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Feeding Barki Sheep in South Sinai on Some Agro-Industrial By-Products By Using Combined Biological Treatments

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ABSTRACT

Effect of using two by-products (sugar beet pulp and date seeds) untreated or biologically treated with different combinations of fungi, bacteria and yeast in sheep feeding on digestibility coefficients, rumen fermentations and rumen microbes, and blood biochemicals was investigated. The study included laboratory experiment, digestibility trial and *in vitro* gas production. Digestibility trial included six experiments: T (1): Concentrate feed mixture (CFM) + berseem hay (BH) (Control). T (2): CFM contained untreated sugar beet pulp (SBP) and date seeds (DS) + BH. T (3): CFM contained SBP and DS treated with *T. viride* and *S. cerevisiae* + BH. T (4): CFM contained SBP and DS treated with *T. viride* and *C. cellulasea* + BH. T (5): CFM contained SBP and DS treated with *C. cellulasea* and *S. cerevisiae* + BH. T (6): CFM contained SBP and DS treated with *T. viride* with *C. cellulasea* and *S. cerevisiae* + BH. Biological combination treatments improved chemical analysis and fiber constituents, while reduced anti-nutritional factors of DS. T6, T4, T3 and T5 increased ($P < 0.05$) digestibility coefficients, nutritive values, nitrogen utilization, microbial protein, rumen fermentations parameters and microbes numbers, gas production at different incubations times and blood serum total proteins and albumin were improved ($P < 0.05$) in T6, T4, T3 and T5, respectively, compared with control and untreated groups. Control group showed higher values than untreated group. Inclusion of sugar beet pulp and date seeds untreated or treated with biological treatments to replace a part of 70% of common concentrate feed mixture had remarkable improved influence on sheep feeding.

Keywords: sugar beet pulp, date seeds, Biological treatments, digestibility, rumen, sheep.

INTRODUCTION

Improving the utilization of non-conventional feeds is the main area of research in Egypt, due to shortage of conventional feeds. Resources of non-conventional feeds are rich in anti-nutritional factors, have unpalatable and a low digestibility and nutritive value. Therefore, many efforts are in progress to improve efficient use of non-conventional feedstuffs by removal or reduce its content of anti-nutritional factors and increasing their nutritive value by chemical, biochemical or biological treatments.

In Sinai, where livestock depend mainly upon grazing the natural pastures, animals suffer from a shortage of feed during most of the year. In such areas, the possibility of using this agro-industrial by products as a part of concentrate mixture, in addition to the native range forages is of more than passing interest.

Sugar beet pulp is the by-product of sugar extracting industry from sugar beet. It is considered as a carbohydrate rich by-product, but its protein content is considered low (Israilides *et al.*, 1994), and its crude fiber content is considered high, and the content of fast fermentable carbohydrates and ether extract are much lower than those of high energy grains (Haakma, 1982).

The annual amounts of SBP are about 385686 ton (Statistics of ministry of agriculture, 2019). A high proportion of SBP is dried, also, it is available in the local market in a dry cubes and it is usually used as an energy source feedstuff for ruminants.

Date seeds also called pits, pip, stones and kernels. The seed forms about 10 to 30% of fruit weight. Hence, big amounts of date seeds; about 242.378 tons can be

excreting as a by-product per year. Dates packing carry out after an industrial operation in which tremendous amounts of seeds excreted as a by-product. The annual production of from dates in Egypt is estimated by 1.121.890 tons (Statistics of ministry of agriculture, 2019). Date seeds are commonly used in desert areas that are available throughout the year as a source of energy. It is cheap and can be offered to animals in crushed or ground form.

Biological treatments can be employed for improving the feeding value and digestibility of low quality fibrous crop residues and agro-industrial by-products resulted from the processing of crops (El-Ashry *et al.*, 1997 and Khorshed, 2000). By biological treatments as white rot fungi, yeast and bacteria, the nutritional quality and digestibility of different lignocellulosic by-products could be increased and be used in ruminant nutrition. This was observed in increasing protein content, improving dry roughage palatability and dry matter intake (Abd El-Aziz, 2002), improving digestibility of nutrients and nutritive value (El-Ashry *et al.*, 2003, Kholif *et al.*, 2005 and Sabbah-Allam *et al.*, 2006), improving nitrogen balance and rumen fermentations (Gado *et al.*, 2007 and Abd El-Galil, 2014), increasing microbial protein synthesis (Yang *et al.*, 1999 and Nsereko *et al.*, 2002), stimulate the growth of rumen protozoa (Kumar *et al.*, 2013), and cellulolytic bacteria (Marghany *et al.*, 2005), improving the energy balance (De Frain *et al.*, 2005).

The objective of the present study was the effect of using two by-products (sugar beet pulp and date seeds) untreated or biologically treated with different combinations of fungi, bacteria and yeasts in sheep feeding on digestibility coefficients, microbial protein,

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rumen fermentations parameters, protozoal count, total and cellulolytic bacteria numbers and blood biochemicals.

MATERIALS AND METHODS

This experiment was carried out at Ras Sudr Experimental Research Station, Desert Research Center, located in southern Sinai governorate, in 2018. The study included laboratory experiment, digestibility trail and *in vitro* gas production.

Laboratory experiment:

A laboratory experiment was designed to study the effect of using a combination of biological treatments (fungi with yeast, fungi with bacteria, bacteria with yeast, and fungi with bacteria and yeast) on chemical composition and fiber constituents of sugar beet pulp and date seeds. Also, the effect of using a combination of biological treatments on anti-nutritional factors (condensed tannins, alkaloids, flavonoids and saponins) in date seeds was studied.

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 laboratorial experiment was designed as follow:

- T (1): Untreated sugar beet pulp (SBP).
- T (2): SBP inoculated with *Trichoderma viride* and *Sacharomyces cerevisiae*.
- T (3): SBP inoculated with *Trichoderma viride* and *Cellulomonas cellulasea*.
- T (4): SBP inoculated with *Cellulomonas cellulasea* and *Sacharomyces cerevisiae*.
- T (5): SBP inoculated with *Trichoderma viride* with *Cellulomonas cellulasea* and *Sacharomyces cerevisiae*.
- T (6): Untreated date seeds (DS).
- T (7): DS inoculated with *Trichodermaviride* and *Sacharomyces cerevisiae*.
- T (8): DS inoculated with *Trichoderma viride* and *Cellulomonas cellulasea*.
- T (9): DS inoculated with *Cellulomonas cellulasea* and *Sacharomyces cerevisiae*.
- T (10): DS inoculated with *Trichoderma viride* with *Cellulomonas cellulasea* and *Sacharomyces cerevisiae*.

Amount of 200 g of each air-dried sugar beet pulp or date seeds were moistened for 60 % moisture and inculcated with the biological treatments for 14 days at 30 ±2 °C. The used fungi, bacteria and yeast were added at a ratio of 1:1:1 (1.5 ml medium of each microbe to 100 g ration plus 10 % molasses solution from the dry matter). Moisture was kept at 60%. At the end of inoculation period samples were oven dried at 70 °C. Product recovery (PR) was calculated according to Nigam (1994).

Anti-nutritional factors (ANF) analysis:

Approximately 200 mg (DM) of ground samples of dried date seeds were extracted in 10 ml of aqueous acetone (7:3 v/v) in a water bath maintained at 39-40°C for 90 min (Makkar, 2000). Condensed tannins (CT) were determined according to Porter *et al.*, (1986), saponins (SAP) were extracted and isolated according to Ahmad *et al.*, (1990), alkaloids (ALK) were determined according to Arambewela and Ranatunge (1991), flavonoids (FLA)

determination done according to Boham and Kocipai (1994).

Digestibility trail:

The objective of this experiment was to study the effect of feeding rations contained sugar beet pulp and date seeds untreated or biologically treated with different combinations of fungi, bacteria and yeasts on digestibility coefficients, microbial protein, rumen fermentations parameters, protozoa count, total and cellulolytic bacteria numbers, blood biochemicals for Barki male sheep. Six experiments were designed as follow:

- T (1): Concentrate feed mixture (CFM) + berseem hay (BH) (control).
- T (2): CFM contained untreated SBP and DS + BH.
- T (3): CFM contained SBP and DS treated with *T. viride* and *S. cerevisiae* + BH.
- T (4): CFM contained SBP and DS treated with *T. viride* and *C. cellulasea* + BH.
- T (5): CFM contained SBP and DS treated with *C. cellulasea* and *S. cerevisiae* + BH.
- T (6): CFM contained SBP and DS treated with *T. viride* with *C. cellulasea* and *S. cerevisiae* + BH.

The concentrate feed mixture consisted of yellow corn (55%), wheat bran (20%), soya bean meal (15%), molasses (5%), limestone (3%), salt (1.5%), minerals premix (0.5%), while the concentrate feed mixture of T2, T3, T4, T5 and T6 consisted of yellow corn (20%), sugar beet pulp (35%), date seeds (35%), molasses (5%), limestone (3%), salt (1.5%), minerals premix (0.5%). The ratio of CFM to BH was 60%: 40% in all treatments.

Twenty four adult Barki male sheep about 2 years old and 47 kg live body weight (four animals for each treatment) were randomly divided into six groups and fed on control and experimental treatments for 50 days. The first 30 days were as a palatability and adaptation period for treatments. Then rams were placed in metabolic cages for 20 days, the first 15 days were considered as an adaptation and preliminary period, followed by 5 days as collection period. Daily amount of feed consumed, residuals, feces and urine were estimated over the collection period for each animal. Rams weighed at the start and the end of the trial.

Proximate analysis:

The proximate analysis for feeds, feces and urine were determined according to the AOAC (1995). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to the procedures of Van Soest (1994).

Rumen liquor parameters:

Rumen liquor samples were obtained at 0, and 4 hours post feeding. Microbial protein in the rumen fluid was estimated as described by Makkar *et al.* (1982), ammonia nitrogen, non-protein nitrogen and total nitrogen concentrations were determined according to AOAC (1995), while true protein nitrogen was calculated by subtracting the non-protein nitrogen content from total nitrogen content. The concentration of total volatile fatty acids were determined according to Warner (1964), pH value was immediately measured using a digital pH meter.

Number of ruminal ciliate protozoa was determined as described by Ogimoto and Imai (1981). Identification of genera and species was according to the description published by Dehority (1993). Dilution series were prepared under O₂-free CO₂ by the anaerobic method of Bryant (1972) using the anaerobic diluents described by

Mann (1968) to determine total bacteria and cellulolytic bacteria numbers.

Blood sampling:

Blood samples were collected from four animals from each group via jugular vein from each dietary treatment just before morning feeding and 4 hours post-feeding. Blood samples were left to coagulate at room temp, then centrifuged at 4000 turn for 15 min to separate serum and kept it frozen at -20°C till analyses for the total proteins using electronic apparatus, albumin according to Doumas and Biggs (1971), while globulin was calculated by subtracting the albumin from total protein, urea according to Patton and Crouch (1977), AST and ALT according to Wikison *et al.* (1972).

In vitro gas production:

The same six experiments were carried out in digestibility trial were used to investigate *in vitro* gas production by using the method described by Menke and Steingass (1988). Buffer and mineral solution were prepared and placed in a water bath at 39°C under continuous flushing with CO₂. Both solid and liquid rumen fractions were collected before the morning feeding from three fistulated Barki sheep fed twice daily with diet containing berseem hay (60%) and concentrate mixture (40%) ration divided into two equal meals at 8a.m. and 2p.m. daily. The sheep were supplemented with minerals and had free access to water throughout the experiment. Rumen contents were collected into pre-warmed insulated bottles, pooled among sheep, homogenized in a laboratory blender, filtered through two layers of cheesecloth and flushed with CO₂. The well mixed and CO₂ flushed rumen fluid was added to the buffered rumen fluid solution (1:2 v/v), which was maintained in a water bath at 39°C and mixed.

