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EFFECT OF BIOLOGICALLY TREATED SUGAR BEET PULP ON CHEMICAL COMPOSITION, NUTRIENTS DISAPPEARANCE, DIGESTIBILITY, RUMEN FERMENTATION, RUMEN MICROBES AND SOME BLOOD COMPOSITION IN ADULT SHEEP

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ABSTRACT

In this study three experiments were conducted, the 1st (a laboratory experiment) included nine treatments (T1-T9) to study the effect of using biological treatments (fungal, bacterial, yeast or yeast combined with fungi or bacteria) on chemical composition and fiber constituents of sugar beet pulp (SBP) to choose the best biological treatments for testing in the 2nd experiment (*in vitro* experiment). In the 2nd experiment, seven rations containing the best five biologically treated SBP as well as control and untreated SPB rations were used to study the effect of the experimental rations on *in vitro* chemical composition and nutrients disappearance. These rations included R1 (control): concentrate feed mixture (CFM) + berseem hay (BH); T2: CFM + untreated SBP+ BH; T3: CFM + SBP treated with *S. cerevisiae*+ BH; T5: CFM + SBP treated with *T. viride* + BH; T5: CFM + SBP treated with *T. viride* + *S. cerevisiae* + BH; T6: CFM + SBP treated with *C. cellulasea* + BH, and T7: CFM + SBP treated with *C. cellulasea* + *S. cerevisiae* + BH. In the 3rd experiment (digestibility experiment) was carried out to study the effect of feeding the same rations on digestibility coefficients, rumen fermentations parameters, microbial protein, protozoal count, number of total bacteria and cellulolytic bacteria and some blood parameters of adult rams. Results revealed that biological treatments increased (P<0.05) CP content and decreased CF, NDF, ADF, ADL content. Digestibility coefficients, concentrations of total volatile fatty acids (TVFA's), total nitrogen, true protein, microbial protein and microbial count increased (P<0.05), nitrogen and water balances improved (P<0.05) in biologically treated SBP as compared to control and untreated SBP rations.

***Keywords**:* Biological treatment, sugar beet pulp, in vivo and in vitro digestibility.

INTRODUCTION

The shortage of feeds in general attracted the attention of many research workers to use agro-industrial by-products Such as sugar beet pulp (SBP), which is the remaining residues after extraction of sugar beet tubers. This residue comprises 6% of the total fresh weight of harvested sugar beet (Kjaergaard, 1984). A high proportion of SBP is dried and frequently beet molasses is added before drying. Also, it is available in the local market in a dry unmolassed cubes and it is usually used as an energy source feedstuff for ruminants.

In Egypt there is a developing tendency to increase the sugar production from beet since 1982. The annual amounts of SBP are about 385686 ton (SMA, 2011).

Dried beet pulp is a carbohydrate rich by-product. The protein content of SBP is considered low compared with the requirements of most ruminants and monogastric animals (Israilides *et al*., 1994). The crude fiber content of beet pulp is considerably high and the content of fast fermentable

carbohydrates and ether extract are much lower than those of high energy grains (Haaksma, 1982). The cellulose structure of SBP is mainly amorphous, which make it easily hydrolysable (Kjaergaard, 1984) and its pectin content is not covalently linked to a lignified matrix, which make it available source of readily fermentable carbohydrate to enhance the microbial biosynthesis in the rumen (Mansfield *et al*., 1994).

The impact of feeding dried SBP on rumen fermentation was investigated by many studies (Mansfield *et al*., 1994; Chikunya *et al*., 1996; Molina *et al*., 2000), however, the results did not show clear trend and they were contradictory. On the other hand, no available data on the effect of feeding beet pulp on rumen microbial population and microbial enzymatic activity.

The present work aims to evaluate different biological treatments of SBP in terms of *laboratory* chemical composition and cell wall constituents (1st experiment), to study the effect of the best biological treatments on *in vitro* chemical composition and nutrient disappearance of rations containing SBP (2nd experiment), and digestibility coefficients, nitrogen and water balances and ruminal and blood parameters of sheep (3rd experiment).

MATERIALS AND METHODS

The field experiments were carried out at Maryout Research Station, Desert Research Center, located 35 km southwest of Alexandria, Egypt.

1st experiment: "Laboratory study"

This study was designed to evaluate the effect of using various biological treatments (fungal, bacterial, yeast or yeast combined with fungi or bacteria) on chemical composition and fiber constituents of SBP to obtain the best biological treatments for *in vitro* and *in vivo* studies. The used biological treatments were obtained from the Microbial Genetic Department, National Research Center, Dokki, Cairo, Egypt. The microorganisms were maintained on agar medium composed of (g/l) yeast extract, 3.0; malt extract, 30; peptone, 5.0; sucrose 20 and agar 20. The biological treatments included SBP inoculated with *Sacharomyces cerevisiae* (T1), *Trichoderma viride* (T2), *T. viride* + *S. cerevisiae* (T3), *Asarglusorsa* (T4), *Asarglusorsa* + *S. cerevisiae* (T5), *Cellulomonas cellulasea* (T6), *C. cellulasea* + *S. cerevisiae* (T7), *Acetobacter xylinum* (T8) and *A. xylinum* + *S. cerevisiae* (T9).

An amount of 200 g of air-dried sugar beet pulp moistened to 60% and treated with the treatments was incubation for 14 days at 30 ±2 ºC for each treatment and the ratio between the combined microorganisms was 1:1 with a final moisture content of 60%. Moisture was kept at 60% and at the end of the inoculation period, samples were oven dried at 70 ºC. Product recovery rate (PRR) was calculated according to Nigam (1994).

2nd experiment:"In vitro study"

This experiment was designed according to the best biological treatment of SBP (fungal, bacterial, yeast or yeast combined with fungi or bacteria), based on their chemical compositions and fiber constituents (1st experiment) to study the effect of these treatments on chemical composition,

cell wall constituents, and *in vitro* nutrient disappearance of ration including treated or untreated SBP . Seven rations were prepared as follow:

R1 (control): Concentrate feed mixture (CFM) + berseem hay (BH).
R2: CFM + untreated SBP+ BH.
R3: CFM + SBP treated with *S. cerevisiae* + BH.
R4: CFM + SBP treated with *T. viride* + BH.
R5: CFM + SBP treated with *T. viride* + *S. cerevisiae* + BH.
R6 CFM + SBP treated with *C. cellulasea* + BH.
R7: CFM + SBP treated with *C. cellulasea* + *S. cerevisiae* + BH.

The ratio of CFM to SBP and BH was 30:30:40% in all treatments.

Ruminal liquor was collected, two hours post feeding from six adult rams fed CFM and good quality BH. Collected ruminal liquor was kept warm in plastic Jug (35-37 ºC), strained through two layers of cheese cloth and mixed with urea-buffer under the lab conditions for *in vitro* studies. The ruminal liquor with the samples of the seven rations, in two replicates for each sample, was incubated for 24 hours to estimate dry matter, organic matter and other nutrients disappearance according to the method described by Terry *et al*. (1969), modified by Norris (1976).

3rd experiment: ″Digestibility study:

The objective of this experiment was to study the effect of feeding the same rations of the 2nd experiment on digestibility coefficients, rumen fermentations parameters, microbial protein, counts of protozoa, total bacteria, and cellulolytic bacteria, and blood parameters of adult rams.

This experiment lasted 50 days. Twenty eight adult rams were divided into 7 groups (four animals for each) were 7 experimental rations for a month as a palatability and adaptation period for treatments. Then rams were placed in metabolic cages, weighed at the start and the end of the trial. The trial lasted for 20 days from which the first 15 days were considered as an adaptation and preliminary period, followed by 5 days as collection period. Over the collection period, daily amount of feed consumed, residuals, feces, urine and drinking water were individually recorded.

Analytical procedures:

Proximate chemical and cell wall constituents analyses:

The proximate chemical analysis of the experimental rations was carried out according to the A.O.A.C. (1990) to determine DM, CP, CF and EE, while NFE was obtained by the difference. Also, NDF, ADF and ADL were determined according to the procedures of Van Soest *et al*. (1991). However, cellulose and hemicelluloses were calculated by the difference between NDF and ADF for hemicelluloses, and between ADF and ADL for cellulose.

Rumen liquor parameters:

Rumen liquor (RL) samples were obtained at 0, 3 and 6 hours post feeding. In RL, ruminal pH value was immediately measured with pH meter, while concentrations of ammonia nitrogen, total nitrogen and non-protein nitrogen were determined by the modified semi-micro-kjeldahl digestion method according to A.O.A.C (1990). However, true protein nitrogen concentration was calculated by subtracting the non-protein nitrogen content

from total nitrogen content. Concentration of total volatile fatty acids (TVFA's) was determined according to Warner (1964).

