN mmol/L	ALT U/L	AST U/L	ALP U/L	Calcium mmol/L	Phosphorus mmol/L	Magnesium mmol/L
Control	0	64.38	0.6	100.0	3.3	13.2	52.4	179.2	2.48	1.97	0.99
	15	61.10	2.6	88.18	2.6	14.4	59.6	224.4	2.86	2.33	1.10
	30	58.92	2.6	107.40	3.1	15.8	65.4	295.0	2.48	2.26	1.21
	45	55.72	2.8	104.20	2.3	15.6	59.2	310.0	2.78	2.66	1.24
	60	53.14	1.4	107.52	3.2	20.4	53.0	224.4	2.66	2.63	1.28
	Mean		58.65 ^a	2.0	101.48 ^b	2.93	15.88 ^a	57.92	246.6	2.65 ^a	2.37
SB	0	57.88	1.4	118.84	3.2	14.8	55.8	220.6	2.44	2.39	1.04
	15	60.88	2.8	101.40	2.7	15.8	59.2	248.0	2.64	2.40	1.07
	30	61.74	3.0	120.98	3.7	19.2	71.0	328.4	2.44	2.28	1.46
	45	58.78	2.6	120.56	2.8	18.6	65.4	309.6	2.58	2.72	1.25
	60	59.38	0.4	110.66	6.7	20.8	55.4	261.8	2.74	2.57	1.09
	Mean		59.70 ^a	2.04	114.49 ^a	3.86	17.84 ^a	61.33	273.68	2.56 ^a	2.47
EZ	0	55.08	1.4	104.78	2.6	12.6	55.4	209.8	2.22	2.10	1.00
	15	59.92	2.8	97.42	4.4	14.8	54.2	232.4	2.34	2.51	1.50
	30	54.26	2.6	112.06	4.0	15.8	64.2	277.8	2.34	2.23	1.35
	45	50.96	2.2	112.72	3.2	15.0	55.4	296.2	2.38	3.02	1.41
	60	47.56	0.8	101.2	4.3	16.4	58.0	301.6	2.62	3.06	1.61
	Mean		53.55 ^b	1.96	105.64 ^b	3.74	14.92 ^b	57.44	263.56	2.38 ^b	2.58
SBEZ	0	55.88	1.2	91.40	2.4	17.0	97.6	200	2.16	2.03	0.92
	15	54.3	2.8	94.36	3.8	9.8	41.8	234.4	2.34	2.52	1.14
	30	54.74	2.8	103.44	3.7	13.6	58.2	308.6	2.14	2.73	1.31
	45	52.16	2.6	107.78	2.7	11.8	49.4	341.0	2.40	2.78	1.12
	60	54.92	1.0	110.78	4.0	12.8	49.8	333.2	4.60	2.98	1.31
	Mean		54.4 ^b	2.08	101.47 ^b	3.36	13 ^b	59.36	283.44	2.32 ^b	2.61
SEM pooled		0.168	0.0478	0.388	0.0499	0.1191	0.6937	2.56	0.0254	0.0165	0.0086
<i>P</i> -value											
Treatment		0.0456	0.9623	0.0572	0.2804	0.0426	0.9806	0.8041	0.0066	0.3983	0.0227
Time		0.0177	0.0001	0.0001	0.0001	0.0082	0.0213	0.0001	0.0002	0.0001	0.0001
Tr \times Time		0.3681	0.2293	0.0396	0.0004	0.3589	0.5983	0.7513	0.7077	0.6106	0.0127

SB, *S. babylonica* L. extract; EZ, exogenous enzyme; EZSB, *S. babylonica* L. extract + exogenous enzyme; BUN; blood urea nitrogen, ALT, alanine aminotransferase or glutamic pyruvate transaminase; AST, aspartate aminotransferase or glutamic oxalacetic transaminase; ALP, alkaline phosphatase. ^{a, b}Different superscripts following treatments mean in the column indicate differences at $P < 0.05$.

amino acids were determined using 2,4,6-trinitrobenzene sulphonic acid (Lin *et al.* 1969). One DMC-U catalyzes the cleavage of 1 mmol of peptide bond per min from DMC at 25°C and pH 7.0 expressed in terms of newly formed terminal amino groups. Xylanase catalyzes the hydrolysis of xylan from oat spelt, and the reducing groups liberated were determined using alkaline copper reagent (Robyt and Whelan 1972). One unit catalyzes the liberation of 1 mmol reducing groups per h from xylan at 37°C and pH 5.5, expressed as xylose equivalents.

Statistical analyses: Data were analyzed using the MIXED procedure of SAS (2002) with repeated measures (Littell *et al.* 1998). Terms in the model were diet (i.e., control, SB, EZ, EZSB), days of sampling (i.e., 0, 15, 30, 45 and 60 d of the experiment). Tests of simple effects were used to partition (slice) interaction effects by treatment to test effects of period separately for each diet and the interaction treatment \times time (SAS 2002). The significant differences between treatment means, time and interaction treatment \times time were determined with a value to significance of $P < 0.05$.

