ISSN 1819-1878

Asian Journal of Animal Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Asian Journal of Animal Sciences

ISSN 1819-1878
DOI: 10.3923/ajas.2016.202.212

Research Article
Improvement of Rumen Fermentation and Performance of Growing Lambs by Adding Natural Microbial Resources

[1]Ebtehag I.M. Abou Elenin, [2]Etab R. Abd El-Galil, [1]K.E.I. Etman and [1]H.M. El-Shabrawy

[1]Department of Animal Nutrition, Animal Production Research Institute (APRI), Nadi El-Said St., Dokki, Giza, Egypt
[2]Department of Animal Production, Faculty of Agriculture, Ain Shams University, Egypt

Abstract

Objective: The objective was to study effect of three natural microbial sources as additives on lamb performance, digestibility, feeding value some rumen and blood parameters, rumen cellulolytic bacteria counts as well as economical return. **Methodology:** Twenty four weaning Rahmany lambs averaged live 20.0±0.30 kg b.wt. were assigned to 4 similar groups (6 animals each), fed concentrate feed mixture and berseem (60:40%), (as a basal ration); (G1) Fed a basal ration without feed additives as control group, (G2) Fed the basal ration supplemented with 10 g per lamb per day of isolated bacteria (cellulolytic bacteria), (G3) Fed a basal ration plus 10 g per lamb per day of fibrozyme, (G4) Fed a basal ration with 10 g per lamb per day of yeast culture (*Saccharomyces cerevisiae*) during 24 weeks of growing period. **Results:** Indicated that there were significant differences in all nutrient digestibility, showing the lowest values with G1, while the highest values were obtained with G4. Furthermore, the rations with biological additives were ($p<0.05$) higher crude fiber digestibility. Animals fed isolated bacteria recorded the highest significantly values of cellulose, hemicelluloses and all cell wall constituents digestibility (%). Supplementing rations G2, G3 and G4 showed significantly high values of TDN and DCP% as well as digestible energy (Mcal kg^{-1} DMI) but no significant. Feed intake (kg per lamb per day) as DM was no significant difference while feed intake as TDN and DCP appeared to increase with supplemented rations compared with control ration. Data observed that body weight gain, average daily gain, feed efficiency were higher with biological additives rations. Total counts of cellulolytic bacteria in rumen were the highest ($p<0.05$) values in G2. Blood total protein and globulin for animals fed biological additives had higher values than those fed control ration (G1). The net revenue were 320, 228 and 226% (G4, G2 and G3, respectively). **Conclusion:** The biological additives can enhance lamb performance, digestibility and economic income with the superiority effect for adding yeast culture. Moreover, it can be enhance ruminal culture and fiber fraction digestibility with adding isolated bacteria with no side effects on physiological status.

Key words: Enzymes extract, fibrolytic enzyme, yeast, performance, economic, lambs

Received: March 26, 2016 Accepted: April 06, 2016 Published: April 15, 2016

Citation: Ebtehag I.M. Abou Elenin, Etab R. Abd El-Galil, K.E.I. Etman and H.M. El-Shabrawy, 2016. Improvement of rumen fermentation and performance of growing lambs by adding natural microbial resources. Asian J. Anim. Sci., 10: 202-212.

Corresponding Author: Ebtehag I.M. Abou Elenin, Department of Animal Nutrition, Animal Production Research Institute (APRI), Nadi El-Said St., Dokki, Giza, Egypt Tel: 00201015191694

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tropical forages are rich in lignin and silica content, so carbohydrate has limited fermentation, the production of VFA and rumen microbial mass is decreased. The rumen digestion of forages is relatively slow and incomplete that lead to decrease animal performance and increase cost feeding livestock production (Bello and Escobar, 1997).

The manipulating of the microbial ecosystem in the rumen is more important to improve ruminant productive performance (Choi *et al*., 2012). There are various attempts have been used to optimize rumen microbial culture for example Direct Fed Microorganisms (DFM) are defined as a source of naturally microorganism (Krehbiel *et al*., 2003). The term DFM has included yeast, fungi, bacteria, cell fragments, enzymes and extracts (Sullivan and Martin, 1999). Their mode of action are variable on increasing substrate breakdown, enhancement of nutrient intake and promote growth. Although anaerobic bacteria, fungi and protozoa degrade cellulose in the rumen, cellulolytic bacteria plays the most important role for fibrolytic activity.

High concentrate diets affect on rumen pH, while, fiberolytic bacteria growth in rumen is strictly limited at pH (Russell and Wilson, 1996) less than 6.00. So, fibre digestion is not maximal under this dietary conditions. Murillo *et al*. (2000) reported that the benefit of enzyme supplementation is a least partially dependent on ruminal pH and Beauchemin *et al*. (2003) reported that adding enzymes to high-concentrate diets gave more improvement in animal performance than with high-roughage diets, which could be attributed mainly to improvements in ruminal fiber digestion. There are conflict among available researches for benefit or mode of action for natural microbial resources on rumen culture or rumen cellulolytic bacteria.

Therefore, the objective of the present study was to; (1) Evaluate the effect of adding different natural microbial feed resources (isolated bacteria, fibrozyme and yeast culture) on Rahmany lambs performance, (2) Investigate digestibility, feeding value and economic efficiency of the tested rations and (3) Count some strains of celluolytic bacteria in the rumen and determine some rumen and blood parameters.

MATERIALS AND METHODS

Experimental animal and rations: This study was carried out at El-Serw Research Station, belonging to Animal Production Research Institute, Agricultural Research Center. Twenty four growing Rahmany lambs averaged 20.0±0.30 kg LBW after weaning were assigned to 4 similar groups (6 animals each) according to their body weight. The feeding trial lasted 24 weeks. Animals were fed Concentrate Feed Mixture (CFM) and berseem at rate of 60:40% on DM basis, respectively (as a basal ration) according to NRC (1985). The animals were randomly assigned to receive the four respective rations as follow:

G1: Control ration (a basal ration)
G2: A basal ration supplemented with 10 g isolated bacteria per head per day
G3: A basal ration supplemented with 10 g commercial fibrozyme per head per day
G4: A basal ration supplemented with 10 g yeast culture per head per day

The isolated bacteria are cellulytic bacteria which secreting cellulase and xylanase enzymes (6×10^5 viable cells g^{-1} DM or cellulase enzyme 7.1 U g^{-1} DM and xylanase 2.3 U g^{-1} DM) according to Abd El-Galil (2000). Fibrolytic enzyme (fibrozyme) is compounded from *Aspergillus niger*, *Trichoderma longibrachiatum*, fermentation extracts and fermentation soluble. It contains 100 U xylanase g^{-1} according to Kung *et al*. (2000). Yeast culture are *Saccharomyces cerevisiae* 1×10^9 CFU g^{-1} DM.

