



# Effect of using Biological Additives on Performance of Animals (2) Influence of Probiotic Bacteria or Enzymes Supplementation on Productive Performance of Fattening Buffalo Calves

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**Abstract** | The influence of probiotics bacteria and fibrolytic enzyme as feed additives were studied on digestibility coefficients, feeding values, feed intake, daily gain and economical efficiencies with using thirty male buffalo calves in fattening trial. Rumen and blood parameters were also studied. First group (10 animals) were received control ration which contains 60 % concentrate feed mixture (CFM), 30 % corn silage (CS) and 10 % alfalfa hay (AH). The 2<sup>nd</sup> and 3<sup>rd</sup> animal groups were fed control ration supplemented with 5 gm probiotics bacteria and 10 gm fibrolytic enzyme per head, respectively. The experiment lasted about 180 days. Animals fed rations B and C appeared improvement in daily live body weight (LBW) gain with rate of 8.43 % and 13.19%, respectively. Consequently, animals fed supplemented rations showed significant better feed utilization efficiency as kg DM, TDN or DCP per kg gain. The feed cost per kg weight gain recorded 28.874, 27.899 and 26.815 LE with animals fed ration A, B and C, respectively. So, the improvement of economical efficiencies were found and recorded 3.46 and 7.76 % with animals fed ration B and C, respectively. Using probiotics bacteria and fibrolytic enzyme in rations of fattening buffalo calves tended to improve nutrient digestibility coefficients, feeding values and increase LBW gain. Moreover, these feed additives appeared to decrease feed cost per kg weight gain and increase net revenue with higher economical efficiencies. Blood and ruminal parameters were not affected by feed additives and they were within the ranges without side effects.

**Keywords** | Probiotic bacteria, Fibrolytic enzymes, Buffalo calves, Digestion, Growth performance, Economic efficiency.

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## INTRODUCTION

The increase demand for dairy products, meat, eggs and aquaculture were driven by the massive increase in population, income growth and cultural growth as reported by Thornton (2010). Elghandour et al. (2015) showed that the probiotics bacteria tended to improve the rumen environmental, enhance the quantity of dry matter intake (DMI), feed efficiency (FE) and average weight gain

(AWG) in ruminants. It may be also decrease the activity of undesirable microorganisms, enhancing immunity and maintain the situation of microbial system in the digestive canal (Khan et al., 2016). Also, probiotics can decrease the demand of using antibiotics and led to increase the average live weight gain in animals by improving the digestibility of feed nutrient, enhancing retention of nitrogen and reducing the losses of basic nutrients (Callaway et al., 2004). Moreover De Ondarza et al. (2010) found that fourteen

variable studies on supplemented tested cows with live yeast in ration increased efficiency of feed conversion by 3% (i.e., 1.75 vs. 1.70 for animals fed supplemented feed and group without supplementation, respectively). This improvement in nutritional efficiency is due to the better use of the existing nutrients compounds in diet (Khalid et al., 2011). The same trend was found by Robinson (2002) who observed improve FCR in the small ruminants with probiotics supplementation. Moreover, Saleem et al. (2017) found improvement in the body weight (BW) before slaughtering (+3.16 kg), ADG (+25.2 g/lamb), TG (+2.11 kg), and FCR (-1.18) of lambs taken feed including *Pediococcus acidilactici* and *Pediococcus pentosaceus* probiotics additives compared with those fed no supplemented rations during post weaning period. Hillal et al. (2011) showed increase in average daily weight gain by 7.2% with growing lambs fed diet including bacterial probiotic and yeast (i.e., *S. cerevisiae*, *Lactobacillus*, *Streptococcus*, *Aspergillus*). Similarly, rearing buffalo calves fed rations with *L. acidophilus* recorded an increase by 31.4% in the average daily weight gain at the 1<sup>st</sup> month (Mudgal and Baghel, 2010). Many searches showed that one of the most effective methods to improve the performance of animal is enhancing of microbial activities in the rumen. Although there is a relationship between forage degradation and the efficiency of production, the relation between enzymatic activity and utilization of forage has not been clarified in the rumen system (Eun et al., 2007). Moreover, results obtained from researches based on using EFE in ruminants, digestive systems were different and led to difficulty in estimating metrics their biological response (Beauchemin et al., 2003; Colombatto et al., 2003). Some studies have shown substantial enhancement of feed digestibility and animal performance traits (Cruywagen and Goosen, 2004; Bala et al., 2009), while others recorded negative effects or there were no effect at all (Baloyi, 2008). The purpose of this experiment was to study the effect of probiotic bacteria and enzymes as feed supplementations on feed intake, digestibility of feed nutrients, activity of rumen fermentation, some blood measurements, body weight gain, feed conversion and economic efficiency of fattening buffalo calves.