Approximately 200 mg DM of finely ground samples were accurately weighed into calibrated glass syringes (100 ml). Buffered rumen fluid (30 ml) was pipetted into each syringe, containing the feed samples, and the syringes were immediately placed into the water bath at 39°C (Blummel and Qrskov, 1993). Three Syringes with only buffered rumen fluid were incubated and considered as the blanks. The gas production was recorded after, 2, 4, 6, 8, 12, 16, 24 and 48 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 200 mg of dry matter and corrected for blanks. Cumulative gas production gas (Y) at time (t) was fitted to the exponential model of Qrskov and McDonald (1979) as follows: Gas production (Gp) = a+ b (1-e-ct), where, a = the gas production from the immediately soluble fraction, b =the gas production

from the insoluble fraction, c= the gas production rate constant for the insoluble fraction (b), t = incubation time.

Statistical analysis:

Statistical analysis system of SAS (2004) was used to analyze data statistically. The data of feed intake, digestibility coefficients, nitrogen balance, water utilization and *in vitro* gas production were analyzed by one-way analysis and the model was: $Y_{ij} = \mu + T_i + e_{ij}$.

The used design for rumen fermentations, rumen microbes and blood parameters was two-way analysis, the model was:

$$Y_{ij} = \mu + T_i + I_j + TI_{ij} + e_{ij}$$

Where: Y_{ij} = experimental observation, μ = general mean.
 T_i = effect of treatment (i =1:6), I_j = effect of time of sampling (j=0 and 4)
 TI_{ij} =effect of interaction of treatment and time of sampling.
 e_{ij} = experimental error.

Separation among means was carried out by using Duncan's multiple tests (Duncan, 1955).

RESULTS AND DISCUSSION

Laboratorial experiment:

Chemical composition:

Data of Table (1) showed that biological treatments improved chemical composition of sugar beet pulp and date seeds compared with untreated SBP and DS. Treatments with the combination of *T. viride*, *S. cerevisiae* and *C. cellulasea* (T5 and T10) improved the contents of DM, OM, EE CP, NFE and NFC in SBP and DS more than other combinations, followed by treatments with the combination of *T. viride* and *C. cellulasea* (T3 and T8), then treatments with the combination of *T. viride* and *S. cerevisiae* (T2 and T7), and treatments with the combination of *S. cerevisiae* and *C. cellulasea* (T4 and T9). These combinations had decreased the contents of ash and CF. The increase of CP content in SBP by biological treatments ranged between 12.09 and 13.44%, while the increase of CP content in DS ranged between 10.94 and 14.84 %. The decrease of CP content in SBP by biological treatments ranged between 3.30 and 6.44 %, while the decrease of CP content in DS ranged between 6.73 and 9.42 %.

The combination of *T. viride*, *S. cerevisiae* and *C. cellulasea* (T5 and T10) showed the best product recovery of microbes on SBP and DS (52.48 and 50.88 %) followed by the combination of *T. viride* and *C. cellulasea* (T3 and T8), while the lowest product recovery was for the combination of *S. cerevisiae* and *C. cellulasea* (T4 and T9).

Table 1. Effect of treatments on chemical composition (%) of sugar beet pulp and date seeds.

Treatments	Item								Product recovery
	DM	OM	Ash	EE	CP	CF	NFE	NCF	
T1	91.05	92.88	7.12	1.16	8.87	23.68	59.17	23.03	-
T2	92.88	93.85	6.15	2.45	21.73	18.42	51.25	16.56	50.68
T3	93.04	94.14	5.86	2.57	21.96	17.96	51.65	17.09	51.34
T4	92.55	93.11	6.89	2.3	20.95	20.38	49.48	15.37	48.56
T5	93.49	95.02	4.98	2.67	22.31	17.24	52.80	20.03	52.48
T6	92.00	91.90	8.10	3.50	6.87	26.24	55.29	18.41	-
T7	92.78	93.08	6.92	3.78	18.22	18.31	52.77	14.17	47.49
T8	93.11	93.88	6.12	3.92	20.18	17.28	52.50	16.31	49.34
T9	92.65	92.66	7.34	3.67	17.81	19.51	51.67	14.27	47.12
T10	93.54	94.04	5.96	4.10	21.71	16.82	51.41	17.01	50.88

T (1): Untreated sugar beet pulp (SBP). T (2): SBP inoculated with *T. viride* and *S. cerevisiae*. T (3): SBP inoculated with *T. viride* and *C. cellulasea*. T (4): SBP inoculated with *C. cellulasea* and *S. cerevisiae*. T (5): SBP inoculated with *T. viride* with *C. cellulasea* and *S. cerevisiae*. T (6): Untreated date seeds (DS). T (7): DS inoculated with *T. viride* and *S. cerevisiae*. T (8): DS inoculated with *T. viride* and *C. cellulasea*. T (9): DS inoculated with *C. cellulasea* and *S. cerevisiae*. T (10): DS inoculated with with *T. viride* with *C. cellulasea* and *S. cerevisiae*.

Results in the present study are in agreement with Israilides *et al.* (1994), who showed that sugar beet pulp

CP content was increased by 10% due to fungal treatments. Also, Abedo *et al.* (2005) found that treatment with

Trichoderma reesei increased the CP and EE content of SBP and decreased NDF and hemicellulose. Moreover, El-Ashry *et al.* (2002 and 2003), Kholif *et al.* (2005) and Aziz (2014 and 2019) reported that the biological treatments led to increase CP and decreased CF and OM content.

Cell wall constituents and anti- nutritional factors:

Data of Table (2) indicated a decrease of NDF, ADF, ADL, cellulose and hemicellulose content in sugar beet pulp and date seeds by biological treatments compared with untreated SBP and DS. Treatments with the combination of *T. viride*, *S. cerevisiae* and *C. cellulasea* (T5 and T10) had the lowest contents compared with other combinations, followed by treatments with the combination of *T. viride* and *C. cellulasea* (T3 and T8), then treatments with the combination of *T. viride* and *S. cerevisiae* (T2 and T7), and treatments with the

combination of *S. cerevisiae* and *C. cellulasea* (T4 and T9).

The data of anti-nutritional factors compounds indicated that SBP is free of condensed tannins (CT), alkaloids (ALK), flavonoids (FLA) and saponins (SAP), although it contains small level of acid detergent lignin (ADL) as reported by Aziz (2019). Untreated date seeds had a considerable content of CT, ALK, and ADL, although it is free of SAP. The biological treatments for DC decreased anti-nutritional factors content, the combination of T10 showed the best efficiency in reducing all anti- nutritional factors content in DS, followed by T8 then T7, while T9 had the highest values. The decreases of CT were 65.07, 57.20, 49.71 and 45.49 %; respectively. The decreases of ALK content were 81.63, 73.47, 68.37 and 57.14 %; respectively. The decreases of FLA were 77.11, 66.95, 59.32 and 48.30 %; respectively.

Table 2. Effect of treatments on cell wall constituents and product recovery (%) of sugar beet pulp and date seeds.

Treatments	Item				Anti- nutritional factors				
	NDF	ADF	ADL	Cellulose	Hemicellulose	CT	ALK	FLA	SAP
T1	59.82	28.79	2.72	26.07	31.03	-	-	-	-
T2	53.11	22.78	2.14	20.64	30.33	-	-	-	-
T3	52.52	22.46	1.82	20.64	30.06	-	-	-	-
T4	54.49	25.07	2.37	22.70	29.42	-	-	-	-
T5	50.01	21.61	1.8	19.81	28.40	-	-	-	-
T6	63.12	47.90	15.26	32.64	15.22	5.21	0.98	1.18	-
T7	55.14	42.34	12.15	30.19	12.80	2.62	0.31	0.48	-
T8	53.47	41.11	11.42	29.69	12.36	2.23	0.26	0.39	-
T9	56.91	43.54	12.57	30.97	13.37	2.84	0.42	0.61	-
T10	51.22	38.91	10.21	28.70	12.31	1.82	0.18	0.27	-

CT=Condensed tannins, ALK =Alkaloids, FLA =Flavonoids, SAP= Saponins.

T (1): Untreated sugar beet pulp (SBP). T (2): SBP inoculated with *T. viride* and *S. cerevisiae*. T (3): SBP inoculated with *T. viride* and *C. cellulasea*. T (4): SBP inoculated with *C. cellulasea* and *S. cerevisiae*. T (5): SBP inoculated with *T. viride* with *C. cellulasea* and *S. cerevisiae*. T (6): Untreated date seeds (DS). T (7): DS inoculated with *T. viride* and *S. cerevisiae*. T (8): DS inoculated with *T. viride* and *C. cellulasea*. T (9): DS inoculated with *C. cellulasea* and *S. cerevisiae*. T (10): DS inoculated with *T. viride* with *C. cellulasea* and *S. cerevisiae*.

Anti-nutritional factors compounds such as, tannins, alkaloids, flavonoids and saponins are considered organic compounds. Makkar (1993) stated that anti-nutritional factors in livestock feeds may be interfere with food utilization and affect negatively the health and performance of animals, protein utilization, depressing digestion, and slowing the passage rate of digesta resulted in higher rumen fill. Barry and Malcolm (1997) reported that alkaloids causing reduction in feed intake and poor feed conversion. The absence of saponins in DS encourage the increase of DS in diets of ruminants that saponins might reduce the pH and ammonia-N concentration in the rumen, and decrease feed intake (Lovett *et al.*, 2006).

The present results of the content of condensed tannins, alkaloids and flavonoids in untreated date seeds are close to those obtained by Khattab *et al.* (2008), but ADL content in the present study was lesser. Many authors

found that biological treatments decreased condensed tannins in by products (Awawdeh and Obeidat, 2013 and Abdou 2018).

Digestibility trail:

Chemical composition and cell wall constituents:

Comparison among treatments (Table 3) showed that all biological combinations treatments for diets contained SBP and DS (T3, T4, T5, T6) had fewer contents of DM, ash, NFE and NFC than control (T1). All biological combinations increased OM, EE and CP contents more than control. Diet contained untreated SBP and DS (T2) had less content of DM, ash, CP, NFE and NFC than control, although it had higher content of OM, EE, CF, NDF, ADF, ADL, cellulose and hemicellulose compared with control.

Table 3. Chemical composition and cell wall constituents (%) of treatments.

Item	CFM	Hay	Treatments					
			T1	T2	T3	T4	T5	T6
Chemical composition (%):								
DM	93.70	91.20	93.80	91.77	92.40	92.73	92.68	93.00
OM	91.90	87.90	91.92	92.89	93.46	93.88	92.93	94.01
Ash	8.10	12.10	8.08	7.11	6.54	6.12	7.07	5.99
EE	3.20	2.60	3.20	3.30	5.95	6.39	5.84	6.74
CP	12.52	14.10	12.53	11.27	16.30	16.94	16.11	17.36
CF	11.40	26.70	11.42	19.32	17.68	16.94	17.87	16.23
NFE	64.78	44.50	64.77	59.00	53.53	53.61	53.11	53.68
NFC	45.16	8.17	45.15	24.90	20.75	21.81	22.55	21.17
Cell wall constituents (%):								
NDF	31.02	63.03	31.04	53.42	50.46	48.74	51.38	47.36
ADF	17.82	44.52	17.86	30.27	28.35	27.13	28.92	26.16
ADL	4.91	7.20	4.93	11.19	10.55	10.18	10.77	9.68
Cellulose	12.91	37.32	12.93	19.08	17.80	16.95	18.15	16.48
Hemicellulose	13.20	18.51	13.18	23.15	22.11	21.61	22.46	21.20

T (1): Concentrate feed mixture (CFM) + berseem hay (BH) (control). T (2): CFM (contains untreated SBP and DS) + BH. T (3): CFM (contains SBP and DS treated with *T. viride* and *S. cerevisiae*) + BH. T (4): CFM (contains SBP and DS treated with *T. viride* and *C. cellulasea*) + BH. T (5): CFM (contains SBP and DS treated with *C. cellulasea* and *S. cerevisiae*) + BH. T (6): CFM (contains SBP and DS treated with *T. viride* with *C. cellulasea* and *S. cerevisiae*) + BH.