The concentrate feed mixture consists of 34.0% zeamaize, 32.3% wheat bran, 20.0% undecorticated cotton seed meal, 10.0% soybean meal, 2.0% limestone, 1.0% sodium chloride, 0.5% minerals and vitamins mixture and 0.2% dicalcium phosphate. The CFM was offered to animals in 2 times daily just at 7 am and at 4 pm. While, the amount of berseem was divided into two equal parts and offered daily at 9 am and 6 pm. Fresh water and salt blocks were available for each group along the day. Amount of CFM and berseem were adjusted bi-weekly for each group according to increase in body weight. Each group was kept in separate shaded pen and fed as group feeding. Animals were weighed bi-weekly, while feed consumption, live weight gain, feed efficiency and feed costs per kg live body weight gain were calculated.

Digestibility trials and analytical methods: Four digestibility trials were conducted using three lambs from each group of feeding trial. Composite samples of feed and feces were analyzed according to AOAC (2000). Digestible Energy (DE) was calculated as according to NRC (1985):

$$DE\ (Mcal\ kg^{-1}\ DMI) = 0.04409\times TDN\%$$

where, DMI is dry matter intake and TDN is total digestible nutrients.

Rumen liquor samples were collected at 0, 3 and 6 h after morning feeding. Ruminal pH was immediately determined after rumen liquor was collected with a digital pH meter (pH ep®, pocket-sized pH meter Hana instruments, Italy). Concentration of NH_3-N was immediately determined using micro-diffusion method of Conway (1963). Frozen rumen liquor samples were analyzed for Total Volatile Fatty (TVF's) acids by steam distillation according to Warner (1964). Blood plasma samples were taken from jugular vein at the end of feeding trial at 0, 3 and 6 h post feeding from experimental animals and stored at -20°C till analysis. Plasma total protein, albumin and AST and ALT transaminase activities and creatinine were analyzed using commercial kits of Bio-Merieux, lab, France.

Bacterial cultures: Six strains of cellulolytic bacteria were isolated from rumen fluid of lambs after 0, 3 and 6 h from morning feeding then grown as pure culture. Rumen fluid was collected by stomach tube. The separated strains were *Cellulomonas cellulasea*, *Bacillus* sp., *Thermonospora fusca*, *Acetobacter xylinum*, *Ruminococcus albus* and *Clostridium cellulovorans*. The isolation of species used the pour-plate technique for pure preparation of cultures according to ATCC (1992). The rumen samples were immediately gassed with CO_2 and viable counts of rumen cellulolytic bacteria were determined according to the method described by Moir (1951) and Gall *et al.* (1949) and their classification were done according to Pounden and Hibss (1948).

Economic evaluation: Economic efficiency was calculated according to the following equation:

Net revenue (LE per lamb per day) = Money output-Money input

Statistical analysis: Data were statistically analyzed using general linear model program of SAS (1999). Digestibility and performance data were analyzed as one way analysis of variance according to the following model:

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

where, Y_{ij} is observation, μ is mean, t_i is effect of treatment and e_{ij} is experimental error. The significance differences among treatments were tested by Duncan (1955).

RESULTS AND DISCUSSION

Chemical composition of experimental rations: Chemical composition and cell wall constituents of CFM, berseem and basal ration as control group (percentage on DM basis) are presented in Table 1. The data indicated that the crude fiber, crude protein and the ether extract were 16.43, 16.26 and 6.13% in the basal ration. It could be noticed that cell wall constituents were higher in basal ration than CFM. Percentage of NDF, ADF, hemicelluloses and cellulose content were 34.99, 18.44, 16.55 and 10.88%, respectively in the basal ration.

Table 1: Chemical composition and cell wall constituents of feed ingredients and calculated chemical composition of basal ration (% on DM basis)

Items	Concentration	Berseem	Basal ration
Dry Matter (DM)	91.21	14.24	60.42
Organic Matter (OM)	85.74	84.38	85.20
Crude Protein (CP)	17.76	14.00	16.26
Crude Fiber (CF)	13.57	20.71	16.43
Ether Extract (EE)	8.60	2.42	6.13
Ash	14.26	15.62	14.80
NFE	45.81	47.25	46.39
Cell wall constituents (%)			
Neutral Detergent Fiber (NDF)	31.25	40.72	34.99
Acid Detergent Fiber (ADF)	16.42	21.53	18.44
Acid Detergent Lignin (ADL)	9.36	4.81	7.56
Hemicellulose	14.83	19.19	16.55
Cellulose	7.06	16.72	10.88

Table 2: Nutrients digestibility and feeding values of experimental rations for growing lambs

	Experimental rations				
Items	G1	G2	G3	G4	±SE
Digestibility coefficient (%)					
DM	54.86^{c}	59.26^{b}	64.28^{a}	67.13^{a}	5.96
OM	56.72^{c}	60.42^{c}	65.83^{b}	69.35^{a}	6.19
CP	73.53^{c}	78.20^{b}	78.70^{b}	83.96^{a}	4.77
CF	54.63^{b}	69.33^{a}	68.10^{a}	67.52^{a}	6.37
EE	71.40^{b}	73.38^{b}	75.35^{b}	78.35^{a}	3.53
NFE	49.61^{c}	49.32^{c}	59.25^{b}	63.63^{a}	6.66
NDF	38.17^{c}	85.12^{a}	60.90^{b}	73.35^{ab}	4.02
ADF	43.72^{c}	84.35^{a}	63.61^{b}	58.76^{b}	3.33
ADL	23.96^{c}	84.91^{a}	55.72^{b}	44.78^{b}	6.22
Hemicellulose	31.99^{c}	85.98^{a}	57.88^{b}	61.89^{b}	5.38
Cellulose	57.69^{b}	83.96^{a}	69.11^{ab}	68.48^{ab}	5.09
Feeding values (%)					
TDN (%)	53.78^{c}	57.10^{c}	61.85^{b}	65.06^{a}	5.53
DCP (%)	11.95^{c}	12.71^{b}	12.79^{b}	13.65^{a}	0.78
DE (Mcal kg^{-1} DMI)^{A}	2.37	2.52	2.73	2.87	

^{A}DE (Mcal kg^{-1} DMI) = 0.04409×TDN%, SE: Standard error, $^{a-c}$Means in the same rows with different superscripts are significantly different at ($p<0.05$), G1: Control, G2: Isolated bacteria, G3: Fibrozyme and G4: Yeast culture

Digestion coefficient and feeding value: Data in Table 2 indicated that supplementing rations with isolated bacteria (G2), fibrozyme (G3) and yeast culture (G4) were significantly ($p<0.05$) increased DM and CF digestibility as compared with those of control ration (G1). These results are in the same line with those obtained by Pinos *et al.* (2000) who illustrated that NDF digestibility was increased in lambs fed xylanase and cellulose enzyme. The increase in digestibility coefficients, especially for CF digestibility might be due to increase in rumen microbial population (Newbold *et al.*, 1996) and/or activity of rumen cellulolytic bacteria (Dawson, 1993).

