

Effect of feeding chemical and microbial treated barley straw on performance and some serum biochemical attributes of Karadi lambs

Shaker A. HASSAN^{1*} Sarwar M SADIQ² Khasraw M. HASSAN²

¹Dept. of Anim. Res. College of Agric. Univ. of Baghdad, Iraq

² Dept. of Anim. Prod. College of Agric. Univ. of Sulaimani, Iraq

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ABSTRACT : Fifteen Karadi male lambs (live-weight 25 ± 0.377 kg and 5 months old) were used to study the effect of feeding lambs with untreated barley straw (US) or urea treated barley straw (UTS) or fungi treated barley straw (FTS) on daily dry matter intake (DMI), live weight gain (LWG), some liver and kidney functions such as glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and urea-N(SUN) as well as serum glucose (SG), total protein (STP) and cholesterol (SC) of karadi lambs were studied. Results revealed that FTS treatment improved DMI and LWG as compared with US or UTS treatments. No significant differences in SG were observed between experimental groups. SUN pointed out to ($P < 0.05$) differences among treatments in favor of chemical treatment which recorded the highest ($P < 0.01$) SUN concentration in comparison with the other treatments. The SUN level ranged from 10 to 25 mg/dl. STP was significant ($P < 0.01$) differences among treatments in favor of fungal treatment which recorded the highest ($P < 0.01$) STP (4.20 mg/dl) in comparison with the other treatments. SC values indicate a significant ($P < 0.05$) differences among treatments in favor of urea and fungi treatments which was the lowest ($P < 0.05$) in comparison with the control group (untreated). GOT and GPT values showed a significant differences among different experimental groups. However, higher ($P < 0.05$) GOT and GPT values were assessed for urea group (10.3 and 28 IU/L), respectively. While, fungi treatment had an intermediate values for GOT and GPT (86 and 24 IU/L) respectively. On contrast, the control groups recorded the lowest ($P < 0.05$) GOT and GPT values, (65 and 20 IU/L). In conclusion, according to the results it seems that the possibility of biological methods of barley straw treatment has a great appeal as an alternative to the use of expensive chemicals.

Keywords: Lambs, chemical and microbial treatments, performance, blood parameters, barley straw

INTRODUCTION

In Iraq, agricultural and industrial by-products are considered as a stable source of ruminant feeds (Al-Ani et al., 1991; Hassan et al., 1998ab; Hassan et al., 1999ab; Hassan 2005; Hassan et al., 2009a). The major limitations of using these residues as ruminant feeds are their poor in nutrients such as protein content and vitamins and they are rich in fibers with low digestibility, or low palatability and high lignin contents. Nowadays interest in their effective utilization is increasing all over the world due to economical factors and pollution. Therefore, chemical and biological treatments are used for increasing the nutritional value of many by-products, because they have significant concentrations of simple carbohydrates, such as mono- and disaccharides. The possibility of biological methods of straw treatment has a great appeal as an alternative to the use of expensive (in terms of money and energy) chemicals. Pollution would also be reduced. As well as biological treatment of barley straw was reduced rumen phenolic compound and increase rumen aerobic bacteria (Hassan et al., 2008). In contrast, blood parameters such as serum urea nitrogen (SUN), serum glucose (SG), blood total protein (STP), cholesterol (SC), Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) are the most parameters shown to vary with different basal

diets and chemical and microbial treatments (Fouda 2008; Hassan and Hassan, 2009a; Hassan and Salim, 2009; Hassan et al., 2009ab; Hassan et al., 2010ab). SUN level in excess of 18 to 20 mg/dl can be associated with lower reproductive performance, higher feed costs, health problems, and poor production (Hansen, 2003). Therefore, the objective of this study was to evaluate the effect of feeding lambs with untreated, treated barley straws with urea or fungi (*Pleurotus ostreatus*, P.O) on daily dry matter intake (DMI), crude protein intake (CPI), live weight gain (LWG) and serum biochemical parameters (SG, Urea-N, SUN, STP, Cholesterol, SC, GOT and GPT) of Karadi lambs related to liver and kidney functions.

MATERIALS and METHODS

The trial was carried out at the animal production field, Dept. of Anim. Prod. College of Agric. Univ. of Sulaimani, Bakrigo, Sulaimani, Iraq.

Animal and diets

Fifteen individual Karadi male lambs with an average live-weight 25 ± 0.377 kg and 5 months old were divided into three equal groups and used to test the effect of feeding untreated (US) or treated barley straws with urea (UTS) or fungi (FTS) on serum biochemical parameters of lambs related to liver and

*Corresponding author: Shaker, A. H., shakeratar@yahoo.com

kidney functions. Five lambs for each treatment were penned individually indoors and the concentrate diet was supplied as a basal diet once daily in equal quantity (2% of the body weight) for all lambs at 9:00am while, US, UTS and FTS were given *ad libitum*. The concentrate diet consisted of 49% barley, 39% yellow corn, 10% soybean meal and 1% of salt and 1% mineral and vitamin mixture and containing (% of DM basis): 94% DM, 91.8% OM, 13.25% CP, 5% CF, 70% NFE, 3.4% EE and finally contain 12.7 MJ of ME/kg DM

according to MAFF (1975). The chemical composition of US, UTS and PTS are shown in table 1. The lambs were weighed at the beginning and the end, and once every 7 days during the experimental period. Daily feed intake and refused were measured and sampled for 9 weeks. The sample of feed and refusal were dried at 65 C° for 48 h until constant weight. The dried samples were ground through 1 mm mash and analyzed chemically.

