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The impact of probiotic (Biovet®) on some clinical, hematological and biochemical parameters in buffalo-calves

H. A. Bakr*, E. M. Said, M. M. Abd El-Tawab, M. S. Hassan

Department of Animal Medicine, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef Egypt

This study was conducted on 25 male buffalo-calves, with age range, 6-7 months old (158 – 165 kg bwt), belonged to a private farm in Beni-Suef governorate. The animals were divided into three groups; control group (5 buffalo-calves) received probiotic-free ration, Group I and Group II (10 buffalo-calves in each). Buffalo-calves in groups I and II were orally administered with 15 and 25 g (Biovet®)/animal/day with respectively. The experiment lasted for 84 days. The effect of probiotic (Biovet®) supplementation on clinical, hematological and biochemical parameters as well as on the body weight gain in growing buffalo-calves were investigated. Hemoglobin concentrations, packed cell volume (PCV %), erythrocyte counts (RBCs) and total leucocytes counts (WBCs) of group I, and II revealed insignificant alterations comparing to control group. Insignificant variations of aspertate aminotransferase activities (AST), alanine aminotransferase activities (ALT), albumin, globulin, urea and creatinine levels of groups I and II were also recorded. The activities of alkaline phosphatase (ALP) in groups I and II buffalo-calves were significantly increased comparing to that in control animals. The levels of the total protein and the glucose levels in the probiotic-treated buffalo-calves increased significantly ($P \leq 0.05$) comparing to that in control animals starting from 28th and 42th day till the end of the experiment respectively. The levels of triglycerides, cholesterol, HDL-cholesterol decreased insignificantly in (Biovet®) whereas LDL-cholesterol levels significantly decreased ($P \leq 0.05$) in treated groups comparing to that in control animals. The T3 and T4 concentrations and body weight gain in probiotic-treated buffalo-calves significantly increased ($P \leq 0.05$) in comparison to control group. The study declared that the probiotic (Biovet®) has obvious effect on body weight gain in buffalo-calves without any deleterious effect on animal health.

The probiotics are quite unique biological active substances that increase the daily live body weight gain and improve animal growth via improving digestion by balancing the gut flora and helps the animal to fulfill its genetic potential in sheep, calves and cattle (Ghorbani *et al.* , 2002, krehbiel *et al.,* 2003; FEFANA, 2008). Morrill, (1995) found that the probiotics supplementation had insignificant effect on red blood cell counts, white blood cell counts and serum total protein in calves. Also, Sadiek and Bohm (2001) recorded the same results in sheep supplemented with probiotics. Sayed (2003) reported that kids supplemented with probiotics had significant increase in hemoglobin concentration, PCV %, erythrocyte count, and blood serum total protein, while total leukocyte count, blood serum AST, serum urea and serum creatinine levels were not significantly altered. Antunovic *et al.* 2005 recorded that probiotic pioneer PDFM® significantly reduce serum glucose and urea levels and activities of ALT, AST and CK but significantly increased the levels of total bilirubin and triglycerides in lambs. Conflicting reports were recorded regarding the effect of probiotics supplementation on average daily gain, some showed improvement on body weight gain in calves and cattle by 6 – 24 % (Saha *et al.,* 1999; Lema *et al.,* 2001; Rao *et al.,* 2003; Isk *et al.,* 2004) while other reports stated that supplementation in calves and cattle had no effect on body weight (Windschtile, 1991; Haryanto *et al.,* 1994; Morrill *et al.,* 1995; Orpeza, *et al.,*1998).

* Corresponding author. Tel.: +20 0822322066;
fax: +20 0822327982.
E-mail address: hossam.bakr@bsu.edu.eg
(H. A. Bakr).

Bacterial enzymatic hydrolysis may enhance the bioavailability of protein and fat (Friend and Shahani, 1984) and increases the production of free amino acids and short chain fatty acids that when absorbed contribute to the available energy pool of the host (Rombeau *et al.,* 1990). Blood cholesterol level was decreased in probiotics supplementation (Zacconi *et al.,* 1992; Vasiljevic and Shah, 2008) and reduced cholesterol and low density lipoprotein-cholesterol in human were also found upon using probiotics(Mohan *et al.,* 1990; Agerholm-Larsen *et al.,* 2000). This study aimed to investigate the effect of the most commonly used probiotic (Biovet®) on clinical, some hematological, and biochemical parameters, as well as on the body weight gain in buffalo-calves under field condition in Beni-Suef governorate in Egypt.

Materials and Methods

Animals. The study was carried out on 25 male-buffalo-calves, selected to fulfill the requirements of fatting, their ages ranged from 6 to 7 months old and their body weights ranged from 158 to 165 kg. The selected animals were raised in open yard, belonged to a private farm in Beni-Suef governorate. The calves were approved apparently healthy after clinical examination according to (Radostits *et al.,* 2000) and were free from internal and external parasites according to Urquhart*, et al.* (1996). These animals were divided into three groups; Control group consisted of five buffalo-calves, received probiotic-free ration, and Groups I and II; consisted of 10 animals in each and received 15 g and 25 g of *Biovet*®/animal/day, respectively. The animals were succumbed to periodical clinical examination along the period of experiment which lasted for 84 days.

