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CONTENTS

Effect of probiotics and growth stimulants on haematological status in Murrah buffalo

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ABSTRACT: The present study was undertaken to investigate the effect of probiotics and growth stimulants on the haematological status of Murrah buffalo. Eighteen Murrah buffalo were selected from LRC (Livestock Research Center) at Sardar Vallabhbhai Patel University of Agriculture & Technology Modipuram, Meerut and divided into three groups. Group T_0(control)was fed normal basal diet, group T_1 and T_2 was fed one and two boluses of probiotics and growth stimulants, respectively. The blood parameters (TEC, TLC, Haemoglobin, DLC, Packed cell volume, Albumin, Total protein, SGOT/ALT and SGPT/AST) were estimated. The supplementation of probiotics and growth stimulants had increase TEC, lymphocyte percent and PCV in group T_1 and T_2. The monocytes and neutrophils percent decreased in group T_1 and T_2. Plasma protein level increased by probiotics and growth stimulants supplementation whereas, albumin, ALT, AST and haematological variable were not affected. "Ecotas bolus" A combination of probiotics and growth stimulants improved haematology and blood biochemical variables effectively.

Key words: Albumin, haemoglobin, Murrah buffalo, total protein, SGOT/ALT

Amongst the livestock, buffaloes (*Bubalus bubalis*) play an important role in the socio-economic statuses of the rural population being a triple purpose animal providing milk, meat, and draft. They are reported to be better in converting poor quality roughage into superior quality meat and also have a five percent higher digestibility of crude fiber than high yielding cows (Sharma *et al.,* 2010) and are one of the most efficient utilizers of lignocellulosic feeds (Kamra, 2010). The world buffalo population is estimated to be approximately 177.24 million spread in some 42 countries of which 171 million (97%) are found in Asia, while approximately 5.38 million (3%) are found in the rest of the world. India has 108.7 million buffalo and they are approximately 55.7% of the total world buffalo population (B.A.H.S., 2016).

MATERIALS AND METHODS

Eighteen Murrah buffalo (female) calves between 12 to 18 months age groups were selected from LRC (Live-stock Research Center) Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. The Ecotasboluses which contained probiotics and growth stimulants were procured from Intas Pharmaceuticals Ltd. and stored at room temperature for further use. All animals were kept under a conventional housing system consisting of 18 normal pens of almost similar sizes.

The feeding of animals was adopted as per the standard feeding schedule. The ingredient composition of the concentrate mixture fed to all animals was the same. Clean and fresh drinking water was given freely to all animals twice a day at 10.00 am and 5.00 pm. Green roughages *viz.*, Maize (*Zea maize*), berseem (*Trifolium alexandrium*), and barley (*Hordeum vulgare*) were provided to the animals during the whole period. The animals were divided into three groups, group $1^{st}(T_0)$ was kept as control, and group $2^{nd}(T_1)$, $3^{rd}(T_2)$ treated with probiotics and growth stimulants (Table 1).

Blood samples were collected from the jugular vein at 30 days intervals *i.e.* day 0, 30, 60, 90 days in EDTA impregnated tube. These blood samples were

Table 1: Probiotics and growth stimulants/animal/day

Feed additive	Groups		
	T_0 (Control group)	T_1 (treatment group)	T_2 (treatment group)
Lactobacillus sporogenes	-	10×10^6 CFU	20×10^6 CFU
Saccharomyces cerevisiae	-	12.5×10^9 CFU	25×10^9 CFU
Aspergillus oryzae	-	10×10^6 CFU	20×10^6 CFU
Zinc sulphate	-	100 mg	200 mg
Cobalt sulphate	-	20 mg	40 mg
Copper sulphate	-	50 mg	100 mg
DL-Methionine	-	0.5 g	1 g
Fructo-oligosaccharide	-	125 mg	250 mg
Biotin	-	5 mg	10 mg

centrifuged at 3,000 rpm for 15 min at 35°c and plasma was collected into cryovials and stored at "20°C until analyzed. Collected plasma samples were analyzed for blood biochemicals using auto-analyzer (Mindray) calibrated according to the specification of span diagnostic kit (Span Diagnosis Ltd) for total protein, plasma albumin, SGOT/ALT, SGPT/AST. The blood smears for DLC were stained using Giemsa stain and Leishman stain, counting was done using the battlement method of counting. Percentage of each type of cell was calculated as (Number of cells/ total number) ×100.