## MATERIALS AND METHODS

The aim of this work was to study the influence of probiotic (active bacteria) and fibrolytic enzyme as feed additive on digestibility coefficients, nutritive values, daily gain and feed efficiency with fattening buffalo calves. This work was carried out at Al-Manar company station, desert road and at Animal House of Animal Production Research Institute during year 2018.

### EXPERIMENTAL ANIMALS AND RATIONS

Thirty male buffalo calves aged 15 months with initial live

body weight of  $280.3 \pm 5.01$  kg were chosen and divided randomly into three similar groups (ten calves in each) and allowed to randomly received the experiment rations. The 1<sup>st</sup> group was fed control ration (A), while the 2<sup>nd</sup> and the 3<sup>rd</sup> animal groups were fed control ration plus probiotic bacteria and fibrolytic enzyme as supplementation with ration (B) and (C), respectively. The control ration contained concentrate feed mixture (CFM), corn silage (CS) and alfalfa hay (AH) with rate of 60, 30, and 10 %, respectively. The allowance of CFM was fed at 8.00 am and 3.00 pm followed by corn silage and alfalfa hay. Fresh water was available all day. The feed additives were taken with rate of 5 gm probiotic bacteria and 10 gm fibrolytic enzyme per head per day with animals in groups B and C, respectively. Animals with ration (B) were fed bacteria isolated which included *Bacillus Subtilis* ( $3 \times 10^8$  CFU) and *Bacillus Licheniformis* ( $1 \times 10^9$  CFU), while animals with ration (C) were fed fibrolytic enzyme which contained phytase, protease, cellulose, xylanase, beta glucanase, amylase and pectinase.

### MANAGEMENT

Animals were individually fed according to Abou-Raiya (1967) recommendations allowances for growing male buffalo calves. Rations were calculated to body weight changes every two weeks. Concentrate feed mixture was given twice daily at 8 a.m. and 3 p.m., followed by corn silage and alfalfa hay. Probiotic bacteria and allzyme were orally added to calves daily before feeding, while water to drink were available all day. Chemical composition of feed ingredients are shown in Table (1).

### DIGESTIBILITY TRIALS

Three digestibility trial were carried out using nine animals (three animal in each group) to determine the digestibility coefficients and feeding value of experimental rations during the middle period of trial using Acid Insoluble Ash (AIA) procedure as a natural marker according to Van Keulen and Young (1977). Samples of feed and feces were collected to be analyzed according to A.O.A.C. (2000) Neutral detergent fiber (NDF), Acid detergent fiber (ADF) and Acid detergents Lignin (ADL) were determined according to Van Soest et al. (1991).

### RUMEN LIQUOR SAMPLES

Rumen Liquor samples were collected from calves's rumen (the same animals of digestibility trials at the same middle period of trial) by stomach tube before providing feed (zero time) and 3 hours after providing. Samples were filtered by using two layers of cheese cloth. Value of pH was measured directly in liquor collected from rumen using Orian 680 digital pH meter. The concentration of total VFA's was evaluated in rumen liquor samples by the steam distillation method (Warner, 1964) using markham micro-distillation

unit. The percentage of NH<sub>3</sub>-N was measured according to the method of AOAC (2000).