Probiotic. The probiotic used in this study was (*Biovet*®). Each gram of probiotic (*Biovet*®) contains *Lactobacillus sporogenes* 7.5 X 10^6, *Lactobacillus acidophilus* 3 X 10^7 and *Saccharomyces cerevisae* SC-47 1.25 X 10^8.

Ration. A fatting ration for buffalo-calves was prepared according to the National Research Council (NRC, 2001). The quantity of the ration for each animal under the experiment was adjusted biweekly according to the body weight.

Samples. Triple venous blood samples were collected pre-prandial from each animal, at 0 (before treatment), 3rd day, 7th day, and 14th day of experiment, then biweekly until the 84th day of the experiment; the first blood sample was received on sodium floride for estimation of blood serum glucose (mg/dl), the second blood sample was received on acetate EDTA as anticoagulant for estimation of hemoglobin concentration (g/dl), (PCV %), erythrocyte count (N X 10^6/ul), and total leukocyte counts (N X 10^3/ul), and the third blood sample was collected in clean dry tubes without anticoagulant to obtain clear sera for estimation of AST activity, ALT activity, ALP activity, serum total protein, serum albumin, serum triglycerides, serum cholesterol, serum low density lipoprotein-cholesterol (LDL-cholesterol), serum high density lipoprotein-cholesterol (HDL-cholesterol), serum urea, serum creatinine, serum tri-iodothyronine (T_3), and serum thyroxin (T_4).

Clinical examination of the experimental animals. All the buffalo-calves under the experiment were clinically examined according to (Radostits *et al.,* 2000).

Estimation of the packed cell volume (PCV %). PCV was estimated by microhematocrite methods according to (Coles, 1986).

Estimation of hemoglobin concentration (Hb g/dl). It was carried out according to the method described by (Wintrobe, 1965).

Estimation of erythrocyte count and total leukocyte counts: was carried out using improved new-Bauer chamber after the methods described by (Jain, 1986).

Estimation of serum aspartate aminotransferase (AST) and alanine aminotransferase activities (ALT) (U/l). Using a test kits according to (Reitman and Frankel,1957).

Estimation of serum alkaline phosphatase activity (ALP) (U/l). Using test kits according to (Kind and King, 1954).

Estimation of serum total protein (g/dl). Using test kits according to the method described by (Peters, 1968).

Estimation of serum albumin (g/dl). Using test kits according to (Drupt, 1974).

Estimation of serum globulin (g/dl). It was calculated mathematically.

Estimation of serum glucose (mg/dl). Using test kits according to (Trinder, 1969).

Estimation of serum triglycerides (mg/dl). Using test kits according to (Fossati and Prencipe, 1982).

Estimation of serum cholesterol (mg/dl). Using test kits according to (Richmond, 1973).

Estimation of serum low-density lipoprotein-cholesterol (LDL-cholesterol) (mg/dl): using test kits according to (Steinberg, 1981).

Estimation of serum high density lipoprotein-cholesterol (HDL-cholesterol) (mg/dl). Using test kits according to (Burstein *et al.,* 1970).

Estimation of serum urea (mg/dl). Using test kits according to (Patton and Crouch, 1977).

Estimation of serum creatinine (mg/dl). Using test kits according to (Houot, 1985).

Estimation of serum tri-iodothyronine (T3) (ng/ml). Using test kits according to (Chopra *et al.,* 1972).

Estimation of serum thyroxin (T4) (ug/dl). Using test kits according to (Tietz, 1976).

Estimation of the body weight. Body weight of each animal was estimated biweekly.

Statistical analysis of the data. It is carried out by PC-State computerized program according to (Mohan *et al.,* 1985).

Results and Discussion

Recorded clinical examination sheets of the experimental animals revealed no abnormalities along the experimental period. The obtained results as shown in Table (1) revealed the effect of probiotic (Biovet®) on the levels of hemoglobin, PCV%, erythrocyte counts and total leucocytes counts along the period of the experiment. Table (2) clarified the activities of AST, ALT, and ALP in control and Group I and group II buffalo calves along the period of the study. Table (3) demonstrated the effect of probiotic (Biovet) ® on serum total protein, albumin, globulin, and glucose levels in along the period of the experiment. Table (4) is showing the effect of the (Biovet) ® on lipid profile (Triglycerides, cholesterol, HDL-cholesterol, and LDL-cholesterol levels) in buffalo-calves of group I and group II. The results of the effect of the probiotic (Biovet) ® on the levels of serum urea, serum creatinine, T_3 and T_4 were summarized in Table (5). The effect of the probiotic (Biovet) ® on the body weight in groups I and II along the period of the experiment was illustrated in Table (6).

Various biological active substances, added to the feed of beef cattle, have been used for a long time in order to promote growth. Based on growing effect over the use of antibiotics and other growth promoters in animal feed industry, there is a great interest in the effect of microbial feed additives on animal performances and animal health.

