UPGRADING NUTRITIONAL VALUE OF MORINGA STALKS BY USING TRICHODERMA REESEI, *CELLULOMONAS CELLULASEA AND SACCHAROMYCES CEREVISIAE IN SOLID STATE* FERMENTATION SYSTEM

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ABSTRACT

Twenty Barki rams with average body weight 42.8 kg were used in a complete randomized design with five treatments to evaluate the nutritive value of treated moringa stalks (MS) under solid state conditions with fungi (Trichoderma reesei), yeast (Cellulomonas cellulasea) and bacteria (Cellulomonas cellulasea) comparing with untreated MS as a positive control and berssem hay as a negative control. Results revealed that DM, OM, CF, EE, NFE, and ADL contents in biological treated MS were lower than untreated MS. However, the CP, ash, NDF, hemicellulose, and cellulose contents were higher in treated MS compared with untreated MS. DM intake was higher in group fed treated MS than untreated MS. All biological treatments resulted in a significant ($P<0.05$) higher digestibility of all nutrients compared with untreated MS. The impact of biological treatments was more obvious with the yeast treatment on TDN and DCP comparing with the other treatments and untreated MS. All groups showed positive nitrogen retained value except that group fed untreated MS showed negative value. Water utilization, rumen fermentation and biochemical blood constituents were also investigated.

Key words: Moringa, sheep, intake, digestibility, water, rumen, blood

INTRODUCTION

Forages have an important role in ruminant nutrition as it provides energy, protein and minerals as well as fiber for chewing and rumination (Ranjabar, 2007). Recently, the agriculture policy in Egypt was directed to increase the cultivated area with *Moringa oleifera* Lamarck, a member of the Moringaceae family, which resulted in producing surplus of crop residues in such areas. *Moringa oleifera* has several economic importance uses for industrial and medicinal applications. Moringa fresh foliage has been included into the diet of different animals with positive effects on feeding goat (Manh *et al*., 2005) and sheep (Ben Salem and Makkar, 2009). However, stalks as the main by-product for moringa crop has not utilized in animal feeding although it represent 30% of plant (Dechasa *et al*,. 2006). Using MS as animal feed could participate in solving the problems of feed shortage in Egypt which is particularly realized at drought seasons and hence the selling price of animals products. Few studies have been conducted on utilization of MS in fattening lambs (Mahmoud, 2013).

Recent years, much interest has been forwarded to develop new bio-techniques for improving the nutritive value of lignocelluloseics fibrous using biological treatment in solid substrate fermentation (SSF) under non-sterile conditions (Leopold *et al*., 2008). The SSF of agricultural and agro-industrial residues could increase their value, example, by increasing protein content and nutritional value or by production of useful enzymes (Rudravaram *et al*., 2006).

In Egypt, supplementing the available low quality residues with microbial cultures and their effects on growth performance and feed utilization by livestock have been investigated by many nutritionists (El-Ashry *et al*., 2003 and Bassuny *et al*., 2005, El-Sheikh, 2007, El-Mahy, 2009). However, ensiling (SSF) of bacteria, fungus and yeast culture with MS for a period before feeding to ruminants has not been studied yet. So, the object of taking up this investigation was to study utilization of Solid-state fermentation by bacteria, fungus and yeast for bioconversion of MS, in order to increase nutritional value and palatability for animal feed.

MATERIALS AND METHODS

The present study was conducted at the farm of Maryout Research Station, Desert Research Center. The chemical analysis of the experimental samples was carried at the department of Animal Production, Faculty of Agriculture, Ain Shams University. The experiment was undertaken through February and March of the year 2015.

Animals, experimental design and management:

Twenty Barki rams with average body weight 42.8 kg ± 0.46 were used in a complete randomized design with five treatments and two consecutive experimental periods. The first period lasted for 25 days, during this period the rams were housed in 5 shaded pens (4animals/group) for recording daily voluntary feed intake. The animals

were thereafter housed in individual metabolic cages for 20 days to conduct digestibility trials for determining the nutritive value of tested diets. The animals were daily provided with fresh clean water twice through the duration of the experiment.

