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Research Article

Growth Performance, Rumen Fermentation and Selected Biochemical Indices in Buffalo Calves Fed on *Basillis subtilus* Supplemented Diet

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ABSTRACT

This study was conducted to evaluate the effect of *Basillis subtilus* supplementation in diet of buffalo calves on growth performance criteria, rumen fermentation properties and certain biochemical indices. Twelve growing buffalo calves, of (4-6) month's age and 125 kg live body weight were assigned into two equal groups (6 animals each): the control group (T1) was fed as T1 plus 0.3 gm FMS®/1 kg diet daily. The study was extended for fifteen weeks. Live body weights were individually recorded biweekly in both group. Rumen and blood samples were collected from each animal in both groups at the end of study. Results showed an increase ($P \leq 0.05$) in dry matter intake (DMI), digestion coefficients, average daily gain, total body weight gain and feed conversion ratio for treated group in comparing with control group. Also treated group revealed ruminal significant increase ($P \leq 0.05$) in total volatile fatty acids (TVFA's), ammonian concentration and significant decrease ($P \leq 0.05$) in total protozoa count (TPC) in comparing with control group. Blood biochemical analysis in treated group showed significant increase ($P \leq 0.05$) in total protein, albumin, triglycerides, AST and ALT levels and significant decrease ($P \leq 0.05$) in urea and creatinine levels compared with control group. The results obtained from this study suggested that B. subtilis may be a potentially useful probiotic in buffalo calves that improved DMI, digestibility, average body weight gain and feed conversion ratio with a recommended effective daily dose 0.3 gm /1 kg in the diet.

Key words: *Basillis subtilus*, Buffalo calves, Digestibility, Rumen fermentation, Biochemical profile, Growth performance

INTRODUCTION

The use of feed additives as probiotics (Dried Fed Microbes) is one of the strategies commonly *used to improve the animal production, health and performance (*Saleem *et al.*, 2017: Soliman *et al*., 2016: Bahari, 2017). Use of probiotic in young ruminant accelerate development of stomach which reflect on increase the animal growth rate (Radzikowski, 2017). FAO/WHO (2002) defined probiotics (DFM) as 'live microorganisms that may beneficially affect the host upon ingestion by improving the balance of the rumen microflora". The terms probiotics and (DFM) are synonymous with each other (Soliman *et al*., 2016, Heyman and Menard, 2001).

The most interesting probiotics preparation can be used as feed additives for ruminants are those of live microorganisms such as *Lactobacillus (L. acidophilus, L. plantarum), Bacillus (B.subtlius . B.cereus)* (Markowiak and Śliżewska, 2018: Saleem *et al.*, 2017). Probiotics exhibit a greet beneficial effect on the animals health and performance (Retta, 2016; Bahari, 2017; Markowiak and Śliżewska, 2018) by stimulating appetite, improving the balance of the intestinal m.o and digestion, enhancing nutrient synthesis and their bioavailability which lead to higher growth performance, increase rumen cellulaytic bacteria population and so increase feed intake and FCR and nutrient absorption.

Bacillus subtilis administration have gained attention for their role in controlling infectious diseases, thereby improving productive performance in animals (Alexopoulos *et al*., 2004; Chen *et al*., 2009) To date, only limited research relating to the dietary utili- zation of B. subtilis in ruminants has been completed (Jenny *et al.*, 1991; Kritas *et al*., 2006). So, this study was conducted to evaluate the effect of inclusion of Five-Men Song (FMS) ® *(Bacillus subtlius)* on buffalo calves performance, digestability, rumen fermentation and certain biochemical indices.

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MATERIALS AND METHODS

This study was carried out in the Experimental Research Station belongs to Faculty of Agriculture, Ain Shams University located in Shalakan village, Qalubia Governorate, Egypt, during the months of February, 2018 through June, 2018.