Concerning the effect of the probiotic (Biovet) ® on the hemogram of the buffalo-calves, Table (1), showed that the results of hemoglobin concentration, PCV %, and erythrocyte counts fluctuated insignificantly among the animals groups in different time of sampling. The results of hemoglobin concentration, PCV%, and erythrocyte counts were within the normal physiological ranges recorded by (Benjamin, 1984). On the other hand the results of total leucocytes counts showed a clear trend, Table (1). The total leucocytes counts increase insignificantly and gradually in animal groups, reaching the maximum levels at end of the experiments as they were $13.07 \pm 1.23\ X10^3$, $13.45 \pm 0.87\ X\ 10^3$, and $13.11 \pm 0.78\ X10^3$/ul in control, group I and group II, respectively. The increase in the total leucocytes counts in the experimental animals may be attributed to the effect of advancing of the animal age but still within the physiological ranges (Benjamin, 1984).

The results of the hemogram described in Table (1) were within the physiological ranges and such findings coincide with that obtained by (Morrill *et al.,* 1995; Sadiek and Boehin 2001).

The results in Table (2) clarified that the activities of AST and ALT were insignificantly changed along the period of the experiment. The activities of AST and ALT in animals of the control group, group I and group II along the period of the experiment were in harmony with that detected by (Nahashon *et al.*, 1992; Bohm and Srour, 1995; Sadeik and Bohm, 2001), who demonstrated that the activities of AST and ALT were normally and nearly the same in control and probiotic-treated animals indicating that probiotic had no side effects on the animal health.

Concerning the results of ALP as shown in Table (2), the activities of ALP were increased gradually with advancing of the age of buffalo-calves along the period of experiment in control, group I, and group II. The activities of ALP were significantly increased ($P \leq 0.05$) in animals of group I and group II in comparing with that of control group at 7th, 14th, 28th, and 84th day of the experiment and at 42th, 56th, and 70th day the activities of ALP were significantly increased ($P \leq 0.01$) in group I and group II comparing with that that in control animals. It is worth to mention that there is significant increase ($P \leq 0.05$) in the activities of ALP in group II in comparing with that in group I at 56th and 70th day of the experiment. The results of ALP in the present study are consistent with the previous observations

Table 1: Effect of probiotics (Biovet®) on hemoglobin concentration, PCV %, RBCs counts, and total WBCs counts in buffalo-calves (Mean±SD).

Time PT (days)	Hemoglobin concentration g/dl			PCV (%)			RBCs counts x 10^6/ul			WBCs counts x 10^3/ul		
	Control	Group I	Group II	Control	Group I	Group II	Control	Group I	Group II	Control	Group I	Group II
Zero	12.28±0.38	12.30±0.88	12.25±0.67	33.20±1.48	33.40±2.20	33.40±0.55	11.07±0.66	11.13±0.97	11.00±0.86	10.28±0.16	10.42±0.51	10.52±1.26
3	12.30±0.70	12.26±0.65	12.15±0.47	33.40±1.94	33.40±0.89	33.20±0.83	11.15±0.64	11.10±0.75	10.97±1.34	11.77±1.03	11.71±1.03	12.09±1.12
7	12.25±0.68	12.00±0.80	12.00±0.36	33.20±2.23	33.10±1.81	33.10±1.51	11.00±0.75	10.80±1.09	10.85±0.76	11.02±1.19	11.62±0.61	12.11±0.48
14	12.32±1.36	12.10±0.71	11.17±0.89	33.40±1.78	33.20±1.70	33.30±2.11	11.23±0.74	11.26±1.14	11.00±1.12	11.95±0.79	12.02±1.34	12.11±0.48
28	12.31±0.85	12.30±0.78	12.15±0.53	33.40±1.30	33.40±0.44	33.30±1.99	11.35±0.92	11.06±1.02	10.93±1.23	12.36±1.26	12.03±0.30	12.00±0.60
42	12.28±0.85	12.10±0.80	12.10±0.82	33.30±1.14	33.10±2.00	33.10±0.44	11.00±0.92	11.30±1.19	10.97±0.75	12.44±1.43	12.61±0.75	12.31±1.27
56	12.30±0.46	12.10±0.21	12.10±0.55	33.40±0.89	33.20±0.83	33.10±1.89	11.44±0.56	11.43±0.50	10.97±0.56	12.34±0.55	12.82±0.43	12.89±1.47
70	12.33±0.72	12.10±0.81	12.29±0.87	33.30±0.89	33.10±1.46	33.20±1.67	11.33±0.99	11.40±0.71	10.91±0.88	13.05±0.94	13.06±0.43	13.10±0.64
84	12.35±0.88	12.20±0.86	12.15±0.68	33.40±1.30	33.20±1.99	33.20±1.58	11.34±0.54	11.45±0.48	10.99±1.22	13.07±1.23	13.54±0.87	13.11±0.78

P. T: Post-treatment

Table 2: Effect of probiotic (Biovet®) on AST, ALT, ALP activities in buffalo-calves (Mean ±SD).