Table 1. Chemical composition and in vitro digestibility of untreated, urea, fungi treated barley straw and concentrate diet (% of DM basis).

Items %	Treatments			
	T3 FTS	T2 UTS	T1 US	Concentrate
DM	95.1	94.5	96.2	94.6
OM	82.7	84.7	87.8	91.8
CP	12.4	19.9	4.1	13.25
CF	-	-	-	5.0
NFE	11.9	9.8	8.4	70
EE	-	-	-	3.4
NDF	74.16	75.8	81.3	-
ADF	50.2	50.1	54.3	-
Lignin	9.13	9.2	11.1	-
Hemi-cellulose	23.96	25.7	27.0	-
Cellulose	41.07	40.9	43.2	-
IVDMD	57.5	53.8	45	-
IVOMD	56.9	51.3 ^b	44	-
ME MJ/ kg DM [#]	8.54	7.7	6.6	12.7

[#] MAFF 1975; ME (MJ/Kg DM) = IVOMD × 0.15 for roughages

and ME(MJ/Kg DM) = 0.012CP+0.031EE+0.005CF+0.014 NFE for concentrate

Barley straw preparation

Chemical treatment

The barley straw used in this experiment was chopped and treated with urea at rate of approximately 70g/kg DM dissolved into 60% moisture on a DM bases (Hassan and Muhamad, 2009). Urea was applied by spraying equal weight of urea solution on straw to provide a treatment level of 70g urea per kg straw DM. The sprayed straw was mixed well to bring urea solution into contact with straw as completely as possible. The freshly-made material was covered with polyethylene nylon for approximately 4 weeks to absorb moisture and ammonia that formed during the heating process. At the end of the incubation period the polyethylene nylon was removed and sun dried and stored in nylon bags for chemical analysis.

Biological treatment

The barley straw was spread on a polyethylene sheet and spread with 75% water content 2% formaldehyde and 1% urea (source of nitrogen) for 24h in fermentation room. The wheat grain spawns of *Pleurotus ostreatus* (P.O), obtained from Hassan et. al., (2008) was used to inoculate the barley straw. The

pasteurized straw was spread again on a polyethylene sheet and mixed with the spawn at the rate of 3 kg spawn per 100 kg straw (on dry matter basis) in spawning room. Then the inoculated straw was packed in black polyethylene bags (80cm length and 40cm diameter and 100 gauge thickness). Each bag that contained approximately 15kg of straw (fresh weight) was tightened up with nylon thread and transferred to the fermentation room where the temperature of 26 ± 3C° and the relative humidity of 70-75% maintained by means of heater during the 21days of incubation, when the mycelium run started, all sides of the bags were crushed, to provide a uniform distribution of mycelium for all substrate. After 21 days of incubation, the bags were removed from the fermentation room and sun dried and stored in cotton bags for chemical analysis.

Blood samples

Ten ml of blood samples were collected from lambs by jugular vein puncture on day-zero before initiation of experiment, at the middle of the experiment (30 days) and at 2-3 days before the end of the of experiment period (60 days). At each period blood samples were taken at zero time (before morning feeding) and at 3,6

and 24 hrs. post concentrate feeding, into plain vacutainer tubes for serum separation. The blood samples were centrifuged at 4000 r.p.m. for 20 minutes, and blood serum was separated and preserved in clean and sterile a plastic tube which was stored at -20°C for biochemical assay. SG was determined as soon as possible after sampling Serum were analyzed for SG, SUN, TP, SC, GOT and GPT .

Mean serum concentration were calculated for each period, all times and for each animal within each treatment group, SG was measured by spectrophotometrically (Spectronic Instruments, USA) utilizing standard kits (Bio-Merieux, France). SUN was measured photo metrically in the serum fraction using a Digital Spectrophotometer (PD-303). APEL-JAPAN, the kit that used to analyze SUN was marked as (Urea-kit S180, France) and according to the method of Coulombe and Faveran (1963). TP was measured in the serum using a Spectrophotometer according to the method described by Peters (1968). The concentration of SC was determined calorimetrically by using commercial kits according to Schmidt-Nielsen (1964). The kit that used to analyze total protein was marked as (BIOLABO REAGENTS 02160, France). Serum GOT and GPT activities were determined calorimetrically by using commercial kits according to Armstrong and Carr (1964).

Chemical analysis

Proximate chemical analysis of raw and treated barley straw and concentrate samples was carried out for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash content according to the AOAC (1995). The nitrogen free extract (NFE) was calculated by subtracting the summation percentages of CP, EE, CF and ash from one hundred. Untreated and treated barley straw samples were analyzed according to Goering and Van Soest (1970) to determine neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). The *In Vitro* digestibility of DM and OM for untreated and treated barley straw were determined using the method of Telley and Terry (1963).

