

Impact of Microbial Feed Supplements on the Productive Performance of Lactating Nubian Goats

¹H.H. Azzaz, ²Hend A. Aziz, ¹Eman S.A. Farahat and ¹H.A. Murad

¹Department of Dairy Sciences, National Research Centre, Dokki, Giza, Egypt

²Department of Animal Nutrition, Desert Research Center, Cairo, Egypt

Abstract: This study was conducted to investigate the effect of *Aspergillus awamori* (Tomoko[®]) and *Lactobacillus acidophilus* (Veta-Zyme Plus[®]) as microbial feed supplements on *in vitro* and *in vivo* feed nutrients digestibility, rumen fermentation characteristics, blood parameters, milk yield and milk composition of lactating Nubian goats. In the *in vitro* trial, the rations supplemented separately with Tomoko[®] and Veta-Zyme Plus[®] at 3 levels (1, 2 and 3 g / kg DM) compared with the control. Increasing Tomoko[®] and Veta-Zyme Plus[®] supplementation levels up to 2 g/kg DM gave the highest values of dry and organic matter disappearance of tested rations. In digestibility and lactation trials, 30 Nubian lactating goats after 7 days of parturition were randomly assigned into 3 groups of 10 animals each using complete random design. The first group was fed on 60% CFM, 20% berseem hay and 20% rice straw (control ration), the second group was fed the control ration + Tomoko[®] at 2g/Kg DM (T₁), while the third group was fed the control ration + Veta-Zyme Plus[®] at 2g/Kg DM (T₂). Microbial feed supplementation significantly ($P<0.05$) increased all nutrients digestibility (except EE digestibility), all rumen parameters (except ruminal pH) and total protozoal count for treated groups compared to those of the control. Goats fed microbial supplemented rations had higher blood plasma total protein, albumin and glucose concentrations than those of the control group. Milk, 4% fat corrected milk and the other components yields were higher for goats fed microbial supplemented rations than those of the control group, while milk composition was not affected. Rations supplemented with *Aspergillus awamori* and *Lactobacillus acidophilus* enhanced Nubian lactating goat's performance with no deleterious effects on their health.

Key words: Microbial feed supplements • Nutrients digestibility • Blood parameters • Milk production and Nubian goats

INTRODUCTION

Improved dairy animals health and their productive performance has always remained a primary goal of researchers associated with dairy animal production. Several preparations have been used to improve dairy animal's performance either by manipulation of the rumen ecology (e.g., buffers) or by altering the rumen microbe's composition and metabolic activities (e.g., ionophore antibiotics). But, with the growing concerns towards the use of antibiotics in animals feed industry, more emphasis has been given to use microbial feed supplements as natural growth promoters.

The definition of microbial feed supplements is very broad and may include specific and nonspecific yeast, fungi, bacteria, cell fragments and filtrates [1]. Yoon and Stern [2] listed several possible modes of action for bacterial and fungal feed supplements including enhancement of the ruminal microbial growth, stabilization of the ruminal pH, improved the ruminal fermentation patterns, improved nutrients retention, digestibility and their flow to the small intestine and they also reduced the animal's stress.

The effect of microbial feed supplementation on dairy animal performance or rumen fermentation has been reviewed by several researchers [3-6]. Although microbial

feed supplementation has improved milk yield and milk composition [7], feed efficiency and animals health [8], animal response to microbial feed supplementation have been inconsistent. Moreover, results of microbial feed supplementation studies which conducted on dairy cattle are difficult to compare because of several factors such as the microbial strain and its viability, microbial inclusion level in the diet, diet composition, feed intake and feeding frequency, animal age, type, health and its physiological and stress status [9].

A comprehensive analysis of variables indicative of the complex of influence of microbial feed preparations is most lacking. Therefore, the present study was conducted to investigate the effect of two commercial products of bacterial feed supplement (*Lactobacillus acidophilus*) and fungal feed supplement (*Aspergillus awamori*) on *in vitro* dry and organic matter disappearance of lactating goats rations, nutrients digestibility, rumen fermentation characteristics, rumen ciliate protozoal count, blood parameters, milk yield and milk composition.

MATERIALS AND METHODS

This work was carried out at Alsttar farm for animal production (a private farm), Cairo - Alexandria Saharan Road nearby the Khatatba city, Monofia governorate, Egypt. Chemical and microbiological analyses were carried out at the Laboratories of Dairy Department, National Research Center, Dokki, Giza, Egypt and Animal Nutrition Department, Desert Research Center, Cairo, Egypt.

Microbial Products Source: Veta-Zyme Plus® bacterial feed supplement preparation produced by Vetagri Consulting Inc, Canada, each gram of it contains 200 million colony forming unit (CFU) of *Lactobacillus acidophilus* on calcium carbonate as a carrier, 400 unit of cellulase, 550 unit of amylase and 2000 unit of protease. Tomoko® fungal feed supplement preparation produced by Biogenkoji Research Institute, Japan, each gram of it contains 3 million cell of *Aspergillus awamori*, using wheat bran as fermentation base, 1000 unit of acidic protease, 30 unit of pectinase, 25 unit of xylanase, 20 unit of α -amylase, 10 unit of phytase, 5 unit of glucoamylase and 4 unit of cellulase.

