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Effect of Enzyme Supplementation on Nutrient Utilization and Production Performance in Lactating Murrah Buffaloes

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ABSTRACT

Background: Enzyme stimulates metabolic and digestive processes leading to increased feed intake and improved nutrient availability in animals. This study was conducted to investigate the effect of enzyme supplementation on nutrient utilization and production performance in lactating Murrah Buffaloes during winter season.

Methods: Twenty four lactating Murrah buffaloes were selected for the study from Livestock Research Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut (UP). All the selected animals were randomly allocated into four treatment groups having six animals in each group on the basis of body weight, lactation length and milk yield. The daily nutrient requirements of lactating Murrah buffaloes in term of DM, DCP and TDN were provided as per the feeding standards of Indian Council of Agricultural Research, New Delhi (2013). Total Mixed Ration for buffaloes was prepared by mixing of roughage and concentrates in the ratio of 60:40 after grinding/chaffing. Roughage part was consisting of available winter season green fodder *e.g.* oat and wheat straw available at research centre. Feeding trial was planned into four treatment groups *viz.* T_1 (Basal diet without enzyme supplementation), T_2 (Basal diet with supplementation of Xylanase @ 800000 IU), T_3 (Basal diet with supplementation of Cellulase @ 240000 IU) and T_4 (Basal diet with supplementation of Xylanase @ 800000 IU and Cellulase @ 240000 IU).

Result: From the present study, it was observed that the average milk yield (5.34 litre/day) was higher in T_4 as compared to other treatment groups, but the difference was non-significant. The average milk fat percent was recorded as 7.52, 7.58. 7.60 and 7.65 in T_1, T_2, T_3 and T_4 treatment groups, respectively. Although the results of T_4 group animals were comparatively higher in all major milk constituents the differences were found to be statistically non-significant. The average DM intake (kg/d, kg/100 kg BW and g/kg $W^{0.75}$) and CP intake (g/d, g/100 kg BW) were almost similar in all treatment groups. The TDN intake (kg/d, kg/100 kg BW and g/kg $W^{0.75}$) was significantly ($P<0.05$) higher in enzyme supplemented groups. The digestibility coefficient of CP, CF, NDF and ADF were higher ($P<0.05$) in buffaloes of T_4 over control (T_1) group. So, it was concluded from the above study that the milk production and milk composition could be improved with the combine use of Xylanase and Cellulase enzyme in murrah buffaloes due to improved CP and fibre digestibility.

Key words: Buffaloes, Cellulase supplementation, Milk production, Murrah, Winter, Xylanase supplementation.

INTRODUCTION

Animal husbandry is an important component of India's agricultural production system. India possesses the world's highest population of bovines (302.79 million). According to the 20th Livestock Census, buffaloes contribute 20.47% of the total bovine population in the country. According to the census, the overall number of buffaloes in India is 109.85 million, with Uttar Pradesh state alone accounting for 33.0 million (BAHS, 2019). According to a breed survey report, the overall number of Murrah buffaloes in India was 47 million, accounting for 42.8 percent of the entire buffalo population. Out of this, Uttar Pradesh had 17.3 million Murrah buffaloes (Breed Report, 2022). The frequent changes of climatic conditions in the region affect the production and reproductive abilities of dairy animals, including Murrah buffaloes.

The persistent difficulty of low productivity in Indian dairy cows has long been a concern for breeders and scientists in the country, especially when compared to other nations. Achieving the Genetic production potential of an animal greatly depends upon its environment including its nutritional management. Enzymes are biological catalysts