In addition, count of ruminal ciliate protozoa (Ogimoto and Imai, 1981). Identification of genera and species was according to the description published by Dehority (1993). Dilution series were prepared under O_2 –free CO_2 by the anaerobic method of Bryant (1972) using the anaerobic diluents described by Mann (1968) to determine count of total bacteria and cellulolytic bacteria.

At the end of digestibility trails, blood serum samples were collected pre-feeding and 4 h post-feeding to determine the concentration of total proteins, albumin, and urea as well as activity of AST and ALT using commercial kits. However, concentration of globulin was obtained by the difference between total protein and albumin.

Statistical analysis:

Data was statistically analyzed according to statistical analysis system of SAS (2000). Data of chemical composition, cell wall constituents analysis, nutrient disappearance, digestibility coefficients, nitrogen balance and water balance were analyzed by one-way analysis and the model was:

$$Yij = M + Ti + eij$$

The used model for rumen fermentation parameters and microbial count was two-way analysisas follows: $Y_{ij} = \mu + T_i + l_j + Tl_{ij} + e_{ij}$

Where: Y_{ij} = experimental observation, μ = general mean, T_i = effect of treatment (i =1-7 rations), l_j = effect of sampling time (j=0, 3 and 6 h), Tl_{ij}=effect of interaction between treatment or ration, and sampling time and e_{ij} = random error. Separation among means was carried out using Duncan's multiple test (Duncan, 1955).

RESULTS AND DISCUSSION

1st experiment: "Laboratory study"

Chemical and cell wall constituents analyses of various treatments:

Data presented in Tables (1 and 2) indicated significant difference among treatments on chemical composition and cell wall constituents. Results presented in Table (1) showed that T3 had the highest (P<0.05) DM, OM, EE and CP contents, followed by T7, T1, T2 and T6, respectively. However, the highest (P<0.05) values of CF content was for T4 and T8, and the lowest (P<0.05) content was for T3, followed by T7 and T9.

Data of Table (2) showed that NDF, ADF, ADL, cellulose and hemicelluloses decreased (P<0.05) to the lowest values in T7 as compared to other treatments, followed by T9, while T4 showed the highest (P<0.05) values, followed by T8 and T5, respectively. The best (P<0.05) product recovery rate was for T3 (45.07%), followed by T7 (47.42%), while the lowest (P<0.05) rate was for T4 (61.37%), followed by T8 (60.11%).

Table (1): Effect of various biological treatments on chemical composition of sugar beet pulp.

Treatment	DM	Chemical composition (%)					
		OM	Ash	EE	CP	CF	NFE
T1	93.02^{b}	92.93^{b}	7.06^{e}	2.22^{c}	20.83^{d}	19.95^{e}	50.05^{h}
T2	92.85^{c}	92.75^{cd}	7.25^{cd}	2.12^{d}	20.27^{e}	19.99^{ed}	50.48^{g}
T3	93.44^{a}	93.10^{a}	6.89^{f}	2.68^{a}	22.33^{a}	17.27^{h}	50.92^{e}
T4	92.17^{g}	92.07^{f}	7.92^{a}	1.79^{f}	16.89^{i}	21.07^{a}	52.42^{b}
T5	92.45^{f}	92.55^{e}	7.45^{b}	1.89^{e}	17.25^{h}	20.07^{c}	53.48^{a}
T6	92.81^{cd}	92.90^{b}	7.09^{e}	2.11^{d}	20.15^{f}	20.06^{cd}	50.75^{f}
T7	93.01^{b}	92.91^{b}	7.09^{e}	2.36^{b}	21.87^{b}	17.46^{g}	51.36^{d}
T8	92.63^{e}	92.72^{d}	7.27^{c}	1.93^{e}	19.07^{g}	20.19^{b}	51.65^{c}
T9	92.74^{d}	92.82^{c}	7.18^{d}	2.23^{c}	21.15^{c}	18.65^{f}	50.93^{e}
±MSE	0.025	0.025	0.025	0.016	0.021	0.023	0.022

Means with different litters with each column are significantly different (P<0.05).T1: SBP with *S. cerevisiae*. T2: SBP with *T. viride*. T3: SBP with *T. viride* + *S. cerevisiae*.
T4: SBP with *A. orsa*. T5: SBP with *A. orsa* + *S. cerevisiae*. T6: SBP with *C. cellulasea*.
T7: SBP with *C. cellulasea* + *S. cerevisiae*. T8: SBP with *A. xylinum*.
T9: SBP with *A. xylinum* + *S. cerevisiae*.

Table (2): Effect of various biological treatments on cell wall constituents and product recovery (%) of sugar beet pulp.

Treat.	Cell wall constituent					Product Recovery (%)
	NDF	ADF	ADL	Cellulose	Hemicellulose	
T1	54.02^{f}	24.37^{e}	1.94^{e}	29.64^{b}	22.43^{c}	55.12^{e}
T2	54.15^{e}	24.97^{d}	2.02^{d}	29.18^{c}	22.95^{b}	57.68^{d}
T3	50.05^{i}	22.65^{g}	1.82^{f}	27.40^{e}	20.83^{e}	45.07^{i}
T4	56.37^{a}	26.30^{a}	2.36^{a}	30.07^{a}	23.94^{a}	61.37^{a}
T5	54.48^{c}	25.25^{c}	2.28^{b}	29.23^{c}	22.97^{b}	50.82^{f}
T6	54.25^{d}	25.08^{cd}	2.17^{c}	29.17^{c}	22.90^{b}	58.40^{c}
T7	50.42^{h}	22.42^{h}	1.86^{f}	28.00^{d}	20.56^{f}	45.60^{h}
T8	55.25^{b}	25.96^{b}	2.19^{c}	29.29^{c}	23.77^{a}	60.11^{b}
T9	51.98^{g}	23.91^{f}	1.93^{e}	28.07^{d}	21.98^{d}	47.32^{g}
±MSE	0.022	0.062	0.015	0.061	0.053	0.036

Means with different litters with each column are significantly different (P<0.05).

Similar results were obtained by El-Ashry *et al*. (2002 and 2003) and Kholif *et al*. (2005), who indicated that the fungal treatment led to increase CP and decreased CF and OM contents. Based on these results, six treatments beside control were used in the following in *vitro* and *in vivo* studies.

2nd treatment: "*In vitro* study"

Chemical composition and cell wall constituents:

Data presented in Table (3) revealed significant (P<0.05) effect of treatment on chemical composition and cell wall constituents. All biological treatments of SBP increased DM, OM, EE and CP contents as compared to untreated SBP; the highest (P<0.05) contents were in R5, followed by R7. It

is important to show that all biological treatments that showed marked increase (P<0.05) in CP content and pronounced decrease (P<0.05) in CF, NDF, ADF, ADL, cellulose and hemicellulose contents as compared to ration containing untreated SBP (R2). Similar results were recorded by Israilides *et al*. (1994), who found that CP content of beet pulp was increased from 9.96 to 19.50% by fungal treatments. Also, Abedo *et al*. (2005) found that fungal treatment with *Trichoderm aressei* increased the CP content of SBP from 9.94 to 19.37% and ether extract from 0.64 to 0.88%. While CF, ADF, ADL and cellulose contents increased and NDF and hemicellulose were decreased by fungal treatment.

Table (3): Chemical composition and cell wall constituents of rations containing biologically treated sugar beet pulp during *in vitro* study.

Item	R1	R2	R3	R4	R5	R6	R7	±MSE
DM (%)	92.80^{a}	89.02^{g}	90.72^{d}	90.05^{e}	91.75^{b}	89.94^{f}	91.40^{c}	0.021
Chemical composition (%):								
OM	88.19^{c}	86.44^{g}	88.04^{d}	87.49^{f}	90.45^{a}	87.74^{e}	90.00^{b}	0.021
CP	13.36^{f}	10.26^{g}	17.28^{c}	16.68^{d}	18.67^{a}	16.47^{e}	18.15^{b}	0.011
CF	20.06^{c}	23.40^{a}	20.06^{c}	20.93^{b}	18.26^{f}	19.77^{d}	18.66^{e}	0.011
EE	2.09^{f}	2.55^{e}	2.88^{c}	2.85^{c}	3.88^{a}	2.69^{d}	3.66^{b}	0.018
NFE	52.83^{a}	50.41^{b}	47.97f	47.25^{g}	49.78^{c}	48.95^{e}	49.70^{d}	0.011
Ash	11.80^{e}	13.55^{a}	11.96^{d}	12.51^{b}	9.55^{g}	12.25^{c}	10.00^{f}	0.021
Cell wall constituents (%):								
NDF	54.23^{f}	71.95^{a}	63.45^{c}	64.15^{b}	56.48^{e}	62.36^{d}	54.26^{f}	0.011
ADF	25.08^{g}	43.31^{a}	38.16^{d}	40.07^{b}	32.66^{e}	39.30^{c}	31.32^{f}	0.027
ADL	2.17^{f}	6.98^{a}	5.46^{c}	5.86^{b}	4.87^{d}	5.47^{c}	4.21^{e}	0.016
CS	29.15^{a}	28.64^{b}	25.28^{c}	24.07^{d}	23.82^{e}	23.06^{f}	22.93^{g}	0.031
HCS	22.90^{g}	36.33^{a}	32.70^{d}	34.21^{b}	27.78^{e}	33.83^{c}	27.11^{f}	0.029

Means with different litters with each row are significantly different (P<0.05).
R1 (control): CFM+BH. R2: CFM + untreated SBP+BH. R3: CFM+BH+SBP treated with *S. cerevisiae*. R4: CFM+BH+SBP treated with *T. viride*. R5: CFM+BH+SBP treated with *T. viride* + *S. cerevisiae*. R6: CFM+BH+SBP treated with *C. cellulasea*. R7: CFM+BH+SBP treated with *C. cellulasea*+*S. cerevisiae*. CS: Cellulose. HCS: Hemicellulose.