Time PT (days)	AST (i.u/l)			ALT (i.u/l)			ALP (u/dl)		
	Control	Group I	Group II	Control	Group I	Group II	Control	Group I	Group II
Zero	107.00±7.85	113.44±12.55	101.00±10.24	89.60±8.35	86.80±14.29	80.80±9/31	177.74±20.92	187.60±20.37	177.65±5.13
3	106.00±5.47	104.00±4.19	107.00±5.70	97.80±7.46	90.80±5.76	90.60±10.69	198.66±15.39	194.32±18.25	191.87±12.19
7	105.00±3.53	103.00±2.73	106.00±8.94	88.20±5.02	86.00±9.87	83.60±10.43	194.40±15.42 ^{a}	226.92±8.25 ^{b}	218.16±7.75 ^{b}
14	110.00±3.53	105.00±6.12	106.00±4.08	82.20±7.82	89.53±10.57	88.00±3.28	197.92±17.26 ^{a}	220.49±8.32 ^{b}	219.38±10.86 ^{b}
28	114.00±5.47	108.00±4.47	111.00±5.47	91.20±12.23	88.60±11.23	88.40±10.13	196.09±13.61 ^{a}	223.25±17.53 ^{b}	229.02±9.38 ^{b}
42	120.00±6.12	116.00±6.51	114.00±6.51	87.20±9.80	82.20±7.82	84.60±6.84	197.95±16.08 ^{A}	233.48±13.93 ^{B}	238.48±16.76 ^{B}
56	117.00±4.18	126.00±8.21	106.00±9.61	84.60±5.36	85.80±5.02	84.60±3.23	197.45±11.22 ^{A}	228.54±14.02 ^{aB}	250.34±18.24 ^{Bb}
70	114.00±2.23	113.45±2.23	104.11±2.73	93.74±4.47	89.53±7.79	85.60±5.47	198.01±15.19 ^{A}	229.77±5.68 ^{Ba}	253.45±18.72 ^{Bb}
84	117.33±3.53	113.00±4.47	104.10±4.47	93.70±7.79	89.53±10.13	88.60±12.61	222.71±13.02 ^{a}	242.86±11.77 ^{b}	256.35±9.35 ^{b}

P. T: Post-treatment

$^{A, B}$ Means in the same row with different superscript are significant different at ($P \leq 0.01$).

^{a,b} Means in the same row with different superscript are significant different at ($P \leq 0.05$).

Table 3: Effect of probiotic (Biovet®) on total protein, albumin, globulin and glucose levels in buffalo-calves (Mean ±SD).

Time PT (days)	Total protein (g/dl)			Albumin (g/dl)			Globulin (g/dl)			Glucose (mg/dl)		
	Control	Group I	Group II	Control	Group I	Group II	Control	Group I	Group II	Control	Group I	Group II
Zero	6.27±0.12	6.34±0.22	6.36±0.63	3.35±0.13	3.20±0.20	3.30±0.29	2.92±0.21	3.14±0.27	3.06±0.82	59.61±3.73	59.95±3.52	60.83±3.97
3	6,31±0.21	6.34±0.36	6.45±0.43	3.41±0.16	3.38±0.19	3.24±0.06	2.90±0.12	2.96±0.19	3.21±0.40	59.89±3.69	63.74±3.00	66.24±2.32
7	6.38±0.19	6.29±0.22	6.46±0.46	3.46±0.62	3.34±0.14	3.31±0.15	2.91±0.14	2.95±0.26	3.15±0.56	64.35±3.37	64.61±4.76	66.37±3.07
14	6.44±0.14	6.43±0.31	6.48±0.26	3.30±0.12	3.17±0.22	3.20±0.06	3.14±0.22	3.26±0.33	3.28±0.30	62.43±2.45	60.92±3.19	61.60±2.97
28	6.31±0.56 ^{a}	6.65±0.15 ^{b}	6.64±0.32 ^{b}	3.54±0.08	3.27±0.27	3.46±0.18	2.77±0.48	3.38±0.22	3.18±0.29	64.14±1.86	67.06±2.47	66.80±3.20
42	6.21±0.25 ^{a}	6.69±0.20 ^{b}	6.61±0.20 ^{b}	3.50±0.25	3.63±0.23	3.48±0.15	2.69±0.40	3.13±0.23	2.47±0.27	65.56±2.12 ^{a}	71.92±5.31 ^{b}	69.67±3.81 ^{b}
56	6.30±0.37 ^{a}	6.65±0.16 ^{b}	6.62±0.38 ^{b}	3.48±0.15	3.53±0.12	3.63±0.09	2.91±0.41	3.12±0.09	3.23±0.35	61.13±4.12 ^{a}	69.72±3.74 ^{b}	70.63±3.48 ^{b}
70	6.38±0.34 ^{a}	6.93±0.18 ^{b}	6.94±0.40 ^{b}	3.79±0.21	3.85±0.16	3.91±0.07	2.56±0.30	3.08±032	3.03±0.40	63.04±3.42 ^{A}	72.98±1.49 ^{A}	72.64±2.73 ^{B}
84	6.32±0.24 ^{a}	6.94±0.13 ^{b}	6.95±0.31 ^{b}	3.79±0.23	3.73±0.12	3.75±0.09	2.53±0.93	3.21±0.24	3.20±0.30	66.34±1.79 ^{A}	78.00±2.40 ^{B}	75.38±3.71 ^{B}

P. T: Post-treatment

$^{A, B}$ Means in the same row with different superscript are significant different at ($P \leq 0.01$).