***In vitro* Study:** The *in vitro* dry matter and organic matter disappearance (IVDMD and IVOMD) for microbial supplemented rations were determined. One gram sample

of total mixed ration (0.6 g concentrate feed mixture, 0.2 g berseem hay and 0.2 g rice straw) was accurately weighed into 250 ml incubation bottles, these bottles were separately supplemented with solution of Veta-Zyme Plus® and Tomoko® at different levels (1, 2 and 3 g/Kg DM) using 5 bottles per each supplementation level. The *in-vitro* technique was carried out according to Fondevila and Pérez-Espés [10]. The procedures were using bottles filled with 140 ml of incubation solution prepared under a CO₂ atmosphere, including a buffer solution, macro-mineral and trace mineral solution, a reduction solution and rumen inoculum. Rumen inoculum was obtained from rams fed berseem hay ration using stomach tube, squeezed through four layers of gauze and fluid was collected in a pre-warmed thermos oxygen-free plastic jar. The bottles were sealed and maintained at 40°C in a shaking water bath (25 oscillations/min) for 48 h.

Digestibility and Lactation Trials

Animals, Feeding and Management: Thirty Nubian lactating goats (about 3 years old and weighting on average 34 ± 0.4 kg) after 7 days of parturition randomly assigned into three groups of ten animals each using complete random design. The entire experimental period was 63 days (9 weeks). Goats were fed dry matter according to 4% of their body weight changed continuously based on animal weight changes. The first group was fed on 60% concentrate feed mixture (CFM), 20% berseem hay and 20% rice straw (control ration). The two microbial preparations were supplemented at the optimum rate which obtained from the *in vitro* trial results. Accordingly the second group was fed the control ration supplemented with Tomoko® at 2g/Kg DM (T₁), while the third group was fed the control ration supplemented with Veta-Zyme Plus® at 2g/Kg DM (T₂). The concentrate feed mixture (CFM) was offered once daily at 8.00 a.m., berseem hay and rice straw were offered once daily at 9.00 and 11.00 a.m., respectively. Tomoko® and Veta-Zyme Plus® were mixed with small amount of CFM and introduced to the animals once per day. Fresh water was available all the time for all experimental groups. Chemical composition of feed ingredients and calculated total mixed ration are shown in Table (1).

Apparent Digestibility: A grab sample method was applied at which acid insoluble ash (AIA) was used as an internal marker for determination of nutrient digestion coefficients according to Ferret *et al.* [11]. At the end of

Table 1: Chemical composition of feed ingredients (on DM basis)

Item	CFM	Berseem hay	Rice straw	Experimental ration (calculated)
Chemical composition, %				
OM	91.59	91.09	82.20	89.61
CP	13.97	15.57	2.42	11.98
EE	4.38	4.85	3.67	4.33
CF	8.64	13.51	29.34	13.75
NFE	64.60	57.16	46.77	59.55
Ash	8.41	8.91	17.80	10.39
Cell wall constituents, %				
NDF	33.16	45.67	69.61	42.95
ADF	12.77	32.47	53.64	24.88
ADL	3.73	5.51	12.97	5.93
Hemicellulose	20.39	13.20	15.97	18.07
Cellulose	9.04	26.96	40.67	18.95

CFM= concentrate feed mixture consisted of 50% yellow corn, 10% soybean meal, 20% wheat bran, 15% cotton seed meal, 3% minerals-vitamins premix and 2% molasses. Hemicellulose = NDF-ADF, Cellulose = ADF-ADL

each month of the experimental period, fecal grab samples were collected in cloth bag connected to the animal back at 12 p.m., for three successive days from five animals of each group. The collected feces were dried at 60°C for 48 h and then ground for chemical analysis. The digestibility coefficient of nutrient was calculated according to the following formula [11].

Digestion co-efficient =

$$100 - \left[100 \times \frac{\% \text{indicator in feed}}{\% \text{indicator in feces}} \times \frac{\% \text{nutrient in feces}}{\% \text{nutrient in feed}} \right]$$

Feed and Fecal Analysis: Chemical analysis of feed stuffs and feces samples were carried out to determine the percentage of dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash content using the methods of A.O.A.C. [12]. The nitrogen free extract (NFE) was calculated by difference. The neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent Lignin (ADL) contents were determined using the methods described by Van Soest *et al.* [13].