Table 3: Counts of total cellulolytic bacteria and some cellulolytic bacteria species separately from rumen animals fed experimental rations

	Experimental rations				
Items	G1	G2	G3	G4	±SE
Cellulolytic bacteria ($n \times 10^5$ mL^{-1})					
Total count	6.98^{d}	9.34^{a}	7.85^{c}	8.17^{b}	0.27
Cellulomonas					
0	1.18	1.85	1.39	1.62	
3	1.47	2.32	1.84	1.92	
6	1.91	2.68	2.60	2.21	
Mean	1.52^{c}	2.28^{a}	1.94^{b}	1.92^{b}	0.03
Bacillus					
0	0.12	0.24	0.15	0.21	
3	0.18	0.28	0.17	0.21	
6	0.19	0.32	0.19	0.27	
Mean	0.16^{c}	0.28^{a}	0.17^{c}	0.23^{b}	0.11
Thermonospora					
0	0.61	0.64	0.65	0.60	
3	0.64	0.67	0.66	0.61	
6	0.62	0.73	0.73	0.76	
Mean	0.62	0.69	0.68	0.66	0.26*
Acetobacter					
0	1.09	1.68	1.18	1.47	
3	1.37	1.89	1.69	1.67	
6	1.70	2.57	2.06	2.13	
Mean	1.39^{d}	2.05^{a}	1.64^{c}	1.76^{b}	0.15
Ruminococcus					
0	2.00	2.46	1.95	2.03	
3	2.33	2.66	2.42	2.47	
6	2.57	2.90	2.76	2.73	
Mean	2.31^{c}	2.67^{a}	2.38^{c}	2.41^{b}	0.14
Clostredium					
0	0.72	1.00	0.75	0.85	
3	0.92	1.43	0.85	1.17	
6	1.30	1.67	1.53	1.57	
Mean	0.98^{d}	1.37^{a}	1.04^{c}	1.19^{b}	0.01

$^{a-d}$Means with different superscripts within each row for each parameter are significantly different ($p<0.05$), G1: Control, G2: Isolated bacteria, G3: Fibrozyme, G4: Yeast culture and *NS

It could be noticed that ration supplemented with yeast culture (G4) attained to be significantly ($p<0.05$) higher for CP and EE digestibility compared with those of control (G1) and the other supplemented rations G2 and G3. On the other hand, there were no significant differences in EE among animals fed rations G1, G2 and G3. While, value of CP digestibility for microbial additives groups (G2 and G3) had significant differences with G1, but the differences between G2 and G3 were not significant. These results are confirmed with Zinn and Salinas (1999) who reported that adding fibrolytic enzyme increased ruminal digestion of feed nitrogen by 5% which reflected on daily gain comparing with control ration. Furthermore, G3 and G4 supplementation had significant the highest digestibility values of DM, OM and NFE. Generally, all rations supplemented with feed additives recorded higher digestibility coefficients for all nutrients compared to no supplemented one (control).

These results indicated that adding yeast for ration (G4) increased value of CP digestibility, this may be due to consider yeast culture as a microbial protein. Thus, the improvement of protein digestibility with yeast culture supplementation may be due to the stimulation of rumen protelytic bacteria (Williams *et al*., 1991).

Digestibility coefficients of cell wall constituents are illustrated in Table 2. Data indicated that digestibility of NDF, ADF, ADL, hemicellulose and cellulose were achieved the highest ($p<0.05$) values in group supplemented with isolated bacteria (G2) being 85.12, 84.35, 84.91, 85.98 and 84.96%, respectively than others. On contrary, control ration (G1) showed the lowest ($p<0.05$) values, as shown in Table 2. Decreasing in cell wall constituents digestibility with G4 than G2 might be due to consider yeast as microbial protein and stimulate proteolytic bacteria so, it had slightly negative effect on digestion of fiber and cell wall constituents comparing with adding cellulolytic enzymes in G2 that stimulate the digestion of fiber and cell wall constituents. This confirmed with increasing of numbers of some strains of cellulolytic bacteria which isolated from rumen and total count of cellulolytic bacteria (Table 3) in group 2 compared with others. These results are in harmony with those recorded by Zinn and Salinas (1999) who showed that supplementing fibrolytic enzyme increased the ruminal digestion of NDF by 23%. While, yeast supplemented group (G4) was the lowest value of NDF, ADF, ADL, hemicellulose and cellulose than other supplemented groups with significant ($p<0.05$) differences. Furthermore, according to Gomez-Alarcon *et al*. (1987) who reported that improving CF digestibility may be due to the increasing number of rumen cellulolytic bacteria. Also, Yoon and Stern (1996) found that increasing DM, OM, CP and EE digestibility with animals fed microbial supplemented rations might be due to adding microbial supplements stimulated the growth and activity of certain ruminal microorganisms.

Robinson (1997) found that Yeast Culture (YC) improved the digestibility of most nutrients and reported that supplementation of YC in the diet increased net digestion in the four stomach, particularly of fiber leading to increase energy output. Chademana and Offer (1990) found that yeast culture increased the initial rate of forage digestion in the rumen.

Table 2 observed that the supplementation rations (G2, G3 and G4) recorded ($p<0.05$) high values compared with G1for feeding values as TDN% and DE (Mcal kg^{-1} DMI) showing significant difference for G3 and G4 in TDN% comparing with G1 and G2. El-Ashry *et al*. (2003, 2001) noticed that supplemented rations with yeast ($p<0.05$) increased feeding value of ration. Abd El-Galil (2014) found that feeding values as TDN for rations supplemented either

fibrozyme or mix (fibrozyme+yeast) were significantly higher than that supplemented with yeast culture and the lowest values observed with ration without supplements. The DCP values were significantly ($p<0.05$) increased with G4 (13.66%) than those of other rations (G2, G3 then G1). Ration supplemented with yeast culture (G4) had the highest DCP% followed by those supplemented with fibrozyme and isolated bacteria, while the control ration (with no supplemented) had the lowest value. Abd El-Galil (2014) recorded that DCP% had significantly higher values with adding fibrozyme or mixed fibrozyme with yeast (8.15 and 8.01%) and ration with yeast (7.37%) than without additives (6.59%). Increasing DCP for rations supplemented with biological additives might be due to increase the number of bacteria in the rumen and increase the digestibility and feeding values in experimental diets and this reflected from results of CP digestibility which confirmed by Yoon and Stern (1996) who illustrated that proteolytic bacteria counts were stimulated by yeast culture.

Improving feed intake seems to be driven partly by an improved rate of fiber breakdown (Wallace and Newbold, 1992) and partly by an improved duodenal flow of absorbable amino nitrogen (Williams *et al*., 1990). The most effect of microbial feed additives is that increasing the viable count of anaerobic bacteria recovered from ruminal fluid by 50-100% (Wallace and Newbold, 1993). Cellulolytic bacterial numbers are increased (Wallace and Newbold, 1993) thus explaining the improvement in fiber breakdown and increasing stability of the fermentation in animals receiving yeast and *A. oryzae* (Harrison *et al*., 1988; Williams *et al*., 1991). Nitrogen balance and metabolism was found to be improved due to the inclusion of YC in the diet of sheep. This may have been due to the increase in N digestibility as well as a better utilization of the dietary, N. Proteolytic bacteria count was increased and the flow of non-microbial non-ammonia N tended to be higher for cows fed YC (Yoon and Stern, 1996; Putnam *et al*., 1997).