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that enhance the rate of chemical processes and improve metabolism in animals. It can increase the digestibility of feed and fodders. This process involves the enzymatic hydrolysis of complex carbohydrates and proteins included in the feed, resulting into the formation of smaller molecules that are more readily digestible within the gastrointestinal tract of animals. The advantages of orally supplementing enzymes include reducing particle size, enhancing digestion and the rate at which ingested feed passes through the rumen, decreasing the viscosity of consumed feed material, increasing absorption and improving feed efficiency while reducing production costs. The application of Exogenous Fibrolytic Enzymes (EFE) to improve the digestibility of fibrous forage is close to providing tangible advantages to ruminant production systems. Cellulase and Xylanase are two prominent classes of enzymes responsible for breaking ß1-4 links that connect sugar molecules in Cellulase. Xylanase is specifically present in plant cell wall components (Beauchemin *et al*., 2003). Enzyme preparations with specific activity are recognised for their capacity to stimulate targeted metabolic and digestive processes in the gastrointestinal system. This can enhance the natural digestive processes, leading to improved nutrient availability and increased feed intake in animals. This study aimed to examine the effect of enzyme supplementation on nutrient utilization and production performance in lactating Murrah buffaloes during winter season.

MATERIALS AND METHODS

The materials and methods followed for the above study have been described as under.

Approval from animal ethics committee

All of the procedures carried out and animal welfare were reviewed and approved by the Institutional Animal Ethics Committee of the Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, India (IAEC/SVPUAT/2022/127).

Location of the study

This experiment was conducted at Livestock Research Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut (U.P.). Geographically Meerut is situated between 29°01″N latitude in the north and 77°45″E longitudes in the East. The climate of this region is sub-tropical with maximum temperature generally ranging from 37-42°C during summer season (April to September) and minimum temperature of about 5-15°C during winter season (November to March). The monsoon generally begins during the first week of July and ceases by the end of September. The average annual rain fall in this region is about 862.7 mm and the annual relative humidity varies from 67 to 83 per cent.

Source of experimental animals

Twenty-four lactating Murrah buffaloes were selected for the research trail from Livestock Research Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut (U.P.).

Animal selection and experimental design

All the selected animals were randomly allocated into four treatment groups having six animals in each group on the basis of body weight, lactation length and milk yield. The daily nutrient requirements of lactating Murrah buffaloes in term of DM, DCP and TDN were provided as per the feeding standards of Indian Council of Agricultural Research, New Delhi. Total Mixed Ration for buffaloes was prepared by mixing roughage and concentrates in the ratio of 60:40 after grinding/chaffing. The roughage part consisted of available winter season green fodder and wheat straw available at the research center. Feeding trial was planned into four treatment groups *viz*. T_1 (Basal diet without enzyme supplementation), T_2 (Basal diet with supplementation of Xylanase @ 800000 IU), T_3 (Basal diet with supplementation of Cellulase @ 240000 IU) and T_4 (Basal diet with supplementation of Xylanase @ 800000 IU and Cellulase @ 240000 IU.

Management of animals

All the lactating Murrah buffaloes under study were maintained in uniform housing and management condition with well-ventilated covered sheds which was well equipped with cemented floor and brick- lined/kachha floor in open paddocks at Livestock Research Centre of the university. During milking time even during morning or evening the buffaloes of all four treatments groups were tied individually. The animal sheds were washed twice daily and thoroughly cleaned to remove faeces and dirt. All the animals were checked for any ailment before enrolment in the experiment. During the experimental period routine health and other management of the farm was followed.

A commercially preprepared enzyme in powder form was procured from the market *i.e*. Xylanase having 400000 IU/g and Cellulase having 100000 IU/g activity. Enzyme was supplemented to each treatment groups individually at the time of feeding by preparing a premix with concentrate with required quantity of enzyme. Animals were fed two times daily at 9:00 and 16:00 h. Clean and fresh drinking water was available for 24 hrs in the shed.