Sampling and Analysis of Rumen Liquor: At the last day of each month of experimental period, rumen liquor samples were collected by stomach tube from two animals each group at 4 h post-feeding of the concentrate feed mixture. Samples were strained through two layers of gauze cloth to remove feed particles and immediately used for determination of ruminal pH using digital pH-meter. Rumen liquor samples were stored in glass bottles with drops of toluene and thin layer of paraffin oil and stored in a deep freeze (-20°C) until analysis. Ammonia

nitrogen concentration (NH₃-N), total nitrogen (TN) and non-protein nitrogen (NPN) were determined by the modified semi-micro-kjeldahl digestion method according to A.O.A.C [12] while true protein nitrogen (TP) was calculated by subtracting the non-protein nitrogen content from total nitrogen content. The total volatile fatty acids (TVFA's) were determined according to method of Warner [14]. The ruminal microbial protein was estimated as described by Makkar *et al.* [15]. For classification and determination of ruminal ciliate protozoal count, the filtered rumen liquor were fixed and stained with 4 times volume of methyl-green formalin saline solution as described by Ogimoto and Imai [16], then stoked in dark place until examination. After gentle mixing of fixed rumen liquor sample, one drop was poured on hemocytometer slide, covered with a cover slip and examined under a light microscope for identification of protozoal genera and species according to the description published by Dehority [17].

Sampling and Analysis of Blood Plasma: Blood samples were taken from jugular vein of two animals each group at the last day of each month of the experimental period at about 4 h after feeding of CFM and directly collected in glass tubes containing EDTA as an anticoagulant. Blood plasma samples were separated by centrifuge at 5000 r.p.m. /15 min. and kept frozen for later analysis. Plasma total protein was determined according to method of Armstrong and Carr [18], albumin [19], globulin was calculated by subtracting the albumin from total protein, urea [20], glucose [21] total lipids [22], plasma Aspartate aminotransferase (AST) and Alanin aminotransferase (ALT) [23].

Sampling and Analysis of Milk: The goats were milked twice a day at 8.00 a.m. and 5.00 p.m. during the last two days of each month of experimental period. Milk samples were immediately collected from each animal after morning and evening milking and milk yield was recorded. The sample of each animal represented a mixed sample of constant percentage of the evening and morning yield. Milk samples were analysed for total solids, fat, total protein and lactose by Bentley¹⁵⁰infrared milk analyzer (Bentley Instruments, Chaska, MN, USA) according to A.O.A.C. [12] procedures. Solids-not-fat (SNF) was calculated by subtracting fat from total solids percentage. Fat corrected milk (4% fat) was calculated by using the following equation according to Azzaz *et al.* [24]: FCM = 0.4 M + 15 F, Where: M= milk yield (g) and F= fat yield (g).

Statistical Analysis: Data obtained from this study were statistically analysed by IBM SPSS Statistics for Windows [25] using the following general model procedure:

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

where Y_{ij} is the parameter under analysis of the ij bottles of *in vitro* and rumen liquor trails or goats of digestibility and lactation trails, μ is the overall mean, T_i is the effect due to treatment on the parameter under analysis, e_{ij} is the experimental error for ij on the observation, the Duncan's multiple range tests was used to test the significance among means [26].

RESULTS AND DISCUSSION

In vitro Study: All levels of Tomoko[®] and Veta-Zyme Plus[®] supplementation significantly increased ($P < 0.05$) *in vitro* dry matter disappearance (IVDMD) and *in vitro* organic matter disappearance (IVOMD) of treated rations compared with control one (Table 2). Increasing Tomoko[®] and Veta-Zyme Plus[®] supplementation levels up to 2 g/kg DM gave the highest values of both IVDMD and IVOMD of tested rations. Our results are in line with those obtained by Azzaz *et al.* [24] who found that all levels of Tomoko[®] supplementation improved *in vitro* degradation of sugar beet pulp and 2g/kg DM of supplementation exhibited the highest ($P < 0.05$) values of IVDMD and IVOMD. Similarly, Farahat [27] reported that, all levels of Veta-Zyme Plus[®] supplementation enhanced *in vitro* disappearance of date Kernels and 1 g/kg DM is the most beneficial level of supplementation.

These positive results could be attributed to success of Tomoko[®] and Veta-Zyme Plus[®] supplementation to alter the chemical composition of tested rations or activate ruminal microbial fermentations. This hypothesis supported by number of studies, Varel *et al.* [28] suggested that *Aspergillus* extracts may improve fibre digestion because they contain esterase enzymes. Also, Arambel and Kent [29] reviewed that in the case of supplemented microbial feed not survives in the rumen, their lysis would provide protoplasm which in turn represent a source of nutrients for the rumen microbes and subsequently faster ruminal microbial colonization and fermentation.

Digestibility and Nutritive Values: Apparent total tract digestibilities of DM, OM, CP, CF and NFE for microbial supplemented goat's rations (T_1) and (T_2) showed significant improvement compared with those of the control, while EE digestibility was not affected by treatments (Table 3).