^{a,b} Means in the same row with different superscript are significant different at ($P \leq 0.05$).

Table 4: Effect of probiotic (Biovet®) on Triglycerides, Cholesterol, HDL-cholesterol, and LDL-cholesterol levels in buffalo-calves (Mean ±SD).

Time PT (days)	Triglycerides (mg/dl)			Cholesterol (mg/dl)			HDL-cholesterol (mg/dl)			LDL-cholesterol (mg/dl)		
	Control	Group I	Group II	Control	Group I	Group II	Control	Group I	Group II	Control	Group I	Group II
Zero	27.88±3.23	27.43±3.77	25.80±2.17	50.37±7.61	51.62±4.23	49.58±7.53	28.55±3.65	29.52±1.94	29.65±1.75	17.89±5.32	17.61±2.05	16.90±3.38
3	27.27±2.29	29.25±2.27	27.93±2.55	58.24±6.24	55.87±4.89	50.12±8.89	26.54±1.14	24.99±3.07	23.94±3.01	24.24±3.60	20.63±2.38	21.79±3.03
7	29.89±4.91	25.63±2.38	24.67±0.97	63.60±6.04	52.25±4.11	55.24±6.56	24.92±1.86	24.70±1.37	22.45±1.74	29.97±4.78 ^{A}	21.66±2.94 ^{B}	21.68±2.01 ^{B}
14	36.00±1.65	25.55±3.44	27.14±2.17	53.12±3.14	41.42±4.68	43.44±5.40	23.59±0.68	23.17±1.56	23.03±1.74	26.55±3.33 ^{a}	19.15±4.48 ^{b}	19.19±2.34 ^{b}
28	38.14±1.68	25.64±2.55	24.67±1.65	53.12±4.57	50.24±3.38	41.37±4.58	23.89±1.09	23.06±2.30	23.44±1.71	24.69±4.50 ^{a}	21.84±3.03 ^{b}	21.12±4.12 ^{b}
42	36.17±7.23	27.70±3.38	24.68±3.04	56.40±3.31	53.12±2.46	44.37±4.40	23.85±1.05	23.39±1.35	23.45±1.45	25.32±3.90 ^{a}	23.77±5.28	20.99±2.61 ^{b}
56	36.25±4.99	29.85±4.95	24.55±5.01	62.35±2.70	58.59±2.70	59.99±5.77	23.97±0.59	23.23±1.04	22.82±0.74	29.70±4.10 ^{Aa}	23.60±3.11 ^{b}	20.62±3.14 ^{B}
70	36.34±3.99	29.80±5.02	25.00±2.39	70.87±4.38	52.37±7.75	59.57±1.02	24.29±0.71	23.19±1.76	23.31±0.71	35.98±3.03 ^{A}	23.41±2.31 ^{Ba}	20.25±0.98 ^{Bb}
84	36.18±2.49	29.78±3.12	25.32±1.02	74.37±5.91	59.02±3.41	47.51±5.51	24.08±0.92	23.80±1.25	23.40±1.02	39.27±6.39 ^{A}	24.95±3.00 ^{B}	21.65±4.08 ^{B}

P. T: Post-treatment

$^{A, B}$ Means in the same row with different superscript are significant different at ($P \leq 0.01$).

^{a,b} Means in the same row with different superscript are significant different at ($P \leq 0.05$).

Table 5: Effect of probiotic (Biovet®) on blood serum urea, creatinine, T_3 and T_4 levels in buffalo-calves (Mean ±SD).

Time PT (days)	Serum Urea (mg/dl)			Serum Creatinine (mg/dl)			Serum T_3(ng/ml)			Serum T_4 (ug/dl)		
	Control	Group I	Group II	Control	Group I	Group II	Control	Group I	Group II	Control	Group I	Group II
Zero	31.10±2.07	33.37±2.03	32.25±3.75	0.82±0.11	0.82±0.11	0.75±0.03	4.27±0.50	4.36±0.82	4.11±0.12	12.26±0.73	12.37±0.86	12.20±0.94
3	36.37±4.86	33.56±3.09	32.25±2.52	0.92±0.06	0.82±0.8	0.95±0.11	3.63±0.17^{a}	4.27±0.24^{b}	4.45±0.29^{b}	14.59±0.53	14.97±0.39	13.83±1.56
7	36.02±2.88	31.42±4.66	37.66±5.19	1.00±0.03	1.00±0.08	1.00±0.04	3.72±0.21^{aA}	4.78±0.50^{ab}	6.46±0.28^{Bb}	13.80±0.48^{A}	13.65±0.7 5^{a}	16.21±0.77^{Bb}
14	36.12±4.04	31.87±3.72	38.35±3.13	0.74±0.02	0.76±0.05	0.71±0.02	4.25±0.37^{aA}	4.60±0.24^{ba}	5.53±0.85^{Bb}	13.50±0.07^{Aa}	14.15±0.73^{ba}	15.85±1.25^{Bb}
28	33.28±1.78	31.63±3.50	32.63±3.54	0.96±0.08	0.89±.10	0.91±0.03	4.47±0.09^{aA}	4.86±0.53^{ba}	6.23±0.22^{Bb}	13.10±0.45^{A}	13.47±0.53^{A}	17.27±0.70^{B}
42	31.00±1.44	31.52±2.33	29.16±1.93	1.04±0.09	1.01±0.09	0.97±0.08	4.67±0.28^{A}	4.88±0.31	6.29±0.28^{B}	14.32±1.38^{A}	13.15±0.42^{A}	17.11±0.62^{B}
56	30.54±4.34	26.36±1.97	26.22±3.14	1.16±0.04	1.13±0.04	1.06±0.08	4.37±0.22^{A}	5.15±0.18^{B}	6.45±0.20^{B}	18.15±0.29^{a}	19.33±1.24^{b}	21.43±0.64^{b}
70	27.13±2.04	26.08±1.61	27.78±3.51	1.13±0.03	1.19±0.04	1.25±0.07	4.67±0.16^{A}	5.11±0.46^{aB}	6.04±0.26^{bB}	18.15±1.27^{a}	19.17±1.56^{b}	21.75±1.25^{b}
84	21.21±1.95	21.71±2.40	21.65±1.95	1.13±0.06	1.17±0.06	1.23±0.06	4.12±0.53^{A}	4.91±0.47^{a}	5.66±0.34^{Bb}	17.33±0.3 0^{a}	19.18±0.15^{b}	19.66±0.83^{b}