There were five experimental basal diets, namely: 1) Berseem hay as a negative control diet (BH); 2) MS without treatment as a positive control diet (MS); 3) MS treated with bacteria (*Cellulomonas cellulasea*) (MSB); 4) MS treated with fungus; *Trichoderma reesei* (MTF); 5) MS treated with yeast; *Saccharomyces cervisiae* (MSY)

During the voluntary feed intake measurement phase, the animals were fed *ad libitum* and feed refusal were collected each day and weighed to assess intake before any new feed was offered. A relative palatability index (RPI) was calculated for each diet by dividing all consumption values by that of the highest value, and multiplying the result by 100. The animals were restricted to 90% of their average dry matter intake during the faecal collection phase to minimize/eliminate feed refusal.

Biological treatments:

Microbiological studies were carried out at laboratory of microbiology, desert research center, Al-Mattaria, Cairo, Egypt. Facultative microbial strains were bacteria (*C. cellulasea*), fungus (*T. reesei*) and yeast culture (*S. cervisiae*).

Preparing growth cultures of microorganisms: The microorganisms was maintained on Czabek Dox agar medium (Oxoid, 1982) for *T.*

reesei, Yeast extract malt agar (YMA) medium (Pridham *et al.*,, 1958) for *S. cervisiae* and carboxy metgyl cellulose (CMC) agar medium (Someya, 1980) for *C. cellulasea* at 30˚C until used. Three different culture medium were prepared, and used to inoculate a sterilized broth medium. The inoculated medium (cultures) were incubated at 30 ºC for 7 days for fungus and 3 days for yeast or bacteria.

Farm application of solid state fermentation for moringa stalks: Large scale application of each strain culture to chopped moringa stalks were prepared by adding 36 liters of one of the previous growth culture to 360 liters of tap water and mixed with 300 kg chopped residues. All mixed well and then bagged and pressed in clean plastic bags holding 50kg and sealed and kept in chamber for 21 days at room temperature. At the end of incubation period, lasted for 21 days, the treated materials were solar dried to stop activity of fungi and moisture content reached less than 10% then packed and stored until used in feeding trials.

Digestibility and nitrogen balance trials: *In vivo* digestibility trials were conducted to evaluate the nutritive value of the experimental roughages fed as sole diet using twenty Barki rams (4 animals/group). Rams placed in the metabolic cages for the complete separation of faeces and urine after adapted for an initial period of 15 days. The adaptation period followed by 5 days of feed, faecal and urine collection phase consecutively. Daily collection from each animal was

composited and sampled for subsequent analysis. All feed and fecal samples were ground through 1-mm screen and mixed before analysis.

Sampling of rumen liquor and analysis: Rumen liquor samples were taken by stomach tube from three animals of each group at the day next to the digestibility trials. The samples were taken before morning feeding (zero-time) and at 3 and 6h post feeding. The rumen samples were filtered through two layers of cheese-cloth and used quickly as possible for measurement of pH by using Beckman pH meter. Strained rumen liquor was stored in plastic bottles with a few drops of toluene and paraffin oil just to cover the surface and stored at a deep freeze (-20˚C) till it was analyzed for ammonia nitrogen (NH_3-N) by using Markham micro-distillation apparatus Markham (1942) and total volatile fatty acids (TVFA's) according to Warner (1964).

***Sampling of blood and analysis*:** Blood serum was analyzed to determine the following parameters: total protein (TP) and albumin as described by Doumas *et al.* (1971). Globulin concentration was calculated by difference. Creatinine was determined using the method of Henery *et al.*, (1974), urea (Beale and Croft, 1961). Alanaine aminotransferase (ALT), aspartate aminotransferase (AST) were measured according to Reitman and Frankel (1957).

Chemical analysis: The samples of feed, feces and urine were analyses for proximate analysis according to AOAC (1995). Cell wall constituents (NDF, ADF and ADL) were determined according to Van Soest *et al.* (1991). Cellulose and hemicelluloses were calculated by

difference. Metabolizable energy values were predicted from the equations of Abate & Meyer (1997);

ME (MJ kg^{-1} DM) = 20.27 − 0.1431CF − 0.111 NFE − 0.2200 ASH.

Statistical analysis: The data were statistically analyzed by analysis of variance using SPSS (2010) program version 19. Treatment significance was determined by the new multiple range test of Duncan (1955).

