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Probiotics supplementation effect on physical body measurement and haemato-biochemical parameters in buffalo calves reared under different housing systems

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Abstract
Present investigation was conducted on sixteen Murrah buffalo calves of either sex with an average age of 3±1 months. These calves were divided into four groups (T_1, T_2, T_3 and T_4) having four calves in each group based on their average weight and age. On-farm trial of 10 days preliminary feeding with housing and 90 days experimental period was conducted between January 2017 to April 2017, on Buffalo Farm, COAS, LUVAS, Hisar. There was no significant difference recorded in other growth parameters (i.e. Body height, Body length, Heart girth, abdominal girth and Pin width) in between any treatment group. Haemato-biochemical examination showed no significant difference was recorded in any of the haemato-biochemical parameters (i.e. Hb, TEC, DLC, Serum urea, Serum lactate dehydrogenase, Serum Albumin: Globulin, Serum Ca and Phosphorous).

Keywords: buffalo calves, probiotic supplementation, physical body parameters, haemato-biochemical changes

1. Introduction
India is the largest producer of milk in the World. Dairy animals in India are the most overlooked and under-managed assets of an individual or a farm. Affording good quality concentrate is becoming more and more difficult for a marginal farmer due to high cost. Straw are mainstay of diet for these animals. Feed intake, microbial production and digestibility on such diets needs improvement (Srinivas *et al.*, 2002) [1] and feed additives play a greater role in tropical countries like India (Hoffmann, 2009) [2].
FAO/WHO, 2002 define probiotics as "live organisms which when administered in adequate amounts confer a health benefit to the host." A proposed definition for probiotics is 'a preparation or a product containing viable, defined micro-organisms in sufficient numbers, which alter the micro-flora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host' (Schrezenmeir and de Vrese, 2001) [3]. In recent years, due to increased consumer's concern about safety, quality of animal products and environmental issues, the current purpose of using feed additives is not only to increase the productivity but also to lower the risk of ruminant digestive carriage of human pathogens and to decrease excretion of polluting outputs like nitrogen-based compounds and methane. Probiotics exhibit potential benefits by improving intestinal microbial balance (Fujiwara *et al.*, 2009) [4] and promoting intestinal digestion, and increasing animal growth performance (Jenny *et al.*, 1991) [5].
The present investigation was planned to study the effect of probiotics supplementation on physical body measurement and haemato-biochemical parameters in buffalo calves.

2. Materials and Methods
2.1 Location
Present investigation was conducted at Buffalo farm, Department of Livestock Production Management, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar.

2.2 Study design
For the study 16 Murrah buffalo calves of 3±1 month average age were selected in which nine were females and seven were male. These were grouped into four groups T_1, T_2, $T_3$and T_4. Each group had four calves based on completely randomized design (CRD) of their body weight. Each group had equal average body weight. All calves were identified by ear tags

containing calf number and farm number. All calves were dewormed before conducting trial. Following treatments were conducted on these groups:-

T_1: Conventional housing + feeding as per ICAR standards (2013)

T_2: Conventional housing + feeding as per ICAR standards (2013) + probiotic supplementation

T_3: Loose housing + feeding as per ICAR standards (2013)

T_4: Loose housing + feeding as per ICAR standards (2013) + probiotic supplementation.

2.3 Physical body measurement and haemato-biochemical parameters

Calves were taken to the separate barn where they were weighted one by one. After that other body parameters (body length, heart girth, paunch girth, body height at shoulder point and pin width) were recorded at the starting of the experiment then at monthly interval. Blood sampling was done two times during whole experiment (i.e. at the starting and the end). Blood sample were collected in 15ml vial using 18G needle from jugular vein puncture two times during the whole experiment, in the beginning and the last week of experiment. Three ml blood was separated immediately for blood tests into EDTA containing collection vial. Hb, TEC, DLC and TLC were counted using haemo-autoanalyzer. Blood serum was separated using centrifugal machine at 3000 rpm for 30 minutes. After that it was deep freeze at -10 degree Celsius. Serum test performed were: Total protein, Albumin, Globulin, Urea, Lactate dehydrogenase, Calcium, Phosphorous. Minerals were estimated in serum through Heat digestion method and Bio chemical analyzer.

2.4 Statistical analysis

Data was analyzed by Independent student't' test using SPSS computer software package to compare the changes in buffalo calves reared under different conditions.

3. Results and Discussion

All the physical measurements of all the groups were taken at one month interval.

3.1 Body Length

There was constant and similar increase in the avg. body length in all treatment groups (Table 1). The rate of increase in body length was higher in the later months of the experiment in all the groups. There was no significant difference obtained in between housing systems.

3.2 Body Height

There was constant and similar month wise increase in the avg. body height in all treatment groups (Table 2). Likewise body length, the rate of increase in body height was higher in the later months of the experiment in all the groups.