### BLOOD SAMPLES

Samples of blood were drawn from the jugular vein of three animals of each group during the middle period of trial of using sterile needle into clean dry heparin zed tubes. The samples were collected from blood centrifuged spent at 4000 r.p.m. for 15 minute. Blood serum was tested to determine total protein, albumin, creatinine, AST and ALT by calorimetrically by using commercial diagnostic kits (Test-combination, Pasteur lap.). Globulin concentration was determined by difference.

### FEED CONVERSION

Feed conversion was calculated as the quantity of feed as DM, TDN and DCP consumed by kg / kg live body weight gain.

### ECONOMIC EFFICIENCY

Average feed cost per day, price of average weight gain per day, net income, and economic efficiency was calculated as a percentage of income to feeding cost. Economic efficiency explained as a percentage of net income to feeding cost accorded to 2020 market prices.

### STATISTICAL ANALYSIS

Data were subjected to statistical analysis as one-way ANOVA, using general linear model (GLM) procedure of SAS (2000). According the following model:

$$Y_{ij} = M + T_i + e_{ij}$$

Where:

$y_{ij}$  = the observation  $M$  = over all mean

$T_i$  = effect of treatment  $e_{ij}$  = experimental error

The significant differences among means were tested using Duncan multiple rang test (Duncan 1955).

## RESULTS AND DISCUSSION

### CHEMICAL COMPOSITION OF FEED INGREDIENTS

Data presented in Table (1) showed the chemical composition of feed ingredients and control ration. It could be noticed that the concentrate feed mixture (CFM) and alfalfa hay (AH) have higher NFE contents with low CP for corn silage. The opposite trend was shown with CF content, while EE content was rarely equal in the three different ingredients. On the other hand the control ration contained 60 % CFM, 30 % corn silage and 10 % alfalfa hay. So, the proximate analysis for control ration was related to the incorporated levels of previous ingredients showing 15.42, 3.72, 22.42, 47.93 and 10.51% for CP, EE, CF, NFE and ash content, respectively.

With respect to cell wall constituents, it could be noticed that concentrate feed mixture recorded the highest ADF and ADL values compared with the others, while NDF values showed nearly equal in CFM, corn silage and alfalfa hay. On the other hand, control ration have the lowest ADF and NDF values. Generally, the cell wall constituents of control ration were affected by those values of different ingredients, as shown in Table (1).

### DIGESTION COEFFICIENTS AND FEEDING VALUES

The results obtained in Table (2). showed significantly ( $P < 0.05$ ) the highest digestibility coefficients of ration (B) than those of ration (A), being 67.12, 68.90, 64.10, 79.30, 67.42, and 70.60 % for DM, OM, CP, EE, CF, and NFE digestibility for ration (B) versus 61.87, 64.77, 61.30, 77.81, 64.71 and 69.45 % for corresponding values of ration (A). At the same time, digestibility coefficients of DM, CP and NFE for ration (C) containing fibrolytic enzymes were higher ( $P < 0.05$ ) significant than those of control ration (A). However, the significant different between ration (B) and (C) were found in OM, EE and CF of digestibility. The data revealed that supplemented ration with bacteria isolated ration (B) or fibrolytic enzymes ration (C) showed significant ( $P < 0.05$ ) higher digestibility coefficients of all nutrients. The results were agreement with those reported by Abou-Elenin et al. (2016) p.16. Moreover, Etman et al., (2020) reported significant ( $P < 0.05$ ) higher DM, OM and CF digestibility with Frieson calves fed Probiotic bacteria and fibrolytic enzymes as supplementation.

Data reported in Table (2) showed that the nutritive values expressed as TDN, DCP, DE and ME for rations supplemented with bacteria or fibrolytic enzymes were significant ( $P < 0.05$ ) higher than those of control ration (A). The ration (B) (supplemented with bacteria) recorded 68.12, 9.88% TDN and DCP, respectively. Versus 68.77, 9.81 % TDN and DCP, respectively with ration (C) (supplemented with fibrolytic enzymes). The same significant increase in DE and ME (Mcal/ kg DM) was found with supplemented rations. The results were agreement with those reported with Etman et al. (2020) and Zeid et al. (2008). At the same time, Qiao et al. (2009). Showed higher digestibility of NDF, ADF and OM with supplemented ration with B-Licheniformis, while Pinos- Rodri guez et al. (2002). recorded improvement in digestion of fiber with using fibrolytic enzymes as feed additives.