RESULTS AND DISCUSSION

Nutrients composition:

Nutrient compositions of the experimental roughages are as shown in Table (1). Results revealed that DM, OM, CF, EE, NFE, and ADL contents in biological treated MS were lower than untreated MS. However, the CP, Ash, NDF, hemicellulose, and cellulose contents were higher in treated MS compared with untreated MS. Fungus treatment resulted in higher ADF however; yeast and bacteria treatments showed lower ADF values compared with untreated MS. The high value of CP content was observed with MSY followed by MSF and then MSB treatments.

These results of CP agree with increasing CP content with biological treatment could be due to microbial protein resulted from growing yeast, fungus and bacteria (El-Mahy,2013, Phillip *et al.*, 2014) Otherwise, Chandra *et al.* (1991) found that increase CP was reflected by a decrease in CF content.

Table (1): Nutrient composition of experimental diets fed to Barki sheep

Items	**MS**	**MSF**	**MSY**	**MSB**	**BH**
DM%	92.53	90.43	90.65	90.18	89.29
Nutrients % on DM basis					
OM	92.17	89.21	90.70	89.23	87.87
CP	7.32	10.02	13.94	9.91	12.44
CF	37.81	35.60	34.87	35.97	25.64
EE	3.40	2.85	2.16	3.10	1.73
NFE	43.64	40.74	39.73	40.25	48.06
Ash	7.83	10.79	9.30	10.77	12.13
NDF	58.20	65.81	61.16	62.94	38.79
ADF	47.58	48.71	46.73	42.35	21.52
ADL	11.60	6.78	6.99	7.01	5.42
Hemicellulose	10.62	17.10	14.43	20.59	17.27
Cellulose	35.98	41.93	39.74	35.34	16.10
ME, MJ/kg DM	8.29	8.28	8.82	8.29	8.60
Cost, LE/ton	1000	1200	1200	1200	2000

MS: Moringa stalks; MSF: fungi treated Moringa stalks ; MSY: yeast Moringa stalks ; MSB: bacteria Moringa stalks ; BH: berseem hay;CFM: concentrate feed mixture.

Bassuny *et al.* (2003a) found that OM and CF contents were decreased, while, CP and ash contents were increased as a result of fungi or bacteria treatments of corn cobs and sugar cane bagasse. Also, Belewu and Popoola (2007) showed that fungus treated sawdust based diets recorded the highest CP content compared to the control diet. Fungal, yeast and bacteria treatments decreased ADL content, whereas, hemicelluloses and cellulose contents were increased. These findings are agreed with those reported by Phillip *et al.* (2014). Reduction in lignin content as a result of biological treatment presumably because of

enzymatic action of microbial strains which could have degraded and solubilized the various fiber fractions for own their growth (Akinfemi and Ladibo, 2013). Baraghit *et al.* (2009) found that biological treatments with different fungal and bacteria strains decreased cell wall constituents of different crop residues.

Voluntary Feed intake:

Feed intake by Barki sheep fed the experimental roughages are shown in Table (2). Sheep fed biological treated moringa stalks showed the highest DM intake than untreated MS. Mean values of DM feed intake in absolute values or expressed as a percentage of body weight followed the same trend of average feed DM intake. The variation in feed intake may attributed to improvement in nutrients resulted from the biological treatment. These results were agree with those findings by Deraz (1996) who observed that chemical and biological treatments increased markedly voluntary feed DM intake of corn stalks compared with mechanically treated corn stalks. The lower DM feed intake of MS diet than the other four diets is a result of higher lignin content.

Feed unit intake expressed as TDN or DCP revealed that biological treatment improved intake of MS to become high quality roughage and covered the maintenance requirements of TDN and DCP that recommended by Kearl (1982). El-Ashry *et al.* (1997) found that, TDN content increased from 63.93 and 63.35% in untreated rice straw and corn stalk to 72.31 and 72.88% in fungal treated ones, respectively.

Table (2): Voluntary of feed intake of moringa stalks untreated or biologically treated with different strains.

Group Ration	LBW[1] kg	Dry matter intake		Feed unit intake, g		% of MR[2]		Palat. Index[3]
		Av., g	% BW	TDN	DCP	TDN	DCP	
BH	42.9	953	2.22	532	73.6	121	188	100
MS	43.7	688	1.57	270	19.7	60	50	72.2
MSF	43.1	909	2.11	448	44.9	101	115	95.4
MSY	43.3	927	2.14	502	84.0	113	216	97.3
MSB	42.6	894	2.10	439	41.7	100	109	93.8

[1]live body weight; [2]Maintenance requirement as Kearl (1982); [3]palatability index:

It is interesting to note from data of % of maintenance requirement (Table, 2) that, the availability of maintenance TDN and DCP in BH, MSF, MSY and MSB diets was more than the requirement, however the availability of these two items throw MS was much less as compared to Kearl (1982). Fortunately, the present results clearly show that, biological treated MS could be used as a basal diet to cover maintenance requirements however; it could not be for untreated one.