Statistical analysis

The obtained data were analyzed according to Statistical Analysis System user's Guide (SAS, 2001) for one way analysis of variance. Separations among means were carried out by using Duncan's (1955) multiple range tests. According to the following model: $Y_{ij} = M + T_i + e_{ij}$. When Y_{ij} = The observations values for each treatment. M = the mean value. T_i = The effect of treatment. e_{ij} = The random error

RESULTS and DISCUSSION

Daily intake and live weight gain

The main effect of treatments on overall mean values of daily DMI, CPI, LWG and final live weight (FLW) of karadi male lambs are presented in Table (2).

Lambs fed Urea and fungal treated straw had significantly ($P < 0.01$) increased the daily CP intake (213 and 176g/day respectively) as compared with those lambs fed untreated straw (101 g/day). As well as fungi treatment showed higher ($P < 0.01$) FLW, LWG and daily DMI (39.76 kg, 28 and 1377g/h/day) as compared with untreated (34.8 kg, 154 and 1220 g/h/day respectively) and urea treated straw (35.5 kg, 166 and 1264g/h/day respectively) without significant differences with both untreated and urea treated groups. Softness improvement of digestible intake could be due to the physical (of the straw structure) and chemical (cell wall degradation) changes of barley straw through solubilization of structural polymers by urea (Boyle et al., 1992), which made it more accessible to the rumen micro organisms or by solid state fermentation process by fungi. In addition urea and fungal treatments increased the DM and OM digestibility of straw, which increased the voluntary intake. Yamakawa et al., (1992) reported an increase in DM intake of P.O. treated rice straw in sheep. Recently Sadiq (2010) reported that digestible OM intake and nutritive value index (NVI) of barley straw were the highest for fungi treated straw and the lowest for untreated straw, while digestible OM intake and NVI for urea treated straw were in between. Higher LWG and FLW values in PTS group may be attributed to the effect of fungal treatments in improving ration digestibility, metabolizable energy content and palatability, in addition to enrich the untreated material with surplus CP content. In this respect, numerous fungal species were used for biological treatments of roughages, particularly *Pleurotus ostreatus* (Abdelhamid et al., 2006 & 2007). Such increases in growth rate, might be related to improvement in roughage palatability (Abdelhamid et al., 2006), crude protein and energy contents (Bassuny et al., 2003 a&b), digestibility and voluntary intake and thus nutritive values (Bader, 2001) and live weight gain (El-Ashry et al., 2001).

Serum biochemical parameters

As well as the main effect of US, UTS and FTS basal diets on values of serum biochemical parameters are presented in Table (2), and the mean values of serum biochemical parameters for day zero, at the 30 and 60 days post experiment period are demonstrated in figures 1- 6.

SG concentration: There were no significant ($P > 0.05$) differences among different experimental groups and times of sampling on SG concentration; except that the lambs of US group shown significantly ($P < 0.05$) lower glucose concentration (66.6 mg/dl) at day zero before initiation of experiment as compared with UTS and FTS treatments (Figure 1). Generally all the concentration means of serum glucose were within the normal range of sheep. Similar observation was reported by Hassan and Hassan, (2010a).

Table 2. The main effect of treatments on some serum biochemical parameters during the experimental periods

Items %	Treatments			
	T3 FTS	T2 UTS	T1 US	Concentrate
Total DMI (g /day)	26.14	1377 ^a	1264 ^b	1220 ^c
Total CP (g /day)	12.12	176.1 ^b	212.8 ^a	101 ^c
Initial live weight (LW, Kg)	0.377	25.4	25.1	25.08
Final LW (Kg)	0.959	39.76 ^a	35.54 ^b	34.8 ^b
Live weight gain (g/day)	11.54	228 ^a	166 ^b	154 ^b
Blood parameters				
Blood sugar mg/dl	3.60 ^{NS}	78.82	75.72	71.65
Blood urea N mg/dl	0.847	13.47b	18.55a	11.47b
Total protein gm/dl	0.185	5.35a	4.55b	4.46b
Cholesterol mg/dl	2.10	45.97b	44.71b	57.29a
G.O.T (U/ L)	5.10	86.27b	103.5a	64,8c
G.P.T (U/ L)	1.77	21.13b	28.33a	20.57b

^{aabc} Means with the different superscripts within row are significantly ($P < 0.05$) different