**Table 1:** Chemical composition of feed ingredients and calculated the composition of basal ration (on DM basis).

Items	Concentrate feed mixture (CFM) *	Corn silage (CS)	Alfalfa hay (AH)	Control ration (A) **
Proximate Analysis % :				
DM	90.40	22.18	96.60	78.00
OM	95.38	80.11	81.21	89.49
CP	15.65	10.03	20.10	15.42
EE	4.43	3.79	4.14	3.72
CF	15.84	34.67	29.80	22.42
NFE	59.46	31.62	27.17	47.93
Ash	4.62	19.89	18.79	10.51
Cell wall analysis % :				
ADF	30.54	27.52	32.18	22.58
NDF	44.86	46.84	40.35	36.84
ADL	23.40	19.37	10.43	13.62
Cellulose	7.14	8.15	21.75	8.96
Hemicellulose	14.32	19.32	8.17	14.26

\* concentrate feed mixture (CFM) consisted of 40% yellow corn, 20% Gluto feed, 17.5 % wheat bran, 10% soybean meal, 5% dry bean, 3 % broken lentils, 3 % sesame meal, 0.5 % salt, 0.5 % bicarbonate, 0.3 % limestone and 0.2 % molasses.

\*\* control ration consisted of 60 % CFM, + 30 % corn silage + 10 % alfalfa hay.

**Table 2:** Average of digestion coefficients and feeding values for the different experimental rations.

Items	Experimental rations *			±SE
	(A)	(B)	(C)	
Digestion coefficients % :				
DM	61.87 <sup>b</sup>	67.12 <sup>a</sup>	65.15 <sup>a</sup>	0.32
OM	64.77 <sup>b</sup>	68.90 <sup>a</sup>	66.37 <sup>b</sup>	0.39
CP	61.30 <sup>b</sup>	64.10 <sup>a</sup>	63.61 <sup>a</sup>	0.28
EE	77.81 <sup>b</sup>	79.30 <sup>a</sup>	77.89 <sup>b</sup>	0.18
CF	64.71 <sup>b</sup>	67.42 <sup>a</sup>	66.94 <sup>a</sup>	0.34
NFE	69.45 <sup>b</sup>	70.60 <sup>ab</sup>	71.23 <sup>a</sup>	0.15
Feeding values % :				
TDN	64.63 <sup>b</sup>	68.12 <sup>a</sup>	68.77 <sup>a</sup>	0.26
DCP	9.45 <sup>b</sup>	9.88 <sup>a</sup>	9.81 <sup>a</sup>	0.05
**DE (Mcal/ kgDM)	2.942 <sup>b</sup>	3.003 <sup>a</sup>	3.032 <sup>a</sup>	
ME(Mcal/ kgDM)	2.402 <sup>b</sup>	2.452 <sup>a</sup>	<sup>a</sup>	

\*ration A is control without additive, while ration B and C are basal ration containing bacteria and fibrolytic enzyme, respectively,

\*\*DE and ME were calculated according to Church and pond (1982).

a ,b, c: means in the same row with different superscripts are significantly (P<0.05) different.

Average daily feed intake was expressed as the amounts of DM, TDN or DCP per head, 100 kg LBW or w<sup>0.75</sup> as shown in Table (3). It could be noticed that the animals fed ration (B) (supplemented with bacteria) showed higher feed intake than those fed ration A (control ration). Average daily feed intake with ration C (supplemented with fibrolytic enzymes) recorded higher TDN and DCP intake per head 100 kg LBW or w<sup>0.75</sup> than the control ration with no significant differences, however, the DM intake

was not significant (P<0.05) affected by supplementation. These results are agreement with those reported by Zeid et al. (2008), and Etman et al. (2020), Moreover, Dyaa El-Din et al. (2013) observed no significant change in DM intake with animals fed ration with or without probiotic supplementation.