The relative palatability index (RPI) is shown in Table (1). Sheep preferred in descending order of magnitude BH, MSY, MSF, and MSB to MS. The relative palatability index observed in BH, MSY, MSF, and MSB was corroborated by crude fiber contents of 25.64, 34.87, 35.60, and 35.97% observed in this group. The following of MSY to BH according to the RPI ranking in this study is an interesting observation. Calculation of daily feed cost reveal that treated MS was lower than BH diet by 43.4, 42.4 and 44.1% for MSF, MSY MSB diets; respectively.

Digestibility and nutritive value of experimental diets

Nutrients digestibility and nutritive values of the experimental diets are presented in (Table 3). Excluding BH diet, all biological treatments resulted in a higher ($P<0.05$) digestibility of all nutrients compared with untreated MS. Generally the highest values of DM, OM, CP, CF. NFE, NDF, ADF and cellulose digestibilities were observed with MSY diet. However, MSB showed highest digestibility values for EE and hemicelluloses. The observed increase in digestibilies of all nutrients of biological treated moringa stalks may be attributed to its high metabolizable energy content compared to their content of control moringa stalks. These results are in agreement with those obtained on biological treatment for different residues (Hassan *et al.*, 2005, El-Shafie *et al.*, 2007, Mahrous and Khorshed, 2012, El-Mahy, 2013 and Phillip *et al.*, 2014). Regarding the effect of biological treatments on the nutritive values of the experimental diets (Table 3), the impact was more obvious with the yeast treatment on TDN and DCP comparing with the other treatment and untreated moringa stalks. Also calculated metabolizable energy content and nutritive ratio showed the same trend. These results are in agreement with that reported by Deraz and Ismail (2001), Mahrous (2005), Kholif *et al.* (2005), Salman *et al.* (2011) and Fayed *et al.* (2012). Calculating improvement of TDN and DCP for treated moringa stalks when related to untreated MS recoded that yeast treatment was superior followed by fungus and bacteria. However, when BH was the basal to calculation MS represent only 70.13 and

37.05% of the nutritive value of BH and improved to be 96.97 and 117.36%, 88.24 and 63.99, 87.85 and 60.36 when MS treated with yeast, fungi and bacteria, respectively.

Table (3): Digestibility coefficients and nutritive values of moringa stalks untreated or biologically treated with different strains.

Items	Experimental diets					SEM
	BH	MS	MSF	MSY	MSB	
Digestibility coefficients (%)						
DM	62.34^{a}	38.90^{d}	51.49^{c}	56.95^{b}	50.91^{c}	1.89
OM	62.18^{a}	40.16^{c}	52.13^{b}	57.52^{a}	51.55^{b}	1.81
CP	61.25^{a}	39.10^{c}	49.37^{b}	65.00^{a}	47.08^{b}	2.42
CF	51.70^{a}	40.91^{c}	48.70^{ab}	49.79^{ab}	47.06^{b}	1.01
EE	58.85^{c}	50.94^{d}	78.51^{ab}	74.53^{b}	79.46^{a}	2.77
NFE	68.20^{a}	38.85^{c}	53.97^{b}	60.75^{ab}	54.53^{b}	2.59
NDF	43.39^{c}	39.11^{c}	51.56^{ab}	56.67^{a}	50.20^{b}	1.58
ADF	24.65^{d}	36.82^{c}	50.23^{a}	55.03^{a}	43.73^{b}	2.56
Hemicellulose	66.74^{a}	49.36^{c}	55.34^{bc}	63.03^{ab}	65.03^{ab}	1.78
Cellulose	48.27^{b}	43.01^{b}	62.71^{a}	67.17^{a}	61.61^{a}	2.24
Nutritive value (%)						
TDN, %	55.87^{a}	39.18^{c}	49.30^{b}	54.18^{a}	49.08^{b}	1.46
DCP, %	7.72^{b}	2.86^{d}	4.94^{c}	9.06^{a}	4.66^{c}	0.52
ME, Mcal/kg DM	2.01^{a}	1.40^{d}	1.73^{b}	1.94^{a}	1.61^{c}	0.05
NR	6.34^{a}	12.74^{c}	8.98^{b}	4.99^{a}	9.76^{b}	0.65
NQI	4.56	3.90	7.18	5.64	6.33	-
TDN improvement, %						
Based on MS	-	100	126	138	125	-
Based on BH	100	70.13	88.24	96.97	87.85	-
DCP improvement, %						
Based on MS	-	100	173	317	163	-
Based on BH	100	37.05	63.99	117.36	60.36	-