The experimental animals and rations

Twelve growing buffalo calves of (4-6) month's age and 125 kg of live body weight were assigned into two similar groups (6 animals each). Each group was assigned randomly to feed on one of the following two dietary treatments:

T1; were fed according to the feeding regime of the farm system (NRC, 2001).

T2; were fed according to the feeding regime of the farm system plus 0.3 gm FMS/1 kg of animal concentrates diet.

The basal diet consisted of concentrate feed mixture (CFM), wheat straw and berseem. The CFM consisted of 38% ground maize, 15% soybean meal, 34% wheat bran, 5% rice bran, 3% molasses,1% mineral salts, 2% limestone powder, 1% sodium bicarbonate and 1% sodium chloride.

All animals were treated for internal and external parasites and vaccinated for common infectious diseases before the experiment started. Chemical compositions of feed stuffs are illustrated in Table 1.

Feed additive (FMS)

Five-Men Song (FMS)®: This product is a water soluble powder using as a feed supplement with probiotics vitamins, whereas each 100 gm contains:

- Bacillus subtilis ($8.4*10^6$ CFU)
- Sorbitol Sodium (400 mg)
- Vitamin B1 (200 mg)
- Glucose up to 100 gm

Digestibility trial

Through the entire of study period, digestibility trial was performed for all the experimental buffalo calves using a grab sample method where acid insoluble ash (AIA) was used as an internal marker according to Schneider and Flatt (1975) for determining the nutrients digestibility.

Rumen fluid samples

Rumen liquor samples were taken from each animal in each group at the end of experiment by using stomach tube 4 hrs post feeding and filtered through four layers of cheese cloth. The pH was immediately recorded using digital pH metter, then samples were stored at -20°C until chemical analysis. Ruminal ammonia nitrogen (NH3-N) concentration was determined according to Conway (1957), Ruminal total volatile fatty acids (TVFA's) concentration was determined by steam distillation procedure according to Warner (1964) and total protozol count was estimating according to Dehorety (1986)

Blood plasma samples

Blood samples were taken from were taken from each animal in each group at the end of experiment. The blood samples were taken at three hours post morning feeding. A sample of 15 ml of blood per animal was withdrawn from the jugular vein. The blood was directly collected into a clean dried glass culture tubes after adding EDTA. The blood plasma was obtained by centrifuging the blood samples soon after collection at 4000 (rpm) for 15 minutes. Blood plasma was transferred into a clean dried glass vials and then stored in deep freezer at -20º C for subsequent specific chemical analysis. Blood plasma was analyzed for total protein (Armstrong and Carr 1964), albumin (Doumas *et al.*, 1971), urea (March 1965), creatinine (Husdan 1968). AST, ALT (Reitman and Frankel 1957) and triglyceride (Fassati 1982). Globulin was calculated by difference.

Analytical methods

The chemical composition of the feedstuffs and feaces were analyzed according to the A.O.A.C (1995) methods to determine moisture, DM, OM, CP, CF, EE, and ash contents, while NFE content was calculated by difference.

Statistical analysis

The obtained data were analyzed according to statistical analysis system of SAS (2001). Separation among means was carried out by using Duncan multiple tests, (1955).

RESULTS AND DISCUSSION

Dry matter intake

Data of Table 2 clearly showed the positive effect of adding *Bacillus Subtlius* as an oral probiotic on dry matter intake. Average DMI throughout the experimental period was significantly increased ($P \leq 0.05$) in treated group compared to control group (4.66 vs 4.30). The same findings were recorded by (Desnoyers *et al.*, 2009; Kowalski *et al.*, 2009; Rezende *et al.*, 2012) in calves and adult cattle. (Chiofalo *et al.*, 2004; Payandeh and Kafilzadeh, 2007; Ataşoğlu. *et al.*, 2010) in sheep and goats, (Degirmencioglul *et al*, 2013) in Anatolion buffalos.

These results may be due to that FMS play a role to improve cellulolytic bacteria number and fiber degradation because of reduction the activity of more ammonia producing microbes (Chadmana and offer, 1990).