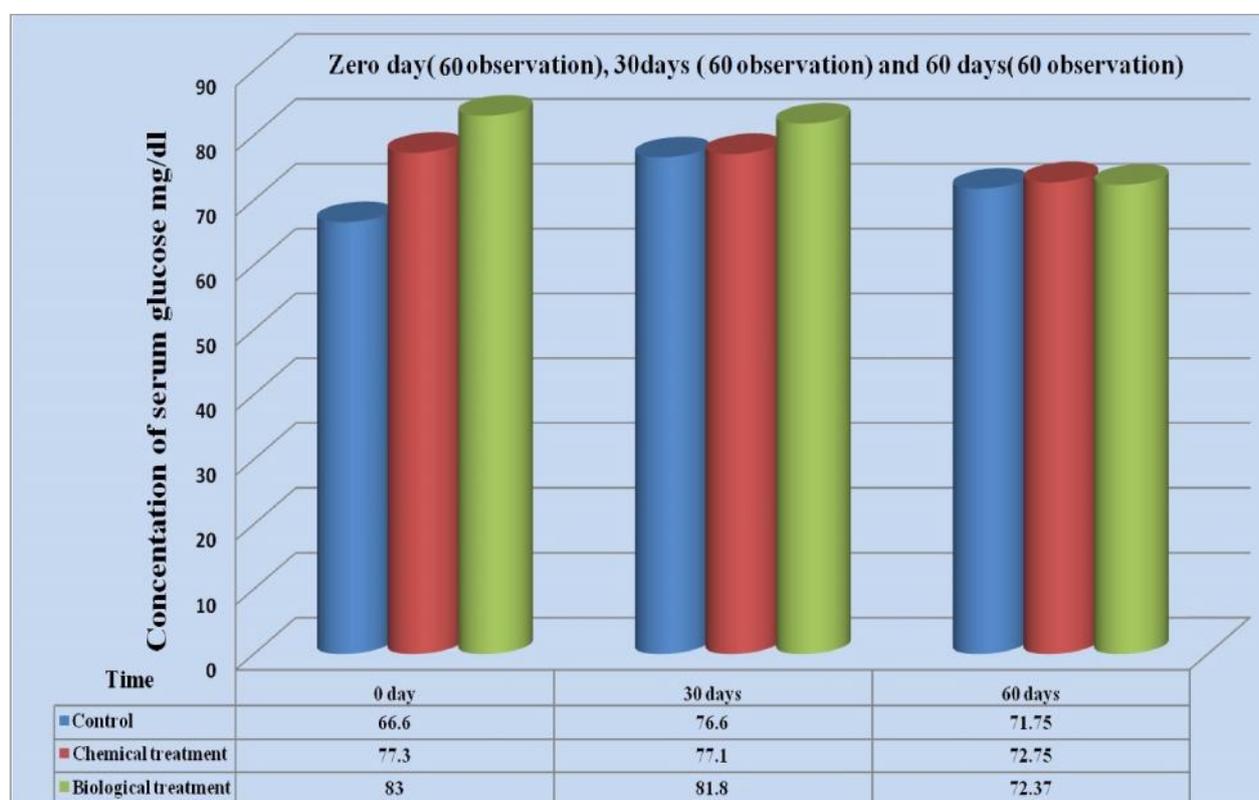


Figure 1. The diurnal patterns of serum glucose concentration (mean of zero time, 30 and 60 days post experiment period) during 24 hrs after morning feeding