RESULTS AND DISCUSSION

In all experiments the animals were in good conditions and had no signs of ill health. Serological data were used as an indication of health status of the experimental animals (Table 2). Interactions between treatment \times time were occurred on the levels of creatinine ($P = 0.0396$), BUN ($P = 0.004$) and magnesium ($P = 0.013$). No interactions were observed for the other measured parameters. Treatments of SB, EZ, and SBEZ had not affected blood glucose, creatinine, BUN, AST, ALP, and phosphorus content, while the levels of total proteins were modified. The highest values total proteins were obtained with control and SB animals. The SB treatment tended to increased ($P = 0.057$) the levels of creatinine *versus* other treatments. For ALT, the highest ($P = 0.043$) value was observed in SB animals. Control and SB had increased ($P = 0.007$) blood calcium content, while EZ treatment increased ($P = 0.023$) the level of magnesium *versus* other groups.

The sampling time produced significant differences in all measured parameters. At day 0, the lowest concentrations of glucose, ALT, AST, ALP, calcium, phosphorus and magnesium were observed in control animals *versus* other group. The same results were observed with total proteins, ALT, and calcium for SB groups; BUN, and ALT for EZ group; ALP for SBEZ group. Total proteins, glucose, Creatinine and urea had the highest concentrations in days 0 and 15 ($P = 0.018$), 15 and 30 ($P = 0.001$), 30 and 45 ($P = 0.001$), 30 and 60 ($P = 0.001$). Enzymes of kidney and liver functions also had the highest values in 30 and 60 (ALT), 0 and 30 (AST), 30 and 45 (ALP). Serum calcium, phosphorus and magnesium were highest in days 60, 45 and 60, 30 and 60, respectively.

For total proteins the reference values are between 60–75 g/L, and a hypoproteinemia was observed in all treatments. Value lower than reference range is dangerous

because the role of protein in numerous physiologic process as integrity of body tissues, enzymes, hormones, carriers for others plasma constituents, etc. (Smith 2007). There are reports to indicate that total proteins and albumin can be lowered due to infection by blood-spoiling parasites, such as *Haemonchus contortus*, but in this case the values decreasing until 15g/L (Braun *et al.* 2010) and our no value was lower to about 50 g/L. Serum total proteins reflect the nutritional status of the animal, and it has a positive correlation with dietary protein (Kumar *et al.* 1980). So, the observed decreased blood total proteins may be due to the decreased protein intake as the dietary protein affected the concentration of plasma protein especially when animals were fed low protein diets (Allam *et al.* 2009, Rowlands 1980).

The level of total proteins is the highest in group SB indicating that tannins presents in the extract has a protector effect on protein. Tannins have an ability to form strong insoluble complexes with proteins that are resistant to stomach pH, where tannins have a large number of free phenolic hydroxyl groups that form strong hydrogen bonds with proteins and carbohydrates. Animals fed on tannin-rich forages, tannin-protein complexes can reduce the digestion of forage protein (Imik *et al.* 2008, Mahgoub *et al.* 2008b, Lu 2011, Durmic and Blache 2012).

In the other two groups of EZ and SBEZ, the levels of serum total proteins were statistically lower. The commercial enzyme powder contained about 29.2 unit/g to proteases (Gado *et al.* 2011, Salem *et al.* 2011a) that was not sufficient to increase the quantity of crude protein ingested by the lambs of this group as the quantity of protein in the diet is relation with the plasma protein concentration (Smith 2007) we did not observe any increase in this parameter. However, in the group SBEZ the lowered value of serum total proteins may be due to the antagonist effect between exogenous enzyme and secondary compounds, because the enzyme decreasing the activity of secondary compounds as shown the study of Gado *et al.* (2011) in which all secondary metabolites in the orange pulp were reduced with the addition of commercial enzyme powder during its ensiling. Salem *et al.* (2011) add 10 g of commercial enzyme powder /animal/day to the diet of lambs as a feed additives and observed an increased diet crude protein content but did not determined the total proteins concentration in serum (Salem *et al.* 2011a).

Results of serum total protein levels were different to other studies in which the effect of secondary compounds had been evaluated, and in which the authors did not observe any increase in this parameter when sheep or goats were fed with diets with high levels of secondary compounds or when the animals ingest these compounds as feed additives (Silanikove *et al.* 1996, Imik *et al.* 2008, Mahgoub *et al.* 2008a, Valizadeh *et al.* 2010, Olafadehan 2011). It may be due to the kind of forage used, concentration of secondary compounds, age of the animals and adaptation time, etc. Ramirez- Restrepo (2010a,b) conducted an experiment with *Salix* spp. as fodder blocks in sheep and did not observe

changes in the levels of serum total proteins but he demonstrated the stimulate effect of condensed tannins in this forage on cell-mediated immune response.