Calculation

$$\text{Total protein concentration (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \text{ X } 6.5$$

- Concentration of standard = 6.5 g/dl

$$\textit{Plasma albumin (g/dl)} = \frac{\textit{Absorbance of Test}}{\textit{Absorbance of Standard}} \times 4$$

AST activity (IU/ml) =ΔAsb/minute× kinetic factor
ALT activity (IU/ml) = ΔAsb/minute × kinetic factor

Statistical Methodology

Statistical analysis was done as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Changes in Haemoglobin (g/dl) concentration of animals during feeding trail are presented in Table 2. Probiotics and growth stimulants supplementation have no significant ($p>0.05$) improvement on haemoglobin in calves. The mean Hb concentration was 11.39, 11.68 and 11.76 for T_0, T_1 and T_3 groups, respectively.

However, Hb concentration improved linearly with progress of feeding period in T_1 and T_2 animals. The improvement was greater in T_2 than T_1 animals.

Table 2: Haemoglobin (g/dl) of Murrah buffalo at different feeding interval of 30 days

Days	Treatments		
	T_0	T_1	T_2
0	11.45±0.57	11.50±0.23	11.48±0.21
30	11.31±0.56	11.56±0.30	11.63±0.18
60	11.33±0.52	11.77±0.24	11.73±0.22
90	11.48±0.66	11.90±0.39	12.20±0.21
Mean±SE	11.39±0.58[a]	11.68±0.29[a]	11.76±0.20[a]

Present findings are in agreement with the previous reports, where Rajikkannu *et al.* (2015) reported that the *Bacillus pumilus* treated group had maximum hemoglobin concentration, similar- findings were reported by Ghazanfar*et al.* (2015) and Otzule and Ilgaza (2015). However, Dutta *et al.,* (2006) and Prasanna Kumar *et al.* (2015) reported no significant difference between treatment groups and the period of the experiment.

Effect of probiotics and growth stimulants on packed cell volume (%)

Packed cell volume was not significantly ($p<0.05$) influenced by feeding probiotics and growth stimulants. Higher PCV (%) were in the T_0 group than T_1 and T_2. Maximum PCV (33.47 %) was recorded in the Murrah buffalo in the T_0 control group and minimum (32.71 %) in the T_1 group.

Table 3: Packed cell volume (%) of Murrah buffalo at different feeding interval of 30 days

Days	Treatments		
	T_0	T_1	T_2
0	33.22±1.05	32.61±0.22	32.84±0.36
30	33.54±1.09	32.57±0.26	32.90±0.25
60	33.64±1.04	32.49±0.43	32.90±0.27
90	33.48±1.06	32.99±0.25	33.02±0.27
Mean+SE	33.47±1.06[a]	32.71±0.29[a]	32.91±0.29[a]

The value of PCV in all three groups lies within the normal range of variations. The observations of the present study in agreement with reports of Dutta *et al*. (2006), Prasanna Kumar *et al*. (2015), and Adjei-Fremah *et al*. (2018). However, Otzule and Ilgaza (2015) and Hussain Dar *et al.* (2017) report improved packed cell volume in crossbred calves by probiotics supplemention.

Effect of probiotics and growth stimulants on Total Erythrocytes Count or Red Blood cell (10^6/µl)

Probiotics and growth stimulants supplementation did not affect RBC in experimental animals. The levels of RBC were similar among the three groups, which varied from 6.93 to 7.30 (106/µl) in T0, 6.79 to 7.44 (106/µl) in $T_1$and 6.64 to 7.61 (106/µl) in the T_2 group.

Table 4: Total Erythrocytes Count (RBC 10^6/µl) of Murrah buffalo at different feeding interval of 30 days

Days	Treatments		
	T_0	T_1	T_2
0	6.76±0.11	6.85±0.12	6.64±0.19
30	7.02±0.17	6.79±0.14	6.88±0.21
60	6.93±0.21	7.10±0.13	7.28±0.17
90	7.30±0.29	7.44±0.14	7.61±0.20
Mean+SE	7.00±0.19[a]	7.04±0.13[a]	7.10±0.20[a]

Although mean RBC levels were not significantly different among the three groups, however a linear improvement in RBC was observed in T_1 and T_2 over an increased period of the feeding of probiotics and growth stimulants (Table 4).

The results in the present study are in agreement with those of Chen *et al*. (2005), Rajikkannu *et al*. (2015), and Ghazanfar *et al*. (2015) who reported increased RBC concentration whereas Dutta *et al.* (2006) report no effect on RBC with the probiotic and growth supplementation.

Effect of probiotics and growth stimulants on total leucocytes count or White Blood Cell (10^3/ ml)

Table 5 reveals that the total leucocytes count (WBC) of Murrah buffalo. There was a non-significant variation in WBC among different groups and no specific trend was observed. The highest value 7.92 (10^3/ml) of WBC observed in the T_0 (control) group and the lowest value 7.72 (10^3/ml) of WBC was observed in the T_2 group.