Growth performance: Table 4 showed that consumption of CFM and berseem were almost similar in all the tested rations. There was a slightly difference in daily total feed intake (kg per lamb) as DM between supplemented and control rations. However, intake as TDN was markedly increased with supplemented rations (0.894, 0.974 and 1.024 kg TDN for G2, G3 and G4, respectively) compared with control ration. With corresponding (0.822 kg TDN) observed for DCP values that being 0.183, 0.199, 0.201 and 0.215 kg for rations G1, G2, G3 and G4, respectively. These results are in harmony with results of Allam *et al*. (2001) who reported that there was a significant improvement in DM intake when yeast culture was given to lactating animals. But disagreement with that reported by Ebtehag *et al*. (2011) who found that feed intake as DM, TDN and DCP did not affected with supplemented rations by yeast culture compared with control ration. Also, Hadjipanayiotou *et al*. (1997) reported that no effect of YC on DM intake.

Table 4: Feed intake, body weight gains (kg) and feed efficiency for growing lambs fed experimental rations

	Experimental rations				
Items	G1	G2	G3	G4	±SE
Number of animals	6	6	6	6	
Experimental period (week)	24	24	24	24	
Feed intake (kg per lamb per day) as fed					
Concentrate Feed Mixture (CFM)	1.07	1.10	1.10	1.10	
Berseem	3.89	3.97	4.03	4.03	
Feed additives	-	0.01	0.01	0.01	
Feed intake (kg per lamb per day) on DM basis					
Concentrate Feed Mixture (CFM)	0.98	1.00	1.00	1.00	
Berseem	0.55	0.57	0.57	0.57	
Total DM intake	1.53	1.57	1.57	1.57	
Feed additives (kg)	-	0.01	0.01	0.01	
TDN intake	0.822	0.894	0.974	1.024	
DCP intake	0.183	0.199	0.201	0.215	
Roughage per oncentrate	40:60	40:60	40:60	40:60	
Body weight (kg)					
Initial	20.00	20.30	20.10	20.20	0.47*
Final	43.30^{d}	49.10^{c}	52.10^{b}	53.60^{a}	4.04
Total gain (kg)	23.30^{d}	28.80^{c}	32.00^{b}	33.40^{a}	3.99
Average daily gain (g)	138.7^{d}	171.6^{c}	190.5^{b}	198.9^{a}	0.02
Feed efficiency as					
kg gain kg^{-1} DM feed intake	0.091	0.110	0.121	0.126	
kg gain kg^{-1} TDN feed intake	0.169	0.192	0.196	0.194	
kg gain kg^{-1} DCP feed intake	0.759	0.862	0.946	0.926	

$^{a-d}$Means in the same rows with different superscripts are significantly different at ($p<0.05$), G1: Control, G2: Isolated bacteria, G3: Fibrozyme, G4: Yeast and *NS

Feeding animals with ration supplemented with isolated bacteria, fibrolytic enzymes or YC increase feed intake, however, differences were not tested statistically due to the group feeding system applied in the present study. However, Bowman *et al*. (2002) reported that the effects of fibrolytic enzymes on DMI appear to be vary among enzymes products and the method of applying enzymes.

Table 4 revealed that animals fed supplemented rations (G2, G3 and G4) had significantly higher in total and daily body weight gains than those fed control (G1). Average daily weight gains recorded the highest values with group fed yeast culture (G4) followed by those fed fibrozyme (G3) and isolated bacteria (G2), being 198.9, 190.5 and 171.6 g day^{-1}, respectively. While, animals fed control ration (G1) recorded the least one 138.7 g day^{-1} as daily weight gain. These results were attributed to increase TDN and DCP intake. Thus, increasing the Digestible Energy (DE, Mcal kg^{-1} DMI) value and CP, CF digestibility (Table 2).

On average, microbial additives may benefit ruminant nutrition (in terms of live body weight gain) by a similar magnitude to ionophores with 7 or 8% improvement

(Wallace and Newbold, 1993), in this case by increasing feed intake and feed efficiency (Williams and Newbold, 1990). The effects are highly variable, however and much remains to be established about the dose and diet-dependence of the effects. Increasing ADG resulting from adding enzymes was attributed to an increase in DMI and improvement in digestibility.

Table 4 noticed that feed efficiency as 1 kg gain per 1 kg DMI, TDNI and DCPI were higher with those fed supplemented ration with isolated bacteria, fibrozyme and yeast culture ration than those fed control ration being 0.192, 0196 and 0.194 kg gain kg^{-1} TDN feed intake and 0.862, 0.946 and 0.926 kg gain kg^{-1} DCPI, respectively, comparing with control ration (0.169 kg gain kg^{-1} TDNI and 0.759 kg gain kg^{-1} DCPI). The highest value of kg gain kg^{-1} DM feed intake (0.126) was recorded with yeast culture ration. These results were confirmed by Mohamed *et al.* (2013) who reported that improving feed efficiency indicates better utilization of nutrients when adding enzymes with the magnitude of improvement being a linear function of enzymes dosage. These results came on line with those obtained by Abd El-Galil (2008).

The DFM improves the intestinal microbial balance of host animal in favor of beneficial gut microflora (Cruywagen *et al.*, 1996). It may also help prevent ruminal acidosis (Nocek *et al.*, 2000) and can improve the feed efficiency and average daily weight gain of feedlot cattle (Rust *et al.*, 2000). Abd El-Galil (2014) recorded that biological additives on basal diet for feedlot Baladi goats with fibrozyme and a combination of yeast culture and fibrozyme enhanced ruminal digestion and thereby enhanced dry matter intake and growth performance. Efficacy of adding biological additives on ration may due to growth types of microorganisms which improve efficiency of using diets.

Rumen parameters and cellulolytic bacterial count: Rumen parameters are illustrated in Table 5. Ruminal pH value is one of the most important factors, which affect microbial fermentation in the rumen and influenced its functions. The pH values were within the normal range with significant differences among tested rations at 0, 3 and 6 h post feeding, being the highest ($p<0.05$) values were occurred with G3 (fibrozyme) and G4 (yeast culture) over all sampling times comparing with other treatments. While, the pH values tend to decrease by prolongation of time post-feeding, reaching lowest at 3 h post-feeding then increased after 6 h feeding.

Ruminal pH affects fiber digestion through its influence on the specific growth rates of cellulolytic bacteria. Growth of cellulolytic bacteria is optimal at ruminal pH greater than 6.5. Between pH of 6.5-6.0, the specific growth rate decreases 14% h^{-1} for every 0.1 U decrease in ruminal pH. Cellulolytic bacteria do not grow at ruminal pH below 6.0 (Zinn and Salinas, 1999).