**Table 3:** Average of daily feed unite intake for the different experimental rations.

Items	Experimental rations			±SE
	(A)	(B)	(C)	
Av. Daily feed intake expressed % :				
Kg DM / head	7.405	7.480	7.230	NS
Kg TDN/ head	4.955 <sup>b</sup>	5.095 <sup>a</sup>	4.972 <sup>b</sup>	(P<0.05)
Kg DCP/ head	0.702 <sup>b</sup>	0.739 <sup>a</sup>	0.709 <sup>b</sup>	(P<0.05)
KgDM/100 kg LBW	2.000	2.002	1.998	NS
KgTDN/100kgLBW	1.338 <sup>b</sup>	1.364 <sup>ab</sup>	1.374 <sup>a</sup>	(P<0.05)
KgDCP/100kgLBW	0.190	0.198	0.196	NS
Kg DM / W <sup>0.75</sup>	1.688 <sup>a</sup>	1.698 <sup>a</sup>	1.653 <sup>b</sup>	(P<0.05)
Kg TDN/ W <sup>0.75</sup>	1.130 <sup>b</sup>	1.157 <sup>a</sup>	1.137 <sup>b</sup>	(P<0.05)
Kg DCP/ W <sup>0.75</sup>	0.160	0.168	0.162	NS

a ,b, c: means in the same row with different superscripts are significantly (P<0.05) different.

### DAILY GAIN AND FEED UTILIZATION EFFICIENCY

The average total live body weight gain (kg) recorded 166.5, 180.5 and 188.5 kg for animals fed ration A, B and C, respectively, indicating significantly (P<0.05) higher with animals fed rations C and B than that of ration A, as shown in Table (4). Corresponding values of average daily live body weight gain were 0.925, 1.003 and 1.047 kg, respectively, showing the same previous significant trend. Moreover, animals fed ration supplemented with feed additives as Probiotic bacteria or fibrolytic enzymes in ration B and C, respectively appeared to improve and higher daily gain with rate 8.43 and 13.19 %, respectively. Etman et al. (2020) showed higher daily gain of Frisian calves fed ration supplemented with probiotic bacteria or fibrolytic enzymes. This improvement might be attributed to enhance rumen fermentation activity and increase feeding values of tested rations. Balci et al. (2007), Hillal et al. (2011) and Soliman et al. (2016) recorded improvement in daily gain with animals fed ration supplemented with probiotic bacteria or fibrolytic enzymes as feed additives.

Feed utilization efficiency was expressed as kg DM, TDN, or DCP per kg gain, as shown in Table (4). It could be noticed that , animals fed ration C (supplemented with fibrolytic enzymes) were the best feed utilization efficiency, showing significant (P<0.05) differences than that fed control ration (ration A without supplementation), while the differences between animals fed ration B and C with utilization efficiency were not significant, as shown in Table (4). The result revealed that the rations supplemented with feed additives as Probiotic bacteria or fibrolytic enzymes in ration B and C, respectively, showed better feed utilization efficiency. Beauchemin et al. (2003) reported improvement feed utilization efficiency with ration supplemented with fibrolytic enzymes with rate up to 12 % . The same trend was shown by Cruz et al. (2014). Moreover, Etman

et al. (2020) recorded better feed conversion as kg feed per kg gain with animals fed rations supplemented with feed additives as Probiotic bacteria or fibrolytic enzymes .