Sample analysis

Feeding materials *e.g*. concentrate ingredients, wheat straw, green fodders, left over feed and faecal matter were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF) and total ash following standard procedure (AOAC, 2005). Fibre fraction of feed, fodder and faecal samples were analysed by using detergent method of fibre estimation (Van Soest *et al*., 1991). Chemical composition (on % DM basis) of feed ingredients used during the trial in winter season is given in Table 1. Buffaloes were milked manually and milk yield of buffaloes was individually recorded. Milk samples were collected from each buffalo and pooled in proportion

to the milk yield of individual buffalo and analyzed for its composition through milk analyzer (Lasctoscan). Monthly average temperature (°C) and humidity (%) in the animal shed were recorded and related data is presented in Table 2. The temperature humidity index (THI) was calculated through equation given by Kendall and Webster (2009).

$$THI = (1.8\ AT + 32) - [(0.55\text{-}0.0055\ RH) \times (1.8\ AT\text{-}26)]$$

Statistical analysis

Statistical package for the social sciences (S.P.S.S., 2017) was used to analyse the data using a perfectly random design and the simple analysis of variance method (Snedecor and Cochran, 1994). The multiple range test described by Duncan was used to differentiate homogenous groupings (1955), when $P<0.05$, differences between treatments were considered significant.

RESULTS AND DISCUSSION

The results of the different experimental groups have been presented and discussed under the following subheads.

Body weight and nutrient intake

Effect of enzyme supplementation on body weight and nutrients intake on lactating Murrah buffaloes during winter season has been presented in Table 3. Body weight (BW) and metabolic body weight remained similar ($P>0.05$) in different treatment groups. Average body weight was 547.88, 551.00, 552.76 and 554.45 kg in T_1, T_2, T_3 and T_4

Table 1: Chemical composition (on % DM basis) of feed ingredients used in the experiment.

Items	Oats fodder	Wheat straw	Concentrate	Total mixed ration
Dry matter	19.35	87.88	88.68	68.12
Organic matter	87.41	88.74	89.50	88.89
Total ash	12.20	11.28	10.44	11.14
Crude protein	9.45	3.02	21.02	13.90
Ether extract	4.60	0.49	3.99	3.49
Crude fibre	21.81	37.61	6.84	17.59
Nitrogen free extract	51.94	47.60	57.71	53.88
Neutral detergent fibre	58.64	83.65	33.09	50.86
Acid detergent fibre	50.50	54.16	17.42	34.71
Hemicellulose	8.14	29.49	15.67	16.15
Acid detergent lignin	2.38	3.81	1.32	2.15
Cellulose	48.12	50.35	16.10	32.56

Table 2: Monthly temperatures (mean, max. and min. in °C), mean relative humidity (RH %) and mean temperature humidity index (THI) during the experiment.

Month	Temperature (°C)			Mean	Mean
	Avg.	Max.	Min.	RH%	THI
December	20.56	27.00	14.34	40.93	65.39
January	17.78	23.18	12.37	57.09	62.74
February	23.09	31.29	16.51	45.40	69.84
Overall	20.57	26.93	14.22	48.31	65.78

Table 3: Effect of enzyme supplementation on body weight and nutrient intake in lactating murrah buffaloes.

Parameters	Treatment groups				SEM	P value
	T_1	T_2	T_3	T_4		
Body weight (kg)	547.88	551.00	552.76	554.45	6.894	0.918
Metabolic body weight (kg)	113.23	113.71	113.98	114.25	1.069	0.918
Dry matter intake, kg/day	16.15	16.36	16.32	16.34	0.224	0.903
Dry matter intake, kg/100 kg BW	2.93	2.95	2.95	2.95	0.008	0.103
Dry matter intake (g/kg $W^{0.75}$)	142.61	143.85	143.19	143.01	0.696	0.654
Crude protein intake, g/day	1446.69^a	1475.22^b	1479.66^b	1486.77^b	6.665	0.002
Crude protein intake g/100 kg BW	253.72^a	261.78^{ab}	268.02^b	273.00^b	3.817	0.012
Total digestible nutrient intake, kg/day	7.43^a	7.83^b	7.92^b	7.97^b	0.081	<0.001
Total digestible nutrient intake/100 kg BW	1.24^a	1.37^b	1.39^b	1.41^b	0.018	<0.001
Total digestible nutrient intake, g/kg $W^{0.75}$	65.60^a	68.95^b	69.85^b	70.20^b	1.044	0.021

Means bearing different superscript in a row differ significantly ($P<0.05$).