Nutrient disappearance:

Data in Table (4) revealed a significant (P<0.05) differences in nutrient and cell wall constituents between different experimental rations. It is worthy noting that biological treatments of SBP increased (P<0.05) disappearance of DM, OM, EE, CP, CF, NFE, NDF, ADF, ADL, cellulose and hemicellulose as compared to untreated SBP and control. In this respect, R5 had the highest disappearance of DM, OM, EE, CP, NFE, NDF and cellulose, while R7 had the highest disappearance of CF, ADF, ADL and hemicellulose.

Results revealed also that combination of yeast with fungi or bacteria enhanced the disappearance of most nutrients as compared to each one alone. Similar results were obtained by El-Ashry *et al*. (2003), who reported that biological treatment of poor quality roughages by *T. viride, Pencillium funiculosium and S. cerevisiae* increased DM and OM *in vitro* disappearance. Also, Colombatto *et al*. (2003) found that fibrolytic enzymes secreted by

cellulolytic bacteria enhanced the fermentation of cellulose and xylan. Moreover, Gado and Abd El-Galil (2009) showed that cellulolytic bacteria strains isolated from sheep was more effective in increased the *in vitro* DM disappearance because these active strains were secreted cellulase enzymes most effective on roughage than other strains.

Table (4): Nutrient disappearance (%) of ration containing biologically treated sugar beet pulp during *in vitro* study.

Item	Treatment							±MSE
	R1	R2	R3	R4	R5	R6	R7	
DM	63.68^{f}	61.22^{g}	69.43^{d}	70.20^{c}	80.89^{a}	66.84^{e}	79.88^{b}	0.010
Chemical composition (%):								
OM	64.25^{f}	64.38^{f}	69.97^{e}	71.75^{d}	84.41^{a}	73.70^{c}	77.31^{b}	0.073
EE	50.27^{e}	56.10^{d}	65.44^{c}	63.63^{c}	79.90^{a}	63.08^{c}	76.58^{b}	10.78
CP	78.44^{g}	85.42^{d}	87.62^{c}	84.57^{f}	90.65^{a}	85.20^{e}	89.05^{b}	0.022
CF	69.80^{f}	55.06^{g}	71.79^{d}	71.19^{e}	83.09^{b}	75.98^{c}	85.55^{a}	0.024
NFE	59.31^{f}	64.80^{d}	63.52^{e}	67.91^{c}	82.87^{a}	69.50^{b}	69.94^{b}	0.167
Cell wall constituents (%):								
NDF	67.86^{f}	64.96^{g}	75.71^{e}	76.51^{d}	83.18^{a}	76.86^{c}	81.41^{b}	0.025
ADF	75.58^{f}	69.04^{g}	76.75^{e}	77.57^{d}	81.32^{b}	80.01^{c}	87.85^{a}	0.017
ADL	53.54^{f}	52.40^{g}	67.76^{d}	66.56^{e}	84.59^{b}	69.40^{c}	86.30^{a}	0.022
CS	61.31^{f}	58.84^{g}	74.07^{c}	74.63^{b}	85.80^{a}	71.59^{e}	72.65^{d}	0.029
HCS	77.63^{f}	72.21^{g}	78.21^{e}	78.50^{d}	84.40^{b}	81.74^{c}	88.07^{a}	0.022

Means with different litters with each row are significantly different (P<0.05).
CS: Cellulose. HCS: Hemicellulose.

3rd experiment: "Digestibility study"

Chemical composition and cell wall constituents:

Data in Tables (5 & 6) indicated the same trend of chemical composition and cell wall constituents of the experimental rations as obtained in the 2nd study. The control ration (R1) showed the highest (P<0.05) contents of DM, OM, EE and NFE as compared to untreated or treated SBP rations. However, contents of DM, EE and CP were higher (P<0.05) in all biological treated rations than in untreated SBP ration (R2). On the other hand, contents of OM, CF, NDF, ADF, ADL, cellulose and hemicellulose decreased (P<0.05) in treated SBP rations more than in untreated one. Generally, R5 and R7 had the highest content of CP and the lowest content of CF and its fraction. The increased CP content by biological treatments may be due to the increase in rumen microorganisms (protozoa and bacteria), which are consume CP of the diet to convert it into microbial protein. While, the observed decrease of CF content by progressed time of incubation may be due to the microbial digestion by cellulolytic bacteria which secreted cellulase enzymes to degrade crude fiber, or due to the utilization of CF by fungi for their growth since fungi among the microorganisms have been proved its capability in decomposing the agricultural by-products as several strains of fungi were used by many researchers for lignocellulosic hydrolyses such as *Aspergillusniger*, *Funsarium moniliforme* and *Trichoderma viride*.

The present results are in agreement with Allam *et al*. (2006), who found that replacing 100% of corn grains in the CFM of lambs by SBP treated with *Trichoderma viride* and *Sacharomyces cerevisiae* decreased contents of OM, CF, hemicellulose, cellulose, lignin and pectin, while CP and true protein contents were increased as compared to control ration. Also, El-Badawi *et al*. (2007) reported that SBP treated with *T. ressei* decreased OM content and increased CP content.

Table (5): Effect of treatments on chemical composition during digestibility trails.

Item	DM (%)	Chemical composition (%)					
		OM	Ash	CP	CF	EE	NFE
Ration:							
R1	93.80^{a}	91.96^{a}	8.04^{g}	12.51^{b}	11.37^{d}	3.15^{a}	65.11^{a}
R2	89.07^{g}	90.69^{b}	9.31^{f}	10.88^{b}	17.90^{a}	2.17^{d}	59.91^{b}
R3	92.75^{d}	89.00^{e}	10.99^{c}	19.04^{a}	17.18^{ab}	2.49^{c}	50.51^{c}
R4	92.36^{e}	89.75^{d}	10.25^{d}	16.40^{a}	15.69^{bc}	2.61^{c}	55.23^{bc}
R5	93.62^{b}	87.04^{g}	12.96^{a}	17.45^{a}	14.30^{c}	2.92^{ab}	52.56^{c}
R6	91.99^{f}	89.93^{c}	10.06^{e}	16.35^{a}	15.69^{bc}	2.62^{c}	55.46^{bc}
R7	93.39^{c}	88.82^{f}	11.18^{b}	17.20^{a}	14.39^{c}	2.75^{cb}	54.65^{c}
±MSE	0.020	0.007	0.007	0.883	0.576	0.073	1.396
Feedstuff:							
CFM	93.80	92.00	8.00	12.49	11.32	3.10	65.09
Hay	91.24	88.01	11.99	14.00	26.61	2.55	44.85
USBP	91.10	95.60	4.40	9.20	24.40	1.18	60.82

Means with different litters with each column are significantly different (P<0.05). CFM: Concentrte feed mixure. USBP: Untreated SBP.

Table (6): Effect of treatments on cell wall constituents during digestibility experiment.

Item	Cell wall constituents (%)				
	NDF	ADF	ADL	Cellulose	Hemicellulose
Ration:					
R1	31.01^{f}	17.80^{g}	4.89^{a}	13.20^{f}	12.91^{f}
R2	47.61^{a}	24.18^{a}	4.16^{b}	23.43^{a}	20.02^{a}
R3	42.53^{c}	21.07^{d}	3.45^{e}	21.46^{b}	17.63^{c}
R4	42.59^{b}	21.37^{c}	3.49^{d}	21.21^{c}	17.88^{b}
R5	40.55^{e}	20.20^{e}	3.36^{f}	20.35^{e}	16.83^{d}
R6	42.61^{b}	21.45^{b}	3.55^{c}	21.16^{c}	17.90^{b}
R7	40.71^{d}	20.10^{f}	3.38^{f}	20.61^{d}	16.72^{e}
±MSE	0.010	0.013	0.008	0.021	0.018
Feedstuff:					
CFM	30.98	17.75	4.86	13.23	12.89
Hay	62.96	44.44	7.13	18.52	37.31
USBP	60.42	29.05	2.84	31.37	26.21

Means with different litters with each column are significantly different (P<0.05). USBP: Untreated SBP.