P. T: Post-treatment

$^{A, B}$ Means in the same row with different superscript are significant different at ($P \leq 0.01$).

^{a,b} Means in the same row with different superscript are significant different at ($P \leq 0.05$).

Table 6: Effect of probiotic (Biovet®) on body weight of buffalo-calves (Mean ±SD).

Time PT (days)	Body weight (Kg)		
	Control	Group I	Group II
Zero			
3	163.6±5.77	164.40±4.93	164.4±6.22
14	178.2±5.63	180.20±6.22	179.6±5.17
28	193.00±5.14	196.80±4.86	196.00±5.61
42	207.8±4.60	214.80±5.35	213.00±9.09
56	223.00±4.63^{a}	232.40±5.12^{b}	230.00±4.84^{b}
70	238.40±4.03^{A}	249.80±4.81^{B}	247.40±3.64^{B}
84	253.80±3.96^{A}	264.80±3.70^{B}	264.20±4.74^{B}

P. T: Post-treatment

$^{A, B}$ Means in the same row with different superscript are significant different at ($P \leq 0.01$).

^{a,b} Means in the same row with different superscript are significant different at ($P \leq 0.05$).

recorded by Antunovic, et al. (2005) whosuggested that the increase in ALP activities in probiotic treated lambs as an indicator to reinforced activity of bone cells. It is worth mentioning that ALP is a microsomal enzyme being intracellular except in the case of osteoblasts, where it acts mainly in an intracellular location. Osteoblasts are responsible for elevating ALP activity during the period of rapid bone growth in young animals (Benjamin, 1984). On the light of these facts, we could emphasize that the rate of bone growth in probiotic-treated buffalo-calves was higher than that of control animals.

In general, the results of AST, ALT, and ALP activities in the probiotics-treated animals in this study were within the normal values and in agreement with that observed by (Sayed, 2003; Antunovic *et al.,* 2005).

The levels of the serum total protein in control animals were fluctuating between 6.21 and 6.44 g/dl (Table 3) while the levels of total protein gradually increased in animals of group I and group II along the period of the experiment and increased in the level of serum total protein became significant (P ≤0.05) at 28th, 42th, 56th, 70th, and 84th day of the experiment and the increases in the mean values of serum total protein of the probiotic-treated groups (I & II) could be attributed to the increases of the serum globulins of the probiotics-treated groups (I & II); in group I (2.95±0.26 – 3.38±0.22 at 7th, 28th, day) and in group II (3.03±0.40 – 3.28±0.30 mg/dl at 70th, and 14th) comparing with that of control group (2.53±0.93 – 2.92±0.21 mg/dl) at 84th and zero day. Similar results were obtained by (Sayed, 2003), who suggested that the significant increase in the blood serum levels of total protein in probiotics treated animals may be attributed to the improvement in the animal appetite and feed utilization in that animals.

Concerning the serum albumin and globulin levels (Table 3), there were no significant changes in the levels of serum albumin and globulin in control and probiotic-treated buffalo-calves. In this context, our results are paralleled with that recorded by Sayed (2003) in probiotic-treated kids.

Regarding to the levels of serum glucose illustrated in Table (3), there were non-significant changes in the levels of serum glucose in control, group I, and group II at the 3rd, 7th, 13th and 28th day of experiment. Significant increases (P≤0.05) in serum glucose levels of probiotics-treated buffalo-calves of group I and group II in comparing with that of control animals at 42th, and 56th day of the experiment. Serum glucose levels increased significantly (P≤0.01) in animals of group I and group II comparing with that of control group at 70th, 84th day of the experiment. There were no significant changes in the levels of serum glucose in animals of group I and that of group II. The increase in the serum glucose levels in Biovet-treated buffalo-calves may be attributed to gluconeogensis enhancement, as the gluconeogensis in the ruminants is the main source of glucose and has a decisive influence on its level in the blood (Huntington and Eisemann, 1988). Our results were in contrary with that of (Antunovic *et al.*, 2005) who recorded that low glucose levels in probiotics treated lambs.