All biological combinations improved DM, OM, EE, CP, NFE and NFC contents, although, it had lower content of CF, NDF, ADF, ADL, cellulose and hemicellulose compared with diet contained untreated SBP and DS. Biological combination of T6 had the best chemical composition and cell wall constituents among all combination followed by T4, this result related to T6 and T4 had the best product recovery on by-product surface. The effect of biological treatments on CP content of diets causing an increase of its content, this is may be due to the increase of microorganisms number that grow on by-product surface, which consume CP of the diet to convert it into microbial protein. The decrease of CF content by biological treatments for diets may be due to the secretion of enzymes by fungi or bacteria to degrade crude fiber to be utilized for their growth. It is known that, yeast benefit from CF degradation by fungi or bacteria to increase their numbers and producing microbial protein.

Similar results for the effect of biological treatments were obtained by Gado *et al.* (2007), Fayed (2008), Aziz (2009) and Farahat (2014), who reported that biological treatments of poor quality roughages improved content of DM, CP, EE and NFE and decreased content of CF, NDF, ADF and ADL.

Feed intake and feed costs:

The data of Table (4) indicated non-significant difference among treatments in initial and final body

weight, but biological combinations (T3, T4, T5 and T6) showed significantly ($P<0.05$) higher body weight change than control (T1) and diet contained untreated SBP and DS (T2), being the highest for T6. Feed intake, as DMI and OMI (g/h/d) increased in T6 followed by T1 then T4 with no significant difference among them; also the difference between T3 and T5 was not significant. Biological combinations of T6 showed the highest ($P<0.05$) intakes of DDMI, DOMI, DOMR, EEI, CPI, DCPI and TDNI, followed by T4 then T3 and T5, the difference among T3, T5 and T1 was not significant, except for intakes of EEI, CPI and DCPI. While, the lowest ($P<0.05$) intakes at all was for T2.

The present results come on the same line with those obtained by Kholif *et al.* (2005) with *C. cellulasea*, Aziz, (2009) with *T. viride* and *S. cerevisiae*. The later authors reported that DMI increased by biological treatments more than untreated group. Muhamad (2012) found that adding yeast culture to the rations of lambs increased feed intake more than control group. However, Aziz (2014 and 2019) indicated no significant difference in feed intake among sheep fed biologically treated sugar beet pulp and control group, as they had higher values than sheep fed untreated SBP group.

Table 4. Live body weight, feed intake and feed costs of Barki sheep fed on experimental treatments.

Item	Treatments						±SEM
	T1	T2	T3	T4	T5	T6	
Number of animals	4	4	4	4	4	4	
Initial body weight	46.86	46.76	47.05	47.13	47.02	47.31	1.404
Final body weight	47.68	47.35	48.15	48.35	48.01	48.61	1.306
Body weight change	0.82 ^b	0.59 ^c	1.10 ^a	1.22 ^a	0.99 ^a	1.30 ^a	1.302
Feed intake g/h/d	1113.25 ^{ab}	972.50 ^d	1047.50 ^c	1100.00 ^b	1038.75 ^c	1155.00 ^a	17.002
DMI g/h/d	1002.66 ^{bc}	892.46 ^d	967.89 ^c	1020.02 ^b	962.71 ^c	1074.15 ^a	16.021
DDMI g/h/d	673.13 ^c	572.29 ^d	663.71 ^c	711.09 ^b	657.91 ^c	757.52 ^a	12.066
OMI g/h/d	921.64 ^{bc}	829.01 ^d	904.59 ^c	957.60 ^b	894.65 ^c	1009.80 ^a	14.894
DOMI g/h/d*	625.60 ^c	557.25 ^d	637.25 ^c	681.67 ^b	621.51 ^c	726.96 ^a	11.308
DOMR g/h/d**	406.64 ^c	362.21 ^d	414.21 ^c	443.08 ^b	403.98 ^c	472.53 ^a	7.349
EEI g/h/d	32.08 ^d	29.45 ^e	57.59 ^c	65.17 ^b	56.22 ^c	72.39 ^a	0.857
CPI g/h/d	125.63 ^d	100.58 ^e	157.76 ^c	172.79 ^b	155.09 ^c	186.47 ^a	2.445
DCPI g/h/d	79.09 ^d	71.08 ^e	116.82 ^c	132.01 ^b	114.74 ^c	144.67 ^a	2.173
TDN g/h/d	663.63 ^{bc}	580.68 ^d	689.34 ^b	729.69 ^{ab}	595.56 ^{cd}	783.73 ^a	24.161
Total feed costs, (EL) kg /h/day	4.61	3.54	3.84	4.05	3.82	4.26	

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

T (1): Concentrate feed mixture (CFM) + berseem hay (BH) (control). T (2): CFM (contains untreated SBP and DS) + BH. T (3): CFM (contains SBP and DS treated with *T. viride* and *S. cerevisiae*) + BH. T (4): CFM (contains SBP and DS treated with *T. viride* and *C. cellulasea*) + BH. T (5): CFM (contains SBP and DS treated with *C. cellulasea* and *S. cerevisiae*) + BH. T (6): CFM (contains SBP and DS treated with *T. viride* with *C. cellulasea* and *S. cerevisiae*) + BH.

*Digestible OM intake. ** Digestible OM fermented in the rumen, calculated as $0.65 \times \text{DOM intake (ARC, 1984)}$.

Treatments contained untreated SBP and DS or biologically treated decreased total feed costs (EL, kg /h/day), T2 recorded the lowest costs followed by T5 then T3, T4 and T6, being 3.54, 3.82, 3.84, 4.05, 4.26 and 4.61 EL, kg /h/day; respectively. The present results are supported by those obtained by Abd El-Aziz (2002), El-Shafie *et al.* (2007), Aziz (2009) and Abdou (2018) who reported that biological treatments for by-products reduced feed costs and improved economic efficiency. Moreover, Sabbah-Allam *et al.* (2006) reported that growing lambs fed concentrate feed mixture included treated sugar beet pulp with *T.viride* and *S. cerevisiae* had the best relative

economic efficiency. Aziz (2019) showed that inclusion of sugar beet pulp and olive cake by 40% and 30% (untreated or biologically treated) in concentrate feed mixture decreased feed costs, untreated group had the lowest feed costs (3.73 L.E./kg) followed by biological treatments (3.93 L.E./kg) compared with control (4.9L.E./kg). Abo El-Nasr (1985) calculated a reduction of feeding costs by 67% than control when lambs were fed a diet containing 22% olive pulp and 22% date seeds. Also, Youssef and Fayed (2001) reported that feeding goats concentrate feed mixture contained 35% date seeds reduced feed costs of kg BW (2.47 EL) more than control that fed CFM(4.33 EL).

Digestibility coefficients and nutritive values:

The data of Table (5) indicated that treatments with biological combinations (T6, T4, T3 and T5) significantly increased ($P<0.05$) digestibility coefficients of DM, OM, CP, , NDF, ADF, ADL, cellulose and hemicellulose more than control, except that T3 was less digestibility of ADL and hemicellulose compared with control. Digestibility coefficient of CF was higher ($P<0.05$) in T6 and T4 with no significant difference between them followed by T1, T3 and T5 with no significant difference among them. Biological combinations (T6 and T4) increased ($P<0.05$) digestibility coefficient of EE compared with control, while T3 increased ($P<0.05$) digestibility coefficient of NFC compared with control. All biological treatments and control increased ($P<0.05$) digestibility coefficients of all nutrients more than untreated SBP and DS (T2). T6 had the highest ($P<0.05$) digestibility coefficients followed by T4 then T3 and T5.

The better palatability of biologically treated groups comparing with untreated group might be the reason of the improvement of DM and other nutrients digestibility. While, the high level of fiber fraction and low content of

CP in untreated group might be the reason of the reduction of the digestibility coefficients.

As for nutritive value, the data of Table (5) indicated that treatments with biological combinations significantly ($P<0.05$) increased TDN and DCP (% of DMI) compared with T1 and T2. In this respect, T6 had higher values followed by T4 then T3 and T5. TDN (% of DMI) values showed non-significant difference between T6 and T4, also the difference between T4 and T3 was not significant, also T5 and T1 did not significantly differ. DCP (% of DMI) showed non-significant difference between T3 and T5. Control group was significantly higher than T2 for TDN value, although they did not significantly differ for DCP value.

The positive effects of biological treatments on total nutrients digestion and nutritive value might be due to that biological treatments alter the nature of rations as a result of producing enzymes which causing acceleration in ruminal fermentation as a result of increasing total ruminal protozoal count which in turn causes improvement in fiber degradation and nitrogen turnover in the rumen (Eugene *et al.*, 2004).

Table 5. Digestibility coefficients and nutritive value of the experimental treatments.

Item	Treatments						±SE
	T1	T2	T3	T4	T5	T6	
Digestibility%:							
DM	67.13 ^c	64.12 ^d	68.56 ^b	69.70 ^a	68.33 ^b	70.50 ^a	0.320
OM	67.88 ^d	67.21 ^d	70.42 ^{bc}	71.17 ^{ab}	69.47 ^c	71.97 ^a	0.366
EE	77.41 ^b	73.68 ^c	77.63 ^b	80.67 ^a	76.82 ^b	82.16 ^a	0.577
CP	62.97 ^d	70.66 ^c	74.03 ^b	76.39 ^a	73.98 ^b	77.57 ^a	0.602
CF	65.92 ^b	61.83 ^c	65.04 ^b	67.43 ^a	64.86 ^b	67.61 ^a	0.935
NFE	69.83 ^a	69.08 ^a	69.31 ^a	66.62 ^c	54.02 ^d	67.56 ^b	3.078
NFC	83.74 ^c	89.74 ^b	96.37 ^a	82.99 ^c	82.31 ^c	82.91 ^c	2.803
NDF	59.27 ^c	54.66 ^d	62.36 ^b	64.00 ^{ab}	62.31 ^b	64.47 ^a	0.544
ADF	59.88 ^c	53.51 ^d	62.05 ^b	65.73 ^a	64.02 ^a	65.10 ^a	0.630
ADL	62.67 ^c	55.01 ^e	60.57 ^d	65.36 ^{ab}	63.49 ^b	66.87 ^a	1.548
Cellulose	58.82 ^b	52.64 ^c	62.93 ^a	65.96 ^a	64.35 ^a	64.06 ^a	1.031
Hemicellulose	58.45 ^d	56.17 ^e	56.51 ^e	61.82 ^b	60.10 ^c	63.69 ^a	1.778
Nutritive value:							
TDN % of DMI	66.22 ^c	61.86 ^d	71.06 ^b	71.52 ^{ab}	65.06 ^c	72.90 ^a	1.795
DCP % of DMI	7.89 ^d	7.96 ^d	12.06 ^c	12.94 ^b	11.92 ^c	13.46 ^a	0.096

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

T (1): Concentrate feed mixture (CFM) + berseem hay (BH) (control). T (2): CFM (contains untreated SBP and DS) + BH. T (3): CFM (contains SBP and DS treated with *T. viride* and *S. cerevisiae*) + BH. T (4): CFM (contains SBP and DS treated with *T. viride* and *C. cellulasea*) + BH. T (5): CFM (contains SBP and DS treated with *C. cellulasea* and *S. cerevisiae*) + BH. T (6): CFM (contains SBP and DS treated with *T. viride* with *C. cellulasea* and *S. cerevisiae*) + BH.

Several authors observed an improvement in DM, CP and CF digestibility coefficients and nutritive value over a wide range of low quality roughages treated by biological treatments (El-Ashry *et al.* (2003), Kholif *et al.* (2005), Gado and Abd El-Galil (2009), Aziz (2009) and Abd El-Galil (2014). Also, Sabbah-Allam *et al.* (2006) reported that treated sugar beet pulp with *T. viride* and *S. cerevisiae* increased DM, OM, CF, NDF, ADF, ADL and cellulose digestibilities. Azzaz *et al.* (2013) showed significant ($P<0.05$) higher DM, OM, CF, NFE digestibility and TDN values of lactating buffaloes fed biologically treated sugar beet pulp compared with those fed the control ration. Aziz (2014 and 2019) found an increase ($P\leq 0.01$) in digestibility coefficients of sheep fed biologically treated sugar beet pulp compared with control and untreated SBP. Moreover, Eid (1998) reported that TDN and DCP were higher in lambs fed diet contained 35% date seeds than control (66.03 and 9.41 vs. 63.4 and

9.15%), respectively. Aziz (2004) showed that including 30 % of date seeds treated with *S. cerevisiae* in sheep diets increased digestibility coefficients and nutritive values more than control. Farahat (2014) reported that biological treatments for date seeds in lactating goats diets significantly ($P<0.05$) improved all nutrients digestibility and nutritive values compared with those of control.