Digestibility coefficients and nutritive values:

Data in Table (7) showed that biological treatments decreased ($P<0.05$) feed intake compared to untreated and control rations, being the lowest ($P<0.05$) for R5, followed by R7, but the differences among R7, R6, R3, and R4 were not significant. Conflicted results were obtained by several authors. In this line, Kholif *et al*. (2005) and Aziz (2009) reported that biological treatment slightly increased DM intake, while, Rode *et al*. (1999) and Yang *et al*. (1999) reported that fungal or enzymatic treatments did not alter DM intake.

Regarding the results of digestibility trails (Table 7), it seems that biological treatments, particularly in R5 and R7 significantly ($P<0.05$) increased digestibility coefficients of all nutrients and most cell wall constituents as compared to control and untreated rations. The improvement of DM digestibility in treated rations might be due to the better palatability of biologically treated SBP compared with untreated SBP and/or better utilization by the host animal. In this respect, Khampa *et al*. (2009) reported higher nutrient digestibilities as a result of yeast supplementation, which could be related to the microbial activities which solubilizing of carbohydrate esters of phenolic monomers in the cell wall. Also, Zadrazil (1984) mentioned that white rot fungi are able to increase the digestibility of plant residues without chemical and physical pretreatment through selective lignin degradation.

In addition, several authors observed an improvement in DM, CP and CF digestibility coefficients over a wide range of low quality roughages treated by biological treatments (Deraz and Ismail, 2001; Mahrous and Abou Ammou, 2005; Aziz 2009). Moreover, Allam *et al*. (2006) reported that SBP treated with *Trichoderma viride* and *Sacharomyces cerevisiae* increased DM, OM, CF and fiber fraction (NDF, ADF, cellulose and ADL) digestibilities, while CP and EE digestibility coefficients were not affected.

On the other hand, data of nutritive values (Table 7) showed significant ($P<0.05$) differences among treatments. Control ration (R1) showed the highest TDN (g/h/d, g/kg BW and g/kg $BW^{0.75}$), followed by untreated SBP ration (R2), but the differences among R2 and biologically treated SBP rations were not significant ($P\leq0.05$). Only R5 and R7 showed the highest ($P<0.05$) TDN% of DM intake as compared to untreated ration (R2), but did not differ from R1.

Data in Table (7) showed that R7 significantly ($P<0.05$) increased nutritive values of DCP (g/h/d, g/h/BW and g/kg $BW^{0.75}$) as compared to control and untreated SBP rations. However, nutritive value in term of DCP% of DMI was significantly ($P<0.05$) the highest for R5, followed by R7. However, the differences in metabolic energy (ME/g TDN) among the experimental rations were not significant.

Based on the foregoing results, biological treatments of SBP increased nutritive values (TDN and DCP). These improvements are associated with the increased digestion in fibrous materials particularly hemicellulose in addition to the increased bacterial digestion of cell wall content (Hassan *et al*., 2005). Also, these results reflected the values

obtained for rations digestibility which were higher for treated rations compared with the untreated rations

Similar results were obtained by Khorshed (2000); Hassan *et al*. (2005); Gado *et al*. (2006) and Aziz (2009), who reported that the nutritive value as TDN and DCP were significantly higher (P<0.05) in biologically treated agriculture by-products.

Nitrogen balance:

Data in Table (8) showed that biological treatments increased (P<0.05) nitrogen intake (NI) and digested nitrogen (DN) values (g/h/d) more than control and untreated SBP rations. The highest (P<0.05) NI and DN values (g/h/d) were recorded for R7, followed by R6 and R3 with insignificant differences. Although, the differences in NI and DN values as g/kg BW or g/kg $BW^{0.75}$ were not significant (P<0.05) among control and biologically treated rations, DN as a percentage of NI showed the same trend, whereas R5 had the highest (P<0.05) value (92.71%) of DN % of NI, followed by T7 (90.54%), while, the lowest one was for untreated SBP (72.51). Fecal and urinary nitrogen excretion (g/h/d, g/kg BW, g/kg $BW^{0.75}$ and % of NI) were the highest (P<0.05) for control, followed by untreated SBP ration.

Therefore, they also had the highest total nitrogen excretion values as g/h/d, g/kg BW, g/ kg $BW^{0.75}$, % of NI . While, R5 had the lowest value % of NI (27.38%). Biological treatments increased (P<0.05) nitrogen balance (g/h/d, g/kg BW, g/kg $BW^{0.75}$,% of NI and % of DN) more than control and untreated SBP, being the highest for R5 and R7.

It is clear that biological treatments of SBP increased nitrogen balance more than untreated USB and control rations containing 60% CFM and 40% BH. This improvement was attributed to less nitrogen excretion, the improvement in rumen fermentation especially ruminal ammonia, NPN, total nitrogen and true protein nitrogen. These results are in agreement with those obtained by Allam *et al*. (2006), who reported that biologically treated SBP with *Trichoderma viride* and *Sacharomyces cerevisiae* had the highest value of nitrogen balance and NB/IN.

7

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Water balance:

Data in Table (9) showed insignificant (P<0.05) differences in free drinking water and total water intake (ml/h/d or ml/Kg $W^{0.82}$), although R1 and R2 had the highest (P<0.05) values of combined and metabolic water (ml/h/d or ml/Kg $W^{0.82}$). Biological treatments decreased (P<0.05) urinary water, fecal water and total water execration (ml/h/d or ml/Kg $W^{0.82}$) more than R1 and R2. The lowest values were for R5, followed by R7. Water balance showed insignificant (P<0.05) differences among all treatments, although, biological treatments had slightly higher values. Both R5 followed by R7 had the highest water balance as a percentage of water intake, being 90.26and 89.00% of intake, respectively.

Subhash *et al*. (1991) reported that the values of water intake (liters/day) were varied between (3.17 and 4.15) for diets which contained paddy straw and fungal treated paddy straw.

Rumen parameters:

Data in Table (10) showed that biological treatments decreased (P<0.01) ruminal pH values and increased (P<0.05) total volatile fatty acids (TVFA's) ruminal liquor (RL) as compared to control and untreated rations. In this way, R, R5 showed the lowest pH (6.37, P<0.05) and the highest TVFA's concentration as compared to control and untreated SBP rations.

These results indicated the negative relationship between pH value and TVFA's concentration for each ration. Fouad (1991) concluded that the rumen pH in general decreased with increasing the TVFA's concentration in lambs rumen.

Results of molar proportions of individual TFVA's (%) (Table 10) showed that biological treatments significantly (P<0.05) increased molar percentage of acetic, propionic and butyric compared with control and untreated SBP rations. Also, R5 exhibited significantly (P<0.05) the highest values, followed by R7. While, untreated SBP showed the lowest values.

The overall means of TVFA’s concentration and molar proportions of acetic, propionic and butyric at the different sampling times were higher (P<0.05) 3 h post- than per-feeding, then significantly (P<0.05) decreased 6 h post-feeding.

Acetic to propionic ratio showed significant decrease (P<0.01) in biological treatments as compared to untreated SBP and control rations, being the highest in R2, followed by R2, and nearly similar in all biological treatments. Overall mean of acetic/propionic ratio showed the same trend of TVFA's at different sampling times. The present data indicated an increase in propionate production and low acetic/propionic ratio which means an increase in propionate production. Such increase is favorable in animal growth since propionate plays a very important role as a major precursor of hepatic gluconeogensis.

9

10

Total nitrogen, true protein nitrogen, non-protein nitrogen, ammonia nitrogen and microbial protein concentrations:

Data in Table (11) showed that biological treatments significantly (P<0.05) increased (P<0.05) total nitrogen (TN), true protein (TP), non-protein nitrogen (NPN), ammonia nitrogen (NH_3-N) and microbial protein (MP) concentrations in RL as compared to control and untreated SBP rations. Rams fed R5 showed significantly (P<0.05) the highest values of TN, TP, NPN, NH_3-N and MP concentrations, followed by R7, while the lowest one was for R2.

The overall means of all values showed an increase (P<0.01) 3 h post-feeding, then decreased (P<0.01) 6 h post-feeding. The increment in microbial protein by biological treatments is may be due to the improvement in microbial population. Microbial protein plays an important role as it analyzed by animal enzymes in the abomasum and small intestine to produce free amino acids which absorbed from the small intestine and used by the host animal (Aziz, 2009).