Table 5: Rumen liquor parameters of growing lambs fed experimental rations

	Experimental rations				
Items	G1	G2	G3	G4	±SE
pH					
0 h	6.73^{b}	6.75^{b}	6.87^{a}	6.90^{a}	0.09
3 h	5.82^{c}	6.03^{b}	6.43^{a}	6.48^{a}	0.29
6 h	6.00^{c}	6.16^{b}	6.53^{a}	6.56^{a}	0.25
Mean	6.18^{b}	6.31^{b}	6.61^{a}	6.65^{a}	0.21
NH_3-N (mg/100 mL)					
0 h	18.10^{b}	19.59^{a}	19.85^{a}	19.88^{a}	0.78
3 h	28.13^{b}	29.89^{ab}	31.23^{a}	31.71^{a}	1.71
6 h	20.23^{b}	22.59^{a}	23.11^{a}	23.35^{a}	1.38
Mean	22.15^{b}	24.02^{b}	24.73^{a}	24.98^{a}	1.41
TVFA's (mequ/100 mL)					
0 h	7.43^{ab}	7.46^{ab}	7.63^{a}	7.18^{b}	0.24
3 h	10.10^{d}	10.72^{c}	11.43^{b}	12.13^{a}	0.82
6 h	8.55^{c}	9.25^{b}	9.87^{a}	9.89^{a}	0.62
Mean	8.69^{c}	9.14^{b}	9.64^{a}	9.73^{a}	0.61

$^{a-c}$Means followed by different letters in the same row are significantly different ($p<0.05$), G1: Control, G2: Isolated bacteria, G3: Fibrozyme and G4: Yeast culture

There were significant differences in TVFA's value among the supplemented rations at different times compared with control ration. In the present study, increasing TVFA's concentration in rumen liquor for lambs fed rations containing biological additives (isolated bacteria, fibrozyme and yeast culture) may be due to increase in DM, OM, CF, CP and NFE digestibility than those of control ration. These results showed that the effect of biological additives on microbial fermentation for protein and fiber in the rumen could be reflected on microbial protein synthesis.

Consistently, the same trend was observed for rumen NH_3-N which recorded significantly higher ($p<0.05$) values for G4 and G3 than G2 and G1. Over all observation, TVFA's and NH_3-N values were higher in groups with biological additives (G2, G3 and G4) than control ration (G1), these means increasing rumen activity. These values were similar to that reported by Abd El-Galil (2006), who found that the highest ammonia N concentration recorded at 3 h post feeding. However, it is well recognized that the ammonia N concentration found in the rumen at any given time presented the net concentration value of its production after utilization by rumen microbes and absorption across the rumen wall, the dilution by other factor and passage to the lower gut. The above categorization on the basis of pH corroborates with the reports of Choudhuri *et al.* (1981). Yoon and Stern (1996) reported that control group (without YC) was the lowest values of NH_3-N and TVFA's. This may have been due to the mode of action of YC in ruminal microbial activity that increasing bacterial counts and activity and the stability of the ruminal environment.

Cellulolytic bacteria counts under the different feeding regimens are given in Table 3. Total cellulolytic bacteria counts in rumen content were the highest ($p<0.05$) value in G2 followed by G4 and G3, while the lowest value was recorded with G1 (control diet). These result might be due to supplemented experimental ration with biological additives, causing enhance and stimulate activity of cellulolytic bacteria in rumen. These results clarify increasing values of CF digestibility and cellulose and hemicelluloses digestibilities. The same trend was observed for number of *Cellulomonas*, *Thermonospora*, *Acetobacter*, *Ruminococcus* and *Clostridium* which the highest significant ($p<0.05$) value was observed with G2 comparing to others while the lowest value was recorded for G1. Result revealed no significant differences of *Thermonospora* count in all groups ration, while the lowest value in control ration (G1) for number of all cellulolytic bacteria.

These results indicated that biological additives had effect on ruminal pH and TVF's values, as well as CP, CF, NFE digestibility and cell wall constituents digestibility in supplemented rations which led to growth different bacteria species in rumen particularly cellulolytic bacteria. The rumen microbial population is very sense, containing bacteria, protozoa and fungi, which could be somewhat changed according to the buffering processes by phosphate and bicarbonate from saliva and also bicarbonate from rumen fermentation. The percentage of *Bacillus*, *Acetobacter* and *Clostridium* from total cellulolytic bacteria were recorded the maximum for animals fed isolated bacteria (G2; 2.99%, 21.95 and 14.67%, respectively), while *Cellulomonas* was achieved the maximum at in (G3; 24.7%) which diet contained fibrozyme as additives.

In relation to these results, Weimer (1996) reported that the bacteria *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes* generally are regarded as the predominant cellulolytic microbes in the rumen. They are able to utilize cellulose (or in some cases xylan) and its hydrolytic products as their nearly sole energy sources for growth. Active cellulose digestion involves adherence of cells to the fibers via a glycoprotein glycocalyx, which protects cells from protozoal grazing and adheres cellulolytic enzymes from degradation by ruminal proteases, while it retains-at least temporarily-the cellodextrin products for use by the cellulolytic bacteria. These properties result in different ecological roles for the adherent and no adherent populations of each species, but overall provide an enormous selective advantage to these cellulolytic bacteria in the ruminal environment. However, major constraints to cellulose digestion are caused by cell-wall structure of the plant (matrix interactions among wall biopolymers and low substrate surface area). The degradation of cellulose in particular requires several enzymes that are joined together in a molecular structure known as a cellulosome. The cellulosome to the surface of plant cell walls, providing the initial step in fibre breakdown (Krause *et al*., 2003).

Many of bacteria in the rumen are free-floating in the liquid just before feeding and become attached to new feed particles after feeding. It is very difficult to remove and count the bacteria attached to feed particles and thus may explain the low numbers observed in the rumen after feeding, when fermentation rate is generally at its greatest. Bacterial numbers are generally assumed to be higher on high concentrate diets compared to high forage diets (Hespell *et al*., 1997; Morgavi *et al*., 2010). However, there are more fluid-associated bacteria with high concentrate diets and thus, easier to enumerate. The biggest differences due to diet are in the type of bacteria rather than the total number. Varel and Dehority (1989) reported that the proportions of *F. succinogenes*, *R. flavefaciens* and *R. albus* in the total cellulolytic bacteria in cattle rumen were 33.0, 2.6 and 46.0%, respectively. In addition, the ability of these three species to digest cellulose is much higher than that of other cellulolytic ruminal species. Therefore, *F. succinogenes*, *R. flavefaciens* and *R. albus* have been considered representative cellulolytic bacterial species in the rumen

The ability of yeast to stimulate the viable count in the rumen depends on its respiratory activity (Newbold *et al*., 1993). Regardless of the efficacy or mode of action of microbial feed additives. It is already in widespread use. It may offer new opportunities for manipulation.

Dawson (1993) reported that there were strains of yeast that stimulate the growth of specific types of bacteria, thus leading the development of additives suitable for different dietary circumstances. In addition, the rumen bacteria change qualitatively and quantitatively in response to the changes in chemical composition of diet (Maklad and Bahira, 2001). The main effect of yeast culture is to stabilize the rumen environment. Concentrations of cellulolytic and anaerobic bacteria were higher in *in vitro* and *in vivo* systems.