### FEED COST AND ECONOMICAL EFFICIENCY

The results obtained in Table (5) showed that the cheapest cost per kg weight gain was recorded with animals fed ration C (26.815 LE), supplemented with fibrolytic enzymes followed by those fed ration B (27.988 LE), which supplemented with probiotic bacteria , while the animals fed ration A (control, without feed additives) showed the highest feed cost per kg gain (28.874 LE)

Accordingly, the ration C appeared the highest net revenue either per head or kg gain, being 29.511 and 28.186 LE, respectively, as shown in Table (5). At the same time, the economic efficiency was 1.905, 1.971 and 2.053 with ration A, B and C, respectively, indicating the highest economic efficiency with animals fed ration C following by those fed ration B. So, the improvement in economic efficiency tended to be higher with ration supplemented with probiotic bacteria or fibrolytic enzymes with rate 3.46 and 7.76 %, respectively. The results were agreement with those recorded by Hesham et al. (2013), Soliman et al.(2016), Abou-Elenin et al. (2016) and Etman et al. (2020).

### SOME BLOOD PARAMETERS

Data presented in Table (6) revealed significant (P < 0.05) higher total serum protein of animals feed ration C than those fed ration A, recording 6.80 and 6.40 gm / 100 ml, respectively, while the differences in total serum protein between animals fed ration B and C were not significant. Albumin concentration was lower significant with animals fed ration supplemented with feed additives (ration B and C) than those fed control ration (ration A).

**Table 4:** Average of daily gain (kg) and feed utilization efficiency for animals fed the different experimental rations.

Items	Experimental rations			significant
	(A)	(B)	(C)	
No. of animals	10	10	10	
Experimental period, (day)	180	180	180	
Av. initial LBW, kg	287.00	286.25	287.50	
Av. final LBW, kg	453.50 <sup>b</sup>	466.80 <sup>a</sup>	456.00 <sup>ab</sup>	
Av. total LBW gain, kg	166.50 <sup>b</sup>	180.50 <sup>a</sup>	188.50 <sup>a</sup>	(P<0.05)
Av. daily LBW gain, kg	0.925 <sup>b</sup>	1.003 <sup>a</sup>	1.047 <sup>a</sup>	(P<0.05)
Improvement %	00.00 <sup>b</sup>	8.43 <sup>a</sup>	13.19 <sup>a</sup>	
<b>Av.daily feed unite intake as:</b>				
Kg DM/ head	7.405 <sup>b</sup>	7.480 <sup>a</sup>	7.230 <sup>a</sup>	
Kg TDN/ head	4.955 <sup>b</sup>	5.095 <sup>a</sup>	4.792 <sup>a</sup>	
Kg DCP/ head	0.702 <sup>b</sup>	0.739 <sup>a</sup>	0.709 <sup>a</sup>	
<b>Feed utilization efficiency:</b>				
Kg DM/ kg gain	8.005 <sup>a</sup>	7.458 <sup>ab</sup>	6.905 <sup>b</sup>	(P<0.05)
Kg TDN/ kg gain	5.357 <sup>a</sup>	5.080 <sup>ab</sup>	4.749 <sup>b</sup>	(P<0.05)
Kg DCP/ kg gain	0.759 <sup>a</sup>	0.737 <sup>ab</sup>	0.677 <sup>b</sup>	(P<0.05)

a ,b and c means in the same row with different superscripts differ are significant (P<0.05) different.

**Table 5:** Average of daily feed intake, daily gain, feed cost and economical efficiency for the different experimental groups .

Items	Experimental rations		
	(A)	(B)	(C)
<b>Av. daily feed intake, (kg/h/d) as fed:</b>			
Concentrate feed mixture (CFM)	4.915	4.965	4.798
Corn silage (C.S)	10.014	10.117	9.779
Alfalfa hay (AH)	0.767	0.774	0.748
<b>Total intake (kg/h/d) as DM:</b>			
CFM	4.448	4.488	4.338
C.S	2.221	2.244	2.169
AH	0.741	0.748	0.723
Total DM intake (kg/h)	7.405	7.480	7.230
Av. Daily LBW gain (kg)	0.925	1.003	1.047
<b>Feed cost and economical efficiency</b>			
*Cost of feed consumed/head (LE)	26.708	27.983	28.075
Price of LBW gain (LE)	50.875	55.165	57.585
Feed cost / kg weight gain (LE)	28.874	27.988	26.815
Net revenue (LE/ h/ day)	24.167	27.182	29.511
Net revenue / kg weight (LE)	26.126	27.101	28.186
Economical efficiency	1.905	1.971	2.053
Improvement economical efficiency ( %)	-	3.46	7.76