The present results of rumen parameters are in agreement with those obtained by Khorshed (2000); Gado *et al*. (2006); Abo-Eid *et al*. (2007) and Aziz (2009), who reported that biological treatment for by- products improved ruminal pH value, and concentration of TVFA's, NPN and NH_3-N. They also found that ruminal parameters were at minimum before feeding and increased to maximum level at 3 and decreased 6 h after feeding. Moreover, Chikunya *et al*. (1996) concluded that the microbial protein production was improved on rations containing SBP.

Ruminal microorganisms:

Data in Table (12) represented the identification of ruminal ciliate protozoa species and their density in the rumen liquor and total bacteria and cellulolytic bacteria numbers during all different sampling times. Seven genera with 13 species and 7 sub-species of ruminal protozoa were identified in ruminal liquor of sheep in this study.

These generas (genus) are *Entodinum spp*. [*E. simplex, E. caudatum, E. bursa, E. minimum and E. triacum*], *Dasytrachia rummantium*, *Isotrchia spp.* [*I. intestinalis and I. prostoma*], *Epidiniume caudatum,* Diplodinum *anisacanthum*, *Polyolastron multivesiculatum* and *Ophryoscolox spp.* [*O. caudatus and O. purkynjei*].

Results clearly showed that biological treatments significantly increased (P<0.01) total and differential numbers of ruminal ciliate protozoa ($x10^4$ cell/ml rumen liquor) more than control and untreated SBP rations. It is clear that R5 had the highest (P<0.01) values of total protozoa count (*Entodinum, Isotrchia, Dasytrachia* and *Epidinium spps.),* followed by R7. Meanwhile, *Polyolastron, Ophryoscolox* and Diplodinum *spps.* counts were higher in R7 more than other treatments, followed by R5. Total protozoa count range was 6.22-7.25 x 10^4cell/ml RL. It seems that the highest presence among all species was for *Entodinum spps.* as it ranged between 4.93-5.83 $x10^4$ cell/ml RL, followed by *Dasytrachia* and *Polyolastron spps*. Comparison among different sampling times indicated that protozoa count showed a decrease (P<0.01) at 3 h post-feeding then it showed the highest (P<0.01) numbers 6 h post feeding.

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The values obtained in this study considered as normal level in rumen (Hungate, 1966). The present results are in agreement with Ivan *et al*. (2000), who found that *Entodinium* was the most detrimental of ciliate protozoa species. Also, Aziz (2009) found that biological treatment of poor quality roughage increased total and differential numbers of ruminal protozoa. While, Mohsen *et al*. (1999) found no effect of feeding rations containing 25 or 50% SBP on protozoal count in RL of sheep.

As for total bacteria ($x10^8$ cell/ml rumen) and cellulolytic bacteria ($x10^6$ cell /ml rumen) numbers, biological treatments increased ($P<0.01$) their numbers more than control and untreated SBP rations. It seems that SBP treated with *Cellulomonas cellulasea* (R6) and *C. cellulasea* + *S. cerevisiae* (R7) had the highest ($P<0.01$) number of bacteria and cellulolytic bacteria, as R7 came in the first class, followed by R6 and then R5 (*T. viride* + *S. cerevisiae)* came in the third class.

Blood parameters:

Results shown in Table (13) revealed that biological treatments significantly increased ($P<0.01$) concentration of total proteins and albumin values (g/dl) as compared to control and untreated SBP, being the highest in serum of rams fed R5, followed by R7 . Meanwhile the lowest values were found for those fed R2. On the other hand, globulin concentration decreased ($P<0.05$) in rams fed R5 and R7, and increased ($P<0.05$) in R3, R4 and R6 as compared to control and untreated SBP rations.

Such results were reflected in the highest ($P<0.05$) albumin/globulin ratio only for R5 and R7 as compared to other rations. In addition, biological treatments, in particular for R5 decreased ($P<0.01$) serum urea values mg/dl as compared to untreated SBP and control rations.

It is of interest to note that biological treatments of SBP only in R5 and R7 significantly ($P<0.05$) decreased activity of serum AST and ALT as compared to untreated SBP in R2.

As affected by sampling time all blood parameters 4 h post-feeding was higher ($P<0.01$) than pre-feeding values.

These results showed that biological treatments of SBP did not cause any lesions in liver and kidney functions.

Similar results were obtained with biological treatments by Kholif *et al*. (2001) and Aziz (2009), who reported that biological treatment increased total proteins albumin and globulin concentrations, and decreased urea concentration, AST and ALT activities in blood serum.

CONCLUSION

It could be concluded that, inclusion of dried sugar beet pulp untreated or treated with biological treatments to replace a part of 30% of common concentrate feed mixture had remarkable improved influence on chemical composition and fiber fraction. Biological treatments decreased feed intake more than control and untreated sugar beet pulp groups which may be decreased feed costs, in the same time increased all nutrients digestibility coefficients. Also, improved nitrogen balance, increased ruminal TVFA's, total nitrogen, true protein and microbial protein, all these improvements will enhance animal performance.

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تـأثير تفـل بنجـر السـكر المعامـل بيولوجيـاً علـى التحليـل الكميـائى و اختفـاء المـواد الغذائيـة والهضـم وتخمـرات وميكروبـات الكـرش و بعـض مكونـات الـدم فـى الأغنـام البالغة

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تضـم هـذه الدراسـة ثـلاث تجـارب :١- تجربـة معمليـة تشـمل تسـعة معـاملات لدراسـة تـأثير المعـاملات البيولوجيـة (الفطـر، البكتريـا، الخميـرة أو بأتحـاد الخميـرة مـع الفطـر أو البكتريـا) علـى التحليل الكميـائى و مكونـات جدار الخليـةلتفل بنجـر السـكر لاختيـار أفضـل هذه المعـاملات لاستخدمها فـى تجـارب الهضـم المعملـى و تجـارب الهضـم.٢- تجـارب الهضـم المعملـى تشـمل سـبعة معـاملات بيولوجيـة لتفل بنجـر السـكر بالاضـافة إلـى مخلـوط المركـزات و دريـس البرسـيم لتقدير معـدل أختفـاء المـواد الغذائيـة معمليـاً. ٣- تجـارب الهضـم لدراسـة تـأثير نفس السـبع معـاملات السـابقة علـى معامل هضم المواد الغذائية و تخمرات الكرش و أعداد بروتـوزوا الكرش و العدد الكلـى للبكتريـا و البكتريـا المحللة للسليلوز و بعض مكونات الدم.و قد أظهـرت النتـائج أن المعـاملات البيولوجيـة أدت إلـى زيـادة معنويـة فـى محتـوى البـروتين الخـام ونقص فـى محتـوى الألياف الخـام و مكوناتهـا. و قد زاد معامل هضـم المـواد الغذائيـة فـى تفـل سـكر البنجـر المعامـل زيـادة معنويـة. كماتحسـن ميـزان النيتـروجين و المـاء،و زاد معنويـاً كـلا مـن الاحمـاض الدهنيـة الطيـارة و النيتـروجين الكلـى و البـروتين الحقيقـى و البـروتين الميكروبـى بـالكرش وأعـداد ميكروبـات الكـرش فـى المجـاميع المعاملـة.

Table (7): Effect of treatments on nutrient digestibility and nutritive value of the experimental rations.