groups, respectively. Similar findings in buffaloes (Shekhar *et al*., 2010) and in cattle (Kung *et al*., 2000; Knowlton *et al*., 2007; Lopuszanka and Bilik 2011; Barbadikar, 2012; Dean *et al*., 2013) were also reported by earlier researchers. Yang *et al.* (2000) also reported in cows fed the control diet, enzyme-treated TMR, or enzyme-treated barley-based concentrate had no effect on body weight changes. Similarly, Knowlton *et al.* (2002) observed body weight in early lactating cows that were administered diets supplemented with enzymes. Shojaeian and Thakur (2007) found that addition of enzymes to the urea-treated wheat straw-based TMR fed to dairy cows had no effect on body weight changes. However, because the buffaloes used in the present investigation were in both early and mid-lactation, no significant effect on the change in body weight was observed. Similar findings were also reported by several other workers (Rode *et al*., 1999; Beauchemin *et al*., 2000 and Titi, 2003). The overall average DMI in four treatment groups was 16.15, 16.36, 16.32 and 16.34 kg/d in T_1, T_2, T_3 and T_4 groups, respectively. There was no significant difference in DM intake between the control and the three treatment groups throughout the experimental period. The average DM intake (kg/100 kg body weight) was 2.93, 2.95, 2.95 and 2.95 kg for the T_1, T_2, T_3 and T_4 groups, respectively, which was almost similar and had no significant difference statistically. The difference in dry matter intake among treatment groups was found non-significant and almost similar in all four groups. Those results were similar with some other findings in cattle (Mohamed *et al*., 2013; Dean *et al*., 2013; Dunda, 2015; El-Bordeny *et al*., 2015). In case of buffalo, Shekhar *et al.* (2010) did not find increase in DMI while the Gaafar *et al.* (2010) found 4% higher DMI in enzyme supplemented group of buffaloes. The cumulative average of CP intake g/day and g/100 kg body weight due to the supplementation of the enzyme xylanase and cellulase in four treatment groups was 1446.69, 1475.22, 1479.66 and 1486.77; 253.72, 261.78, 268.02 and 273.00, respectively. The CP intake g/day and g/100 kg was found to be significantly higher in T_3 and T_4 group in buffaloes. The results were in agreement with Romero *et al.* (2016) who reported that supplementation of Xylanase plus @ 1 mL/kg DM of TMR (T1) significantly ($P<0.001$) increased the DCP intake (kg/d) in Holstein cows. Average total TDN intake was 7.43, 7.83, 7.92 and 7.97 kg/day in control (T_1), T_2, T_3 and T_4 groups, respectively. Average TDN intake, kg/100 kg BW was 1.24, 1.37, 1.39 and 1.41 in control (T_1), T_2, T_3 and T_4 groups, respectively. The average TDN intake g/kg $W^{0.75}$ were 65.60, 68.95, 69.85 and 70.20 in control (T_1), T_2, T_3 and T_4 groups, respectively. The Average total TDN intake, kg/100 kg BW and g/kg $W^{0.75}$ was significantly higher ($P<0.05$) in treatment groups supplemented with xylanase and cellulase in lactating Murrah buffaloes. The above findings provided additional support to the findings of similar reports indicating that the average TDN consumption in the enzyme supplemented group of Sahiwal cows, who were fed urea treated wheat straw, was considerably higher ($P<0.01$) as compared to the control group (Shojaeian and Thakur, 2007). Shekhar *et al.* (2010) showed that the intake of total digestible nutrients (kg per day) was considerably greater in the group that received enzyme supplementation.