Nitrogen utilization:

Treatments contained SBP and DS treated with biological combinations of microbes (T6, T4, T3 and T5; respectively) significantly ($P<0.05$) increased nitrogen intake (g/h/d) and digested nitrogen (g/h/d and % of NI) values compared with control and untreated SBP and DS (Table 6). Fecal nitrogen excretion (g/h/d and % of NI) was significantly ($P<0.05$) increased in T1 more than biological combinations treatments, while T2 had the lowest ($P<0.05$) FN excretion. While, T1 followed by T2 had the highest ($P<0.05$) urinary nitrogen excretion and total nitrogen

excretion (g/h/d and % of NI) compared with T6, T4, T3 and T5. Biological combinations treatments significantly (P<0.05) decreased FN, UN and total N excretion, this reflected on nitrogen utilization, as biological combinations treatments significantly (P<0.05) increased nitrogen utilization (g/h/d, % of NI and % of digested N) more than T1 and T2. The highest (P<0.05) nitrogen utilization was for T6 followed by T4 then T3 and T5, while T2 had the lowest (P<0.05) nitrogen utilization. The increase in

nitrogen utilization by biological combinations treatments was 11.89, 9.83, 7.37, 7.01 for T6, T4, T3 and T5; respectively compared with T2, while the increase in nitrogen utilization was 10.61, 8.55, 6.09, 5.73 compared with T2. The improvement in nitrogen balance by biological treatments may be due to the increase in rumen microbes, which enhanced rumen fermentations such as total nitrogen, true protein nitrogen, ruminal ammonia and NPN (Tables 8 and 9).

Table 6. Nitrogen utilization for Barki sheep fed on experimental treatments.

Item	Treatments						±SE
	T1	T2	T3	T4	T5	T6	
Nitrogen intake, g/h/d	20.10 ^d	16.09 ^e	25.24 ^c	27.64 ^b	24.81 ^c	29.83 ^a	0.390
Digested nitrogen							
g/h/d	12.65 ^d	11.37 ^e	18.69 ^c	21.12 ^b	18.35 ^c	23.14 ^a	0.347
% of N intake	62.97 ^d	70.66 ^c	74.03 ^b	76.39 ^a	73.98 ^b	77.57 ^a	
Fecal nitrogen							
g/h/d	7.44 ^a	4.71 ^c	6.55 ^b	6.52 ^b	6.45 ^b	6.69 ^b	0.180
% of N intake	37.02 ^a	29.33 ^b	25.96 ^c	23.61 ^d	26.01 ^c	22.43 ^d	0.602
Urinary nitrogen							
g/h/d	0.39 ^a	0.40 ^a	0.35 ^c	0.31 ^d	0.37 ^b	0.28 ^e	0.041
% of N intake	1.98 ^b	2.52 ^a	1.39 ^d	1.14 ^e	1.50 ^c	0.94 ^f	0.027
Total N excretion							
g/h/d	7.84 ^a	5.12 ^c	6.90 ^b	6.84 ^b	6.83 ^b	6.96 ^b	0.180
% of N intake	39.00 ^a	31.85 ^b	27.35 ^c	24.75 ^d	27.52 ^c	23.37 ^d	0.599
Nitrogen balance							
g/h/d	12.25 ^d	10.97 ^e	18.34 ^c	20.80 ^b	17.98 ^c	22.86 ^a	0.347
% of N intake	60.99 ^d	68.14 ^c	72.65 ^b	75.24 ^a	72.47 ^b	76.63 ^a	0.599
% of digested N	96.85 ^e	96.43 ^f	98.12 ^c	98.50 ^b	97.96 ^d	98.79 ^a	0.043

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

T (1): Concentrate feed mixture (CFM) + berseem hay (BH) (control). T (2): CFM (contains untreated SBP and DS) + BH. T (3): CFM (contains SBP and DS treated with *T. viride* and *S. cerevisiae* + BH. T (4): CFM (contains SBP and DS treated with *T. viride* and *C. cellulasea* + BH. T (5): CFM (contains SBP and DS treated with *C. cellulasea* and *S. cerevisiae*+ BH. T (6): CFM (contains SBP and DS treated with *T. viride* with *C. cellulasea* and *S. cerevisiae*+ BH.

The present results are paralleled with those reported by El-Ashry *et al.* (2003), Kholif *et al.* (2005), Ahmed and Salah (2002), Aziz (2004 and 2009), Gado *et al.* (2007), Abd El-Galil (2014) and Azzaz *et al.* (2013 and 2014), who reported that biological treatments with yeast or fungi or bacteria for a wide range of by-products increased nitrogen utilization compared with control or untreated by-products. Moreover, Sabbah-Allam *et al.* (2006) reported that biological treatment with *T. viride* and *S. cerevisiae* had the highest value of nitrogen balance and NB/IN in diets contained sugar beet pulp. Aziz (2014 and 2019) found that sheep fed sugar beet pulp treated with *S. cerevisiae* or *T. viride* or *C. cellulasea* increased nitrogen balance compared with control and untreated SBP.

Eid (1998) fed lambs on ration containing 35% date seeds and found that NI, FN, UN and TE for treated groups were lower than control, while, N balance was higher than control. Fayed *et al.* (2001) fed bucks on ration containing 20% DS as a replacing of CFM and found that NI, FN, UN and NB % of intake was higher (P>0.05) than control. Aziz (2004) showed that including 30 % of date seeds treated with *S. cerevisiae* increased nitrogen intake and nitrogen utilization of sheep. Farahat (2014) reported that biological treatments for date seeds in lactating goats diets significantly (P<0.05) improved nitrogen balance.

Water utilization:

The data of Table (7) indicated significant (P<0.05) differences among all treatments, control group (T1) followed by T2 increased free drinking water (ml/h/d)

more than biological combinations treatments, although biological combinations treatments except T5 increased (P<0.05) metabolic water (ml/h/d) more than T1 and T2. Also, biological combinations treatments and T2 had higher (P<0.05) Combined water (ml/h/d) than T1. Total water intake (ml/h/d) showed slight difference as T1 had the highest (P<0.05) value followed by T3 and T2 with no significant difference between each, while T6 had the lowest (P<0.05) total water intake followed by T5 and T4 with no significant difference between each. T6, T4, T5 and T3; respectively decreased (P<0.05) fecal water, urinary water and total water excretion (ml/h/d and % of intake) compared with T2 and T1. Biological combinations treatments had lower water intake and water excretion, this reflected on water utilization (ml/h/d), as T6 had the lowest (P<0.05) utilization of water (ml/h/d) followed by T5 then T4 with no significant difference between each, also the difference between T3 and T4 was not significant, while T1 had the highest (P<0.05) utilization of water (ml/h/d). Although, the difference among T1 and biological treatments was not significant for water utilization as % of intake, while T2 was the lowest one.

The present results are on the other hand of these obtained by Fayed *et al.* (2008) and Aziz (2009 and 2014), who stated that total water intake and water balance did not significantly (P<0.05) differ for sheep fed biologically treated agriculture by-products although, biological treatments was slightly higher than control and untreated.

Table 7. Water balance for Barki sheep fed on experimental treatments.

Item	Treatments						±SE
	T1	T2	T3	T4	T5	T6	
Water intake:							
Free drinking water, ml/h/d	3895.00 ^a	3745.00 ^{ab}	3682.50 ^{bc}	3527.50 ^d	3625.00 ^c	3330.00 ^e	29.25
Metabolic water, ml/h/d*	457.91 ^{bc}	400.67 ^d	475.64 ^b	503.49 ^{ab}	410.93 ^{cd}	540.77 ^a	16.67
Combined water, ml/h/d	69.02 ^c	80.03 ^a	79.61 ^a	79.97 ^a	76.03 ^b	80.85 ^a	1.164
Total water intake, ml/h/d	4421.93 ^a	4225.71 ^b	4237.75 ^b	4110.95 ^c	4111.97 ^c	3951.62 ^d	35.57
Water excretion:							
Fecal water							
ml/h/d	86.27 ^b	97.36 ^a	84.29 ^c	80.18 ^d	84.68 ^c	76.99 ^e	0.169
% of intake	1.95 ^c	2.30 ^a	1.99 ^c	1.95 ^c	2.06 ^b	1.95 ^c	0.018
Urinary water							
ml/h/d	415.00 ^{ab}	437.50 ^a	384.00 ^c	369.00 ^c	387.50 ^{bc}	357.50 ^c	9.334
% of intake	9.38 ^b	10.35 ^a	9.06 ^b	8.98 ^b	9.42 ^b	9.04 ^b	0.196
Total water excretion							
ml/h/d	501.27 ^b	534.86 ^a	468.29 ^c	449.18 ^{cd}	472.18 ^c	434.49 ^d	9.328
% of intake	11.33 ^b	12.65 ^a	11.05 ^b	10.93 ^b	11.48 ^b	10.99 ^b	0.195
Water utilization:							
ml/h/d	3920.65 ^a	3690.84 ^{bc}	3769.46 ^b	3661.77 ^c	3639.79 ^c	3517.13 ^d	32.29
% of intake	88.66 ^a	87.34 ^b	88.94 ^a	89.07 ^a	88.51 ^a	89.00 ^a	0.195

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

T (1): Concentrate feed mixture (CFM) + berseem hay (BH) (control). T (2): CFM (contains untreated SBP and DS) + BH. T (3): CFM (contains SBP and DS treated with *T. viride* and *S. cerevisiae* + BH. T (4): CFM (contains SBP and DS treated with *T. viride* and *C. cellulasea* + BH. T (5): CFM (contains SBP and DS treated with *C. cellulasea* and *S. cerevisiae* + BH. T (6): CFM (contains SBP and DS treated with *T. viride* with *C. cellulasea* and *S. cerevisiae* + BH.

Identification and determination of ruminal protozoa species and ruminal bacteria:

The identification of ruminal ciliate protozoa indicated the presence of 7 species in this study (Table 8), which are *Isotrichia spp.*, *Dasytrachia spp.*, *Diplodinium spp.*, *Polyplastron spp.*, *Ophryoscolox spp.*, *Epidinium spp.* and *Entodinium spp.* The density of ruminal protozoa species (count $\times 10^4$ cell/ml rumen liquor) indicated significant differences (P<0.05) among all treatments. It is obvious that biological combinations treatments recorded the highest (P<0.05) densities of all protozoa species in rumen liquor of sheep compared with control and untreated groups. The highest (P<0.05) densities of all protozoa species were for T6 followed by T4 and T5, the differences between T4 and T5 were not significant for all species, except in case of *Polyplastron spp.* they were significantly (P<0.05) differ, but the difference between T4 and T6 was not significant for *Polyplastron spp.* density. This may be due to that *Polyplastron spp.* is the most complex protozoa genera in domestic ruminants and it is more resistance to changes in ruminal pH (Hungate, 1966) as T6 and T4 reduced ruminal pH more than other treatments (Table 9).

The data clearly showed that the lowest (P<0.05) density of all protozoa species were for T2 and T1, although they did not significantly differ. While, no significant difference was detected among T4, T5, T1, T2 and T3 for *Isotrichia* and *Ophryoscolox spp.* Also, the differences between T3, T1 and T2 were not significant for *Dasytrachia*, *Diplodinium* and *Polyplastron spp.*, this may be attributed to that these species have a large range of ruminal pH value.

As for total ruminal protozoa count ($\times 10^4$ cell /ml rumen liquor), sheep received biological combinations treatments recorded the highest (P<0.05) density of total ruminal ciliate protozoa compared with control and untreated groups, as T6 recorded the highest (P<0.05) density followed by T4 and T5 then T3, while no significant difference was detected between T4 and T5.