In addition, goats received supplemented ration with Tomoko[®] showed significant ($P < 0.05$) increase DM, OM and NFE digestibility compared with those received Veta-Zyme Plus[®] supplementation. There were no significant ($P > 0.05$) differences between treated groups (T_1 and T_2) in CP and CF digestibility. Furthermore, the nutritive values of the experimental rations expressed as total digestible nutrients (TDN) and digestible crude protein (DCP) match the digestibility coefficients (Table 3). Goats fed (T_1) and (T_2) rations showed significant ($P < 0.05$) higher TDN and DCP compared to those fed the control ration. These results are supported by the finding of Farahat [27] who stated that supplemented lactating goat's diets with Veta-Zyme Plus[®] significantly ($P < 0.05$) improved all nutrients digestibility and nutritive values compared with those of the control. Also, lactating buffaloes fed Tomoko[®] supplemented rations showed significant ($P < 0.05$) higher DM, OM, CF, NFE digestibility and TDN value compared to those fed the control ration [24]. Available data about the effects of microbial feed supplements on total nutrients digestion is very few. In this study, Tomoko[®] and Veta-Zyme Plus[®] supplementation would be expected to increase total tract digestibility by: 1) Alter the nature of tested rations based on their composition of enzymes activities which causing acceleration in ruminal fermentation, 2) Their positive effects on total ruminal protozoal count (Table 5) which in turn causes improvement in fiber degradation and nitrogen turnover in the rumen as reported by Eugene *et al.* [30] and/or 3)

Table 2: *In vitro* DM and OM disappearance of microbial supplemented ration

Treatments	Supplementation level g/Kg DM	IVDMD%	±SE	IVOMD%	±SE
Control	0	36.41 ^c	1.83	44.90 ^c	1.43
Tomoko [®]	1	45.76 ^b	1.45	61.60 ^{bc}	0.97
	2	61.95 ^a	1.93	74.76 ^a	1.19
	3	45.72 ^b	1.66	63.08 ^b	1.43
Vetazymeplus [®]	1	45.27 ^b	1.29	56.42 ^{cd}	1.30
	2	60.59 ^a	1.30	74.68 ^a	0.75
	3	41.98 ^b	0.92	59.27 ^{bcd}	1.13

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

Table 3: Effect of treatments on digestion coefficients and nutritive values of experimental rations fed to goats

Item	Treatments			±SE
	Control	T ₁	T ₂	
Initial body weight (kg)	34.40	34.10	33.60	0.36
Dry matter intake (g)	1480	1467	1445	19.00
Nutrient digestibilities (%)				
DM	64.28 ^c	68.17 ^a	66.59 ^b	0.47
OM	68.40 ^c	73.15 ^a	71.68 ^b	0.56
CP	62.90 ^b	70.96 ^a	68.04 ^a	1.20
CF	63.16 ^b	70.29 ^a	70.72 ^a	1.15
EE	71.77	72.75	72.72	0.73
NFE	68.70 ^c	74.64 ^a	73.14 ^b	0.70
Nutritive values:				
TDN (%)	64.13 ^b	69.70 ^a	68.52 ^a	0.68
DCP (%)	7.54 ^b	8.50 ^a	8.15 ^a	1.44

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

Table 4: Effect of treatments on ruminal parameters of goats groups

Items	Treatments			±SE
	C	T ₁	T ₂	
pH	6.60 ^a	6.26 ^c	6.40 ^b	0.05
TVFA's mg/100 ml	11.80 ^b	15.07 ^a	13.13 ^b	0.50
Ammonia-N mg/100 ml	51.93 ^c	67.18 ^a	57.20 ^b	1.01
NPN mg/100 ml	60.78 ^c	77.43 ^a	65.39 ^b	0.78
Total nitrogen mg/100 ml	156.19 ^c	199.85 ^a	174.63 ^b	1.20
True protein mg/100 ml	95.41 ^c	122.41 ^a	109.24 ^b	1.13
Microbial protein mg/100 ml	111.49 ^c	155.37 ^a	130.55 ^b	18.24

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

Enhancing ruminal microorganism's colonization. Our suggestions are supported by studies of Dawson *et al.* [31] and Harrison *et al.* [32]; they reported that numbers of total ruminal anaerobes especially cellulolytic bacteria have been increased with fungal extracts treatment. Also, *Aspergillus* fermentation extracts have been shown to directly stimulate rumen fungi, which may improve fiber digestion [33]. In addition, Newbold [34] suggested that enzymatic attack of plant fibers by

Aspergillus spp. may be an important factor in the stimulation of fibers degradation by rumen microorganisms.

Rumen Parameters: Ruminal parameters values of goats received the different experimental rations are illustrated in Table (4).