The serum creatinine is no conditioner by the diet. Its concentration in serum provides a measure of glomerular filtration rate in ruminants. This parameter is an indicator of renal failure, small increases in creatinine may be seen with progressively compromised renal function (Turner *et al.* 2005, Ford and Mazzaferro 2007, Smith 2007). In the present study, the levels of creatinine were within the normal range (76–176.8 $\mu\text{mol/L}$) reported for healthy sheep (Ford and Mazzaferro 2007, Smith 2007), but were higher in SB group which indicates that secondary compounds (phenols and condensed tannins) present in the extract were negatively affected glomerular filtration, causing renal damage (Silanikove *et al.* 1996, Mahgoub *et al.* 2008b, Olafadehan 2011, Durmic and Blache 2012). Increasing in protein intake had no effect on plasma creatinine concentration but it can be positively related to the turnover of the protein pool in ruminants (Turner *et al.* 2005). Mahgoub *et al.* (2011) didn't observe any changes in this parameter when provided feeds that contained phenols and condensed tannins to sheep, but they observed a decrease in blood urea nitrogen. This is associated with liver failure. The same author observed a damage functional and structural in gut, liver and kidney of sheep fed non- conventional feeds containing phenols and condensed tannins (Mahgoub *et al.* 2008a, Mahgoub *et al.* 2008b).

In our experiment, BUN was not modified with the treatments. It may be reduced slightly in animals given anabolic steroids or fed diets with low protein content but adequate caloric content. Changes in both BUN and creatinine can be associated to renal failure (Smith 2007, Mahgoub *et al.* 2008a, Braun *et al.* 2010). Although generally BUN tends to be decreased in ruminants fed diets with low protein content or with severe liver disease (Mahgoub *et al.* 2008a).

Liver ALT enzymes are used to assess liver damage (Mahgoub *et al.* 2008a). In the present study, its levels were in normal range (15–40 u/L) as reported by Ford and Mazzaferro (2007) and Smith (2007). The higher levels of ALT in SB group indicated that secondary compound presented in SB caused moderate damage in liver since high values in serum indicate hepatocyte injury and intracellular enzymes output (Ford and Mazzaferro 2007, Mahgoub *et al.* 2008a,b, Olafadehan 2011). It is possible that this negative effect can be caused by ethanol and methanol in extract (Lieber 1990).

For minerals as calcium, phosphorus and magnesium, there are little investigates in sheep. In our experiment, calcium values were in the normal range (2.3–2.9mmol/L) for healthy sheep. This electrolyte maintain neuromuscular excitability, permeability of cell membranes, conduction of nerve impulses, muscle contraction and blood clotting (Ford and Mazzaferro 2007, Smith 2007). Renal excretion, intestinal absorption, mobilization form large bones and

its concentration in the diet, allregulate its concentration in serum (Ford and Mazzaferro 2007, Smith 2007, Braun *et al.* 2010). In the present study, calcium was higher in control group with no significant differences with SB group. It is a good result as secondary compounds in the extract did not produce negative effects on calcium metabolism as occur in the experiment of Olafadehan (2011) who reported a decreased calcium in serum when the goats were fed with *Pterocarpus erinaceus* rich in tannins.

Magnesium, concentration in serum are influenced by food supply (Ford and Mazzaferro 2007, Smith 2007). They reported that the normal range is about 0.8 – 1.15 mmol/L. In the present study, its levels in blood were higher compared with the normal range in the treatment EZ but not in the treatments of control, SB and SBEZ. These results are opposite to results of Olafadehan (2011), who observed an increase in serum concentration of magnesium in goats fed on *Pterocarpus erinaceus*. In our study SB did not produced a significant increase or decrease in serum magnesium concentration.

Our results of serum Ca and magnesium content confirmed that tannins presents in *S. babylonica* extract did not produced any changes in absorption of Ca and magnesium from the gastrointestinal tract with no signs of mineral depletion (Silanikove *et al.* 1996, Mahgoub *et al.* 2008b, Olafadehan 2011). The differences between our results and results of other studies fed diets rich in secondary compounds for serum concentration of Ca and Mg maybe due to kind of plant used, concentration of secondary compound, age of animals, etc (Mahgoub *et al.* 2008b).

The time produced significant differences in all measured parameters for all treatments; but there are no studies in which the time had been considered. It is possible that these differences can be associated with the time of adaptation required for ruminant microorganism and cells for detoxifying, metabolizing and using the diet compounds and drugs (Durmic and Blache 2012).

There were interactions between time and treatment for creatinine, BUN and magnesium. These differences indicated that treatments can cause a damaged renal if used for long time. Damage of kidney would most likely lead to renal failure and to changes in serum urea and creatinine and minerals concentration (Silanikove *et al.* 1996, Olafadehan 2011).

The absence of clinical signs of ill health, toxicity symptoms suggested that secondary compounds present in *Salix babylonica* extract were well tolerated by the sheep. The results indicated that exogenous enzyme had no negative effects on serum parameters. Although, *Salix babylonica* extract produced some significant negative effects, yet the parameters which significantly differed were in normal range. It was thus concluded that the supplementation with *Salix babylonica* extract, exogenous enzymes and their mix did not cause any major health disorders when these were fed for more than 60 days in lambs.

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