Nutrient utilization and digestibility coefficient

The data related to nutrient utilization and digestibility coefficient in lactating Murrah buffaloes among different treatment groups of buffaloes during winter season is presented in Table 4. Dairy animal's daily intake of digestible nutrients, which is a function of both intake and digestibility, determines how well they perform. Forage digestibility was correlated with NDF digestibility and content (Mertens, 2009). Average body weights were 557.62, 560.23, 563.39 and 567.17 kg in control (T_1), T_2, T_3 and T_4 groups, respectively which did not differ significantly among the different treatment groups. Similar trend was observed in case of metabolic body weight ($W^{0.75}$) which was 114.75, 115.15, 115.63 and 116.21 in control (T_1), T_2, T_3 and T_4 groups, respectively. Total DMI as kg/d and kg/100 kg bwt were 14.75 and 2.63 in control group (T_1), 14.80 and 2.69 in T_2 group, 15.08 and 2.68 in T_3 and 15.51 and 2.82 in T_4 group. The CP intake as kg/d and g/100 kg body weight was 1.46 and 255.25 in control group (T_1), 1.50 and 260.03 in T_2 group, 1.59 and 266.21 in T_3 and, 1.68 and 269.63 in T_4 group, respectively. The CP intake of the different enzyme supplemented groups differed significantly ($P<0.05$). Average TDN intakes expressed as kg/d, kg/100 kg body weight and g/kg $W^{0.75}$ were 7.54, 1.07, 65.73 in T_1 group; 7.78, 1.24, 67.56 in T_2 group; 7.88, 1.22, 67.19 in T_3 group and 7.90, 1.28 and 68.16 in T_4 group, respectively. No significant difference in TDN intake between the treatment groups due to enzyme supplementation was found during winter season. The CF, NDF and ADF digestibility improved significantly ($P<0.05$) in enzyme supplemented buffaloes during the winter season. Knowlton *et al.* (2002); Bowman *et al*. (2002) and Gado *et al.* (2009) reported similar results. Titi and Tabbaa (2004) observed that supplementation of fibrolytic enzymes significantly ($P<0.05$) increased NDF digestibility. Gaffar *et al.* (2010) reported that lactating buffaloes supplemented with cellulase and xylanase showed a significant ($P<0.05$) increase in crude fibre digestibility (CF). The results of present study were in agreement with the report of Arriola *et al.* (2011) who found that lactating cows with added fibrolytic enzyme at varying levels of concentrate in diet and groups with higher concentrate proportions and enzyme supplementation had greater digestibility compared to the groups without supplementation. El-Bordeny *et al.* (2017) added enzymes to Barkey lambs' diet which resulted in a notable enhancement in the digestibility of many components such as DM, OM, CP, CF, NFE, NDF, ADF, Cellulase and hemicellulose. According to Shekhar *et al.* (2010), there was a significant ($P<0.05$) increase in the digestion of CF, NDF and ADF. The result showed that the activity of the

rumen bacteria responsible for breaking down fibrous materials might have been enhanced due to the combined action of externally added fibrolytic enzymes and the enzymes produced by the rumen microorganisms themselves. Azam *et al.* (2017) found that increasing the amounts of enzyme supplementation in the diets of lactating Nili Ravi buffaloes considerably improved the digestibility of crude protein and NDF.

Milk yield and composition

The effects of enzyme supplementation on yield and composition of milk in lactating Murrah buffaloes during winter season have been summarized in Table 5. The average milk yield (kg/d) was 4.39, 5.02, 5.11 and 5.34 in T_1, T_2, T_3 and T_4 groups, respectively. The average milk yield (5.34 litre/day) was higher in T_4 as compared to other treatment groups. Similarly, average milk yield was numerically higher in the enzyme supplemented groups as compared to control group. The analysis of variance revealed that there was non-significant difference in milk yield. Similar results were reported by Elwakeel *et al.* (2007) who revealed that effect of enzyme supplementation was non-significant on milk yield. Shojaeian and Thakur (2007) reported in Sahiwal cows fed urea treated wheat straw-based ration supplemented with xylanase and cellulase at 1.5 g/kg DM produced 9.98 per cent more milk. Arriola *et al.* (2011) investigated the impact of fibrolytic enzyme and found that enzyme supplementation increased milk yield at different concentrate amount, but the effect was statistically non-significant between groups. In winter season, it was found that effects among treatment groups on the milk composition of lactating Murrah buffaloes were statistically non-significant and the findings of the present study were in line with the results of Vicini *et al.* (2003); Shojaeian and Thakur (2007) who also reported that the addition of enzyme supplements to dairy cows diets did not have any effect onthe percentage of milk fat that was produced. According to Miller *et al.* (2008) contents of milk protein and milk fat increased quadratically and linearly, upon enzyme supplementation on different dosages of liquid fibrolytic

Table 4: Effect of enzyme supplementation on nutrient utilization and digestibility coefficient in lactating murrah buffaloes.