Item	R1	R2	R3	R4	R5	R6	R7	±MSE
Number of animals	4	4	4	4	4	4	4	-
Live body weight	33.18	33.06	33.43	33.52	33.31	33.01	33.33	1.36
Fee intake g/h/d	1321.36^{a}	1132.28^{b}	1052.94^{c}	1009.01^{c}	917.76^{d}	1076.28^{bc}	1034.51^{c}	21.28
Digestibility%:								
DM	79.50^{b}	77.54^{c}	78.72^{bc}	78.82^{bc}	85.29^{a}	79.72^{b}	83.90^{a}	0.602
OM	80.10^{c}	78.89^{d}	79.77^{cd}	79.92^{c}	86.23^{a}	80.70^{c}	84.79^{b}	0.312
EE	87.34^{bc}	86.34^{c}	86.88^{bc}	87.19^{bc}	90.58^{a}	85.48^{c}	88.60^{b}	0.648
CP	83.47^{d}	78.37^{e}	89.33^{bc}	88.92^{c}	92.13^{a}	89.05^{c}	91.25^{ab}	0.647
CF	60.85^{d}	66.10^{c}	67.15^{c}	66.83^{c}	75.91^{a}	70.69^{b}	75.25^{a}	0.515
NFE	82.74^{b}	81.41^{bc}	80.11^{c}	80.18^{c}	86.06^{a}	80.76^{c}	85.52^{a}	0.503
Fiber fractions:								
NDF	58.06^{c}	72.41^{b}	76.56^{b}	77.32^{b}	82.43^{a}	77.37^{b}	82.48^{a}	0.653
ADF	47.41^{e}	63.49^{c}	61.03^{d}	62.51^{c}	74.07^{a}	65.50^{b}	75.06^{a}	0.460
ADL	54.01^{d}	47.59^{f}	46.56^{f}	49.74^{e}	69.30^{b}	56.17^{c}	71.92^{a}	0.665
Cellulose	52.17^{d}	69.80c^{c}	69.94^{c}	70.53^{c}	79.29^{a}	72.45^{b}	80.32^{a}	0.470
Hemicellulose	65.58^{d}	78.72^{c}	89.26^{ab}	90.99^{a}	88.74^{ab}	87.54^{b}	87.40^{b}	0.769
Nutritive value:								
TDN g/h/d	1021.29^{a}	827.42^{b}	777.21^{bc}	749.56^{c}	716.63^{c}	810.95^{b}	810.59^{b}	605.08
TDN g/kg BW	30.79^{a}	24.99^{b}	23.44^{bc}	22.61^{c}	21.67^{c}	24.69^{b}	24.44^{b}	17.19
TDN g/kg BW$^{0.75}$	73.89^{a}	59.93^{b}	56.22^{bc}	54.20^{c}	51.93^{d}	59.08^{b}	58.63^{b}	41.87
TDN% of DMI	77.30^{ab}	73.03^{d}	73.97^{c}	74.29^{bc}	78.12^{a}	75.70^{b}	78.49^{a}	66.48
DCP g/h/d	138.02^{d}	89.05^{e}	156.50^{ab}	146.40^{cd}	148.04^{bc}	155.81^{ab}	162.41^{a}	2.95
DCP g/kg BW	4.16^{b}	2.69^{c}	4.72^{ab}	4.41^{ab}	4.47^{ab}	4.74^{ab}	4.89^{a}	0.206
DCP g/kg BW$^{0.75}$	9.98^{b}	6.45^{c}	11.32^{a}	10.58^{ab}	10.73^{ab}	11.35^{a}	11.75^{a}	0.405
DCP % of DMI	83.63^{b}	72.51^{c}	89.28^{ab}	88.63^{ab}	92.71^{a}	88.84^{ab}	91.43^{ab}	2.39
Metabolic energy	3.69	2.99	2.81	2.71	2.59	2.93	2.93	2.19

Means with different litters with each row are significantly different (P<0.05).

Table (8): Nitrogen balance of sheep fed experimental treatments.

Balnce	Item	R1	R2	R3	R4	R5	R6	R7	±MSE
Nitrogen intake	g/h/d	26.41^{c}	19.65^{e}	28.04^{b}	26.43^{c}	25.55^{d}	28.06^{b}	28.42^{a}	0.000
	g/kg BW	0.797^{a}	0.595^{b}	0.845^{a}	0.797^{a}	0.772^{a}	0.855^{a}	0.857^{a}	0.031
	g/ kg BW$^{0.75}$	1.91^{ab}	1.43^{c}	2.02^{ab}	1.91^{ab}	1.85^{b}	2.04^{a}	2.05^{a}	0.056
Digested nitrogen	g/h/d	22.08^{c}	14.24^{d}	25.03^{ab}	23.42^{bc}	23.68^{b}	24.62^{ab}	25.73^{a}	0.516
	g/kg BW	0.665^{b}	0.430^{c}	0.755^{ab}	0.706^{ab}	0.716^{ab}	0.750^{ab}	0.776^{a}	0.033
	g/ kg BW$^{0.75}$	1.59^{b}	1.03^{c}	1.81^{ab}	1.69^{ab}	1.71^{ab}	1.79^{ab}	1.86^{a}	0.066
	% of N intake	83.63^{b}	72.51^{c}	89.28^{ab}	88.64^{ab}	92.71^{a}	87.74^{ab}	90.54^{ab}	2.51
Fecal nitrogen	g/h/d	4.32^{a}	4.42^{a}	3.45^{b}	3.64^{b}	2.86^{c}	3.77^{b}	3.71^{b}	0.102
	g/kg BW	0.130^{a}	0.132^{a}	0.105^{b}	0.110^{b}	0.087^{c}	0.115^{b}	0.110^{b}	0.004
	g/ kg BW$^{0.75}$	0.310^{a}	0.320^{a}	0.250^{b}	0.262^{b}	0.205^{b}	0.272^{c}	0.267^{b}	0.010
	% of N intake	16.36^{b}	22.50^{a}	12.30^{de}	13.77^{c}	11.22^{e}	13.45^{cd}	13.05^{cd}	0.405
Urinary nitrogen	g/h/d	6.82^{a}	6.12^{b}	5.35^{c}	5.21^{c}	4.15^{d}	5.33^{c}	4.51^{d}	0.155
	g/kg BW	0.207^{a}	0.185^{b}	0.162^{c}	0.160^{c}	0.125^{d}	0.165^{c}	0.135^{d}	0.006
	g/ kg BW$^{0.75}$	0.500^{a}	0.445^{ab}	0.385^{bc}	0.360^{bcd}	0.290^{d}	0.415^{ab}	0.350^{cd}	0.024
	% of N intake	26.30^{b}	30.54^{a}	19.93^{c}	19.58^{c}	15.87^{d}	20.03^{c}	17.06^{d}	0.404
Total N excretion	g/h/d	11.20^{a}	10.34^{b}	8.98^{cd}	8.82^{cd}	6.99^{e}	9.33^{c}	8.71^{d}	0.155
	g/kg BW	0.335^{a}	0.320^{ab}	0.255^{bc}	0.260^{abc}	0.210^{c}	0.285^{abc}	0.260^{abc}	0.021
	g/ kg BW$^{0.75}$	0.810^{a}	0.760^{ab}	0.620^{bc}	0.620^{bc}	0.505^{c}	0.685^{ab}	0.620^{bc}	0.039
	% of N intake	42.43^{b}	52.64^{a}	32.03^{cd}	33.38^{c}	27.38^{e}	33.27^{c}	30.65^{d}	0.614
Nitrogen balance	g/h/d	15.20^{d}	9.30^{e}	19.06^{b}	17.60^{c}	18.55^{b}	18.72^{b}	19.71^{a}	0.157
	g/kg BW	0.455^{a}	0.285^{b}	0.540^{a}	0.510^{a}	0.560^{a}	0.570^{a}	0.585^{a}	0.041
	g/ kg BW$^{0.75}$	1.10^{b}	0.685^{c}	1.31^{ab}	1.23^{ab}	1.34^{ab}	1.37^{ab}	1.40^{a}	0.076
	% of N intake	57.57^{d}	47.36^{e}	67.96^{bc}	66.61^{c}	72.62^{a}	66.72^{c}	69.34^{b}	0.614
	% of digested N	69.12^{c}	65.70^{d}	76.86^{ab}	75.03^{ab}	78.23^{a}	77.02^{ab}	78.46^{a}	2.72

Means with different litters with each row are significantly different (P<0.05).

Table (9): Water balance for sheep fed experimental treatments:

Balance	Item		R1	R2	R3	R4	R5	R6	R7	±MSE
Water intake	Free	ml/h/d	3640.00	3625.00	3617.50	3622.50	3612.50	3620.00	3612.50	185.28
		ml/Kg $W^{0.82}$	206.00	205.83	204.62	204.33	205.27	206.57	203.80	10.37
	Combined	ml/h/d	81.92^{b}	123.42^{a}	75.81^{c}	76.68^{c}	58.27^{e}	86.20^{b}	68.17^{d}	1.65
		ml/Kg $W^{0.82}$	4.63^{b}	7.01^{a}	4.28^{cd}	4.34^{cd}	3.30^{e}	4.93^{bc}	3.86^{d}	0.172
	Metabolic	ml/h/d	704.69^{a}	570.92^{b}	536.27^{cd}	517.20^{de}	494.47^{e}	559.55^{bc}	559.30^{bc}	10.48
		ml/Kg $W^{0.82}$	39.90^{a}	32.37^{b}	30.36^{bc}	29.27^{bc}	28.05^{c}	31.93^{b}	31.66^{b}	1.04
	Total	ml/h/d	4426.62	4319.34	4229.59	4216.38	4165.24	4265.76	4239.98	186.41
		ml/Kg $W^{0.82}$	250.54	245.22	239.27	237.95	236.63	243.44	239.33	10.72
Water execration	Urinary water	ml/h/d	528.00^{a}	525.75^{a}	442.50^{b}	423.75^{bc}	352.50^{c}	421.25^{bc}	382.50^{c}	65.59
		ml/Kg $W^{0.82}$	29.98^{a}	29.65^{a}	25.11^{b}	23.99^{bc}	19.95^{c}	24.03^{bc}	21.61^{c}	3.82
		% of intake	11.84^{a}	12.00^{a}	10.46^{b}	10.13^{b}	8.38^{c}	9.88^{bc}	9.04^{bc}	1.32
	Fecal Water	ml/h/d	83.29^{ab}	75.19^{ab}	111.97^{a}	79.29^{ab}	56.32^{b}	65.67^{b}	83.90^{ab}	13.60
		ml/Kg $W^{0.82}$	4.71^{ab}	4.27^{ab}	6.25^{a}	4.44^{ab}	3.15^{b}	3.72^{b}	4.68^{ab}	0.690
		% of intake	1.90^{ab}	1.74^{ab}	2.68^{a}	1.92^{ab}	1.35^{b}	1.53^{b}	1.95^{ab}	0.334
	Total water execration	ml/h/d	611.29^{a}	600.94^{a}	554.47^{b}	503.04^{bc}	408.82^{d}	486.92^{bc}	466.40^{c}	67.51
		ml/Kg $W^{0.82}$	34.70^{a}	33.93^{a}	31.36^{c}	28.42^{cd}	23.10^{d}	27.75^{cd}	26.30^{cd}	3.86
		% of intake	13.74^{a}	13.75^{a}	13.15^{a}	12.05^{b}	9.73^{c}	11.41^{b}	10.99^{b}	1.36
Water balance		ml/h/d	3815.32	3718.39	3675.11	3713.34	3756.42	3778.84	3773.58	167.44
		ml/Kg $W^{0.82}$	215.84	211.28	207.91	209.52	213.53	215.69	213.03	9.44
		% of intake	86.25	86.25	86.84	87.94	90.26	88.58	89.00	1.36