3.3 Heart Girth

There was constant and similar month wise increase in the avg. heart girth in all treatment groups (Table 3). The rate of increase in body length was higher in the later months of the experiment in all the groups as was obtained earlier for body length and height.

3.4 Abdominal Girth

There was constant and similar month wise increase in the abdominal girth in all treatment groups (Table 4). As for earlier traits also, the rate of increase in abdominal girth was higher in the later months of the experiment in all the groups that might be due to the development of rumen.

3.5 Hip Width

There was constant and similar month wise increase in the hip width in all treatment groups (Table 5). The rate of increase in hip width was higher in the later months of the experiment in all the groups. Gorgulu *et al.* (2003) [6] also determined that calves of the probiotic group have less health problem than control group. But there were no differences between control and probiotic group with respect to growing performance during pre-weaning period.

3.6 Hemoglobin (Hb)

There was not any significant change obtained. All values are within normal range (Table 6).

3.7 Red blood corpuscles (RBC)

There was non-significant increase observed in red blood cells (Table 6).

3.8 White blood corpuscles (WBC)

Non-significant increase in WBC was observed in the final blood samples in all groups (Table 6). However, lymphocytes count was significantly increased in final sample in T_1 and T_4. In elder buffalo calves (related with developed rumen) Lymphocytes concentration remains normally higher as compare to pre-weaned buffalo calves (related with under developed rumen). This shows there is no relation with probiotic feeding and Lymphocytes count increase.

3.9 Serum proteins

There is slight increase in total serum proteins. Slight increase in total serum proteins, decrease in albumin and increase in globulins is normal phenomenon in growing calves (Table 7).

3.10 Serum minerals concentration

There is non-significant decrease is observed in serum minerals Calcium and Phosphorous (Table 8).

3.11 Serum Urea concentration

It is also remains constant within normal range.

3.12 Lactate Dehydrogenase

LDH concentration is slightly increased in all the final serum samples but non-significant (Table 8).

Both the samples did not show any significant difference in Hb, RBC, WBC, Total Protein, Albumin: Globulin, Urea, Calcium and Phosphorous. Lymphocytes content was also improved in final sample in T_1 and T_4. This shows no relation with probiotic feeding. In developed buffalo calves (developed rumen) Lymphocytes concentration in normally high as compare to pre-weaned buffalo calves (under developed rumen). As slight alopecia was observed in all groups so this increase is could be due to parasitic load. The slight increase in total serum proteins, decrease in albumin and increase in globulins is normal phenomenon in growing calves (More, 2006) [7].

Any significant effect of housing was not recorded in any of these tests.

Table 1: Effect of probiotic supplementation on average monthly body length change

Average body length (cm)				
Groups	**Month 0**	**Month i**	**Month ii**	**Month iii**
T_1	71.75 ±3.30	76.50 ±3.69	81.00 ±3.65	88.50 ±5.92
T_2	68.25 ±6.44	72.75 ±6.2	78.75 ±4.57	83.50 ±6.45
T_3	66.25 ±0.96	73.00 ±2.16	80.25 ±5.44	90.00 ±5.48
T_4	68.75 ±4.11	73.00 ±3.37	77.00 ±4.32	84.25 ±3.77
C.D.	NS	NS	NS	NS

Table 2: Effect of probiotic supplementation on average monthly body height change

Average height (cm)				
Groups	**Month 0**	**Month I**	**Month II**	**Month III**
T_1	77.75 ±1.93	81.75 ±1.93	87.25 ±2.32	94.00 ±2.48
T_2	76.75 ±3.04	78.75 ±2.17	84.50 ±2.02	90.50 ±1.65
T_3	75.50 ±1.25	79.75 ±1.25	84.50 ±0.64	91.25 ±1.38
T_4	74.00 ±1.47	77.75 ±1.43	83.25 ±1.49	88.25 ±1.11
C.D.	NS	NS	NS	NS

Table 3: Effect of probiotic supplementation on average monthly heart girth change

Heart girth average (cm)				
Groups	**Month 0**	**Month i**	**Month ii**	**Month iii**
T_1	88.00 ±2.79	96.75 ±4.09	104.75 ±3.97	112.50 ±3.57
T_2	89.25 ±2.65	96.75 ±4.21	102.37 ±5.65	110.75 ±3.64
T_3	87.25 ±2.28	94.75 ±1.79	102.37 ±1.14	112.00 ±2.27
T_4	92.00 ±0.91	97.25 ±1.49	103.50 ±2.50	111.50 ±2.66
C.D.	NS	NS	NS	NS

Table 4: Effect of probiotic supplementation on average monthly abdominal girth change