Parameters	Treatment groups				SEM	P value
	T_1	T_2	T_3	T_4		
Average body weight (kg)	557.62	560.23	563.39	567.17	3.543	0.744
Metabolic body weight ($W^{0.75}$)	114.75	115.15	115.63	116.21	0.545	0.740
DMI (kg/d)	14.75	14.80	15.08	15.51	0.707	0.865
DMI, kg/100 kg BW	2.63	2.68	2.69	2.82	0.125	0.680
CP intake (kg/d)	1.46^{a}	1.50^{a}	1.59^{b}	1.68^{c}	0.017	<0.001
CP intake (g/100kg BW)	255.25^{a}	260.03^{b}	266.21^{bc}	269.63^{c}	1.385	<0.001
TDN intake (kg/d)	7.54	7.78	7.88	7.90	0.344	0.870
TDN intake (kg/100 kg BW)	1.07	1.22	1.24	1.28	0.094	0.448
TDN intake per $kgW^{0.75}$ (g/d)	65.73	67.56	67.19	68.16	8.368	0.933
Digestibility coefficient						
DM	52.09	53.25	54.36	56.54	2.664	0.684
OM	56.70	57.58	58.78	59.67	2.354	0.819
CP	56.72	60.01	61.30	63.30	2.756	0.359
EE	71.44	73.31	75.43	77.04	2.640	0.494
CF	49.00^{a}	52.96^{ab}	54.50^{b}	55.22^{b}	1.367	0.031
NFE	61.38	62.78	63.22	65.28	2.464	0.737
NDF	51.63^{a}	52.57^{a}	55.31^{ab}	57.79^{b}	1.209	0.015
ADF	48.88^{a}	48.82^{a}	51.06^{b}	53.79^{c}	0.649	<0.001

Means bearing different superscript in a row differ significantly (P<0.05).

Table 5: Effect of enzyme supplementation on milk yield and milk composition in lactating murrah buffaloes.

Parameters	Treatment groups				SEM	P value
	T_1	T_2	T_3	T_4		
Milk yield (kg/d)	4.39	5.02	5.11	5.34	0.768	0.842
Fat (%)	7.52	7.58	7.60	7.65	0.058	0.450
Lactose (%)	5.07	5.19	5.24	5.31	0.102	0.417
SNF (%)	10.54	10.55	10.57	10.59	0.029	0.688
Protein (%)	3.56	3.77	3.76	3.78	0.079	0.188
pH	6.52	6.53	6.55	6.51	0.066	0.968
Total solids (%)	18.07	18.15	18.18	18.25	0.054	0.148

enzyme that were added to the diet of lactating cow. Shekhar *et al.* (2010) did not find any marked increment in milk fat contents in enzyme supplemented Murrah buffaloes. Mohamed *et al.* (2013) and El-Bordeny *et al.* (2015) reported components like lactose, total solids, milk protein and SNF% were not affected by enzyme supplementation in buffaloes. Azam *et al.* (2017) found milk protein, solids not fat, fat and lactose were not affected (P>0.05) by supplementation. Liu *et al.* (2022) observed no statistically significant change in milk composition when tested on dairy cows given a cocktail of fibrolytic and amylolytic enzymes.

CONCLUSION

It was concluded from the present study that the milk production and milk composition could be improved with the combine use of Xylanase and Cellulase enzyme in Murrah buffaloes due to improved CP and fibre digestibility during winter season.

Conflict of interest

The authors declare that there is no conflicts of interest.

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