Means with different litters with each row are significantly different (P<0.05).

Table (10): Effect of treatments on ruminal pH, volatile fatty acids and molar proportion of individual VFA's.

Item	Time, h	R1	R2	R3	R4	R5	R6	R7	±MSE	Overall mean
Ruminal pH value	0	7.42	7.10	7.10	6.87	6.8	7.00	6.82	0.043	7.02^{a}±0.016
	3	6.52	6.25	6.05	6.12	6.07	6.10	6.10	0.043	6.17^{b}±0.016
	6	6.72	6.47	6.25	6.42	6.20	6.35	6.37	0.043	6.40^{c}±0.016
Overall mean		6.89^{a}	6.60^{b}	6.46^{c}	6.47^{c}	6.37^{d}	6.48^{c}	6.43^{cd}	0.025	
TVFA's (ml equiv./100 ml R.L)	0	6.50	6.13	7.00	6.77	6.72	6.27	6.72	0.137	6.58^{c}±0.051
	3	8.12	7.94	9.35	9.35	10.56	9.75	10.17	0.137	9.32^{a}±0.051
	6	7.17	6.97	8.37	8.25	8.36	8.52	8.34	0.137	8.00^{c}±0.051
Overall mean		7.26^{d}	7.01^{e}	8.24^{bc}	8.12^{c}	8.54^{a}	8.18^{bc}	8.41^{ab}	0.079	
Molar proportion of individual VFA's (%):										
Acetic	0	32.01	31.32	36.43	34.97	42.53	34.71	39.13	0.227	35.87^{c}±0.086
	3	37.75	36.58	41.55	40.43	47.43	40.43	44.87	0.227	41.29^{a}±0.086
	6	34.75	34.65	39.26	38.74	44.97	37.88	42.55	0.227	38.97^{b}±0.086
Overall mean		34.84^{e}	34.18^{f}	39.08^{c}	38.05^{d}	44.98^{a}	37.67^{d}	42.18^{b}	0.131	
Propionic	0	16.15	15.78	19.36	20.13	21.46	18.02	20.43	0.157	18.76^{c}±0.059
	3	20.35	17.81	25.41	21.98	29.5	22.61	28.49	0.157	23.74^{a}±0.059
	6	18.10	16.40	22.70	22.69	25.62	20.55	24.52	0.157	21.51^{b}±0.059
Overall mean		18.20^{f}	16.66^{g}	22.49^{c}	21.60^{d}	25.55^{a}	20.39^{e}	24.48^{b}	0.090	
Butyric	0	14.00	13.93	16.44	15.62	17.44	13.90	15.48	0.275	15.26^{c}±0.103
	3	16.36	16.26	18.31	17.62	21.33	16.17	18.49	0.275	17.79^{a}±0.103
	6	14.85	14.85	17.99	17.73	18.33	15.35	17.10	0.275	16.60^{b}±0.103
Overall mean		15.07^{d}	15.01^{d}	17.58^{b}	16.99^{c}	19.03^{a}	15.14^{d}	17.02^{c}	0.158	
A/P ratio	0	1.98	1.98	1.88	1.74	1.98	1.92	1.91	0.023	1.91^{a}±0.008
	3	1.85	2.05	1.63	1.84	1.60	1.78	1.57	0.023	1.76^{c}±0.008
	6	1.91	2.12	1.73	1.70	1.75	1.84	1.73	0.023	1.83^{b}±0.008
Overall mean		1.91^{b}	2.05^{a}	1.74^{d}	1.76^{d}	1.77^{d}	1.85^{c}	1.74^{d}	0.013	

Means with different litters with each row and column are significantly different (P<0.05).

Table (11): Effect of treatments on ruminal pH, volatile fatty acids and molar proportion of individual VFA's (%).

Item	Time (h)	Experimental ration								Overall mean
		R1	R2	R3	R4	R5	R6	R7	±MSE	
Total nitrogen (mg/dl R.L)	0	95.60	89.78	109.20	109.30	126.40	108.84	124.60	1.562	109.10^{c}±0.590
	3	117.32	113.22	128.25	130.55	149.80	127.44	143.40	1.562	130.00^{a}±0.590
	6	108.32	105.22	119.25	120.55	139.52	118.51	133.40	1.562	120.68^{b}±0.590
overall mean		107.08^{d}	102.74^{e}	118.90^{c}	120.13^{c}	138.57^{a}	118.26^{c}	133.80^{b}	0.902	
True protein nitrogen (mg/dl R.L)	0	38.79	33.23	38.30	40.24	45.20	46.74	45.65	1.915	41.16^{b}±0.724
	3	44.32	41.37	41.95	41.76	50.92	45.88	44.25	1.915	44.35^{a}±0.724
	6	40.32	38.37	38.95	37.76	45.65	41.55	41.25	1.915	40.55^{b}±0.724
Overall mean		41.14^{cd}	37.66^{e}	39.73^{de}	39.92^{de}	47.25^{a}	44.72^{ab}	43.71^{bc}	1.10	
NPN (mg/100 ml R.L)	0	56.80	56.55	70.90	69.05	81.20	62.10	78.95	0.915	67.93^{c}±0.345
	3	73.00	71.85	86.30	88.79	98.87	81.56	99.15	0.915	85.64^{a}±0.345
	6	68.00	66.85	80.30	82.79	93.87	76.96	92.15	0.915	80.13^{b}±0.345
Overall mean		65.93^{d}	65.08^{d}	79.17^{b}	80.21^{b}	91.31^{a}	73.54	90.08^{a}	0.528	
Ammonia nitrogen (mg/dl R.L)	0	26.92	26.92	30.57	33.76	38.66	30.67	32.88	0.399	31.48^{c}±0.150
	3	32.53	31.30	40.47	40.57	47.08	34.76	42.28	0.399	38.43^{a}±0.150
	6	28.88	28.23	36.64	38.24	44.70	32.38	39.42	0.399	35.50^{b}±0.150
Overall mean		29.44^{f}	28.81^{f}	35.89^{d}	37.52^{c}	43.48^{a}	32.60^{e}	38.19^{b}	0.230	
Microbial protein (mg/dl RL)	0	62.06	61.70	65.43	66.36	70.95	65.20	70.07	0.326	65.97^{c}±0.123
	3	105.58	105.58	112.55	112.61	129.56	109.88	115.41	0.326	113.02^{a}±0.123
	6	102.51	102.51	109.55	109.55	119.50	107.22	111.66	0.326	108.93^{b}±0.123
Overall mean		90.05^{e}	89.93^{e}	95.84^{c}	96.17^{c}	106.67^{a}	94.10^{d}	99.04^{b}	0.188	

Means with different litters with each row and column are significantly different (P<0.05).

Table (12): Effect of treatments on ruminal ciliate protozoa, total bacteria and cellulolytic bacteria numbers.