Sheep received control group recorded total protozoa density higher (P<0.05) than sheep received untreated SBP and DS. In this concern, results indicated that *Entodinium spp.* recorded the largest density of tested ruminal protozoa species, the improvement of *Entodinium spp.* density was the same of total protozoa density. This can be explained depending on that, *Entodinium spp.* is responsible for utilization of formed lactic acid in the rumen (Khaled and Baraka, 2011) and microbial supplementations produce lactate sustain a tonic level of lactic acid in the rumen, which could potentially stimulate lactic acid utilizing microorganisms (Nocek *et al.*, 2002). Comparing among different sampling times showed that densities of differential and total ciliate protozoa recorded significant increase (P<0.05) at 4hrs Post-feeding compared with the densities of per-feeding. The obtained densities of ruminal protozoa are considered in the normal level in rumen as reported by Hungate (1966).

The present results are in accordance with these obtained by Jouany *et al.* (1998) who reported that live yeast culture addition to the diets of ruminants increased ruminal protozoa count. Shakweer (2003) found that addition of *C. cellulans* to diets contained sugarcane bagasse and rice straw with fermented with yeast (*S. cerevisiae*) plus fungi (*Phanerochaete chrysosporium*) increased protozoa counts at 3h post-feeding in the rumen of mature rams than those treated with yeast plus fungi only. Aziz (2014 and 2019) reported that sheep fed sugar beet pulp treated with yeast, fungi and bacteria recorded an increase in ruminal ciliate protozoa counts compared with control and untreated SBP.

As for total ruminal bacteria count ($\times 10^8$ cell /ml rumen) and cellulolytic bacteria ($\times 10^6$ cell /ml rumen) density (Table 8), biological combinations treatments significantly increased (P<0.05) their densities more than untreated and control groups. It seems that the combination of *T. viride* with *C. cellulasea* (T4) had highest (P<0.05) density of total bacteria and cellulolytic bacteria followed

by the combination of *T. viride* with *C. cellulasea* with *S. cerevisiae* (T6) then the combination of *T. viride* with *S. cerevisiae* and the combination of *C. cellulasea* with *S. cerevisiae*, but the difference between T4 and was not significant for the density of cellulolytic bacteria. It seems that treatment contained untreated SBP and DS (T2) was higher ($P<0.05$) than control group (T1). The overall means of the densities of total and cellulolytic bacteria at

different sampling times showed an increase at 4hrs post-feeding compared with the densities of per-feeding.

These results are in agreement with those obtained by Dawson and Tricarico (2002), Marghany *et al.* (2005), Khaled and Baraka (2011), Kumar *et al.* (2013) and Aziz (2014 and 2019), who found that biological treatments with yeast, bacteria and fungi for ruminants diets increased ruminal cellulolytic bacterial numbers.

Table 8. Ruminal ciliate protozoa and ruminal bacteria for Barki sheep fed on experimental treatments.

Item	Time	Treatments						±SE	Overall mean
		T1	T2	T3	T4	T5	T6		
<i>Isotrichia spp.</i>	0	0.160	0.160	0.157	0.162	0.161	0.152	0.036	0.159 ^b ±0.014
	4	0.197	0.196	0.189	0.200	0.200	0.259	0.036	0.207 ^a ±0.014
overall mean		0.179 ^b	0.178 ^b	0.173 ^b	0.181 ^b	0.180 ^b	0.206 ^a	0.025	
<i>Dasytrachia spp.</i>	0	0.381	0.362	0.368	0.386	0.382	0.381	0.071	0.377 ^b ±0.029
	4	0.384	0.404	0.413	0.432	0.433	0.480	0.071	0.424 ^a ±0.029
overall mean		0.383 ^c	0.383 ^c	0.390 ^c	0.409 ^b	0.407 ^b	0.430 ^a	0.056	
<i>Diplodinium spp.</i>	0	0.094	0.091	0.107	0.101	0.103	0.101	0.026	0.099 ^b ±0.017
	4	0.131	0.126	0.127	0.142	0.142	0.183	0.026	0.142 ^a ±0.017
overall mean		0.113 ^{cd}	0.109 ^d	0.117 ^{bc}	0.121 ^b	0.122 ^b	0.142 ^a	0.018	
<i>Polyplastron spp.</i>	0	0.295	0.286	0.315	0.312	0.311	0.300	0.052	0.303 ^b ±0.021
	4	0.335	0.337	0.321	0.388	0.350	0.384	0.052	0.352 ^a ±0.021
overall mean		0.315 ^c	0.311 ^c	0.318 ^c	0.350 ^a	0.330 ^b	0.342 ^a	0.037	
<i>Ophryoscolox spp.</i>	0	0.144	0.143	0.150	0.150	0.152	0.146	0.013	0.147 ^b ±0.054
	4	0.177	0.186	0.185	0.189	0.188	0.223	0.013	0.191 ^a ±0.054
overall mean		0.160 ^b	0.164 ^b	0.167 ^b	0.169 ^b	0.170 ^b	0.184 ^a	0.094	
<i>Epidinium spp.</i>	0	0.136	0.134	0.140	0.140	0.142	0.140	0.020	0.138 ^b ±0.084
	4	0.168	0.163	0.174	0.176	0.175	0.208	0.020	0.177 ^a ±0.084
overall mean		0.152 ^c	0.148 ^c	0.157 ^b	0.158 ^b	0.159 ^b	0.174 ^a	0.014	
<i>Entodinium spp.</i>	0	5.060	4.955	5.160	5.357	5.595	5.267	0.056	5.232 ^b ±0.022
	4	5.175	5.060	5.367	5.592	5.287	6.450	0.056	5.488 ^a ±0.022
overall mean		5.117 ^d	5.007 ^e	5.264 ^c	5.475 ^b	5.441 ^b	5.858 ^a	0.039	
Total protozoa density	0	6.272	6.132	6.399	6.611	6.846	6.488	0.059	6.458 ^b ±0.024
	4	6.568	6.473	6.777	7.119	6.776	8.189	0.059	6.984 ^a ±0.024
overall mean		6.420 ^d	6.302 ^e	6.588 ^c	6.865 ^b	6.811 ^b	7.338 ^a	0.042	
Cellulolytic bacteria density	0	3.042	3.195	4.662	5.190	4.502	5.050	0.035	4.273 ^b ±0.014
	4	3.607	3.787	5.005	5.900	4.987	5.867	0.035	4.859 ^a ±0.014
overall mean		3.325 ^f	3.491 ^e	4.833 ^c	5.545 ^a	4.745 ^d	5.458 ^b	0.025	
Total bacterial density	0	3.442	3.877	4.725	5.025	4.452	4.475	5.225	4.332 ^b ±0.015
	4	4.325	4.600	4.912	5.585	5.187	5.225	5.225	4.972 ^a ±0.015
overall mean		3.883 ^d	4.238 ^c	4.818 ^b	5.305 ^a	4.820 ^b	4.850 ^b	0.027	

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

T (1): Concentrate feed mixture (CFM) + berseem hay (BH) (control). T (2): CFM (contains untreated SBP and DS) + BH. T (3): CFM (contains SBP and DS treated with *T. viride* and *S. cerevisiae* + BH. T (4): CFM (contains SBP and DS treated with *T. viride* and *C. cellulasea* + BH. T (5): CFM (contains SBP and DS treated with *C. cellulasea* and *S. cerevisiae*+ BH. T (6): CFM (contains SBP and DS treated with *T. viride* with *C. cellulasea* and *S. cerevisiae*+ BH.

Microbial protein and rumen parameters:

The data of Table (9) indicated that that the biological combinations treatments recorded the highest ($P<0.05$) concentrations of ruminal parameters followed by control and untreated groups. The concentrations of microbial protein, ammonia nitrogen, non-protein nitrogen, total nitrogen, total volatile fatty acids (TVFA's) and true protein (mg/100 ml R.L) were significantly ($P<0.05$) higher in T6 followed by T4 then T3 and T5, but the difference between T6 and T4 was not significant for the concentrations of non-protein nitrogen and total nitrogen. Also the difference between T3 and T5 was not significant for the concentrations of total nitrogen and TVFA's, while T5 had higher ($P<0.05$) concentration of true protein more than T3. The lowest ($P<0.05$) ruminal microbial protein and other parameters was for T1 and T2, no significant difference was detected between T1 and T2 for the concentrations of microbial protein, ammonia nitrogen,

non-protein nitrogen and total nitrogen, except that the difference was significant ($P<0.05$) for true protein and TVFA's concentrations. As for ruminal pH values, it seems that treatments contained SBP and DS untreated or biologically treated decreased ruminal pH values more than control group, the difference was not significant among T6, T4,T5, T3 and T2, while T6 and T4 were slightly lower. The values of ruminal pH were within the physiological range of 6.0-7.0 which have no negative effect on bacterial growth (Hoover, 1986).

The overall means of microbial protein and rumen parameters concentrations at different sampling times showed a significant increase ($P<0.05$) after feeding to reach the highest ($P<0.05$) values after 4hrs post-feeding. The overall means of ruminal pH at the different sampling times were at the other hand of other rumen parameters as it showed a significant decrease ($P<0.05$) after 4hrs post-feeding although it was high per-feeding. Similar results

were obtained by El-Ashry *et al.* (1997) who treated rice straw with *P. funiculiformis* fungus and found that ruminal total VFA's concentration was increased at 3h post-feeding but ruminal pH values was decreased at 3h post-feeding.

The increase of TVFA's, microbial protein, NH₃-N, NPN, TN and TP concentrations due to biological treatments may be attributed to the increase in crude protein content and the decrease in fiber content leading to digestibility coefficients increase due to microbial population improvement in the rumen, as rumen microbes are responsible for occurring fermentations in the rumen (Aziz, 2009). While, the reduction of ruminal pH as a result of feeding diets treated with biological treatments could be attributed to protozoa role of slowing down the fermentations by digesting starch and converting soluble sugars to storage polysaccharides (Williams and Coleman, 1997).

Several authors reported an increase of TVFA's, ruminal ammonia nitrogen, total nitrogen, NPN, true

protein nitrogen and microbial protein concentrations by using treatments of fungi, bacteria and yeast or combination of each other with a wide range of by-products (Khorshed, 2000; Kholif *et al.*, 2005, Gado *et al.*, 2007, Aziz, 2009, El-Shabrawy *et al.* 2012, Azzaz *et al.*, 2013 and Abd El-Galil, 2014).

Moreover, El-Badawi (2007) and Aziz (2014 and 2019) reported an increase of microbial protein, TVFA's, NPN, NH₃, TP and TN concentrations with rations containing biologically treated SBP. Aziz (2004) showed that including 30 % of date seeds treated with *S. cerevisiae* in sheep diets increased TVFA's, ruminal ammonia nitrogen, total nitrogen, NPN, true protein nitrogen. Farahat (2014) reported that feeding lactating goats diets contained biologically treated date seeds led to decrease ruminal pH value and increase in total volatile fatty acids and ammonia nitrogen concentrations compared with those of control.

Table 9. Microbial protein and rumen parameters (mg/100 ml R.L) of Barki sheep fed on experimental treatments.