Ruminal pH showed the lowest ($P<0.05$) value by goats fed Tomoko[®] supplemented ration (T₁) followed by Veta-Zyme Plus[®] supplemented ration (T₂). In contrast, rumen fluid for goats feed ration supplemented with Tomoko[®] had the highest ($P<0.05$) total volatile fatty acids (TVFA's), ammonia nitrogen (NH₃-N), non-protein nitrogen (NPN), total nitrogen, true protein and microbial protein concentrations followed by Veta-Zyme Plus[®] supplemented goats, then the lowest values were recorded for control group. These results are in agreement with those obtained by Farahat [27] who found that lactating goats fed diet supplemented with Veta-Zyme Plus[®] showed decrease in ruminal pH value and increase in total volatile fatty acids and ammonia nitrogen concentrations compared to those of the control. Moreover, Khaled and Baraka [35] found that Barky finishing lambs received ration supplemented with Tomoko[®] showed significant decrease in ruminal pH and total protein but significant increases ($P<0.05$) in total volatile fatty acids concentration compared to those of the control. In this concern, Dawson *et al.* [31] reported a decrease in ruminal pH and an increase in cellulolytic ruminal bacterial numbers in steers fed hay supplemented with lactic acid bacteria (*L. acidophilus*). In addition, Osman *et al.* [36] found that ruminal TVFA's were increased significantly in the rumen of supplemented dairy cattle with Bovamine (*Lactobacillus acidophilus* strain NP51 and *Probiobacterium freudenreichii* strain NP24). In this study, the reduction in ruminal pH values and increase TVFA's concentration of microbial feed supplemented

Table 5: Effect of treatments on ruminal ciliate protozoa count ($\times 10^4$ cell/ml rumen liquor) of goats groups:-

Items	Treatments			±SE
	C	T ₁	T ₂	
<i>Entodinium spp.</i>				
<i>E. caudatum</i>	343.33 ^b	463.89 ^a	380.00 ^b	16.34
<i>E. simplex</i>	162.22 ^b	234.44 ^a	178.33 ^b	12.00
<i>E. minimum</i>	275.00 ^b	361.11 ^a	304.00 ^b	12.45
<i>E. bursa</i>	71.113 ^c	169.44 ^a	113.00 ^b	5.45
<i>E. furca</i>	8.33 ^b	23.33 ^a	10.00 ^{ab}	3.80
<i>E. triacum</i>	3.33 ^b	11.11 ^a	1.66 ^b	1.49
<i>Epidinium spp.</i>				
<i>E. ecaudatum</i>	42.77 ^a	46.66 ^a	27.77 ^b	1.16
<i>Polyolastron spp.</i>				
<i>P. multivesiculatum</i>	89.99 ^a	51.11 ^b	44.44 ^b	4.32
<i>Diplodinium spp.</i>				
<i>D. psitaceum</i>	168.55	174.44	186.66	15.42
<i>D. dentatum</i>	37.72 ^b	58.89 ^a	27.77 ^c	0.87
<i>D. elongatum</i>	27.22 ^b	36.66 ^a	29.44 ^b	1.86
<i>Ophryoscolox spp.</i>				
<i>O. caudatus</i>	18.33 ^b	25.55 ^a	19.33 ^b	1.23
<i>O. purkynjei</i>	16.11 ^b	21.10 ^a	18.88 ^{ab}	1.71
<i>Isotrichia spp.</i>				
<i>I. prostoma</i>	29.16 ^b	35.55 ^a	34.39 ^a	0.71
<i>I. intestinalis</i>	24.11	25.66	25.33	1.27
<i>Dasytrachia spp.</i>				
<i>D. rummantium</i>	60.44 ^c	87.22 ^a	74.44 ^b	2.00
Total count	1377.76 ^c	1826.22 ^a	1475.50 ^b	20.10

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

goats may be due to the intensive fermentation process of feed nonstructural and structural carbohydrates or increase in cellulolytic ruminal bacterial numbers as reported by Dawson *et al.* [31]. Increase ruminal $\text{NH}_3\text{-N}$, NPN, total nitrogen, true protein and microbial protein concentrations with microbial feed supplementation may be due to higher protozoal count of microbial supplemented goats (Table 5) which in turn increase net microbial growth yield and positively improve CP digestibility (Table 3).

Ruminal Ciliate Protozoa Count: Seven genus or species with 16 subspecies of ruminal protozoa of goats groups were identified in this study, the identification of ruminal protozoa species and their density in the rumen liquor (count $\times 10^4$ cell/ml rumen liquor) for all treatments are illustrated in Table (5).

It is obvious that Tomoko[®] supplementation (T₁) had the highest ($P < 0.05$) counts of all protozoal subspecies except in case of *Polyolastron multivesiculatum* which was higher in the rumen of the control group than the other groups, this may be due to that *Polyolastron spp.* is the most complex protozoal genera in domestic

ruminants and it is more resistance to changes in ruminal pH [37]. Moreover, protozoal subspecies (*E. bursa*, *I. prostoma* and *D. rummantium*) count recorded significant increase in rumen liquor of Veta-Zyme Plus[®] supplemented goats compared to those of the control, while no significant difference among all goats groups in protozoal subspecies (*Diplodinium psitaceum* and *Isotrichia intestinalis*) and this may be attributed to that these species have a large range of ruminal pH. As for total ruminal protozoa count ($\times 10^4$ cell/ml rumen liquor), goats received ration supplemented with Tomoko[®] (T₁) had the highest ($P < 0.05$) total ciliate densities of ruminal protozoa followed by goats received ration supplemented with Veta-Zyme Plus[®] (T₂) then goats of the control group. In this concern, results indicated that *Entodinium spp.* recorded the largest count of tested ruminal protozoa species and both microbial feed supplements (Tomoko[®] and Veta-Zyme Plus[®]) improved their count compared to the control. This can be explained depending on that, *Entodinium spp.* is responsible for utilization of formed lactic acid in the rumen [35] and direct feed microbials that produce lactate (e.g. *Lactobacillus acidophilus*) sustain a tonic level of lactic acid in the rumen, which could potentially stimulate lactic acid-utilizing microorganisms [38]. Also, Martin and Streeter [39] reported that fungal cultures improve the use of lactate by the ruminal organism *Selenomonas ruminantium* by providing a source of dicarboxylic acids (e.g., malic acid) and other growth factors.