BH: berseem hay; MS: Moringa stalks; MSF: fungal treated Moringa stalks; MSY: yeast treated Moringa stalks; MSB: bacterial treated Moringa stalks.
NR:nutritive ratio= (TDN-DP) / DP.; NQI: Nutritive quality index = CP% X DMD%/100.
Means followed by the same letter are not significantly different at 5%

Nitrogen utilization for Barki sheep fed the experimental diets:

Data of nitrogen balance for sheep fed the experimental diets are presented in Table (4). The N-retention is considered as the most common index of protein nutrition status of ruminants (Owen and Zinn, 1988) All treatments showed positive nitrogen retained value except that group fed untreated moringa stalks (MS) showed negative value. There were significant ($P<0.05$) differences between biological treatment tested. Nitrogen balance (g/d) for MSY (1.48) was higher than MSY (1.10) MSB (0.91). The same trend was detected for nitrogen retained either as percent of nitrogen intake or as percent of nitrogen digested. The present results were in agreement with that of Mahrous (2005).

Table (4): Mean nitrogen balance by Barki sheep fed a sole diet of Moringa stems treated with different strains.

Items	Experimental diets					SEM
	BH	MS	MSF	MSY	MSB	
N intake, g/d	17.06^{b}	7.25^{d}	13.10^{c}	19.37^{a}	13.41^{c}	0.96
Feces N, g/d	6.61^{a}	4.41^{b}	6.64^{a}	6.81^{a}	7.12^{a}	0.29
Urine N, g/d	9.25^{b}	3.94^{d}	5.37^{c}	11.07^{a}	5.38^{c}	0.63
Total N output, g/d	15.86^{b}	8.35^{d}	12.00^{c}	17.88^{a}	12.50^{c}	0.78
N Retained, g/d	+1.21^{ab}	-1.10^{c}	+1.10^{ab}	+1.48^{a}	+0.91^{b}	0.22
NR mg/kg $w^{0.75}$	70.8^{ab}	-65.4^{c}	64.8^{ab}	86.9^{a}	54.7^{b}	13.0
NR/NI, %	7.06^{a}	-15.27^{b}	8.44^{a}	7.72^{a}	6.83^{a}	2.13
NR/ND, %	11.50^{a}	-39.39^{b}	17.14^{a}	11.86^{a}	14.18^{a}	4.94

Means followed by the same letter are not significantly different at 5%

Water utilization:

Data in Table (5) represent the effect of the experimental diets on water utilization expressed as metabolic body mass (kg $W^{0.82}$). Animals under investigation received biological treated moringa stalks showed

higher ($P < 0.05$) values of dietary water intake comparing to untreated one. This result might be due to the higher feed intake and also the higher moisture content where, the process of solid state fermentation that undergo with condition of 60% moisture for treated moringa and sundried before feeding was not enough to make the treated moringa completely dry. Also, BH group showed the highest value comparing with the groups, as berseem hay is the highest moister content than the other diets. Mean values of combined metabolic water intake are mainly related to TDN intake of each diet.

BH group was the highest in free water intake followed by MS and then the three treated moringa groups. This may be due to physical prosperities in term of water holding capacity which is higher for moringa stalks than berseem hay. (More and Sahni ,1981) concluded that dry matter intake is considered to be a major factor affecting water intake and it is customary to express the water intake (the sum of water drunk and water contained in feed). They added that, the inadequate supply of drinking water restricted the dry matter intake. Low feces water output ($P<0.05$) for BH group comparing to their mates received the other four diets might be due to lower faecal moisture and/or lower digesta flow rate for hay than moringa stalks. Regarding the urine output values, it can be seen that the lower ash content in moringa stalks than berseem hay reflect the lower urine output.