Table 1: Chemical composition of the experimental feed stuffs (% on DM basis).

Feed stuff	DM	OM	CF	CP	EE	NFE	Ash	AIA**
Berseem	90.94	95.38	30.15	19.64	3.80	41.79	4.62	4.42
Wheat straw	91.73	81.66	51.92	4.15	1.83	23.76	18.34	15.31
CFM*	91.56	96.24	23.60	16.88	2.99	52.77	3.76	5.62

* CFM: Concentrate feed mixture; ****AIA:** Acid insoluble ash.

Table 2: Effect of *Basillis subtilus* supplementation on dry matter intake (kg/h/d)

Period (days)	Treatments	
	T1	T2
0 – 15	4.17±0.66	4.38±1.16
16 - 30	4.16±0.50	4.41±0.87
31 – 45	4.27±0.55	4.49±0.43
46 – 60	4.24±0.33	4.70±1.23
61 – 75	4.39±0.64	4.74±0.91
76 – 90	4.41±0.65	4.84±0.42
91 - 105	4.43±0.54	5.05±1.29
Average	4.30^{B}	4.66^{A}

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

Table 3: Effect of *Basillis subtilus* supplementation on nutrients digestibility (%)

item	T1	T2
DM	$61.43±1.24^{B}$	$68.65±0.44^{A}$
OM	$67.07±1.08^{B}$	$71.30±0.76^{A}$
CP	$62.88±0.74^{B}$	$68.54±0.89^{A}$
CF	$57.54±1.52^{B}$	$65.02±0.9^{A}$
EE	$80.96±1.73^{B}$	$84.33±0.75^{A}$
NFE	$71.40±1.05^{B}$	$79.54±0.61^{A}$

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

Table 4: Effect of *Basillis subtilus* supplementation on some rumen fermentation parameters

Item	Treatments	
	T1	T2
PH	6.86^{A}	6.76^{B}
Total Volatile Fatty Acids (mmol/l)	81.54^{B}	86.35^{A}
Ammonia (mmol/l)	101.28^{B}	107.10^{A}
Total Protozoa Count (x10^5/ml)	15.19^{A}	13.48^{B}

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

Table 5: Effect of *Basillis subtilus* supplementation on selected plasma biochemical parameters

Item	Treatments	
	T1	T2
Total protein (g /dl)	5.89^{B}	6.26^{A}
Albumin (g /dl)	1.93^{B}	2.31^{A}
Globulin (g /dl)	3.96	3.95
Urea (mg /dl)	28.21^{A}	25.49^{B}
Creatinine (g /dl)	1.26^{A}	1.17^{B}
Triglyceride (mg/dl)	36.94^{B}	47.10^{A}
AST (unit /L)	31.57^{A}	26.58^{B}
ALT (unit /L)	33.26^{A}	29.42^{B}

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

Table 6: Effect of *Basillis subtilus* supplementation on changes of body weights and daily gain (kg/h/d)

Item	Treatments	
	T1	T2
	Animal weight	
Initial weight	125.60±15.40	124.67±25.73
Final weight	187.80±18.70	207.67±32.18
Total gain	$62.20±3.52^{B}$	$83.00±7.00^{A}$
Period (days)	Average daily gain (kg/h/day)	
0-15	0.569±0.10	0.711±0.16
16-30	0.566±0.08	0.733±0.10
31-45	0.564±0.11	0.778±0.12
46-60	0.539±0.06	0.778±0.16
61-75	0.567±0.11	0.800±0.10
76-90	0.581±0.10	0.844±0.08
91-105	0.674±0.13	0.889±0.17
Average	0.581^{B}	0.790^{A}

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

Table 7: Effect of *Basillis subtilus* supplementation on dry matter conversion (kg DM/ kg gain)

Period (days)	Treatments	
	T1	T2
0 – 15	7.36±0.78	6.11±0.51
16 - 30	7.39±0.72	5.92±0.43
31 – 45	8.02±1.23	6.12±1.36
46 – 60	7.93±0.41	5.95±0.49
61 – 75	8.52±1.48	5.83±0.46
76 – 90	8.17±1.05	5.86±0.86
91 - 105	7.09±0.83	5.61±0.51
Average	7.79^{A}	5.92^{B}

A and B Means of treatments within the same row with different superscript letters are significantly different (P≤0.05).