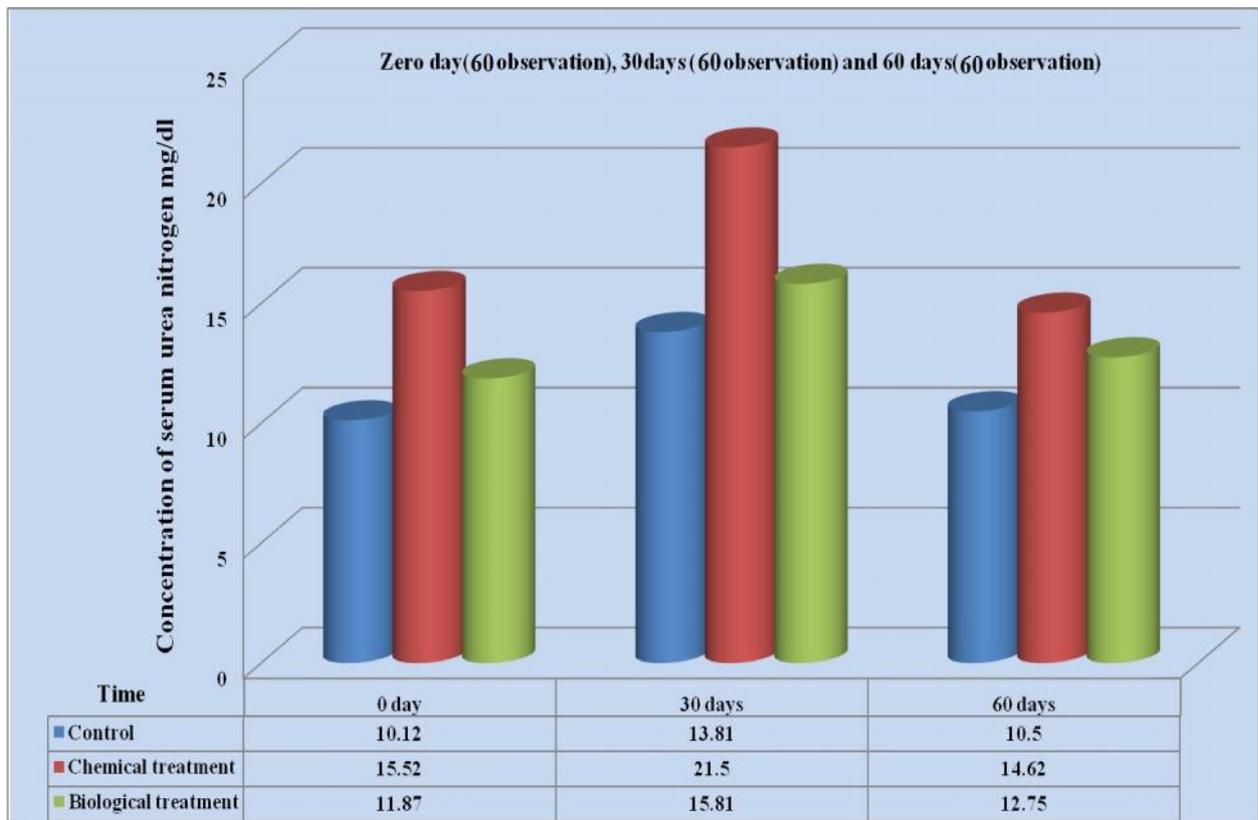


Figure 2. The diurnal patterns of serum urea nitrogen concentration (mean of zero time, 30 and 60 days post experiment period) during 24 hrs after morning feeding

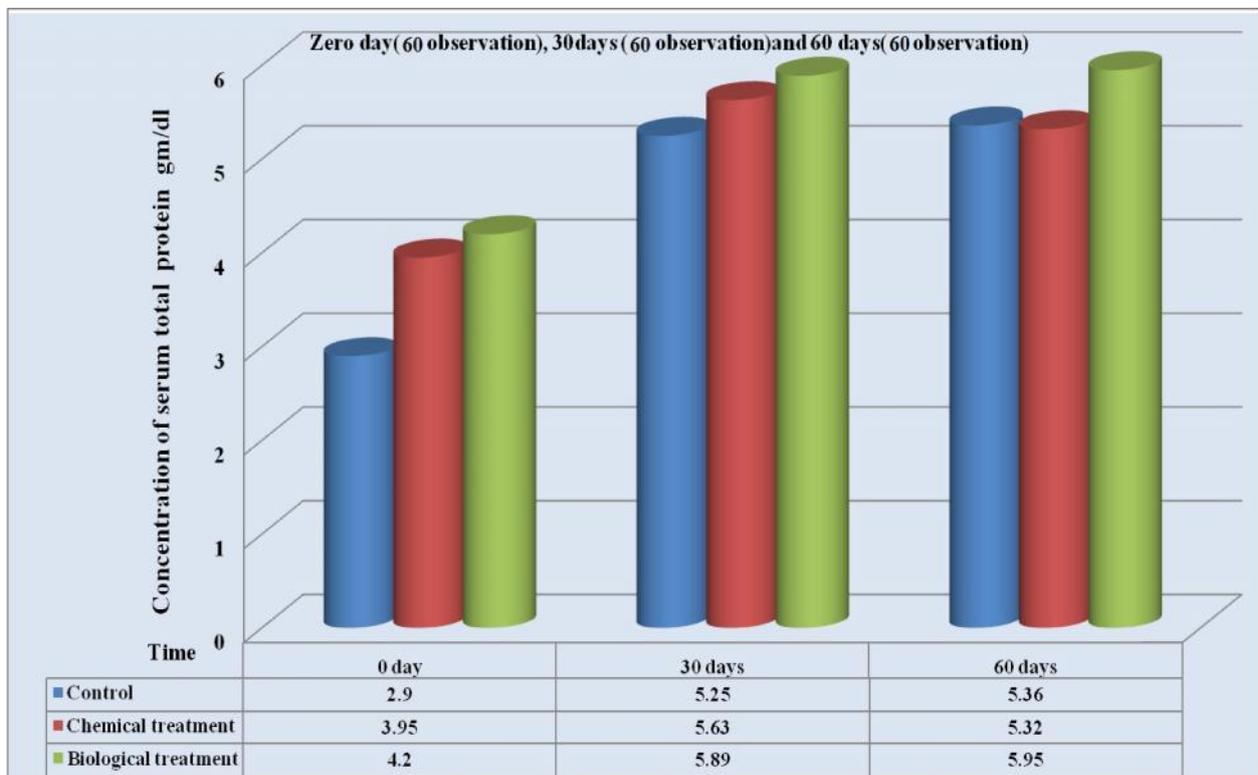


Figure 3. The diurnal patterns of serum total protein concentration (mean of zero-time, 30 and 60 days post experiment period) during 24 hrs. after morning feeding