Blood parameters: Table 6 showed that blood total protein, albumin, globulin, AST, ALT and creatinine. Blood total protein values were\significantly higher ($p<0.05$) records (5.04, 5.14 and 5.40 g dL^{-1}) for animals fed rations containing biological additives (G2, G3 and G4) than control ration (4.55 g dL^{-1}, G1). This was reflects on total body gain. On the other hand, values of blood albumin were no significant difference among tested rations, but values increased with supplemented rations. This might be associated with improved nitrogen absorption (Talha *et al*., 2009). Increasing blood albumin

Table 6: Some parameters of blood from growing lambs fed experimental rations

Items	Time (h)	Experimental rations G1	G2	G3	G4	±SE
Blood proteins						
Total protein (g dL^{-1})	0	3.94	4.11	4.66	4.22	0.258
	3	4.22^{c}	4.39^{bc}	5.10^{ab}	5.49^{a}	0.199
	6	5.49^{b}	6.61^{a}	5.65^{b}	6.50^{a}	0. 163
	Mean	4.55	5.04	5.14	5.40	0.237*
Albumin (g dL^{-1})	0	2.05	2.17	2.21	2.50	0.246
	3	2.49	2.59	2.69	2.73	0.214
	6	2.76	2.87	3.02	2.94	0.205
	Mean	2.43^{b}	2.55^{a}	2.64^{a}	2.72^{a}	0.233
Globulin (g dL^{-1})	0	1.89	1.24	1.64	1.29	0.211
	3	1.73^{b}	1.84^{b}	2.46^{ab}	2.77^{a}	0.196
	6	2.73^{c}	5.37^{a}	4.01^{b}	5.21^{a}	0.185
	Mean	2.12^{b}	2.82^{ab}	2.70^{ab}	3.09^{a}	0.206
Kidney function						
Creatinine (mg dL^{-1})	0	0.81	0.98	0.80	1.09	0.232
	3	0.98	1.05	0.97	0.92	0.229
	6	1.14	1.10	0.75	0.96	0.263
	Mean	0.98	1.04	0.84	0.99	0.237*
Liver function						
AST (IU L^{-1})	0	0.60	0.62	0.61	0.65	0.211
	3	0.60	0.62	0.61	0.63	0.234
	6	0.61	0.62	0.61	0.64	0.230
	Mean	0.60	0.62	0.61	0.64	0.229*
ALT (IU L^{-1})	0	0.80	0.83	0.86	0.81	0.210
	3	0.81	0.83	0.83	0.82	0.213
	6	0.79	0.80	0.85	0.83	0.211
	Mean	0.80	0.82	0.85	0.82	0.212*

$^{a-c}$Means in the same rows with differ rent superscripts are significantly different at ($p<0.05$), *NS

Table 7: Economical efficiency of growing lambs fed experimental rations

Items	Experimental rations G1	G2	G3	G4
Economic efficiency				
Gain price (LE day^{-1})	4.86	6.01	6.67	6.96
Cost feed (LE kg^{-1} per lamb per day)				
Concentrate intake	2.57	2.63	2.63	2.63
Berseem intake	1.56	1.59	1.61	1.61
Price of additive (10 LE g^{-1})	0.00	0.10	0.75	0.35
Total feed cost (LE day^{-1})	4.12	4.32	4.99	4.59
Feed cost kg^{-1} gain	2.26	2.03	2.29	2.18
Net revenue (LE per lamb per day)^{A}	0.74	1.68	1.67	2.37
Increasing rates of net revenue (%)	100	228.00	226.00	320.00
Economic efficiency^{B}	1.18	1.39	1.33	1.52
Improvement	100	118.00	113.00	128.00

Price of feedstuffs and supplementation: 2400 LE t^{-1} of Concentrate Feed Mixture (CFM) and 400 LE t^{-1} on of berseem, 10 LE kg^{-1} of isolated bacteria, 35 LE kg^{-1} of yeast culture, 75 LE kg^{-1} of fibrozyme and price of meat: 35 LE kg^{-1} live body weight, 1$US: 7.20 LE (Egyptian pound), ^{A}Net revenue (LE per lamb per day): Money output-money input, ^{B}Efficiency: Money output/money in put

suggested normal status of liver function, since liver is the main organ of albumin synthesis. In addition, blood globulin (g dL^{-1}) value for G4 were significantly higher than those of G2, G3 and G1. The blood creatinine, AST (IU L^{-1}) and ALT (IU L^{-1}) values had no significant differences ($p>0.05$) among all diets. However, all previous parameters showed slightly higher values with supplemented rations (G2, G3 and G4) than that of control ration (G1). These mean that biological additives not affected on kidney function as creatinine or liver function as AST and ALT concentration. Dimova *et al*. (2013) found that no significant differences ($p>0.05$) in plasma creatinine concentration due to probiotic supplements. Blood creatinine level is a useful indicator of filtration in the kidney and normal concentration of creatinine indicates the optimal physical activity. Generally, the increase in blood constituents might be due to the role of biological additives in improving all nutrient digestibility and rumen parameters of lambs fed supplemented rations (G2, G3 and G4) and also might be probably led to an increase in the absorption rate from the digestive tract, thus blood constituents of animals fed supplemented rations were reflection of the regime of diets.

These results were agreement with those obtained by Abd El-Galil (2008). The Direct-Fed Microbial (DFM) improved the intestinal microbial balance of ruminants microflora (Cruywagen *et al*., 1996). In addition, Hutjens (1991) refers to expected performance changes when animals fed on a feed additive, who mentioned to greater DM intake, stimulate rumen microbial synthesis, increase digestibility, stabilize rumen environment and pH and improve health (less ketosis, reduce acidosis and increase immune response). It can be improve the feed efficiency and average live daily weight gain of cattle fed supplemented feed (Rust *et al*., 2000) and may prevent ruminal acidosis (Nocek *et al*., 2000). The present results of blood parameters were in same line with those obtained by Abd El-Galil (2014) and Abou-Elenin *et al*. (2015).

Economic evaluation: Regarding the economic evaluation (Table 7), results indicated that feed cost to produce 1 kg gain was better with animals fed isolated bacteria ration (G2) followed by those fed yeast culture (G4), comparing with control ration (G1); being 2.03, 2.18 and 2.26 LE kg^{-1} gain, respectively. The highest net revenue (LE per lamb per day) was shown with yeast culture (G4) followed isolated bacteria (G2) ration then fibrozyme (G3) rations in comparison with control ration. The increasing rates of net revenue were 320, 228 and 226% with animals fed supplemented rations (G4, G2 and G3, respectively), comparing with control ration (100%). It could be noticed that, the economic efficiency recorded higher value with yeast culture (G4) followed by isolated bacteria (G2), while control ration (G1) was the lowest value. The present study showed that the inclusion of fibrozyme as a feed additive into the diets of ruminants is currently not economically feasible comparing with tested rations with other biological additives.

Overall, recent data indicated that microbial additives might benefit ruminant nutrition in terms of live weight gain and milk production (Abou-Elenin *et al*., 2015) by a similar magnitude to ionophores being 7 or 8% improvement (Wallace and Newbold, 1993) and by increasing feed intake rather than feed efficiency (Williams and Newbold, 1990). In recent study, Castillo-Gonzalez *et al*. (2014) concluded that the use of additives can manipulate the ruminal ecosystem and ruminal microflora in order to improve production. Because ruminal microorganisms are crucial for proper animal nutrition, it is important to generate innovative knowledge in the study of ruminal fermentation and microbial ecosystems to improve the ruminant feeding process. Therefore, the use of additives in the diet to improve the efficient use of nutrients and thus reduce the final products cost that affect the atmosphere are being important.