Nutrients digestibility

Results of digestion coefficients were summarized in Table 3. The data showed that the treated group (T2) had the higher values (P≤0.05) of all nutrients digestibility than those of T1 (control group). These results are in harmony with those obtained by Soliman *et al*. (2016) on Barki sheep fed on a ration supplemented by FMS as probiotic. They recorded a significant increase in all digestibility coefficients. In Addition, Gomez-Basauri *et al*. (2001) recorded a positive effects of (DFM) on DM, OM, CP and CF digestibilities compared with cows received control ration. Also, Krehbiel *et al.* (2003) illustrated that probiotic (DFM) enhance digestibilities of CP and CF digestibilities. Moreover, Ismaiel *et al.* (2010) found that probiotic improved crude protein and crude fiber digestibility in lambs. This improvement in digestion coefficients can be reflect the role of probiotic in rumen ecology. The positive effects of probiotic on CF digestibility and the other nutrients may be explained by the increase in cellulolytic bacteria growth and numbers which led to enhance nutrients digestibilities especially fiber (Dawson *et al*., 1990); Michael *et al*., 2011 and sallam *et al*., 2014).

Rumen fermentation properties

Data presented in Table 4 showed significant decrease (P≤0.05) in pH and total protozoa count (TPC), while the values of TVFA's and Ammonia concentration were increased (P≤0.05) in treated group (T2) compared with those in control group (T1).

Rumen pH, NH3-N and VFA concentrations are the important indicators that reflect rumen function and stability of the intraruminal milieu. In the present study, oral administration of B. subtilis decreased the rumen pH, but increased NH3-N and total VFA in the ruminal fluid. Similar results were also observed in dairy cattle as reported by Sun *et al*., (2013) and in calves (Sun *et al*., 2011), Soliman *et al*., (2016) showed significant increase in TVFA and decrease in ruminal ammonia level in Barki lambs fed on probiotic supplemented fed. Anjum *et al*., (2018) recorded that yeast supplementated diet increased TVFA's in ruminal fluid of Nili-Ravi buffaloes. This increase might be due to more NDF digestibility. They also reported decrease in rumen ammonia-N of yeast fed buffaloes as a result of incorporation of ammonia into microbial protein (Bakr *et al*., 2015) and/or it may be due to inhibitory effect of yeast on proteolysis (Khattab *et al*., 2003).

Regarding to total protozoa count in rumen fluid results of Table 4 showed significant decrease in comparison with control group. It is known that ruminal microbial population is of great importance for rumen fermentation. Sun *et al.*, (2013) recorded that B. subtilis increased numbers of certain groups of bacteria, such as amylolytic and proteolytic bacteria and reduced protozoal number in dairy cattle.

Blood plasma parameters

Data of Table 5 showed significant increase (P≤0.05) in total protein, albumin, globulin and triglycerides in treated group (T2) compared with those in control group (T1), While there was significant decrease (P≤0.05) in blood urea nitrogen (BUN), AST, ALT and creatinine levels in treated group (T2) compared with those in control (T1).

In the present study, the levels of total protein, albumin and globulin were significant increase (P≤0.05) in treated group in compared with control group, the same results were recorded by (Abdel-Salam *et al.*, 2014) on Najdi male lambs fed basal diet supplemented by synbiotic. on the other hand, ntunovic *et al.*, (2006) El-Katcha *et al.*, (2016) reported non-significant differences in plasma total protein, albumin, globulin, and glucose levels on growing lambs or goats fed diets containing probiotic. Arab *et al.*, (2014) reported that glucose, total protein, albumin, and cholesterol concentrations were decreased significantly (P≤0.05) in lambs receiving 0.5 or 1 g (bacillus subtilis and bacillus licheniformis) as a probiotic/kg feed compared to control group.