Blood Plasma Parameters: Microbial supplemented goats had higher ($P < 0.05$) plasma total protein and albumin than those of the control (Table 6). This may be due to the improvements occurred in metabolic process as a response to Tomoko[®] and Veta-Zyme Plus[®] supplementation and indicate that these goats cover their protein requirements.

These results are in a good agreement with the findings of Farahat [27] who reported that Veta-Zyme Plus[®] increased ($P < 0.05$) serum total protein and albumin of supplemented goats compared to those of the control. Also, Khaled and Baraka [35] found that Tomoko[®] supplementation increased serum total protein but not serum albumin of treated fattening lambs. In contrast, Azzaz *et al.* [24] reported that Tomoko[®] supplementation had no effect on plasma total protein and albumin of treated buffaloes. In addition, plasma globulin and urea concentrations were higher ($P < 0.05$) for goats fed (T₁) ration than those fed (T₂) and control rations (Table 6).

Table 6: Blood plasma parameters of treated lactating goats groups

Items	Treatments			± SE
	Control	T ₁	T ₂	
Total protein (g/dl)	6.37 ^b	7.09 ^a	6.91 ^a	0.11
Albumin (g/dl)	3.48 ^b	3.78 ^a	3.80 ^a	0.07
Globulin (g/dl)	2.89 ^b	3.31 ^a	3.10 ^b	0.07
A/G ratio	1.22	1.16	1.23	0.02
Urea (mg/dl)	19.75 ^b	23.55 ^a	22.27 ^b	0.67
Glucose (mg/dl)	69.70 ^b	74.73 ^a	74.18 ^a	0.85
Total lipids (mg/dl)	465.50	491.61	487.20	38.81
AST (U/ml)	43.57	42.77	43.37	2.20
ALT (U/ml)	21.77	23.40	23.30	0.72

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

Table 7: Treatments effect on goat's milk yield and milk composition

Items	Treatments			± SE
	Control	T ₁	T ₂	
Yield (kg/d)				
Milk	949.27 ^b	1069.53 ^a	1030.70 ^a	13.33
4% FCM	832.94 ^b	976.37 ^a	939.41 ^a	18.23
Total solids	107.16 ^b	127.36 ^a	121.78 ^a	13.21
Fat	30.22 ^b	36.57 ^a	35.14 ^a	0.97
Solids not fat	76.88 ^b	90.79 ^a	86.64 ^a	1.62
Total protein	28.77 ^b	34.22 ^a	32.56 ^a	0.80
Lactose	41.28 ^b	48.55 ^a	46.15 ^a	0.99
Ash	6.83 ^b	8.02 ^a	7.92 ^a	0.19
Milk composition %				
Total solids	11.30	11.89	11.81	0.14
Fat	3.20	3.42	3.40	0.08
SNF	8.10	8.47	8.41	0.08
Total protein	3.04	3.20	3.16	0.06
Lactose	4.35	4.35	4.48	0.06
Ash	0.72	0.75	0.77	0.01

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

This may be due to a higher organic matter and crude protein (CP) digestibility for Tomoko[®] supplemented goats compared to other groups. Our data are supported by results of Khaled and Baraka [35] they found that serum globulin and urea nitrogen concentrations were significantly increased by fattening lambs received Tomoko[®] supplemented diet while, no effect were detected for Tomoko[®] supplementation on plasma globulin and urea concentrations of mild lactating buffaloes [24]. Also, Farahat [27] reported that Veta-Zyme Plus[®] had no effect on serum globulin and urea concentrations of supplemented goats. Furthermore, significant increase in plasma glucose concentrations were detected in microbial supplemented goats compare to those of the control group and this may attributed to higher OM, CF and NFE digestibility (Table 3). In contrast, Tomoko[®] and Veta-Zyme Plus[®] had no effect on blood glucose concentration of supplemented buffaloes

[24] and goats [27] respectively. Moreover, there were insignificant ($P > 0.05$) differences among all groups in the overall means of albumin/globulin ratio, total lipids, AST and ALT (Table, 6). These results were supported by finding of Azzaz *et al.* [24] and Farahat [27]. The concentrations of all tested blood parameters were in the normal range for healthy animals and indicated that microbial feed supplements (Tomoko[®] and Veta-Zyme Plus[®]) were not negatively affected liver activity or general goat's health.