The results of triglycerides, cholesterol, and HDL-cholesterol, and LDL-cholesterol had the same trend in the probiotic-treated buffalo-calves and their mean values were decreased in probiotics-treated animals comparing with that in control. The levels of triglycerides, cholesterol, and HDL-cholesterol were non-significantly decreased (P≤0.05) in probiotic treated animals of groups I and II comparing with that in control one (Table 4). The minimum triglycerides levels in control group was 27.27 ± 2.29 mg/dl recorded at 3rd day of experiment, but the minimum values of triglycerides in group I and II were 25.55±3.44 and 24.55±5.01 mg/dl at 14th and 56th day of experiment, respectively. The maximum values of triglycerides in group I and II were 29.80±5.02 and 27.93±2.55 at 70th and 3rd day of experiment. The range of cholesterol levels in the control animals was 50.37±7.61 – 74.37±5.91 mg/dl, in group I was 41.42±4.68 – 59.02±3.41 mg/dl, and in group II was 41.37±4.58 – 59.99±5.77 mg/dl. The minimum HDL-cholesterol value was 23.85±1.05 mg/dl at 28th day of experiment in control group, 23.06±2.30 mg/dl at 14th day of the experiment in group I, and 22.82±0.74 mg/dl at 56th day of the experiment in group II. The maximum values of HDL-cholesterol were 26.54±1.14, 24.99±3.07, and 23.94±3.01 were recorded in the third day of the experiment in control, group I, and group II animals, respectively. The obvious effect of the (Biovet®)® on LDL-cholesterol levels were illustrated in (Table: 4). Beginning in the third day and till 84th day of the experiment, the level of LDL-cholesterol were decreased in probiotic-treated buffalo-calves comparing with that in control. The levels of LDL-cholesterol were significantly decreased (P ≤0.01) in Biovet-treated buffalo-calves at 7th, 56th, and 70th day of the experiment comparing with that in control group at the same time. Also, significant reduction (P≤0.05) in LDL-cholesterol levels in probiotics-treated buffalo-calves were recorded at 14th, 28th, and 42th day of the experiment comparing with that in control animals at the same period. It was notable that, in group II animals, the levels of LDL-cholesterol were decreased more than that of group I. All the results of lipid profile in the current study were within the normal physiological ranges mentioned by Benjamin (1984) and Kaneko *et al.* (1997). The obtained results of lipid profile in probiotics-treated buffalo-calves were in consistent with that reported by Zacconi, *et al.* (1992) and Taranto, *et al.* (1998) who attributed the low levels of cholesterol in probiotics-treated animals to the inhibition of cholesterol synthesis by direct assimilation. DeSmet, *et al.* (1994) recorded that the lactobacilli and bifidobacteria (the most common used probiotic microorganisms) had the ability to conjugate with bile acids enzymaticaly increasing their rate of excretion and lead to the reduction of serum cholesterol. In this context, Begley, *et al.* (2006) stated that hypocholesterol-emic effect and the reduction of serum lipids concentrations in probiotics treated patients attributed to the de-conjugation of bile by bile salts hydrolase and co-precipitation of cholesterol with the de-conjugated bile, the cholesterol is excreted via fecal route and prior to its secretion the de-conjugation of the bile, results in free bile salts, consequently they are less efficiently absorbed and thus excreted in large amounts in feces; this effect is additionally augmented by poor solubility of lipids by free bile salts, which limits their absorption in the gut leading to further reduction of serum lipid concentration. Our results are supported by the results of Mohan, *et al.* (1990), who recorded that hyperlipidemic patients treated with *lactobacillus sporogen* experienced a mean 32% reduction in total cholesterol and 35% reduction in LDL-cholesterol over three months treatment. Another mechanism had been implicated as a potential mechanism for cholesterol lowering effect of probiotics by Liong and Shah (2006), who recorded that serum cholesterol level was reduced via the alteration of lipid metabolism contributed by short chain fatty acids, this was supported by negative correlation between serum cholesterol levels and cecal propionic acid and positive correlation with fecal acetic acid concentrations.

Serum urea and creatinine levels in the animals of control, group I, and group II (Table 5) were fluctuating and within the normal physiological ranges recorded by (Benjamin, 1984). No significance differences were recorded among the animals groups along the period of the experiment. The obtained results were in agreement with that recorded by (Bohm and Srour, 1995; Sadeik and Bohm, 2001; Sayed, 2003).