In the present study treated group had lower levels of urea in the blood plasma that probably due to better utilization of nitrogen from feed in the rumen with probiotic supplementation. Similar results were observed by Antunovic *et al.*, (2005, 2006); Ding *et al.*, (2008) who found that blood urea was decreased in response to probiotic supplementation compared to the control group. Other researchers observed no differences in the concentration of blood urea nitrogen between diets containing probiotic and the control.

The level of creatinine was significant decreased (P≤0.05) in treated group while triglycerides level was significant increased (P≤0.05) compared to control group. Activity of AST and ALT was significantly decreased (P≤0.05) in treated group (T2) when compared with those in control group (T1).

This finding could be explained by the decrease of gluconeogenesis process which reflect normal metabolic conditions in the liver.

Body weight and growth performance criteria

Data of Table 6 clearly showed the positive significant effect on total and daily gain of supplementing diet by *Basillis subtilus* as a probiotic component in compared with control group. The higher values (P≤0.05) of total gain and average daily gain were recorded for treated group (T2) than the control group (T1). The present results are supported by the results of (Desnoyers *et al*., 2009; Kowalski *et al*., 2009; Rezende *et al*., 2012)in calves and adult cattle, Chiofalo., *et al*., (2004); Payandeh and Kafilzadeh (2007); Ataşoğlu *et al*., (2010) in sheep and goats, Degirmencioglul *et al*., (2013) in Anatolion buffalos. On the other hand Anjum *et al*., (2018) reported non effect on average body weight gain in Nili-Ravi buffaloes received Saccharomyces cerevisiae and Baranwoski *et al*., (2007) stated that use of probiotics in growing lambs had no effect on daily weight gain.

Probiotics improve microbial ecology (Musa *et al*., 2009), nutrient synthesis and absorption, nutrient bio-availability and stabilized ruminal pH and lactate resulting in better weight gain in farm animals (Mountzouris *et al*., 2007; Oyetayo and Oyetayo, 2005). Probiotic supplementation in lambs increased weight gain (Jang *et al*., 2009). Higher weight gain values of treated group as compared to the control could be due to improving microbial protein synthesis leading to more amino acids supply at post-ruminal level (Erasmus *et al*., 1992) and/or to higher consumption Table 2 and better feed efficiency in the probiotics supplemented group (Antunovic *et al*., 2006).

Feed conversion (kg DM/ kg gain)

Results of dry matter conversion Table 7 showed that the better (P≤0.05) DM conversion was recorded for the treated group (T2) compared to the control one. These may be related to the higher gain Table 6 of the treated group. These results are in agreement with those obtained by Chiofalo *et al*., (2004); Antunovic *et al*., (2006) and Whitley *et al*., (2009) in ruminants. Also, Hansen et al., (2017) on lactating buffaloes reported positive effect of live yeast as a probiotic on dry matter conversion. Moreover, Kuss *et al*., (2009) found an enhancement in feed conversion with supplementing diets by probiotic (Saccharomyces cerevisiae) in non-castrated cattle. Same results recorded by Soliman *et al*., (2016) on sheep. In addition to Abdelrahman and Hunaiti (2008) and Robinson (2002) pointed that using probiotics containing yeast in lamb's rations improved feed conversion ratio, which is due to the probiotics effect on rise the amount of cellulose degrading bacteria in the rumen of lambs fed with probiotics.

Conclusions

The obtained results from this study suggested that supplementation of buffalo calves with B. subtilis as a rate of 0.3 gm/kg diet improved dry matter intake, nutrients digestibility, total weight gains, daily weight gains, feed conversion rates and rumen fermentation parameters.

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