Item (mg/100 ml R.L)	Time	Treatments						±SEM	Overall mean
		T1	T2	T3	T4	T5	T6		
Microbial protein	0	65.06	64.70	68.43	73.07	68.20	73.95	0.255	68.90 ^b ±0.104
	4	108.58	108.58	115.55	118.41	112.88	132.56	0.255	116.09 ^a ±0.104
Overall mean		86.82 ^e	86.64 ^e	91.99 ^c	95.74 ^b	90.54 ^d	103.25 ^a	0.180	
Ammonia nitrogen	0	29.92	29.92	33.57	35.88	33.67	41.66	0.367	34.10 ^b ±0.150
	4	35.53	34.30	43.47	45.28	37.76	50.08	0.367	41.07 ^a ±0.150
Overall mean		32.72 ^e	32.11 ^e	38.52 ^c	40.58 ^b	35.72 ^d	45.87 ^a	0.260	
NPN	0	60.80	60.55	74.90	82.95	66.10	85.20	1.150	71.75 ^b ±0.469
	4	77.00	75.85	90.30	103.15	85.56	102.87	1.150	89.12 ^a ±0.469
Overall mean		68.90 ^d	68.20 ^d	82.60 ^b	93.05 ^a	75.83 ^c	94.03 ^a	0.813	
Total nitrogen	0	97.60	91.78	111.20	126.60	110.84	128.40	1.995	111.07 ^b ±0.814
	4	119.32	115.22	130.25	145.40	129.44	151.80	1.995	131.90 ^a ±0.814
Overall mean		108.46 ^c	103.50 ^c	120.72 ^b	136.00 ^a	120.14 ^b	140.10 ^a	1.411	
True protein nitrogen	0	36.79	31.23	36.30	43.65	44.74	43.20	2.401	39.32 ^b ±0.980
	4	42.32	39.37	39.95	42.25	43.88	48.92	2.401	42.78 ^a ±0.980
Overall mean		39.55 ^e	35.30 ^f	38.12 ^d	42.95 ^c	44.31 ^b	46.06 ^a	1.698	
TVFA's ml equiv/100 ml R.L	0	6.90	6.53	7.40	7.12	6.67	7.12	0.083	6.95 ^b ±0.033
	4	8.52	8.34	9.75	10.57	10.15	10.96	0.083	9.71 ^a ±0.033
Overall mean		7.71 ^d	7.43 ^c	8.57 ^c	8.84 ^b	8.41 ^c	9.04 ^a	0.058	
pH value	0	7.52	7.20	7.20	6.92	7.10	6.95	0.040	7.15 ^a ±0.016
	4	6.62	6.35	6.15	6.20	6.20	6.17	0.040	6.28 ^b ±0.016
Overall mean		7.07 ^a	6.77 ^b	6.67 ^b	6.56 ^b	6.65 ^b	6.56 ^b	0.028	

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

T (1): Concentrate feed mixture (CFM) + berseem hay (BH) (control). T (2): CFM (contains untreated SBP and DS) + BH. T (3): CFM (contains SBP and DS treated with *T. viride* and *S. cerevisiae*) + BH. T (4): CFM (contains SBP and DS treated with *T. viride* and *C. cellulasea*) + BH. T (5): CFM (contains SBP and DS treated with *C. cellulasea* and *S. cerevisiae*) + BH. T (6): CFM (contains SBP and DS treated with *T. viride* with *C. cellulasea* and *S. cerevisiae*) + BH.

Blood biochemicals:

The data of Table (10) showed that blood serum total proteins and albumin (g/dl) concentrations were significantly (P<0.05) increased by biological combinations treatments comparing with control and untreated treatment, the highest (P<0.05) concentrations were recorded for T6 followed by T4 then T3 and T5, with no significant difference between T3 and T5. Control group was significantly (P<0.05) higher than T2. Globulin (g/dl) concentrations were higher (P<0.05) in T3 and T5 than T1 and T2, while T6 and T4 showed the lowest values, with no significant difference between both groups. As for albumin: globulin ratio, just T6 and T4 had the highest (P<0.05) value while control and other treatments did not significantly differ. The obtained values of blood

serum metabolites are within the normal range those obtained by El-Ashry *et al.* (1997).

The main reason of the improvement of blood serum total proteins and albumin concentrations by feeding biologically treated sugar beet pulp and date seeds may be due to that SBP contains polysaccharides that rapidly decayed by rumen microbes and converted into glucose which is absorbed from small intestine by blood to be used as a source of blood proteins.

Concerning the kidney and liver functions, the data indicate that T1 and T2 significantly (P<0.05) increased urea (mg/dl) concentration and AST (U/L) activity more than biological combinations treatments, although the difference between T1 and T2 was not significant. The concentration of urea and AST (U/L) activity showed non-significant difference among biological combinations

treatments, as T6 was the lowest one and T3 was the highest one. ALT (U/L) activity showed significant ($P<0.05$) increase by T2, T1, T5 and T3; respectively, and the difference among them was not significant, while T6 followed by T4 had the lowest ($P<0.05$) ALT (U/L) activity and the difference between them was significant ($P<0.05$). The obtained values of serum urea are within normal range recorded by Rakha (1985) for sheep, while AST and ALT activity values are within normal range reported by Mohamed and Abou-Zeina (2008) with biologically treated sugar beet pulp. The overall means of blood biochemicals concentrations at different sampling times showed a significant increase ($P<0.05$) after feeding to reach the highest ($P<0.05$) values after 4hrs post-feeding.

The decrease that occurs by biological treatments in blood serum urea concentration and AST (U/L) activity is a real useful indicator for crude protein and nitrogen metabolism (Valkeners *et al.*, 2008), and might be explained by the better utilization of ammonia nitrogen by rumen microbes (Chaucheyars- Durand and Fonty, 2001).

The present results are coincide with the results reported previously by El-Ashry *et al.* (1997), Khorshed

(2000), Shakweer, (2003), Gado *et al.* (2007), Kholif *et al.* (2005), Aziz (2009), Abou-Eleninet *et al.* (2011), Muhamad (2012), Azzaz *et al.* (2013), Azzaz *et al.* (2014) and Abdou (2018), who reported that biological treatments had a significant effect on blood metabolites of ruminants by using yeast, fungi or bacteria for a wide range of poor quality by-products.

Moreover, El-Badawi *et al.*(2007), Mohamed and Abou-Zeina (2008) and Aziz (2014 and 2019) found that treating sugar beet pulp with *Trichoderma reesei*, *Trichoderma viride*, *Cellulomonas cellulasea* or *Sacharomyce scerevisiae* increased total proteins, albumin and globulin concentrations, and decreased urea concentration, AST and ALT activity in blood serum of sheep and goats. Also, Aziz (2004) showed that including 30 % of date seeds treated with *S. cerevisiae* in sheep diets increased total proteins, albumin and globulin concentrations, and decreased urea concentration, AST and ALT activity. Farahat (2014) reported that feeding lactating goats diets contained biologically treated date seeds increased total proteins, albumin and globulin concentrations but did not affect globulin, urea AST and ALT activities compared with those of control.

Table 10. Blood serum biochemical and enzyme activity of Barki sheep fed on experimental treatments.

Item	Time	Treatments						±SEM	Overall mean
		T1	T2	T3	T4	T5	T6		
Total proteins, g/dl	0	7.52	7.29	8.04	8.30	8.03	8.13	0.075	7.88 ^b ±0.030
	4	8.32	7.70	8.65	9.25	8.43	9.75	0.075	8.68 ^a ±0.030
	Overall mean	7.92 ^d	7.49 ^c	8.34 ^c	8.77 ^b	8.23 ^c	8.94 ^a	0.053	
Albumin, g/dl	0	4.25	3.74	4.44	5.30	4.25	5.44	0.065	4.57 ^b ±0.026
	4	4.54	4.37	4.75	6.15	4.77	6.34	0.065	5.15 ^a ±0.026
	Overall mean	4.40 ^d	4.06 ^c	4.59 ^c	5.72 ^b	4.51 ^c	5.89 ^a	0.046	
Globulin, g/dl	0	3.26	3.54	3.59	2.99	3.77	2.69	0.080	3.31 ^b ±0.032
	4	3.77	3.32	3.90	3.10	3.66	3.41	0.080	3.53 ^a ±0.032
	Overall mean	3.51 ^b	3.43 ^b	3.75 ^a	3.04 ^c	3.72 ^a	3.05 ^c	3.053	
A/G ratio	0	1.30	1.08	1.24	1.77	1.13	2.02	0.045	1.42±0.018
	4	1.20	1.32	1.21	1.99	1.30	1.85	0.045	1.48±0.018
	Overall mean	1.25 ^b	1.20 ^b	1.23 ^b	1.88 ^a	1.22 ^b	1.94 ^a	0.032	
Urea, mg/dl	0	32.02	34.23	25.25	25.15	25.43	24.83	0.448	27.82 ^b ±0.182
	4	41.25	39.74	32.48	31.95	33.50	32.06	0.448	35.16 ^a ±0.182
	Overall mean	36.63 ^a	36.99 ^a	28.86 ^b	28.55 ^b	29.46 ^b	28.45 ^b	0.316	
AST, (U/L)	0	27.12	27.26	26.13	26.00	25.00	25.43	0.244	26.16 ^b ±26.16
	4	29.98	29.99	29.18	29.09	30.15	28.25	0.244	29.44 ^a ±26.16
	Overall mean	28.55 ^a	28.63 ^a	27.66 ^b	27.54 ^b	27.57 ^b	26.84 ^c	0.172	
ALT, (U/L)	0	15.75	15.75	15.62	15.37	15.62	15.00	0.167	15.52 ^b ±15.52
	4	17.36	17.72	17.25	17.20	17.65	16.32	0.167	17.25 ^a ±17.25
	Overall mean	16.55 ^{ab}	16.73 ^a	16.43 ^{ab}	16.28 ^b	16.63 ^{ab}	15.66 ^c	0.118	

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

T (1): Concentrate feed mixture (CFM) + berseem hay (BH) (control). T (2): CFM (contains untreated SBP and DS) + BH. T (3): CFM (contains SBP and DS treated with *T. viride* and *S. cerevisiae*) + BH. T (4): CFM (contains SBP and DS treated with *T. viride* and *C. cellulasea*) + BH. T (5): CFM (contains SBP and DS treated with *C. cellulasea* and *S. cerevisiae*) + BH. T (6): CFM (contains SBP and DS treated with *T. viride* with *C. cellulasea* and *S. cerevisiae*) + BH.

In vitro gas production:

The data of Table (11) showed the values of the gas production as a result of *in vitro* fermentation. The results showed that rate of gas production of control (T1) and treatments contained untreated SBP and DS or biologically treated (T2, T3, T4, T5 and T6) during the first 8 h of incubation was very low. Then the gas production rate of control and all treatments increased after the first 12 h to be almost the double, and then the gas production rate showed gradual increase by advanced time of incubation with faster rate to reach the highest rate of production after 48 h.

All biological combinations treatments significantly ($P<0.05$) increased gas production more than control and untreated groups during different incubations times, starting from 2 -48 h of incubation, being the highest ($P<0.05$) for T6, followed by T4 then T5 and T3. The difference between T6 and T4 was not significant after 2, 6 and 8 h of incubation, while the difference between T5 and T3 was not significant at all different incubations times. Control group (T1) had higher ($P<0.05$) gas production more than treatment contained untreated SBP and DS at all different incubations times and total gas production.

The increase of gas production after the first 12hrs till 24hrs may be attributed to the fiber components, which could be the most degraded component after 24hrs of incubation or might be due to the high sugar or starch content that fermented within the first 24h, then the crude fiber content is the limit factor for gas production after 24h of incubation. Makker (2000) stated that when diets are incubated with buffered rumen fluid *in vitro*, the carbohydrates are fermented to produce short chain fatty acids, gases and microbial cells. The increase of gas production by biological combinations treatments may be

due to the increase of ruminal fermentation of those treatments.

Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate, while gas production from protein fermentation is relatively small as compared with carbohydrate fermentation; however gas production from fat fermentation is negligible. Increasing gas production at later incubation times reflecting less microbial mass production, much microbial lyses and probably increasing microbial energy spilling (Khattab *et al.* 2012, and 2014).

Table 11. *In vitro* gas production at different incubation times and gas production kinetics of the experimental rations.

Item	Treatments						±SEM
	T1	T2	T3	T4	T5	T6	
Gas production, ml/200 mg DM							
2 h	2.20 ^c	1.99 ^d	2.28 ^{bc}	2.41 ^{ab}	2.29 ^{bc}	2.44 ^a	0.037
4 h	4.47 ^d	4.08 ^e	5.75 ^c	6.44 ^b	5.75 ^c	6.75 ^a	0.078
6 h	6.90 ^c	6.13 ^d	8.83 ^b	9.65 ^a	8.87 ^b	9.50 ^a	0.079
8 h	9.21 ^c	8.59 ^d	10.65 ^b	11.70 ^a	10.64 ^b	12.19 ^a	0.147
12 h	16.53 ^d	15.39 ^e	20.05 ^c	21.73 ^b	20.15 ^c	25.53 ^a	0.077
16 h	21.64 ^d	20.19 ^e	23.37 ^c	25.09 ^b	23.48 ^c	26.44 ^a	0.107
24 h	28.04 ^d	26.87 ^e	29.84 ^c	33.60 ^b	29.72 ^c	37.97 ^a	0.082
48 h	40.05 ^d	38.89 ^e	45.08 ^c	47.38 ^b	45.06 ^c	51.55 ^a	0.134
Total gas production	129.05 ^d	122.13 ^e	145.87 ^c	158.01 ^b	145.99 ^c	172.39 ^a	0.415
Gas production, Kinetics							
A ml/g DM	3.49 ^d	3.20 ^e	4.23 ^c	4.49 ^b	4.34 ^{bc}	4.76 ^a	0.058
B ml/g DM	56.42 ^c	53.58 ^d	58.04 ^b	58.66 ^a	58.05 ^b	58.99 ^a	0.156
C (h ⁻¹)	0.045 ^d	0.037 ^e	0.064 ^c	0.071 ^b	0.069 ^b	0.079 ^a	0.001

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

T (1): Concentrate feed mixture (CFM) + berseem hay (BH) (control). T (2): CFM (contains untreated SBP and DS) + BH. T (3): CFM (contains SBP and DS treated with *T. viride* and *S. cerevisiae*) + BH. T (4): CFM (contains SBP and DS treated with *T. viride* and *C. cellulasea*) + BH. T (5): CFM (contains SBP and DS treated with *C. cellulasea* and *S. cerevisiae*) + BH. T (6): CFM (contains SBP and DS treated with *T. viride* with *C. cellulasea* and *S. cerevisiae*) + BH.