Milk Yield and its Composition: Milk, 4% fat corrected milk and the other components yields were higher ($P < 0.05$) for goats fed microbial supplemented rations (T₁) and (T₂) than those of the control, while milk composition was not affected by microbial feed supplementation (Table 7). These results are in line with those of Farahat [27] who reported that although, Veta-Zyme Plus[®] supplemented goats showed significant increase in actual milk, 4% fat corrected milk, milk total solids, milk fat, milk solids not fat and milk lactose yields, their is no effect of Veta-Zyme Plus[®] supplementation on milk composition. In addition, Iwanska *et al.* [40] stated that, fat corrected milk, milk fat, milk protein, casein yields, lactose percentage, total solid, solid-not-fat and somatic cell count were significantly higher in Polish Black and White cows treated with biologically active compounds.

In contrast, Azzaz *et al.* [24] found no significant ($P > 0.05$) differences among control and Tomoko[®] supplemented buffaloes groups in milk composition and milk component's yields. In our study, improve milk production and milk components by microbial feed supplemented goats may be attributed to one or more of the following reasons; 1) Higher nutrients digestibilities by microbial supplemented goats (Tables 2 and 3) higher TVFA's, protein and their derivatives concentrations and ciliate protozoal count in the rumen of microbial supplemented goats (Tables, 4 and 5). Our findings are supported by West and Bernard [41] suggestions. They stated that improved milk yield in the absence of greater DMI suggests that rumen function was improved, through improved digestion, improved rumen environmental conditions, or through greater microbial protein yield.

CONCLUSION

In light of the obtained data and the foregoing discussion it can be concluded that *Aspergillus awamori* and *Lactobacillus acidophilus* supplementation

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 which reflect higher nutrients digestibility coefficient and milk productivity of supplemented Nubian goats.

REFERENCES

1. Knowlton, K.F., J.M. McKinney and C. Cobb, 2002. Effect of a direct-fed fibrolytic enzyme formulation on nutrient intake, partitioning and excretion in early and late lactation Holstein cows. *J. Dairy Sci.*, 85: 3328-3335.
2. Yoon, I.K. and M.D. Stern, 1995. Influence of direct-fed microbials on ruminal microbial fermentation and performance of ruminants: A review. *Asian-australas J. Anim. Sci.*, 8: 533.
3. Krehbiel, C.R., S. R. Rust, G. Zang and S.E. Gilliland, 2003. Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. *J. Anim. Sci.*, 81(E. Suppl. 2): E120-E132.
4. Jouany, J.P. and D.P. Morgavi, 2007. Use of 'natural' products as alternatives to antibiotic feed additives in ruminant production. *Animal*, 1: 1443-1466.
5. Guedes, C.M., D. Gonçalves, M.A.M. Rodrigues and A. Dias-da-Silva, 2008. Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fibre degradation of maize silages in cows. *Anim. Feed Sci. Tech.*, 145: 27-40.
6. Wallace, R.J., D. Colombatto and P.H. Robinson, 2008. Enzymes, direct-fed microbials and plant extracts in ruminant nutrition. *Anim. Feed Sci. Tech.*, 145: 1-4.
7. Wallace, R.J., 1994. Ruminal microbiology, biotechnology and ruminant nutrition. Progress and problems. *J. Anim. Sci.*, 72: 2992-3003.
8. Raeth-Knight, M.L., J.G. Linn and H.G. Jung, 2007. Effect of Direct-Fed Microbials on Performance, Diet Digestibility and Rumen Characteristics of Holstein Dairy Cows. *J. Dairy Sci.*, 90: 1802-1809.
9. Wagner, D.G., J. Quinonez and L.J. Bush, 1990. The effect of corn or wheat-based diets and yeast culture on performance, ruminal pH and volatile fatty acids in dairy calves. *Agri-Practice*, 11: 7-12.
10. Fondevila, M. and B. P'erez-Esp'es, 2008. A new in vitro system to study the effect of liquid phase turnover and pH on microbial fermentation of concentrate diets for ruminants. *Anim. Feed Sci. Technol.*, 144: 196-211.
11. Ferret, A., J. Plaixats, G. Caja, J. Gasa and P. Prió, 1999. Using markers to estimate apparent dry matter digestibility, fecal output and dry matter intake in dairy ewes fed Italian ryegrass hay or alfalfa hay. *Small Ruminant Research*, 33: 145-152.
12. A.O.A.C., 1995. Official Methods of Analysis of AOAC International, Agricultural, Chemicals, Contaminants, Drugs. 16th ed. Washington DC USA.
13. Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74: 3583-3597.
14. Warner, A.C.J., 1964. Production of volatile fatty acids in the rumen: Methods of Measurements. *Nutr. Abst. Rev.*, 34: 339.
15. Makkar, H.P.S., O.P. Sharma, R.K. Dawra and S.S. Negi, 1982. Simple determination of microbial protein in rumen liquor. *J. Dairy Sci.*, 65: 2170-2173.
16. Ogimoto, K. and S. Imai, 1981. Atlas of Rumen Microbiology. Japan Scientific Societies Press, Tokyo.
17. Dehority, B.A., 1993. Laboratory manual for classification and morphology of rumen ciliate protozoa. C.R.C. Press Inc., Florida.
18. Armstrong, W.D. and C.W. Carr, 1964. Physiological Chemistry, 3rd Edn. Laboratory Directions Bures Publishing Co. Minneapolis, Minnesota, USA.
19. Dumas, B., W. Wabson and H. Biggs, 1971. Albumin standards and measurement of serum with bromocresol green. *Clin. Chem. Acta.*, 31: 87.
20. Fawcett, J.K. and J.E. Soctt, 1960. Spectrophotometric and kinetics investigation of the berthelot reaction for the determination of ammonia. *J. Clin. Pathol.*, 13: 156-159.
21. Siest, G., J. Henny and F. Schiele, 1981. Interpretation Des Examens De Laboratoire, pp: 206.
22. Zöllner, N. and K. Kirsch, 1962. Colorimetric method for determination of total lipids. *Journal of Experimental Medicine*, 135: 545-550.
23. Reitman, S. and S. Frankel, 1957. Colorimetric method for the determination of serum glutamic-oxaloacetic and glutamic-pyruvate transaminase. *Ann. J. Clin. Pathol.*, 28: 56.
24. Azzaz, H.H., H.A. Murad, A.M. Kholif, T.A. Morsy, A.M. Mansour and H.A. El-Sayed, 2013. Increasing nutrients bioavailability by using fibrolytic enzymes in dairy buffaloes feeding. *Journal of Biological Sciences*, 13(4): 234-241.
25. IBM Corp. Released, 2011. IBM SPSS Statistics for Windows, Version 20. Armonk, NY: IBM Corp.