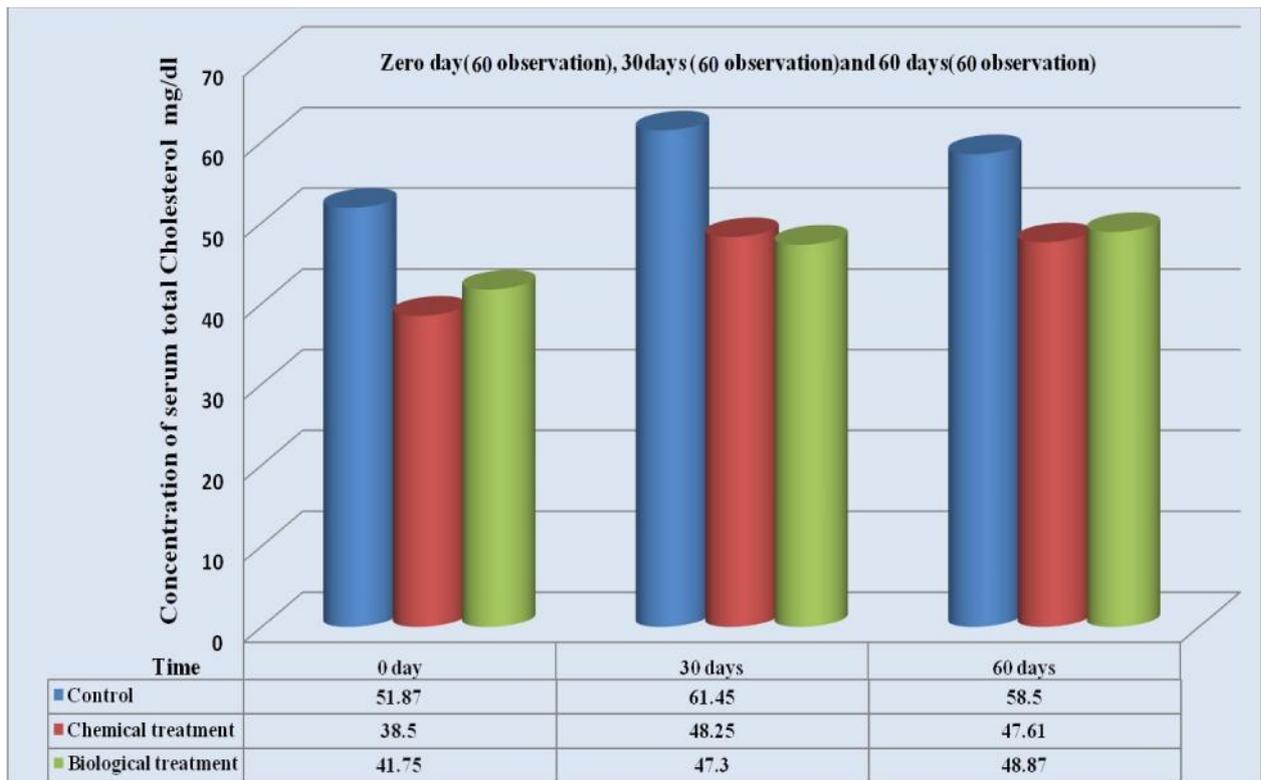


Figure 4. The diurnal patterns of serum total cholesterol concentration (mean of zero-time, 30 and 60 days post experiment period) during 24 hrs. after morning feeding

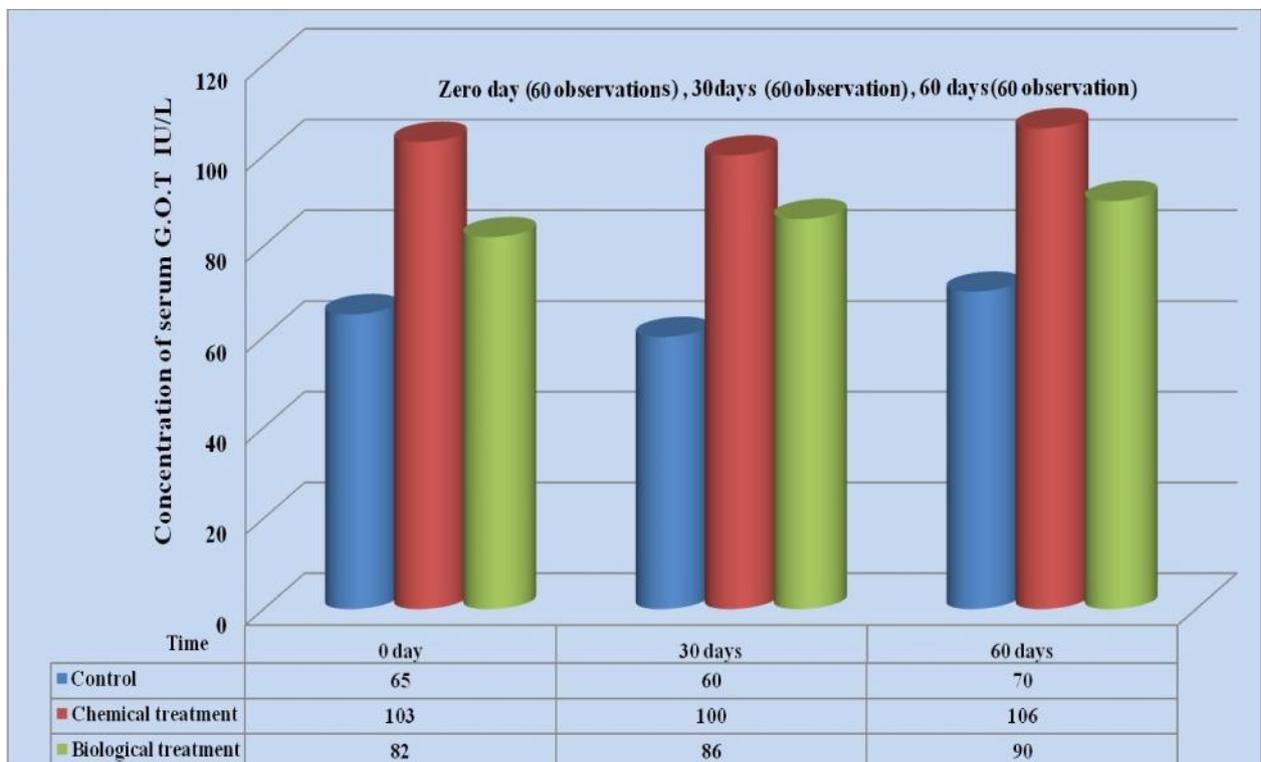


Figure 5. The diurnal patterns of serum glutamate oxaloacetate transaminase concentration (mean of zero-time, 30 and 60 days post experiment period) during 24 hrs. after morning feeding