A= soluble fraction (ml/g DM), B= insoluble fraction (ml/g DM) and C= production rate (h⁻¹).

The present results were close to that reported by Khattab *et al.* (2012) and (2014) who replace yellow corn grain in ruminant diets by azzawi date palm and found that gas production range was 50.0 -59.2 mL/200 mg DM.

The data of kinetics of gas production obtained from the exponential treatments showed significant difference, the higher gas volume from the insoluble fermentation (B) fraction was recorded for T6 and T4 (58.99 and 58.66ml/g DM) while the lowest was for T2 (53.58ml/g DM). Also, the intercept fraction (A) and the rate of gas production (C) were higher for T6 (4.76 and 0.079h⁻¹) and the lower was for T2 (3.20 and 0.037h⁻¹), also the highest potential of gas production (a+c) was for T6 and could be due to the lower content NDF and ADF comparing with other treatments. Mauricio *et al.* (2001) stated that the rate of gas production is associated with the rapid growth phase of microorganism, and in mixed cultures the rate of fermentation will be a result of the interaction between the microorganism and the manner in which they digest the feed particulars.

Similar results of *in vitro* gas production with biological treatments were obtained by Abdou (2018) who reported that the gas production of jojoba meal treated with *Asperigulls oryzae* fungus showed higher gas production starting from 2hrs to 48hrs of incubation than untreated jojoba meal.

CONCLUSION

Inclusion of sugar beet pulp and date seeds untreated or treated with biological treatments to replace a part of 70% of common concentrate feed mixture had remarkable improved influence on sheep feeding. As feeding diets contained sugar beet pulp and date seeds biologically treated with different combinations of *T. viride*, *S. cerevisiae* and *C. cellulasea* enhanced rumen fermentation, improve efficiency of microbial protein synthesis and causes an increase in differential and total count for all species of ruminal ciliate protozoa population and total and cellulolytic bacteria density, also causes an increase in blood serum total proteins and albumin which reflect higher nutrients digestibility coefficient and nutritive value, with addition of reducing feed costs more than control group.

REFERENCES

- Abd El-Aziz, M.Y. (2002). Nutritional studies on biological treatment of agricultural by-products on ruminants. M. Sc. Thesis, Faculty of Agric., Zagazig University, Egypt.
- Abd El-Galil, Etab. R. I. (2014). Using biological additives to manipulate rumen fermentation and improve Baladigoats performance. Egyptian J. Nutrition and Feeds. 17(1):29-42.
- Abo El-Nasr, H. M. (1985). A study on the possibility of using desert agricultural by-products in feeding livestock. Ph. D. Thesis. Fac. of Agric., Cairo Univ.

- Abdou, Ahlam R. (2018). Nutritional evaluation of treated jojoba meal by *Aspergillus oryzae* as sheep feed. Egyptian J. Nutrition and Feeds, 21(1): 35-51
- Abedo, A.A., El-Ashry, M.A., A.Y. El-Badawi, F.I.S. Helal and M. Fadel (2005). Effect of feeding biologically treated sugar beet pulp on growth performance of sheep. Egyptian J. Nutrition and Feeds. 8: 579-590.
- Abou-Elenin, E.I.M., El-Hosseiny, H.M. and El-Shabrawy, H. M. (2011). Comparing effects of organic acid (malate) and yeast culture as feed supplement on dairy cows performance. Nature and Sci., 9: 132-140.
- Ahmad, V.U., Perveen, S. and Bano, S. (1990). Saponins from the leaves of *Guaiacum officinale*. Phytochemistry, 29:3287-3290.
- Ahmed, B.M. and M. S. Salah (2002). Effect of yeast culture as an additive to sheep feed on performance, digestibility, nitrogen balance and rumen fermentation. J. King Saud., Vol. 14, Agric. Sci. (1):1-13.
- AOAC (1995). Association of Official Analytical Chemists. Official methods of analysis. 15th ed. Arlington, Virginia, USA.
- Arambewela, L. and Ranatunge, T. (1991). Indole alkaloids from *Tabernaemontana divaricate*. Phytochemistry, 30:1740-1741.
- ARC (1984). The nutrient requirement of Ruminant Livestock, Supplement 1. Commonwealth Agriculture Bureaux, Slough, UK.
- Awawdeh, M.S. and Obeidat, B.S. (2013). Treated olive cake as non-forage fiber source for growing Awassi lambs: Effects on nutrient intake, rumen and urine pH, performance, and carcass yield. Asian Australas. J. Anim. Sci. may, 26(5)661-667.
- Aziz, Hend A. (2004). Studies on effect of some non-hormonal growth promoters on growth rate and some rumen parameters in small ruminants under desert condition. M. Sc. Thesis, Fac. of Agric., Ain Shams Univ, Egypt.
- Aziz, Hend A. (2009). Effect of feeding olive tree pruning by-products in Sinai on sheep performance. Ph. D. thesis, Fac. of Agric., Ain Shams Univ, Egypt.
- Aziz, Hend A. (2014). Effect of biologically treated sugar beet pulp on chemical composition, nutrients disappearance, digestibility, rumen fermentations, rumen microbes and blood composition in adult sheep. J. Agric Sci. Mansoura Univ., 5(12): 647-671.
- Aziz, Hend A. (2019). Nutritional value of sugar beet pulp and olive cake treated by using monism biological treatment and its effect on sheep feeding. Egyptian J. Nutrition and Feeds, 22(3): 423-438.
- Azzaz, H.H., Hend A. Aziz, Eman S.A. Farahat and Murad, H.A. (2014). Impact of microbial feed supplements on the productive performance of lactating Nubian goats. Global Veterinaria, 14 (4): 567-575.
- Azzaz, H.H., Murad, H.A., Kholif, A.M., Morsy, T.A., Mansour, A.M. and El-Sayed, H.A. (2013). Increasing nutrients bioavailability by using fibrolytic enzymes in dairy buffaloes feeding. Journal of Biological Sciences, 13(4): 234-241.
- Barry, J. B. and Malcolm, R. (1997). Sorghum ergot (*Claviceps africana*) associated with feed refusal and impaired milk production in pigs and cattle in Queensland. Queensland Department of Primary Industries, Australia.
- Blummel M. and Qrskov, E.R. (1993). Comparison of an in vitro gas production and nylon bag degradability of roughages in predicting feed intake in cattle. Anim. Feed Sci. and Technol., 40:109-119.
- Boham, B.A. and Kocipai, A.C. (1994). Flavonoids and condensed tannins from the leaves of Hawaiian *vaccinium vaticulatum* and *V. calycinium*. Pacifici Sci., 48:458-463.
- Bryant, M.P. (1972). Commentary on the Hugate technique for culture of anaerobic bacteria. Ani. J. Clin. Nutr. 25:1324.
- Chaucheyars- Durand, F. and Fonty, G. (2001). Establishment of cellulolytic bacteria and development of fermentative activities in the rumen of gnotobiotically-reared lambs receiving the microbial additive *Sacharomyces cerevisiae* CNCM 1-1077. Reprod. Nutr. Dev. 41: 57-68.
- Dawson, K. A. and Tricarico, J. (2002). The evaluation of yeast cultures 20 years of research. Proceedings of Alltech's 16th Annual Symposium, Alltech Technical Publications, European, Middle Eastern and African lecture Tour.
- DeFrain, J.M., Hippen, A.R., Kalscheur, K.F. and Tricarico, J.M. (2005). Feeding alpha-amylase improves the glycemic status and performance of transition dairy cows. Journal of Dairy Science, 88: 4405-4413.
- Dehority, B. A. (1993). Laboratory Manual for classification and Morphology of rumen ciliate protozoa. CRC. Press Inc., Florida.
- Doumas, Wabson and Biggs. H. (1971). Albumin standards and measurement of serum with bromocresol green. Din. Chem. Acta. 31: 87.
- Duncan, D.B. (1955). Multiple range and multiple F-test. Biometrics. 11:1-42.
- Eid, E. Y. (1998). Effect of organic wastes utilization in ruminants feeding on animal performance under desert conditions. M. S. thesis, Fac. of Agric., Zagazig Univ., Egypt.
- El-Ashry, M.A., Ahmed, M.F., El-Saadany, S.A., Youssef, M.E.S., Gommaa, I.A. and Deraz, T.A.A. (1997). Effect of mechanical vs. mechano-chemical or mechano-biochemical treatments of crop residues on their use in ruminant rations: Digestibility, nitrogen balance and some blood and rumen liquor parameters of sheep. Egyptian J. Nutrition and Feeds, 1: (Special Issue): 173-186.
- El-Ashry, M.A.; El-Sayed, H.M., Fadel, M., Metwally, H.M. and Khorshed, M.M. (2002). Effect of chemical and biological treatments of some crop residues on their nutritive value 2- effect of biological treatments on chemical composition and in vitro disappearance. Egyptian J. Nutrition and feeds, (1): 43-54.
- El-Ashry, M.A., Kholif, A.M., Fadel, M., El-Alamy, H.A., El-Sayed, H.M. and Kholif, S.M. (2003). Effect of biological treatments on chemical composition and in vitro and in vivo digestibilities of poor quality roughages. Egyptian J. Nutr. and Feeds, 6:113-126.
- El-Badawi, A.Y., Abedo, A.A., El-Ashry, M.A., Helal, F.I.S. and Yacout, M.H.M. (2007). Microbial protein enrichment of sugar beet pulp by aerobic fermentation: 2- Reflection of tow dietary replacement levels of SBP or Fungal treated SBP on ruminal degradation Kinetics, rumen fermentation and some Hematological parameters of sheep. Egyptian J. Nutrition and feeds, 10 (2) (Special Issue): 569-584.