Table (5): Water utilization for Barki sheep fed the experimental diets during the digestibility trials

Items	Experimental diets					SEM
	BH	MS	MSF	MSY	MSB	
Feed water intake g/kg$^{0.82}$	4.69^{a}	2.76^{d}	4.18^{c}	4.26^{bc}	4.45^{b}	0.16
Metabolic water, g/kg$^{0.82}$	12.98^{a}	6.72^{d}	10.93^{c}	12.67^{ab}	11.37^{bc}	0.56
Free water intake, g/kg$^{0.82}$	82.3^{e}	90.9^{d}	131.9^{c}	154.7^{a}	144.6^{b}	6.74
Total water intake, g/kg$^{0.82}$	100^{d}	100^{d}	147^{c}	172^{a}	160^{b}	7.01
Feces water, g/kg$^{0.82}$	10.1^{c}	26.1^{a}	21.1^{ab}	19.0^{b}	21.5^{ab}	1.44
Urine water, g/kg$^{0.82}$	42.1^{a}	28.0^{bc}	32.0^{b}	23.8^{c}	28.2^{bc}	1.66
Total water output, g/kg$^{0.82}$	55.2^{ab}	62.0^{a}	59.5^{ab}	48.7^{b}	56.2^{ab}	1.71
Retained water (RW),g/kg$^{0.82}$	75.1^{d}	68.5^{d}	132.0^{c}	175.1^{a}	152.6^{b}	9.74
RW /total water intake,%	57.4^{c}	52.5^{d}	69.0^{b}	78.4^{a}	73.1^{b}	2.31
g RW/ g DMI	1.49^{d}	1.87^{c}	2.75^{b}	3.47^{a}	3.04^{b}	0.17
g RW/ g TDNI	2.66^{c}	4.78^{b}	5.62^{ab}	6.43^{a}	6.21^{a}	0.36
g RW/ g DCPI	19.6^{c}	65.8^{a}	55.9^{a}	38.4^{b}	67.2^{a}	4.47
g RW/ g CFI	5.80^{c}	4.94^{c}	7.74^{b}	9.96^{a}	8.44^{b}	0.43

* Metabolic water: one gm TDNI yields 0.6 gm water (the carbohydrate factor)

Means followed by the same letter are not significantly different at 5%

Within biological treated moringa groups, MSY showed the lowest values like the trend for feces water output. Animal group fed MSY diet showed higher values of retained water either expressed as g/kg w$^{0.82}$ or percentage of water intake, or related to DMI, TDNI and CFI, than the other four experimental groups. The higher ($P<0.001$) retained water for MSY group may be attributed to one or both of the following reasons; first higher water holding capacity, the second is increasing of heat increment that result from diet fermentation which may lead to increase

water needed for body cooling system (Gihad *et al*., 1989, Kewan *et al.*, 2011).

Rumen fermentation

Ruminal pH, NH_3-N, TVFA's values recorded for sheep fed the experimental roughages are shown in Table (6). Sheep fed MSF, MSY and MSB diets showed higher ($P<0.05$) pH values at 0, 3 and 6hrs post feeding than those fed MS or BH. The present results were within the range reported by Choughary and Orga (1979) that was 4.6 to 7.9 and observed during feeding different types of feedstuff. Also, these results were in agreement with Aziz *et al.* (2008) for olive pruning tree by-products treated with *T. viride + S. Cervesiae*. However, Mahrous *et al.* (2011) and Mahrous and Khorshed (2012) reported that biological fungal treatment had no significant effect on pH values. On the other hand, Deraz and Ismail (2001) reported that fungal treatments resulted in lower ($P<0.05$) pH values compared with control diet. All experimental groups showed the lowest pH values at 3hr post feeding except MSY which reveal decreasing trend from 0 to 6 hrs post feeding. These findings might be related to ruminal fermentation process by rumen microorganisms (Aziz *et al.*, 2008). These results are similar to that obtained by Phillip *et al.* (2014).

The present data of ruminal NH_3-N concentration (Table 6) showed gradual increase post feeding to reach maximum values at 3hrs for MSF, MSY, MSB and BH then declined to lower values at 6hrs post feeding. The highest ($P<0.05$) values observed at 3hrs post feeding recorded for sheep fed MSB followed by MSY, MSF, MS comparing

with BH group. These differences of NH_3-N values may be due to the difference in nitrogen sources and concentration among treatments (Bassuny *et al.,* 2003b). Otherwise, the increase in NH_3-N concentration post feeding may be related to higher solubility and degradation of dietary CP or to higher NPN content that converted easily to ammonia during the fermentation process.