\*based as the assumption that the price of one kg of concentrate feed mixture (CFM), corn silage(CS) , alfalfa hay (AH), bacteria and enzymes were 3.90, 0.60, 2.00, 2.00 and 2.00 LE respectively , while the price of one kg of live body weight was 55 LE.

The results showed also significant (P < 0.05) higher of GOT and GPT with animals fed ration C than those fed ration A. the same significant differences were found in creatinine and blood urea nitrogen between animals fed rations A and C , while differences between animals fed ration B and C were not significant, as shown in Table (6).

**Table 6:** Average of some blood parameters for animals fed the different experimental rations

Items	Experimental rations			Significant level
	(A)	(B)	(C)	
Serum protein (g/100 ml)				
Total protein (g/dl)	6.40 <sup>b</sup>	6.57 <sup>ab</sup>	6.80 <sup>a</sup>	(P<0.05)
Albumin (g/dl)	3.63 <sup>a</sup>	3.47 <sup>b</sup>	3.43 <sup>b</sup>	(P<0.05)
Globulin (g/dl)	2.77	3.10	3.37	N S
Albumin: globulin ratio	1.31	1.17	1.06	
Liver function (IU/ L)				
GOT (AST)	42.62 <sup>b</sup>	43.18 <sup>ab</sup>	44.15 <sup>a</sup>	(P<0.05)
GPT (ALT)	30.24 <sup>a</sup>	32.16 <sup>ab</sup>	33.52 <sup>a</sup>	(P<0.05)
Kidney function (IU/ L)				
Creatinine mg/dl	0.79 <sup>b</sup>	1.02 <sup>a</sup>	0.99 <sup>a</sup>	(P<0.05)
Blood Urea-N mg/100 ml	14.15 <sup>b</sup>	15.24 <sup>ab</sup>	16.38 <sup>a</sup>	(P<0.05)
B U N / Creatinine	17.91	141.94	16.55	

a ,b and c: means the same row with different superscripts are significant (P<0.05) differed.

**Table 7:** Average of some rumen parameters for animals fed the different experimental rations during two periods.

Items	Experimental rations			±SE
	(A)	(B)	(C)	
pH value				
Zero time feeding	6.98	6.54	6.48	NS
3 hrs post feeding	5.60	5.37	6.51	NS
Overall mean	6.29 <sup>a</sup>	5.96 <sup>b</sup>	5.99 <sup>b</sup>	(P < 0.05)
NH3-N(mg/100 ml)				
Zero time feeding	17.85	19.90	18.86	NS
3hrs post feeding	19.81	22.62	21.40	NS
Overall mean	18.83 <sup>b</sup>	21.26 <sup>a</sup>	20.13 <sup>a</sup>	(P < 0.05)
TVFs (meq/100ml)				
Zero time feeding	6.00	7.63	7.82	NS
3hrs post feeding	9.15	11.25	11.79	NS
Overall mean	7.58 <sup>b</sup>	9.44 <sup>a</sup>	9.80 <sup>a</sup>	(P < 0.05)

a and b: means in the same row with different superscripts are significantly (P<0.05) differ.

It could be noticed that the animals fed supplemented rations (ration B and C) recorded significant (P < 0.05) higher concentration in live enzymes (GOT and GPT) and some parameters of kidney such as creatinine and blood urea nitrogen. The concentration of GOT and GPT for blood of animals fed ration C was 44.15 and 33.52 IU / L, respectively. Corresponding values of creatinine and blood urea nitrogen were 0.99 mg / dL and 16.38 mg / 100 ml, respectively. The differences in serum protein concentration (Table 6) might be attributed to be higher digestibility of CP and DM of experimental rations (Table 2), Also, a raise of GOT and GPT activity was related to the supplementation rations and the serum creatinine was gradually raised with raising fibrolytic enzymes in ration as mentioned by Kholif et al. (2017). The results were agreement with those reported by Abou-Elenin et al. (2015),

Kholif et al. (2017) and Etman et al. (2020).