- El-Shabrawy, H.M., Hoda, M. El-Hosseiny and Abou-Elenin, I.M. (2012). Role of malic acid, malate salts and yeast culture as feed additives on performance of growing crossbreed Frisian male calves. *Egyptian J. Nutr. and Feeds*, 15(3):471-483.
- El-Shafie, M. H., Mahrous, A.A. and Abdel-Khalek, T.M.M. (2007). Effect of biological treatments for wheat straw on performance of small ruminants. *Egyptian J. Nutrition and feeds*, 10 (2) (Special Issue): 635-648.
- Eugene, M., Archimède, H. and Sauvant, D. (2004). Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminants. *Livest. Prod. Sci.* 85:81-97.
- Farahat, Eman. S.A. (2014). Using biologically treated date kernels in lactating rations. Ph.D. Thesis, Fac. Agric., Cairo Univ., Egypt.
- Fayed, M. Afaf; bdelGany, F.Bouthaina and Emam, S. Shalabia (2008). Nutritional studies on sheep fed some SALT plants treated with bacteria in Sinai. *Egyptian J. Nutrition and Feeds*. 11(1):93-106.
- Fayed, A. M., Yossef, K. M. and Abou El-Nasr, H. M. (2001). Nutritional performance of goats fed non-conventional diets based on olive pulp in Sinai. *Egyptian J. Nutrition and Feeds*. 4:81-89.
- Gado, H.M. and Abd El-Galil, R.I. (2009). Evaluation differences of some ruminal bacteria by in vitro dry matter, cellulose and hemicellulose disappearance rate and extent of bagasse. *Egyptian J. Nutrition and Feeds*. 12 (3):359-372.
- Gado, H.M., Metwally, H.M., Soliman, H., Basony, A.Z.L. and El Galil, E.R. (2007). Enzymatic treatments of bagass by different sources of cellulase enzymes. In: Proceedings of 11th Conference on Animal Nutrition, Al-Aqsor-Aswan, Egypt, November 13-18, 2007, vol. 10: 607.
- Haaksmma, J. (1982). Feeding value of pressed beet pulp compared to other fodders. Proc. Int. Inst. Sugar beet Research, 45th winter Congress, USA.
- Hoover, W.H. (1986). Chemical factors involved in ruminal fiber digestion. *J. Dairy Sci.*, 69:2755-2766.
- Hungate, R.E. (1966). *The Rumen and its Microbes*. Academic Press Inc., New York and London.
- Israilides, C.J., Iconomou, D., Kandylis, K. and Nikokyris, P. (1994). Fermentability of sugar beet pulp and its acceptability in mice. *Bioresour Technology*, 47: 97.
- Jouany, J.P., Mathieu, F., Senaud, J., Bohatier, J. and Mercier, M. (1998). The effect of *Sacharomyce scerevisiae* and *Asarglus orsa* on the digestion of the cell wall fraction of a mixed diet in defaunated and refaunated sheep rumen. *Reproduction Nutrition Development*, 38:401-416.
- Khaled, N.F. and Baraka, T.A. (2011). Influence of TOMOKO® (Direct-Fed Microbials) on productive performance, selected rumen and blood constituents in Barky finishing lambs. *Journal of American Science*, 7, 564-570.
- Khatab, H. M., Sooud, A.O., Salem, A.M. Mansour, A. M. and Younan, B.R. (2008). Agro-industrial by-products for feeding lactating goats. *Egyptian J. Nutrition and Feeds*. (1): 144-158.
- Khatab, I., Abd- El wahed, A. and Kewan, K. (2012). The use of in vitro gas production technique to predict the nutritive value of azzawi date palm as a replacer for yellow corn grain in ruminant diets under Siwa oasis conditions. *Egyptian J. Nutrition and Feeds*. 15 (1) Special Issue: 69 -178.
- Khatab, I. M., Salem, A. Z. M., Camacho, I. M., Abdel-Wahed, A. M. and Kewan, K. Z. (2014). Azzawi dates (*Phoenix dactylifera*) as a substitute for corn as an energy source in sheep diet: In vitro gas production and fermentation. *Animal Nutrition and Feed Technology*, 14: 41-49.
- Kholif, A.M., El-Ashry, M.A., El-Alamy, H.A., El-Sayed, H.M., Fadel, M. and Kholif, S.M. (2005). Biological treatments of banana wastes for feeding lactating goats. *Egyptian J. Nutrition and Feeds*. (2): 149-162.
- Khorshed, M.M. (2000). Different treatments for improving nutritional quality of some crop residues used in ruminant nutrition. Thesis Ph. D. degree. Faculty of Agriculture. Ain Shams University, Egypt.
- Kumar, S., Chigurupati, D., Prasad, S. and Prasad, R. (2013). Effect of yeast culture (*Saccharomyces cerevisiae*) on the ruminal microbial population in buffalo bulls. *Buff. Bull.*, 32:116.
- Lovett, D. K., Stack, L., Lovell, S., Callan, J., Flynn, B., Hawkins, M. and Mara, F.P.O. (2006). Effect of feeding *Yucca Schidigera* extract on performance of lactating dairy cows and ruminal fermentation parameters in steers. *Livestock Science*, 102:23-32.
- Makkar, H.P.S. (1993). Anti-nutritional Factors in Foods for Livestock. Animal Production in Developing Countries. Occasional Publication, 16-Br. Society of Animal Production.
- Makkar, H.P.S. (2000). Quantification of tannins in tree foliage. A laboratory manual for the FaO/laea coordinated research project on the use of nuclear and related techniques to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage. FAO/IAEA Working Document, IAEA, Austria, p.38.
- Makkar H.P.S., Sharma O.P., Dawra R.K., Negi S.S., (1982). Simple determination of microbial protein in rumen liquor. *J. Dairy Sci.*, 65:2170-2173.
- Mann, S.O. (1968). An improved method for determining cellulolytic activity in anaerobic bacteria. *J. Appl. Bacteriol.* 31:241.
- Marghany, M., Sarhan, M. A., Abd El-Hey, A. and El-Tahan, A. A. H. (2005). Performance of lactating buffaloes fed rations supplemented with different levels of baker's yeast (*Sacharomy cescerevisiae*). *Egyptian J. Nutrition and Feeds*. (Special Issue), 8:21.
- Mauricio, R., Owen, E., Mould, F. L., Givens, I., Teodorou, M.K., France, J., Davies, D.R. and Dhanoa, M. S. (2001). Comparison of bovine rumen liquor and bovine faeces as inoculum for an in vitro gas production technique for evaluating forages. *Anim. Feed Sci. Technol.*, 89:33.
- Menke, K. H. and Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analyses and gas production using rumen fluid. *Anim. Res. Develop.*, 28:7.
- Mohamed, M. I. and Abou-Zeina, A. A. Hala (2008). Effect of dietary supplementation with biologically treated Sugar beet pulp on performance and organs function in goat kids. *American-Eurasian. J. Agric. & Environ. Sci.*, 4(4):410-416.
- Muhamad, Suzan M.N. (2012). Effect of adding yeast culture to ration on the performance and some blood parameters of Arabi fattening lambs. *Egyptian J. Nutrition and Feeds*. 15 (1):23-30.

- Nocek, J. E., Kautz, w. P., Leedle, J. A. Z. and Allman, J.G. (2002). Ruminant supplementation of direct-fed microbials on diurnal pH variation and in situ digestion in dairy cattle. J. Dairy Sci. 85,429-433.
- Nigam, P. (1994). Process selection for protein enrichment; fermentation of the sugar industry by-products molasses and sugar beet pulp. Process Biochemistry, 29:337.
- Nsereko, V.L., Beauchemin, K.A., Morgavi, D.P., Rode, L.M., Furtado, A.F., McAllister, T.A., Iwaasa, A.D., Yang, W.Z. and Wang, Y. (2002). Effect of a fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows. Canadian Journal of Microbiology, 48: 14-20.
- Ogimoto, K. and Imai, S. (1981). Atals of Rumen Microbial-Ogy. Japan Scientific Societies Press, ToKyo.
- Patton, C. J. and Crouch (1977). Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem., 49: 464-469.
- Porter, L.J., Hrstich, L.N. and Chan, B.G. (1986). The conversion of procyanidins and prodelphinidins to cyaniding and delphinidins. Phytochemistry 1:223-230.
- Qrskov, E.R. and McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci., 92:499.
- Rakha (1985). Effect of concentrate deprivation on animal health and production. M. SC. Thesis, Fac. of Vet. Medicine, Cairo Univ. Egypt.
- Sabbah-Allam., Al-Bedawi, T.M., Hanaa H. El-Amary and Shereen H. Mohamed (2006). Improving sugar beer pulp through biological treatment and its use in sheep ration. Egyptian J. Nutrition and Feeds. 9(2): 235-247.
- SAS (2004). SAS/STAT 9.1.3 User's Guide: Statistical Analysis systems Institute Inc., Release 8.1, Cary, NC., USA.
- Shakweer, I.M.E. (2003). Effect of biological treatments of rice straw and sugarcane bagass on their digestibility, nutritive value, ruminal activity and some blood parameters in rams. Egyptian Journal of Nutrition and Feeds, 6: 925-940.
- Statistics of Ministry of Agriculture, Egyptian (2019). Economic Affairs, Sector of Agricultural statistics.
- Valkeners, D., Thewis, A., Van Laere, M. and Beckers, Y. (2008). Effect of rumen degradable protein balance deficit on voluntary intake, microbial protein synthesis, and nitrogen metabolism in growing double-musled Belgian Blue bulls fed corn silage-based diet. J. Anim. Sci. 86:680-690.
- Van Soest, P. J. (1994). The Nutritional Ecology of the Ruminant, 2nd edition. Cornell University press. Ithaca, N Y., 476p.
- Warner, A.C.J. (1964). Production of volatile fatty acids in the rumen methods of measurements. Nutr. Abst. & rev. 34:339.
- Williams, A.G. and Coleman, G.S. (1997). The rumen anaerobic fungi. In: The Rumen Microbial Ecosystem (Eds. P.N. Hobson and C.S. Stewart), Kluwer Academic and Publishers, Book News, Inc., Portland, pp. 73-139.
- Wikison, J.H., Barn, D.N., Moss, D.W. and Walker, P.G. (1972). Standardization of clinical enzyme assays. A reference method for aspartate and alanine transaminases. J. Clin. Pathol., 25:940.
- Yang W.Z., Beauchemin, K. A. and Rode, L. M. (1999). Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. J. Dairy., 82:391-403.
- Youssef, K. M. and Fayed, Afaf M. (2001). Utilization of some organic wastes as feed supplement for growing goats under desert conditions. Egyptian J. Nutrition and Feeds, 4:91-99.

تغذية أغنام البرقي في جنوب سيناء على بعض مخلفات التصنيع الزراعي باستخدام المعاملات البيولوجية المتحدة هند أحمد على عزيز

قسم تغذية الحيوان - مركز بحوث الصحراء-القاهرة- مصر

تم دراسة استخدام نوعين من المخلفات (تفل بنجر السكر و نوى البلح) غير معاملان أو معاملان بيولوجياً بخلبطين مختلف من الفطر و البكتريا و الخميرة كجزء من مخلوط العلف المركز لتغذية الأغنام و تأثيره على معاملات الهضم الغذائي، و البروتين الميكروبي، و تخمرات الكرش، و بروتوزوا الكرش، و العدد الكلي للبكتريا، و البكتريا المحللة للسليولوز و قياسات الدم. حيث اشتملت الدراسة على تجربة معملية و تجربة هضم و تجربة إنتاج الغاز معملياً. و اشتملت تجربة الهضم على ستة تجارب و هي معاملة (1): مخلوط مركزات + دريس البرسيم (مقارنة). معاملة (2): مخلوط مركزات يحتوى على تفل بنجر السكر + نوى بلح (غير معاملان) + دريس البرسيم. معاملة (3): مخلوط مركزات يحتوى على تفل بنجر السكر + نوى بلح (معاملان بالفطر و الخميرة) + دريس البرسيم. معاملة (4): مخلوط مركزات يحتوى على تفل بنجر السكر + نوى بلح (معاملان بالفطر و البكتريا) + دريس البرسيم. معاملة (5): مخلوط مركزات يحتوى على تفل بنجر السكر + نوى بلح (معاملان بالفطر و البكتريا و الخميرة) + دريس البرسيم. و قد أظهرت النتائج أن الخليط الناتج من المعاملات البيولوجية أدى إلى تحسن معنوي في محتوى البروتين الخام و انخفاض في محتوى الألياف الخام و مكوناتها في كلا من بنجر السكر و نوى البلح، كما أدى إلى انخفاض في محتوى مضادات التغذية في نوى البلح. و قد أظهر الخليط الناتج من المعاملات البيولوجية في كل من المعاملة السادسة و الرابعة و الثالثة و الخامسة على التوالي زيادة معنوية في معامل هضم المواد الغذائية و القيمة الغذائية و معدل الاستفادة من التتروجين و تخمرات و أعداد ميكروبات الكرش و إنتاج الغاز في أوقات التحضين المختلفة و تركيز البروتين الكلي و الألبومين لسيرم الدم مقارنة بمجموعة المقارنة و المعاملة الثانية. و قد كانت قيم مجموعة المقارنة أعلى من المعاملة الثانية غير المعاملة. إضافة تفل بنجر السكر و نوى البلح غير معاملان أو معاملان بيولوجياً ليحلا محل 70% من مخلوط العلف المركز له تأثير جيد في تغذية الأغنام.