Table (6): Rumen fermentation parameters for Barki sheep fed a sole diet of Moringa stems treated with different strains.

Items	Time	Experimental diets					SEM
		BH	MS	MSF	MSY	MSB	
pH	0	5.55^{b}	5.71^{b}	6.46^{a}	6.62^{a}	6.56^{a}	0.12
	3	5.18^{d}	5.38^{c}	6.25^{b}	6.44^{a}	6.14^{b}	0.14
	6	5.28^{c}	5.50^{c}	6.45^{a}	6.11^{b}	6.27^{ab}	0.13
NH_3-N, mg/dl	0	17.1^{b}	19.2^{b}	25.3^{a}	18.0^{b}	25.7^{a}	1.04
	3	23.9^{c}	27.3^{c}	33.7^{b}	37.1^{b}	48.0^{a}	2.31
	6	21.3^{b}	33.8^{a}	27.9^{a}	32.4^{a}	30.1^{a}	1.36
TVFA's, meq/dl	0	7.66^{a}	6.54^{bc}	7.07^{ab}	7.02^{ab}	6.24^{c}	0.15
	3	8.42	8.94	8.66	9.56	9.74	0.20
	6	9.51^{b}	10.60^{a}	9.59^{b}	10.09^{ab}	10.57^{a}	0.16

Means followed by the same letter are not significantly different at 5%

Similar trend was observed on different types of biological treated residues as reported by Tuen and Dahan (1994), Salman *et al.* (1998), Bassuny *et al.* (2003b) and Mahrous and Khorshed (2012). Also, El-Ashry *et al.* (1997) and Khorshed (2000) found a significant increase in rumen NH_3-N with fungal treated residues and with yeast culture treatment. On the other hand Hassan *et al.* (2005) reported that ruminal NH_3-N was significantly increased ($P<0.05$) for untreated banana

wastes than that of biologically treated feed group. These findings are in agreement with results of Phillip *et al.* (2014) when grape trees by-products treated with either *Trichoderma reesei* or *T. viride*. However, sheep fed MS roughage showed the maximum values of NH_3-N at 6hrs post feeding which revealed that untreated MS has low degradable protein with time progressing. Pant *et al.* (1963) reported the concentration of NH_3-N in rumen fluid reached its peak after 3hrs post feeding then declined after 5hrs post feeding. The present values are within the range 10 - 40mg/100ml rumen fluid that reported by MacDonald (1952).

Concentrations of TVFA's (meq/100 ml) by sheep fed the experimental diets are presented in Table (6). All the experimental diets resulted in gradual increase to peak values at 6hrs post feeding. The highest ($P<0.05$) values of TVFA's were observed with sheep fed MS followed by MSB, MSY, MSF and BH. Bacteria and yeast cultures are higher than fungi culture in fermenting MS in the rumen to produce TVFA's. However the same fungal strain was effective in TVFA's production from pruning trees by-products (Phillip *et al.*, 2014), this emphasis that the effect of the same microbial strain may be differed with differ the treated residues. Bassuny *et al.* (2003b) and Mahrous *et al.* (2011) reported that rumen TVFA's concentrations were significantly increased with fungal and yeast treated residues. The increase of TVFA's may be due to the increase of OM digestiblility (El-Ashry *et al.*, 2003) or correlated with NDF disappearance (Doane *et al.*, 1997). Moreover, Phillip *et al.* (2014) reported that higher

concentration of TVFA's of biological treatment groups may be as a result of altered rumen microbial population and microbial activity. Also, Allam *et al.* (1984) showed that TVFA's concentration is a reflection of different factors such as DM digestibility, rate of absorption, rumen pH, digesta passage flow rate and microbial population in the rumen and their activities.

Blood parameters:

Blood serum parameters presented in Table (7) showed significant (P>0.05) differences among the different experimental groups for serum total protein, urea and createnine concentrations. However, insignificant (P>0.05) differences were observed in serum albumin, globulin, ALT and AST concentrations. Cornelius (1970) reported that the concentration of total protein in the serum of domestic animals ranged between 6-10 g/dl.

Table (7): Blood serum parameters for Barki sheep fed a sole diet of moringa stems treated with different strains.