CONCLUSION

Results of this study concluded that adding isolated bacteria, fibrozyme and yeast culture as feed additives in growing Rahmany lambs rations could be enhancing nutrients digestibility, feeding values, daily gain and feed efficiency with no side effects on physiological status. Moreover, biological additives increased total number of cellulolytic bacteria and some strains of fibrolytic bacteria particularly by using isolated bacteria. While, yeast culture had the significant superiority ($p<0.05$) in improving digestibility, feed efficiency and economic income.

ACKNOWLEDGMENT

Authors are appreciating and thanking for Animal Production Research Institute (APRI) belonging to Agricultural Research Center (ARC), Agriculture Ministry in Egypt to fund, support and help authors to conduct this study.

SIGNIFICANT STATEMENTS

Most forage contains cellulose, hemicelluloses and lignin, it has limited digestion and slow rumen degradation that lead to decrease animal performance and increase cost feeding livestock production.

- There are many biological additives but its cost is the limitation of using it
- So, conducting this study to comparing of effect of using different biological resources: Local isolated bacteria, commercial mixed enzymes or yeast culture on animal performance and rumen culture as well as economically return
- This study revealed that adding yeast culture is the best economically but isolated bacteria was the more effective on fiber digestion and rumen culture with no side effects on physiological state

REFERENCES

AOAC., 2000. Official Methods of Analysis. 17th Edn., Association of Official Analytical Chemistry, Arlington, Virginia, USA.

ATCC., 1992. Catalogue of Bacteria and Bacterophages. 18th Edn., American Type Culture Collection, USA.

Abd El-Galil, E.R.I., 2000. Nutritional factors affecting the performance of small ruminants. M.Sc. Thesis, Faculty of Agriculture, Ain-Shams University, Egypt.

Abd El-Galil, E.R.I., 2006. Effect of biological treatments on silage and feeding value of roughages in ruminants. Ph.D. Thesis, Faculty of Agriculture, Ain-Shams University, Egypt.

Abd El-Galil, E.R.I., 2008. Effect of bacterial treatments on chemical composition, cell wall constituents and digestibility of rice straw. Egypt. J. Nutr. Feeds, 11: 497-510.

Abd El-Galil, E.R.I., 2014. Using biological additives to manipulate rumen fermentation and improve baladi goats performance. Egypt. J. Nutr. Feeds, 17: 29-42.

Abou-Elenin, I.M.E., E.R.I. Abd El-Galil, K.E.I. Etman and H.M. El-Shabrawy, 2015. Impact of feeding natural microbial (enzymes) resources on lactating goats performance. Egypt. J. Nutr. Feeds, 18: 87-100.

Allam, A.M., K. El-Shazly, B.E.A. Borhami and M.A. Mohamed, 2001. Effect of baker's yeast (*Saccharomyces cerevisiae*) supplementation on digestion in sheep and milk response in dairy cows. Egypt. J. Nutr. Feeds, 4: 315-315.

Beauchemin, K.A., D. Colombatto, D.P. Morgavi and W.Z. Yang, 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci., 81: E37-E47.

Bello, M.G.D. and A. Escobar, 1997. Rumen manipulation for the improved utilization of tropical forages. Anim. Feed Sci. Technol., 69: 91-102.

Bowman, G.R., K.A. Beauchemin and J.A. Shelford, 2002. The proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows. J. Dairy Sci., 85: 3420-3429.

Castillo-Gonzalez, A.R., M.E. Burrola-Barraza, J. Dominguez-Viveros and A. Chavez-Martinez, 2014. Rumen microorganisms and fermentation. Archivos Medicina Veterinaria, 46: 349-361.

Chademana, I. and N.W. Offer, 1990. The effect of dietary inclusion of yeast culture on digestion in the sheep. Anim. Prod., 50: 483-489.

Choi, N.G., G.Y. Lee, K.H. Jeong and C.H. Kim, 2012. Isolation of anaerobic cellulolytic bacteria from the rumen of holstein dairy cows to develop feed additives for ruminants. Korean J. Organ. Agric., 20: 327-343.

Choudhuri, P.C., B. Prasad and S.K. Misra, 1981. Note on the use of rumen liquor in the treatment of chronic alkaline indigestion in cows. Indian J. Anim. Sci., 51: 356-360.

Conway, E.J., 1963. Microdiffusion Analysis and Volumetric Error. 1st Edn., Chemical Publishing Co. Inc., Los Angeles, CA., ISBN-13: 978-0820601519, Pages: 482.

Cruywagen, C.W., I. Jordaan and L. Venter, 1996. Effect of *Lactobacillus acidophilus* supplementation of milk replacer on preweaning performance of calves. J. Dairy Sci., 79: 483-486.

Dawson, K.A., 1993. Probiotics and Enzymes in Ruminant Nutrition. In: Enzymes in Animal Nutrition, Wenk, C. and M. Boessinger (Eds.). Institut fur Nutztierwissenschaften, Zurich, Switzerland, pp: 89.

Dimova, N., M. Baltadjieva, V. Karabashev, S. Laleva and Y. Popova *et al*., 2013. Effect of adding of probiotic Zoovit at feeding of lambs from breed synthetic population Bulgarian milk. Bulgarian J. Agric. Sci., 19: 98-101.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.

Ebtehag, I.M.A.E., M.E.H. Hoda and H.M. El-Shabraw, 2011. Comparing effects of organic acid (Malate) and yeast culture as feed supplement on dairy cows performance. Nat. Sci., 9: 132-140.

El-Ashry, M.A., A.M. Fayed, K.M. Youssef, F.A. Salem and H.S. Aziz, 2003. Effect of feeding flavomycin or yeast as feed supplement on lamb performance in Sinai. Egypt. J. Nutr. Feeds, 6: 1009-1022.

El-Ashry, M.A., Z.A. Motagally and Y.A. Maareck, 2001. Effect of live dried baker's yeast and yeast culture on performance of growing buffalo calves. Egypt. Nutr. Feed, 4: 607-617.

Gall, L.S., W. Burroughs, P. Gerlaugh and B.H. Edgington, 1949. Special methods for rumen bacterial studies in the field. J. Anim. Sci., 8: 433-440.

Gomez-Alarcon, R., C. Dudas and J.T. Huber, 1987. Effect of *Aspergillus oryzae* (Amaferm) and yeast on feed utilization by Holstein cows. J. Dairy Sci., 70: 218-218.

Hadjipanayiotou, M., I. Antoniou and A. Photiou, 1997. Effects of the inclusion of yeast culture on the performance of dairy ewes and goats and the degradation of feedstuffs. Livstock Prod. Sci., 48: 129-134.

Harrison, G.A., R.W. Hemken, K.A. Dawson, R.J. Harmon and K.B. Barker, 1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation and microbial populations. J. Dairy Sci., 71: 2967-2975.

Hespell, R.B., D.E. Akin and B.A. Dehority, 1997. Bacteria, Fungi and Protozoa of the Rumen. In: Gastrointestinal Microbiology: Gastrointestinal Microbes and Host Interactions, Volume 2, Mackie, R.I., B.A. White and R.E. Isaacson (Eds.). Chapman and Hall, New York, ISBN: 9780412983719, pp: 59-149.