Although mean WBC levels were not significantly different among the three groups, however a linear improvement in WBC was observed T_1 and T_2 over an increased period of the feeding of probiotics and growth stimulants (Table 4).

Table 5: Total leucocytes count (WBC 10^3/ml) of Murrah buffalo at different feeding interval of 30 days

Days	Treatments		
	T_0	T_1	T_2
0	7.88±0.32	7.75±0.40	7.61±0.21
30	7.85±0.29	7.93±0.27	7.58±0.20
60	8.01±0.29	7.96±0.32	7.78±0.15
90	7.95±0.25	7.90±0.26	7.93±0.26
Mean+SE	7.92±0.29[a]	7.88±0.30[a]	7.72±0.21[a]

The findings in the present study are in agreement with the previous reports of Dutta, *et al*. (2006), Prasanna Kumar *et al*. (2015), Dabiri *et al*. (2016), and Adjei–Fremah *et al*. (2018). There were no significant differences among all groups in mean neutrophils value.

Table 6: Neutrophils (%) of Murrah buffalo at different feeding interval of 30 days

Days	Treatments		
	T_0	T_1	T_2
0	32.16±0.39	32.25±0.27	32.21±0.26
30	32.10±0.31	32.15±0.25	32.01±0.23
60	31.89±0.18	31.92±0.18	31.83±0.22
90	31.84±0.20	31.66±0.26	31.59±0.16
Mean±SE	31.99±0.27[a]	31.99±0.24[a]	31.91±0.22[a]

The findings in the present study are in agreement with the previous report of Hussain Dar *et al.* (2017) who reported no difference in neutrophil on 90th days of supplementation in crossbred calves. But Kalandakanond *et al.* (2008) reported that the probiotic groups showed significant ($p<0.05$) increases in the number of heterophil in supplementation of probiotics.

Effect of probiotics and growth stimulants on Lymphocyte

The lymphocytes did not significantly ($p<0.05$) increase by feeding probiotics and growth stimulants. Table 7 shows that the mean lymphocytes were higher in the T_2 (60.02 %) group and lower in the T_0 (59.87 %) group. In the T_1 group, the lymphocytes were 59.91.

Table 7: Lymphocytes (%) of Murrah buffalo at different feeding interval of 30 days

Days	Treatments		
	T_0	T_1	T_2
0	59.64±0.14	59.54±0.24	59.55±0.25
30	59.75±0.34	59.74±0.25	59.88±0.23
60	60.00±0.18	60.02±0.18	60.15±0.22
90	60.09±0.20	60.34±0.25	60.50±0.17
Mean±SE	59.87±0.28^a	59.91±0.23^a	60.02±0.22^a

The findings in the present study are contrary to the previous report of Hussain Dar *et al.* (2017) who reported a significant increase in lymphocytes due to supplementation of probiotics on 90th days in crossbred calves. But Kalandakanond *et al.* (2008) reported that the probiotic groups showed significant decreases in the number of lymphocytes.

Table 8: Monocytes (%) of Murrah buffalo at different feeding interval of 30days

Days	Treatments		
	T_0	T_1	T_2
0	8.19±0.06	8.20±0.08	8.23±0.06
30	8.14±0.07	8.10±0.07	8.09±0.04
60	8.09±0.06	8.05±0.06	8.01±0.03
90	8.06±0.05	7.99±0.05	7.90±0.02
Mean±SE	8.12±0.06^a	8.08±0.07^a	8.05±0.04^a

Effect of probiotics and growth stimulants on Monocytes

Table 8 reveals that the monocytesnon-significantly ($p<0.05$) decreased by feeding probiotics and growth stimulants. The values of the monocytes in all groups were within the normal range of the species. The mean monocytes were higher in group T_0 (8.12 %) and lower in group T_2 (8.05 %).

The findings in the present study are contrary to the previous report of Hussain Dar *et al.* (2017) who reported a decrease in monocyte due to supplementation of probiotics on 90th days of supplementation in crossbred calves. However, Kalandakanond *et al.* (2008) reported that the probiotic groups showed significant ($p<0.05$) increase in the number of monocytes.

Effect of probiotics and growth stimulants on total protein (g/litre)

Total protein was significantly ($p<0.05$) influenced by feeding probiotics and growth stimulants in experimental animals (Table 9). Higher total protein (g/litre) levels were observed in the T_2 group under probiotics than the T_0 groupwhereas total protein levels were similar in T_2 and T_1 groups. Maximum total protein (70.96 g/litre) was in the T_2 group fed with growth stimulants and minimum (69.49 g/litre) in the T_0 group (control).