Items	Experimental diets					SEM
	BH	MS	MSF	MSY	MSB	
Total protein, g/dl	7.00^{ab}	6.61^{ab}	7.20^{a}	6.90^{ab}	6.13^{b}	0.14
Albumin, g/dl	3.57	3.54	3.57	3.38	3.35	0.08
Globulin, g/dl	3.43	3.07	3.63	3.52	2.78	0.17
Urea, mg/dl	50.9^{ab}	49.4^{bc}	56.8^{a}	44.4^{c}	43.8^{c}	1.34
Createnine, mg/dl	1.13^{b}	1.21^{b}	1.44^{a}	1.43^{a}	1.57^{a}	0.04
ALT, IU/l	67.3	74.1	69.4	72.8	76.2	1.97
AST, IU/l	165.6	161,3	143.6	156.7	160.5	5.38

Means followed by the same letter are not significantly different at 5%

Deraz (1996) reported that the highest value of total protein, albumin and urea were recorded with rams fed ration contained corn stalks with chemi-fungal followed by rations treated with urea and untreated. Khorshed (2000) indicated that values of serum total protein of ration contained biological treatment were higher than the value of the control. Deraz and Ismail (2001) and Mahrous *et al.* (2011) reported that no significant differences ($P<0.05$) were observed in blood constituents as feeding rations contained biological treated roughages. MBS animal group showed the lowest values of serum total protein and urea being 6.13 g/dl and 43.8 mg/dl; respectively. However, it has the highest value of createnine concentration (1.57 g/dl) comparing with the other experimental groups. Result data of serum ALT are higher than those (34.89-35.02 IU/l) found by Abou Ammou *et al.* (2008) for growing lambs fed wheat straw treated with *T. viride*. However, AST concentration were lower the normal range (260-350 IU/l) reported by Mohamed and Selim (1999).

CONCLUSION

From the present study, it was clear that, quality of MS as roughage for ruminants could be clearly improved after treating with fungi and yeast or bacteria and may appeared to be equal to berseem hay under Egyptian conditions and it could be utilized to cover a large part of shortage of animal feeds now a day and in the future, furthermore, it was lower than hay for daily feed cost. Besides of the biological methods of treating MS produce good quality forage for animal

feeding; it indirectly reduces the environmental pollution resulting from burning these residues.

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تحسين القيمة الغذائية لحطب المورينجا باستخدام فطر الترايكوديرما ريساي، بكتريا السليوموناس سليولانس وخميرة الخباز في نظام التخمر في الحالة الصلبة

[١٠]

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المستخلص

استخدم ٢٠ ذكر أغنام برقي بمتوسط وزن ٤٢،٨ كجم في تصميم احصائي كامل العشوائية في خمس مجموعات متساوية لدراسة تأثير المعاملات البيولوجية باستخدام فطر الترايكوديرما ريساي، بكتريا السليوموناس سليولانس وخميرة الخباز في نظام التخمر في الحالة الصلبة على القيمة الغذائية لمخلف حطب المورينجا وذلك مقارنة بالحطب غير المعامل ودريس البرسيم. أظهرت النتائج أن هذه المعاملات أدت الى خفض نسبة المادة الجافة والمادة العضوية والألياف والدهن والكربوهيدرات الذائبة واللجنين في حطب المورينجا وعلى العكس فقد زادت نسبة البروتين والرماد والألياف المتعادلة والسليولوز والهيميسليولوز وذلك مقارنة بالحطب الغير معامل. كما أدت هذه المعاملات الى تحسن في كمية المأكول من المادة الجافة وكذلك معاملات هضم العناصر الغذائية. كانت معاملة الخميرة هي الأعلى تحسنا للقيمة الغذائية للحطب من حيث البروتين المهضوم والطاقة. أظهرت جميع الحيوانات المغذاة على الحطب المعامل ميزان آزوتي موجب بينما كان سالبا في المجموعة التي غذيت على الحطب الغير معامل.

من نتائج هذه الدراسة يتضح أن حطب المورينجا المعامل بيولوجيا يمكن استخدامه كعليقة حافظة للأغنام كما أنه أقل تكلفة من دريس البرسيم علاوة على أن توجيه استخدامه كعلف للحيوانات المجترة يؤدي إلى خفض تلوث البيئة الناتج عن حرق مثل هذه المخلفات.