Item	Time (h)	Experimental ration								Overall mean
		R1	R2	R3	R4	R5	R6	R7	±MSE	
Total protozoa ($x10^4$ cell /ml RL)	0	6.20	6.11	6.51	6.49	6.72	6.21	6.76	0.068	6.43^{b}±0.025
	3	5.91	5.77	6.12	6.14	6.17	6.25	6.25	0.068	6.09^{c}±0.025
	6	6.94	6.78	7.82	7.52	8.87	7.17	7.51	0.068	7.52^{a}±0.025
Overall mean		6.35^{e}	6.22^{f}	6.82^{bc}	6.72^{c}	7.25^{a}	6.54^{d}	6.84^{b}	0.039	
Entodinum spp.	0	4.92	4.81	5.21	5.18	5.34	4.91	5.34	0.061	5.10^{b}±0.023
	3	4.81	4.70	5.01	5.01	5.03	5.11	5.10	0.061	4.97^{c}±0.023
	6	5.43	5.28	6.20	5.91	7.13	5.54	5.61	0.061	5.87^{a}±0.023
Overall mean		5.05^{e}	4.93^{f}	5.47^{b}	5.37^{c}	5.83^{a}	5.19^{d}	5.35^{c}	0.035	
Isotrchia spp.	0	0.187	0.186	0.177	0.181	0.190	0.179	0.190	0.005	0.184^{b}±0.001
	3	0.150	0.150	0.142	0.145	0.151	0.147	0.152	0.005	0.148^{c}±0.001
	6	0.230	0.229	0.249	0.242	0.248	0.234	0.231	0.005	0.237^{a}±0.001
Overall mean		0.189^{ab}	0.188^{ab}	0.189^{ab}	0.189^{ab}	0.196^{a}	0.187^{b}	0.191^{ab}	0.002	
Dasytrachia spp.	0	0.364	0.384	0.404	0.402	0.412	0.393	0.412	0.007	0.396^{b}±0.002
	3	0.361	0.342	0.361	0.360	0.362	0.348	0.366	0.007	0.357^{c}±0.002
	6	0.455	0.455	0.460	0.458	0.537	0.450	0.462	0.007	0.468^{a}±0.002
Overall mean		0.393^{d}	0.393^{d}	0.408^{bc}	0.407^{bc}	0.437^{a}	0.397^{cd}	0.413^{b}	0.004	
Epidinium spp.	0	0.148	0.143	0.147	0.150	0.155	0.154	0.156	0.003	0.150^{b}±0.001
	3	0.116	0.114	0.120	0.116	0.122	0.120	0.120	0.003	0.118^{c}±0.001
	6	0.162	0.160	0.188	0.194	0.197	0.193	0.219	0.003	0.188^{a}±0.001
Overall mean		0.142^{d}	0.139^{d}	0.152^{c}	0.153^{bc}	0.158^{a}	0.155^{bc}	0.165^{b}	0.001	

Means with different litters with each row and column are significantly different (P<0.05).

Table (12): Continued

Item	Time (h)	Experimental ration								Overall mean
		R1	R2	R3	R4	R5	R6	R7	±MSE	
Polyolastron spp.	0	0.325	0.327	0.295	0.310	0.340	0.311	0.378	0.006	0.326^{b}±0.002
	3	0.285	0.276	0.290	0.301	0.301	0.305	0.302	0.006	0.294^{c}±0.002
	6	0.316	0.327	0.374	0.365	0.392	0.396	0.523	0.006	0.385^{a}±0.002
Overall mean		0.308^{e}	0.310^{de}	0.320^{cd}	0.325^{c}	0.344^{b}	0.337^{b}	0.401^{a}	0.003	
Ophryoscolox spp.	0	0.147	0.156	0.151	0.154	0.158	0.155	0.159	0.001	0.154^{b}±0.000
	3	0.114	0.113	0.116	0.120	0.122	0.120	0.120	0.001	0.118^{c}±0.000
	6	0.188	0.184	0.193	0.196	0.198	0.197	0.279	0.001	0.205^{a}±0.000
Overall mean		0.150^{d}	0.151^{cd}	0.153^{c}	0.157^{b}	0.159^{b}	0.157^{b}	0.186^{a}	0.001	
Diplodinum spp.	0	0.111	0.106	0.117	0.116	0.122	0.107	0.122	0.002	0.114^{b}±0.001
	3	0.074	0.071	0.081	0.080	0.083	0.087	0.081	0.002	0.079^{c}±0.001
	6	0.157	0.151	0.163	0.160	0.164	0.159	0.184	0.002	0.162^{a}±0.001
Overall mean		0.114^{d}	0.109^{e}	0.120^{bc}	0.119^{bcd}	0.123^{b}	0.117^{cd}	0.129^{a}	0.001	
Total bacterial ($x10^{8}$ cell /ml RL)	0	2.94	3.37	3.72	3.65	3.95	3.97	4.48	0.031	3.73^{c}±0.011
	3	3.82	4.10	4.41	4.46	4.68	4.72	4.52	0.031	4.39^{a}±0.011
	6	3.67	3.82	4.22	4.40	4.32	4.42	4.28	0.031	4.16^{b}±0.011
Overall mean		3.48^{f}	3.76^{e}	4.12^{d}	4.17^{d}	4.32^{c}	4.37^{b}	4.43^{a}	0.017	
Cellulolytic bacteria ($x10^{6}$ cell/ml RL)	0	2.89	2.74	4.00	3.76	4.20	4.75	4.89	0.095	3.89^{c}±0.036
	3	3.48	3.30	4.70	4.70	4.68	5.56	5.60	0.095	4.57^{a}±0.036
	6	3.21	3.30	4.36	4.48	4.43	5.25	5.35	0.095	4.30^{b}±0.036
Overall mean		3.19^{c}	3.03^{d}	4.35^{b}	4.31^{b}	4.44^{b}	5.19^{a}	5.28^{a}	0.055	

Means with different litters with each row and column are significantly different (P<0.05).

Table (13): Effect of experimental treatments on blood composition:

Item	Time (h)	Experimental ration								Overall mean
		R1	R2	R3	R4	R5	R6	R7	±MSE	
Total proteins (g/dl)	0	7.32	7.09	7.84	7.82	7.93	7.83	8.10	0.083	7.70^{b}±0.031
	4	8.12	7.50	8.45	8.43	9.55	8.23	9.05	0.083	8.47^{a}±0.031
Overall mean		7.72^{c}	7.29^{d}	8.14^{b}	8.12^{b}	8.74^{a}	8.03^{b}	8.57^{a}	0.059	
Albumin (AL) (g/dl)	0	3.95	3.44	4.14	3.78	5.14	3.95	5.00	0.062	4.20^{b}±0.023
	4	4.24	4.07	4.45	4.69	6.04	4.47	5.85	0.062	4.83^{a}±0.023
Overall mean		4.10^{d}	3.76^{e}	4.29^{c}	4.23^{c}	5.59^{a}	4.21^{cd}	5.42^{b}	0.043	
Globulin (GL) (g/dl)	0	3.36	3.64	3.69	4.04	2.79	3.87	3.09	0.090	3.50^{b}±0.034
	4	3.87	3.42	4.00	3.73	3.51	3.76	3.20	0.090	3.64^{a}±0.034
Overall mean		3.61^{b}	3.53^{b}	3.85^{a}	3.88^{a}	3.15^{c}	3.82^{a}	3.14^{c}	0.063	
AL/GL ratio	0	1.17	0.97	1.12	0.93	1.84	1.02	1.62	0.043	1.24^{b}±0.016
	4	1.09	1.19	1.11	1.27	1.71	1.19	1.83	0.043	1.34^{a}±0.016
Overall mean		1.13^{b}	1.08^{b}	1.12^{b}	1.10^{b}	1.78^{a}	1.10^{b}	1.72^{a}	0.030	
Urea (mg/dl)	0	29.92	32.13	23.15	22.99	22.73	23.33	23.05	0.417	25.33^{b}±0.157
	4	39.15	37.64	30.38	31.23	29.96	31.40	29.85	0.417	32.80^{a}±0.157
Overall mean		34.53^{a}	34.89^{a}	26.76^{bc}	27.11^{bc}	26.35^{c}	27.36^{b}	26.45^{bc}	0.295	
AST (U/l)	0	23.12	23.26	22.13	22.25	21.43	21.00	22.00	0.236	22.17^{b}±0.089
	4	25.98	25.99	25.18	25.02	24.25	26.15	25.09	0.236	25.38^{a}±0.089
Overall mean		24.55^{a}	24.63^{a}	23.66^{b}	23.63^{b}	22.84^{c}	23.57^{b}	23.54^{b}	0.167	
ALT (U/l)	0	4.75	4.75	4.62	4.55	4.00	4.62	4.37	0.163	4.52^{b}±0.061
	4	6.36	6.72	6.25	6.37	5.32	6.65	6.20	0.163	6.26^{a}±0.061
Overall mean		5.55^{ab}	5.73^{a}	5.43^{ab}	5.46^{ab}	4.66^{c}	5.63^{ab}	5.28^{b}	0.115	

Means with different litters with each row and column are significantly different (P<0.05).