26. Duncan, D.B., 1955. Multiple range and multiple F-test. *Biometrics*, 11: 1-42.
27. Farahat, Eman, S.A., 2014. Using biologically treated date kernels in lactating rations. Ph.D. Thesis, Fac. Agric., Cairo Univ., Egypt, pp: 126.
28. Varel, V.H., K.K. Kreikemeier, H.J.G. Jung and R.D. Hatfield, 1993. *In vitro* stimulation of forage fiber degradation by ruminal microorganisms with *Aspergillus oryzae* fermentation extract. *Appl. Environ. Microbiol.*, 59: 3171-3176.
29. Arambel, M.J. and B.A. Kent, 1990. Effect of yeast culture on nutrient digestibility and milk yield response in early to mid lactation dairy cows. *J. Dairy Sci.*, 73: 1560-1563.
30. Eugene, M., H. Archimède and D. Sauvant, 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.
31. Dawson, K.A., K.E. Neuman and J.A. Boling, 1990. Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. *J. Anim. Sci.*, 68: 3392-3398.
32. Harrison, G.A., R.W. Hemken, K.A. Dawson, R.J. Harmon and K.B. Barker, 1988. Influence of addition of yeast culture to diets of lactating cows on ruminal fermentation and microbial populations. *J. Dairy Sci.*, 71: 2967-2975.
33. Chang, J.S., E.M. Harper and R.E. Calza, 1999. Fermentation extract effects on the morphology and metabolism of the rumen fungus *Neocallimastix frontalis* EB188. *Journal of Applied Microbiology*, 86: 389-398.
34. Newbold, C.J., R.J. Wallace, A. Chesson, Eds., V.C.H. Weinheim, 1995. Microbial Feed Additives for Ruminants. In *Biotechnology in Animal Feeds and Animal Feeding*, pp: 259-278.
35. Khaled, N.F and T.A. Baraka, 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.
36. Osman, M., J. Stabel, K. Onda, S. Down, W. Kreikemeier, D. Ware and D.C. Beitz, 2012. Modification of digestive system microbiome of lactating dairy cows by feeding Bovamine®: Effect on ruminal fermentation, *Animal Industry Report: AS 658, ASL R2701*.
37. Hungate, R.E., 1966. *The Rumen and its Microbes*. Academic Press Inc., New York and London.
38. Nocek, J.E., W.P. Kautz, J.A.Z. Leedle and J.G. Allman, 2002. Ruminal supplementation of direct-fed microbials on diurnal pH variation and in situ digestion in dairy cattle. *J. Dairy Sci.*, 85: 429-433.
39. Martin, S.A. and M.N. Streeter, 1995. Effect of malate on in vitro mixed ruminal microorganism fermentation. *J. Anim. Sci.*, 73: 2141.
40. Iwanska, S., D. Strusinska and A. Opalka, 2000. Effect of biologically active compounds on milk yield and composition. *Roczniki Naukowe Zootechniki*, 6: 46-50.
41. West, J.W. and J.K. Bernard, 2011. Effects of addition of bacterial inoculants to the diets of lactating dairy cows on feed intake, milk yield and milk composition. *The Professional Animal Scientist*, 2: 122-126.