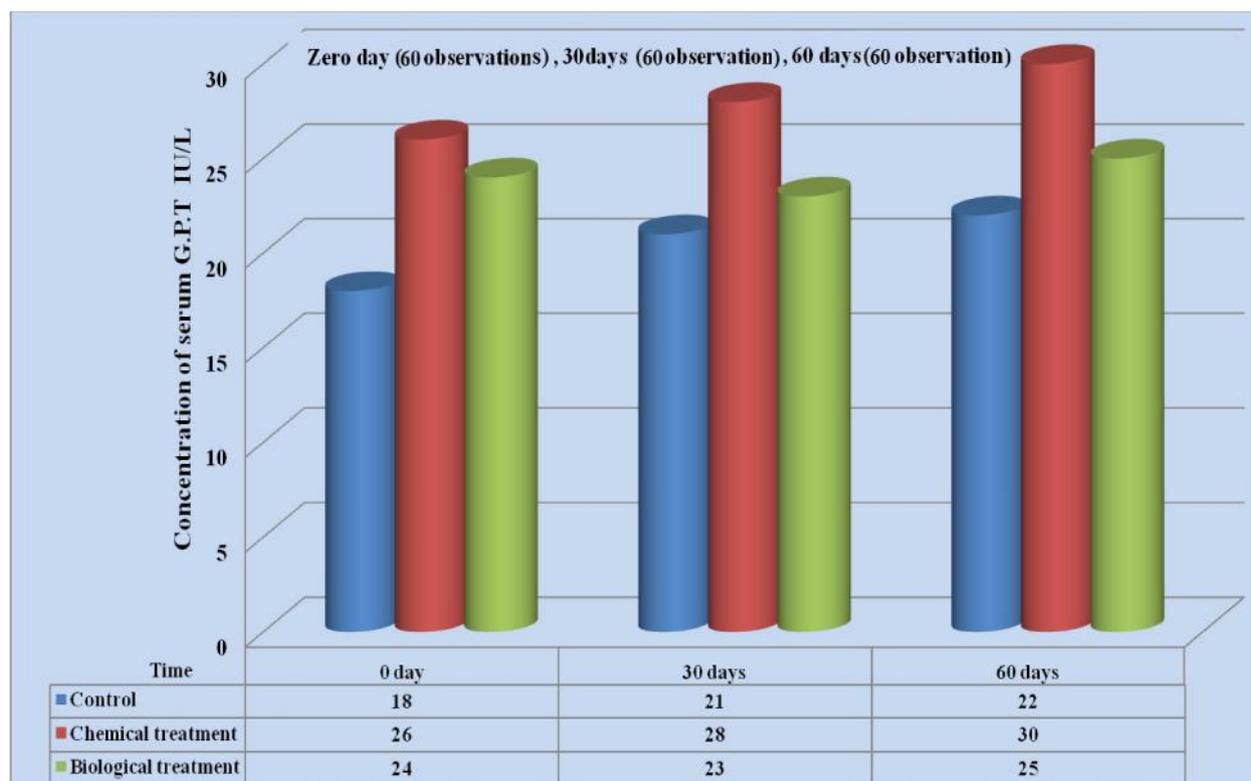


Figure 6. The diurnal patterns of glutamate pyruvate transaminase concentration (mean of zero-time, 30 and 60 days post experiment period) during 24 hrs. after morning feeding

SUN concentration: Data presented in Table (2) for the main effects and in Figure (2) for day zero, at the 30 and 60 days post experiment period were pointed out to, ($P < 0.05$) differences among treatments in favor of chemical treatment which recorded the highest ($P < 0.01$) urea-N concentration in comparison with the other treatments. However, differences between FTS and control group were not statistically significant. The urea-N level ranged from 10 to 25 mg/dl (Figure 2). SUN concentrations were significantly ($P < 0.05$) different among different times of sampling; all the experimental lambs shown significantly ($P < 0.05$) higher SUN concentrations (66.6 mg/dl) at the 30 days post experiment period as compared with day zero and 60 days post experiment period (see figure 2). Rakha, (1985) reported that the normal urea-N level in sheep and goats was ranged from 8 to 40 mg/dl. Changes in serum urea would reflect changes in criminal ammonia-N concentration (Fouda, 2008). Such results may favored urea as a good soluble degradable NPN source which led to enrich animal rumen media with an abundance N released. Thus the excess $\text{NH}_3\text{-N}$ which was more than the ability of microflora to synthesis to microbial protein is being withdrawn via the portal vein to animal livers and in turn raised urea concentrations in serum blood of experimental animals (Hassan and Hassan, 2010b; Hassan et al., 2009b). Our results were in accordance with those of Lewis (1957) who demonstrated that increasing the concentration of plasma urea after feeding was caused by the increasing of ruminal ammonia. Similarly, Yadav and Yadav

(1988) found that, cattle given ration containing urea treated straw showed higher serum urea concentration values than those fed untreated paddy straw. Moreover, blood urea N analyses can be used as a signal red to point out potential problem in the feeding program. The BUN level in excess of 18 to 20 mg/dl can be associated with lower reproductive performance, higher feed costs, health problems, and poor production (Hansen, 2003). Finally, the obtained results indicated that the urea and fungal treated straw had no adverse effect on liver or kidney function. Similar results were reported by Svozil et al., (1989) when the cellulolytic preparation was used in nutrition of lambs.