Hutjens, M.F., 1991. Feed additives. Vet. Clin. North Am.: Food Anim. Pract., 7: 525-540.

Krause, D.O., S.E. Denman, R.I. Mackie, M. Morrison, A.L. Rae, G.T. Attwood and C.S. McSweeney, 2003. Opportunities to improve fiber degradation in the rumen: Microbiology, ecology and genomics. FEMS Microbiol. Rev., 27: 663-693.

Krehbiel, C.R., S.R. Rust, G. Zhang and S.E. Gilliland, 2003. Bacterial direct-fed Microbials in ruminant diets: Performance response and mode of action. J. Anim. Sci., 81: E120-E132.

Kung, Jr. L., R.J. Treacher, G.A. Nauman, A.M. Smagala, K.M. Endres and M.A. Cohen, 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. J. Dairy Sci., 83: 115-122.

Maklad, E.H.M. and K.M. Bahira, 2001. Comparison among the effects of clover hay and corn silages as feed ingredients on the nutritive value, bacterial strains and fermentation in the rumen of sheep. J. Agric. Sci. Mansoura Univ., 25: 7592-7597.

Mohamed, D.E.D.A., B.E. Borhami, K.A. El-Shazly and S.M.A. Sallam, 2013. Effect of dietary supplementation with fibrolytic enzymes on the productive performance of early lactating dairy cows. J. Agric. Sci., 5: 146-155.

Moir, R.J., 1951. The seasonal variation in the ruminal microorganisms of grazing sheep. Aust. J. Agric. Res., 2: 322-330.

Morgavi, D.P., E. Forano, C. Martin and C.J. Newbold, 2010. Microbial ecosystem and methanogenesis in ruminants. Animal, 4: 1024-1036.

Murillo, M., E.G. Alvarez, J. Cruz, H. Castro, J.F. Sanchez, M.S. Vasquez and R. Zinn, 2000. Interaction of forage level and fibrolytic enzymes on digestive function in cattle. Proc.-Am. Soc. Anim. Sci. Western Sect., 51: 324-327.

NRC., 1985. Nutrition Requirements of Sheep. 6th Edn., National Academy Press, Washington, DC., USA.

Newbold, C.J., R.J. Wallace and F.M. McIntosh, 1993. The stimulation of rumen bacteria by *Saccharomyces cerevisiae* is dependent on the respiratory activity of the yeast. J. Anim. Sci., 71: 280-280.

Newbold, C.J., R.J. Wallace and F.M. McIntosh, 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. Br. J. Nutr., 76: 249-261.

Nocek, J.E., W.P. Kautz, J.A.Z. Leedle and J.G. Allman, 2000. Altering diurnal pH and in situ digestion in dairy cows with ruminal supplementation of direct-fed microbial (DFM) and yeast. J. Dairy Sci., 83: 1242-1242.

Pinos, R.J., S. Gonzalez, G. Mendoza, M. Cobo and R. Bacena *et al*., 2000. Effect of fibrolutic enzyme supplement (Fibrozyme) on intake and apparent digestibility of alfalfa and ryegrass fed to lambs. J. Dairy Sci., 83: 275-275.

Pounden, W.D. and J.W. Hibbs, 1948. The influence of the ratio of grain to hay in the ration of dairy calves on certain rumen microorganisms. J. Dairy Sci., 31: 1051-1054.

Putnam, D.E., C.G. Schwab, M.T. Socha, N.I. Whitehouse, N.A. Kierstead and B.D. Garthwaite, 1997. Effect of yeast culture in the diets of early lactation dairy cows on ruminal fermentation and passage of nitrogen fractions and amino acids to the small intestine. J. Dairy Sci., 80: 374-384.

Robinson, P.H., 1997. Effect of yeast culture (*Saccharomyces cerevisiae*) on adaptation of cows to diets postpartum. J. Dairy Sci., 80: 1119-1125.

Russell, J.B. and D.B. Wilson, 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci., 79: 1503-1509.

Rust, S.R., K. Metz and D.R. Ware, 2000. Effects of Bovamine™ rumen culture on the performance and carcass characteristics of feedlot steers. Michigan Agricultural Experiment Station, Beef Cattle, Sheep and Forage Sys. Res. Dem. Rep. No. 569, East Lansing, MI., USA., pp: 22-26.

SAS., 1999. SAS Procedure Guide, Version 6.12. SAS Institute Inc., Cary, NC., USA.

Sullivan, H.M. and S.A. Martin, 1999. Effects of a *Saccharomyces cerevisiae* culture on *in vitro* mixed ruminal microorganism fermentation. J. Dairy Sci., 82: 2011-2016.

Talha, H.M., R.I. Moawd, A.A. Abu El-Ella and G.H. Zaza, 2009. Effect of some feed additive on rearing calves from birth to weaning: 1-Productive performance and some blood parameters. J. Agric. Sci. Mansoura Univ., 34: 2611-2631.

Varel, V.H. and B.A. Dehority, 1989. Ruminal cellulolytic bacteria and protozoa from bison, cattle-bison hybrids and cattle fed three alfalfa-corn diets. Applied Environ. Microbiol., 55: 148-153.

Wallace, R.J. and C.J. Newbold, 1992. Probiotics for Ruminants. In: Probiotics: The Scientific Basis, Fuller, R. (Ed.). Chapman and Hall, London, pp: 317-363.

Wallace, R.J. and C.J. Newbold, 1993. Rumen Fermentation and its Manipulation: The Development of Yeast Culture as Feed Additives. In: Biotechnology in the Feed Industry, Lyons, T.P. (Ed.). Alltech Technical Publications, Nicholasville, KY., pp: 173.

Warner, A.C., 1964. Production of volatile fatty acids in the rumen: Methods of measurement. Nutr. Abstr. Rev., 34: 339-352.

Weimer, P.J., 1996. Why don't ruminal bacteria digest cellulose faster? J. Dairy Sci., 79: 1496-1502.

Williams, P.E., C.A. Tait, G.M. Innes and C.J. Newbold, 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci., 69: 3016-3026.

Williams, P.E.V. and C.J. Newbold, 1990. Rumen Probiosis: The Effects of Novel Microorganisms on Rumen Fermentation and Ruminant Productivity. In: Recent Advances in Animal Nutrition, Haresign, W. and D.J.A. Cole (Eds.). Buttenvorths, London, pp: 211.

Williams, P.E.V., A. Walker and J.C. MacRae, 1990. Rumen probiosis: The effects of addition of yeast culture (viable yeast (*Saccharomyces cerevisiae*) plus growth medium) on duodenal protein flow in wether sheep. Proc. Nutr. Soc., 49: 128A-128A.

Yoon, I.K. and M.D. Stern, 1996. Effects of *Saccharomyces cerevisiae* and *Aspergillus oryzae* cultures on ruminal fermentation in dairy cows. J. Dairy Sci., 79: 411-417.

Zinn, R.A. and J. Salinas, 1999. Influence of fibrozyme on digestive function and growth performance of feedlot steers fed a 78% concentrate growing diet. Proceedings of the Alltech's 15th Annual Symposium on Biotechnology in the Feed Industry, (BFI'99), Lexington, KY., USA., pp: 313-319.