The results of T_3 and T_4 are illustrated in Table (5), the serum concentrations of T_3 and T_4 were significantly increased (P ≤0.01 and P ≤0.05 according the time of sampling) in probiotic-treated buffalo-calves in comparing with that in control buffalo-calves. The minimum concentrations of T_3 in control, group I, and group II were 3.63±0.17, 4.27±0.24, and 4.45±0.29 ng/ml at the 3rd day of the experiment, respectively, while the maximum concentrations were 4.67±0.16, 5.15±0.18, and 6.46±0.28 ng/ml at 70th day of the experiment, respectively. The ranges of T_4 concentrations were 12.26±0.73 – 18.15±1.27 ug/dl, 12.37±0.86 – 19.33±1.24 ug/dl, and 12.20±0.94 – 21.75±1.25 ug/dl in control animals, group I, and group II, respectively. It is worth mentioning that the concentrations of T_3 and T_4 in probiotics-treated buffalo-calves were increased with increase the dose of Biovet (Table 5). The increase in the serum T_3 and T_4 in probiotic treated buffalo-calves can be referred to the probiotics (Biovet®) which minimized the stress on treated animals. As the reduction of stress effect on animals that will stimulate thyroid hormone release, moreover increase iodide uptake because of the reduction of glucocorticoids associated with stress will decrease urinary iodide excretion (Benjamin, 1984).

From Table (6), it was clear that the probiotic improves the body weight gain in probiotic-treated buffalo-calves. At 14th, 28th, and 42th day of the experiment, the body weights were insignificant increased in probiotics-treated animals comparing with that of control animals. At 56th day of the experiment, there was significant increased (P ≤0.05) in body weight gain in animals of group I and II comparing with that in control. At the 70, and 84th day of the experiment, the body weights of the animals in group I and II were significantly increased (P ≤0.01) comparing with that in control group. The improvement in body weight gain in Biovet®-treated buffalo-calves may be attributed to the improvement of the processes of digestion, absorption, and metabolism of the essential nutrients giving rise to the best feed utilization by treated animals (Sisson, 1989) or may be referred to improvement in rumen fermentation that increased degradability of forage and flow of microbial protein from the rumen (Wallace and Newbold, 1992).

In conclusion, this study referred to the probiotic (Biovet®) which has obvious effect on body weight gain in buffalo-calves after considerable time of administration (not less than two months) without any deleterious effect on animal health even when using it by high doses.

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تأثير استخدام البروبيوتك (بيوفيت®) علي الحالة الاكلينيكية والصورة الدموية والبيوكميائية في العجول الجاموسي

تم اجراء هذه الدراسة بغرض دراسة تاثير استخدام اشهر انواع الاحياء الدقيقة المستخدمة كبروبيوتك (بيوفيت®) علي الحالة الاكلينيكية والصورة الدموية والبيوكميائية في العجول الجاموسي النامية وكذلك دراسة تاثيرها على اوزان تلك الحيونات ، وقد اشتملت هذه الدراسة على عدد 25 عجل جاموسي تتراوح اعمارها بين 6 – 7 اشهر واوزانها بين 158 – 165 كجم باحد المزارع الخاصة في محافظة بني سويف. وقد قسمت هذه الحيوانات الى ثلاثة مجموعات : المجموعة الضابطة (5 حيوانات) والمجموعة الاولى (10 حيوانات) والمجموعة الثانية (10 حيوانات) . وقد تم تجريع الحيوانات في المجموعة الاولى و المجموعة الثانية جرعة بالفم يومية مقدارها (15) و (25) جم من (بيوفيت®) علي التوالي ، وقد اوضحت الدراسة عدم حدوث تغيرات معنوية على بعض قياسات صورة الدم (مثل تركيز الهيموجلوبين والنسبة المئوية لخلايا الدم المضغوطة وعدد خلايا الدم الحمراء والعد الكلي لخلايا الدم البيضاء) في الحيوانات التي تم تجريعها البروبيوتك بمقارنتها في المجموعة الضابطة كما انه لم تظهر اي اختلافات معنوية في نشاط انزيمي الاسبرتات امينوترانسفيراز والانالين امينوترانسفيراز ومستويات الالبيومين والجلوبيلين والبولينا والكرياتينين بينما حدثت زيادات معنوية في مستوى البروتين الكلي في حيوانات المجموعتين الاولي والثانية عند اليوم 28 و 42 و 56 و 70 و 84 من التجربة بمقارنتها بمثيلتها في المجموعة الضابطة. كما لوحظ ايضا ارتفاع معنوي في نشاط انزيم الفوسفوكينيز القلوي في حيوانات المجموعة الاولى والثانية بمقارنتها بالمجموعة الضابطة. من الجدير بالذكر ان مستويات الدهون الثلاثية والكلوسترول والليبوبروتين العالي الكثافة قد حدث بها انخفاض غير معنوي في الحيوانات المجموعة الاولى والثانية عن نظيرتها في المجموعة الضابطة في حين حدث انخفاض معنوي في مستوى الليبوبروتينات المنخفضة الكثافة في حيونات المجموعة الاولى والثانية عن نظيرتها في المجموعة الضابطة وعلي العكس من هذا وجد ارتفاع معنوي في تركيزات هرمونات الغدة الدرقية (T4،T3) في حيوانات المجموعة الاولي والثانية . وقد اظهرت الدراسة ايضا وجود زيادات في اوزان حيوانات المجموعة الاولى والثانية عن نظيرتها في المجموعة الضابطة. من هذه الدراسة يمكن ان نستنتج ان استخدام البروبيوتك (بيوفيت®) في العجول الجاموسي له تاثير محفز للنمو ويزيد من الوزن وليس له اية تاثيرات ضارة على الحالة الصحية لتلك الحيوانات.