STP concentration: Data presented in Table (2) for the main effects and in Figure (3) for day zero, at the 30 and 60 days post experiment period were pointed out to a significant ($P < 0.01$) differences among treatments in favor of fungal treatments (T3) which recorded the highest ($P < 0.01$) total protein concentration (4.20 mg/dl) in comparison with the other treatments (2.78 and 3.91 mg/dl in T1 and T2 respectively). Differences between FTS and urea treatment groups were not statistically significant. Total protein values on, 30 and 60 days post experiment were not significantly different among treatments, but revealed slightly higher total protein concentration as compared with day zero. This matter may point out to the positive associative influences of ration total protein content due to both of chemical substances (urea, T2) or fungus (T3). Matching of blood total protein values with the corresponding values of nitrogen intake, SUN and

IVOMD reflected to somehow a positive interaction and correlation ship between both of dietary NI, SUN and serum total protein concentration. Hence, total blood protein may be accounted as a biochemical indicator of experimental animals' physiological state. Higher serum total protein showed by treated bean straw with some fungus strains indicated better utilization of dietary protein and ruminal true protein-N through the digestive tract (Recce, 1991). However, higher total protein values (6-9 g/dl) were reported by Smith et al., (1979), and Hassan et al., (2009b) when animals fed poultry excreta vs. cottonseed meal and reed silage respectively.

SC concentration: Cholesterol values are presented in Table (2) for the main effects and in Figure (4) for day zero, at the 30 and 60 days post experiment period indicate a significant ($P<0.05$) differences among treatments in favor of urea and fungi treatments which was the lowest ($P<0.05$) in comparison with the control group (untreated). The differences between UTS and FTS groups were not statistically significant. The mean concentration of cholesterol (mg/dl) in control group for three time periods ranged from 51.9 to 61.5 compared with 38.5 to 47.6 and 41.8 to 48.9 in UST and FTS groups, respectively. Cholesterol values obtained in the present study were within the normal ranges obtained by Hassan et al., (2009 and 2010) using Awassi and Karadi lambs similar results was reported by Fayed (2009) who found that biological treatment by white fungi was significantly reduced blood cholesterol of lambs fed white fungi treated diet as compared with those fed control diet. Also he concluded that this finding could be attributed to high tannin and saponin contents. Morehouse et al., (1999) found that a number of synthetic saponins have been shown to be cholesterol absorption inhibitors causing reduction in plasma non high-density, lipoprotein cholesterol fraction. Digestibility of fats in ruminants is limited by the lack of emulsifying agents in the rumen (Cheeke, 1999). Other suggested mechanisms of action of saponins include delaying the intestinal absorption of dietary fat by inhibiting pancreatic lipase activity (Han et al., 2000). Although, Fouad et al., (1998), Kholif et al., (2000), Abo-Eid et al., (2007), Fouda (2008) in sheep and Mohamed and Abou-Zeina (2008) in goat kids when fed fungi and urea treatments for low quality roughages obtained higher concentration of cholesterol.

GOT and GPT concentrations: It appears from Table (2) and Figure 5 and 6 that GOT and GPT assessed in blood serum, as an indicators of liver function showed a significant differences among different experimental groups. However, higher ($P<0.05$) GOT and GPT values were assessed for urea group (103 and 28 IU/L, mean of three periods), respectively. Fungi treatment had an intermediate values for GOT and GPT (86 and 24 IU/L.) respectively. On contrast, the control groups recorded the lowest ($P<0.05$) GOT and GPT values, (65 and 20 IU/L) but with significant difference with fungal group (T2), indicating healthy liver functions for such groups. It

could be noticed that, GOT levels were higher than those of GPT for all experimental rations. In general, the values recorded for GOT and GPT are within the normal range reported by Mohamed and Abou-Zeina (2008) in goats' kids. Changes in serum urea would reflect changes in ammonia-N concentration than higher liver activity lead more ammonia cycling to the rumen and increased urinary -N (Table 2). Abd-El-Kareem (1990) and Fouda (2008) found, values ranged from 24 to 65 and 14 to 37 IU/L for GOT and GPT, respectively, in goats and sheep. Several reasons might be explain the higher GOT and GPT values assessed for urea group. First, treated straw with high level of urea (7%) as compared with above references (4-5%). Secondly, this experiment was carried out during the summer season ($45-50^{\circ}\text{C}$) and under heat stress. Finally, the age, body weight and breed of lambs used in this experiment and fed such treatments need more adaptation and feeding practices. All of these speculations were reported by Boots et al., (1969).

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