Table 9: Total Protein (g/litre) of murrah buffalo at different feeding interval of 30 Days

Days	Treatments		
	T_0	T_1	T_2
0	69.25±0.38	69.41±0.39	69.38±0.43
30	69.34±0.42	70.48±0.38	70.52±0.38
60	69.61±0.35	71.24±0.29	71.40±0.39
90	69.76±0.28	72.28±0.22	72.56±0.43
Mean±SE	69.49±0.36^b	70.85±0.32^a	70.96±0.41^a

Data presented in the Table 9 reveals that the total protein (g/litre) level of Murrah buffalo increased on all days of the feeding trial. The findings of the present study are in agreement with the report of Bakr *et al.* (2015) who also reported the higher blood total protein level in the cattle supplemented with probiotics. Fagari-Nobijari *et al*. (2012) also reported higher plasma total protein ($p<0.01$) in animals fed $ZnSO_4$.

Effect of probiotics and growth stimulants on albumin

The albumin was not significantly ($p<0.05$) influenced by feeding probiotics and growth stimulants. While comparing the overall effect of probiotics and growth stimulants, higher values of albumin (g/litre) were observed in the Murrah buffalo in the T_2 group under probiotics and growth stimulants feeding than Murrah buffalo under the normal feeding T_0 group. Maximum albumin (29.79 g/litre) was recorded in the T_2 group and the minimum (29.06 g/litre) in the T_0 group.

Table 10: Albumin (g/litre) of Murrah buffalo at different feeding interval of 30 Days

Days	Treatments		
	T_0	T_1	T_2
0	28.62±0.23	28.73±0.40	28.79±0.45
30	29.03±0.18	29.49±0.42	29.50±0.35
60	29.12±0.15	29.91±0.44	30.06±0.36
90	29.47±0.28	30.43±0.37	30.82±0.33
Mean+SE	29.06±0.21[a]	29.64±0.41[a]	29.79±0.37[a]

The findings in the present study are in agreement with the study of Fagari-Nobijari *et al*. (2012) and Pourakbari *et al*. (2015) who reported linearly increased albumin content with probiotic supplementation. Whereas Bakr *et al*. (2015) reported lower serum albumins in the yeast-treated group.

Effect of probiotics and growth stimulants on SGPT/ALT

The SGPT level non-significantly ($p<0.05$) increase by feeding probiotics and growth stimulants. Higher values of SGPT (IU/L) were observed in the T_0 group than T_1 and T_2 groups. Maximum SGPT (48.96 IU/L) recorded in the Murrah buffalo in the T_0 control group and minimum (48.39 IU/L) in the T_2 group.

Table 11: SGPT (IU/L) of Murrah buffalo at different feeding interval of 30 days

Days	Treatments		
	T_0	T_1	T_2
0	48.04±0.37	47.93±0.37	48.02±0.42
30	48.53±0.52	48.78±0.58	48.66±0.45
60	49.40±0.33	49.39±0.56	48.41±0.52
90	49.88±0.28	49.54±0.28	48.46±0.50
Mean+SE	48.96±0.37[a]	48.94±0.45[a]	48.39±0.47[a]

The findings in the present study are contrary to the previous work of Hatab *et al.* (2016) who reported that the serum ALT activities were significantly lower in probiotics groups as compared to control group.

Effect of probiotics and growth stimulants on SGOT/AST

The SGOT linearly ($p<0.05$) increased in all groups. While comparing the overall effect of feeding type, higher values of SGOT (IU/L) were observed in the T_0 group than T_1 and T_2 groups. Maximum SGOT (131.02 IU/L) were recorded in the T_2 group and minimum (129.15 IU/L) in the T_0 group. In T_1 group the average SGOT was recorded 130.03 (IU/L).

Table 12: SGOT (IU/L) of Murrah buffalo at different feeding interval of 30 days

Days	Treatments		
	T_0	T_1	T_2
0	128.09±0.65	128.12±0.76	128.51±0.76
30	128.79±0.70	129.22±0.56	130.56±0.55
60	129.77±0.80	130.72±0.63	132.36±0.32
90	129.55±0.94	132.06±0.44	132.64±0.56
Mean±SE	129.15±0.77[b]	130.03±0.59[ab]	131.02±0.55[a]

The findings in the present study are in agreement to the previous work of Azizzadeh *et al.* (2005) and Abu El-Ella *et al.* (2014) who reported that the Biogen-Zinc supplementation significantly ($P<0.05$) increased AST.

CONCLUSION

On the basis of the above findings, it may be concluded that the supplementation of probiotics and growth stimulants had a significant effect on Plasma protein level which was increased by probiotics and growth stimulants supplementation whereas, albumin, ALT, AST, and haemologicals variable were not affected. Therefore, it is concluded the preparation containing a combination of probiotics and growth stimulants increases the Plasma protein level.

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