Average abdominal girth (cm)				
Groups	**Month 0**	**Month I**	**Month II**	**Month III**
T_1	103.00 ±3.85	110.50 ±6.02	119.00 ±6.12	133.25 ±4.13
T_2	98.00 ±4.32	108.00 ±6.31	114.50 ±6.41	129.25 ±3.94
T_3	95.75 ±1.31	107.25 ±3.30	113.25 ±1.93	133.25 ±1.70
T_4	97.25 ±2.29	110.00 ±4.81	118.75 ±2.95	135.75 ±4.48
C.D.	NS	NS	NS	NS

Table 5: Effect of probiotic supplementation on average monthly pin width change

Average pin width (cm)				
Groups	**Month 0**	**Month I**	**Month II**	**Month III**
T_1	12.67 ±0.07	13.20 ±0.11	14.25 ±0.26	15.40 ±0.22
T_2	12.10 ±0.50	13.10 ±0.74	13.70 ±0.83	14.80 ±1.15
T_3	12.35 ±0.07	13.40 ±0.25	14.35 ±0.48	15.45 ±0.32
T_4	12.52 ±0.13	13.50 ±0.29	14.40 ±0.35	15.10 ±0.42
C.D.	NS	NS	NS	NS

Table 6: Effect of probiotic supplementation on hematological parameters

Tests	**Hb (g/dl)**		**RBC (m/mm^3)**		**WBC (m/mm^3)**		**Lymphocytes (%)**	
Treatments	Initial	final	Initial	final	Initial	final	Initial	final
T_1	11.76±0.64	11.33±0.44	66.1±4.09	54.07±6.32	17.43±2.00	35.12±10.96	54.07±6.32	78.40±5.61
T_2	11.30±0.64	10.83±0.28	61.1±3.37	50.97±4.33	13.82±1.37	25.82±9.03	50.97±4.33	51.70±11.24
T_3	11.25±0.52	12.25±0.72	66.0±3.34	55.32±2.60	15.65±2.47	19.28±4.09	55.32±2.60	57.07±2.09
T_4	12.03±0.49	10.55±0.50	72.0±3.45	55.60±2.76	12.92±0.41	28.19±9.65	55.60±2.76	87.40±4.34
C.D.	NS	NS	NS	NS	NS	NS	NS	20.97

Mean in column with different superscripts differ significantly (P<0.05)

Table 7: Effect of probiotic supplementation on serum biochemical parameters-I

Tests	**Total protein (g/dl)**		**Albumin (g/dl)**		**Globulin (g/dl)**		**Albumin: Globulin**	
Treatments	Initial	final	Initial	final	Initial	final	Initial	final
T_1	5.88±0.27	7.36±0.03	3.88±0.11	3.33±0.17	2.03±0.20	4.03±0.16	1.91	0.83
T_2	5.93±0.12	7.53±0.28	3.42±0.22	3.31±0.13	2.51±0.18	4.22±0.40	1.36	0.78
T_3	6.08±0.37	7.38±0.08	3.26±0.10	3.54±0.10	2.84±0.34	3.85±0.08	1.15	0.92
T_4	5.70±0.50	7.29±0.19	3.41±0.20	3.37±0.11	2.32±0.41	3.88±0.12	1.47	0.88
C.D.	NS	NS	NS	NS	NS	NS	NS	

Table 8: Effect of probiotic supplementation on serum biochemical parameters-II

Tests	**Calcium (mg/dl)**		**Phosphorous (mg/dl)**		**Urea (mg/dl)**		**LDH (U/L)**	
Treatments	Initial	Final	Initial	final	Initial	final	Initial	final
T_1	9.45±0.45	8.62±0.29	8.08±0.42	7.01±0.50	50.78±1.43	46.22±3.69	2,111.0±161.7	2,419.3±151.3
T_2	9.72±0.23	8.65±0.55	8.12±0.39	6.52±0.34	52.30±3.94	59.88±10.6	1,943.3±171.7	2,302.5±97.02
T_3	8.55±0.12	9.25±0.35	8.23±0.67	7.32±0.50	49.60±3.23	47.03±2.14	2,158.2±143.2	2,218.7±150.3
T_4	9.28±0.30	9.05±0.24	8.34±0.17	7.87±0.32	53.85±3.11	48.13±2.50	2,116.5±100.8	2,470.0±257.1
C.D.	NS	NS	NS	NS	NS	NS	NS	NS

4. Conclusion

There was no significant difference recorded in other growth parameters (i.e. Body height, Body length, Heart girth, Abdominal girth and Pin width) in between any treatment group. Similarly, no significant difference was recoded in any of the haemato-biochemical parameters (i.e. Hb, TEC, DLC, Serum urea, Serum lactate dehydrogenase, Serum Albumin: Globulin, Serum Ca and Phosphorous).

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