#### SOME RUMEN PARAMETERS

Data presented in Table (7) showed non significant differences in rumen pH values among the different experimental groups at both zero and 3 hrs post feeding, while the significant (P < 0.05) differences are found with overall mean of pH value. Result showed decreasing pH values. It could be noticed that the animals fed supplemented rations (ration B and C) appeared to somewhat lower of ruminal pH value with no significant in both periods, while the reducing values in overall mean of pH with animals fed ration B and C were significant (P < 0.05) as shown in Table (7). Result obtained showed reduce pH value with sampling times. These results are agreement with those of Azzaz (2009) who reported that the fibrolytic enzymes

treatment tended to significantly reduced ruminal pH. Also, Kholif et al. (2017), Gado et al. (2007), Muwalla et al. (2007) and Etman et al. (2020) recorded the same previous trend.

The rumen ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) showed non significant differences during zero time and 3 hrs post feeding among the different experimental groups. However, some increasing in  $\text{NH}_3\text{-N}$  concentration were found with animals fed supplemented rations (ration B and C) and also non significant higher concentration was recorded at 3 hrs post feeding samples. At the same time, overall mean of  $\text{NH}_3\text{-N}$  concentrations showed significant ( $P < 0.05$ ) higher with animals fed supplemented rations, being 18.83, 21.26 and 20.13 mg/ 100 ml for animals fed ration A, B and C, respectively. These results are agreement with those reported by Kholif et al. (2017), Abdel-Gawad et al. (2007), Azzaz et al. (2007), Muwalla et al. (2007) and Etman et al. (2020).

The data illustrated in Table (7) showed that animals fed supplemented rations B and C) tended to have higher TVFA's concentration at zero time, being 7.63 and 7.82 meg/ 100 ml, respectively. Corresponding values at 3 hrs were 11.25 and 11.79 meg/ 100 ml, respectively. The overall mean of VFA's concentration took the same previous trend recording 9.44 and 9.80 meg/ 100 ml with animals fed ration B and C, respectively. The data showed significant ( $P < 0.05$ ) differences in overall mean VFA's concentration, while the differences either between zero time or 3 hrs post feeding were not significant. Shafie and Ashour (1997), observed that the pattern of VFA's concentration reflected with the pattern of fermentation activity in the rumen. Azzaz (2009) showed that the fibrolytic enzymes treatment significantly reduced rumen pH and raised VFA's concentration in the rumen. Taie (1993), Kholif et al. (2017) and Etman et al. (2020) pointed out that the increasing of rumen VFA's concentration paralleled with a decreasing in rumen pH.

## CONCLUSION

It could be concluded that probiotic bacteria or fibrolytic enzymes as feed additives in rations of fattening buffalo calves tended to increase all nutrient digestibility coefficients, feeding value and daily live body weight. Moreover, using those biological supplementation appeared to lower feed cost to get one kg weight gain and get higher net revenue and economic efficiency with no adverse effect.

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tion research institute.

## CONFLICT OF INTEREST

The authors announce that this research was performed in absence of any commercial or financial relationships that could be interpreted as a possible conflict of interest.

## NOVELTY STATEMENT

The novelty of this research is the use of probiotics bacteria and fibrolytic enzyme to enhance the nutritive value of rations and improve the productive performance buffalo calves.

## AUTHORS CONTRIBUTION

Kamel Etman Ibrahim Etman. put the idea and the design of the study, the experimental work was carried out by Salah Kamal Sayed and Farouk EL-Sayed Amin, the lab analysis was performed by Abd El-Ghany Hasaneen and Mona Ahmed Al-Sayed Farag, the statistical analysis performed by Mostafa Mohamed El-Nahrawy. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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