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Impact of diet supplementation of enzymes during hot weather conditions on milk production and nutrient utilization in Murrah buffaloes

ANUJ KUMAR[1✉], AMIT KUMAR[1], D K SINGH[1], DEBASHIS ROY[1], MANOJ KUMAR SINGH[1], T K SARKAR[1], ROHIT KUMAR[1] and VIVEK KUMAR YADAV[1]

Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh 250 110 India

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ABSTRACT

An experiment was conducted to evaluate the impact of diet supplementation of enzymes during hot weather conditions on milk production and nutrient utilization in Murrah buffaloes. Twenty four Murrah buffaloes were distributed randomly into four equal groups (T_1, T_2, T_3 and T_4) with six buffaloes in each, on the basis of their body weight, lactation length and test day milk yield. 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). The results revealed that the average milk yield (5.93 L/day) was higher in T_4 as compared to other treatment groups, i.e. 5.47, 5.59 and 5.88 L/day in T_1, T_2 and T_3 group, respectively. Statistically the difference in milk yield was significantly higher in cellulase supplemented groups. The milk fat (%) was significantly higher in enzyme supplemented over control group. The results were shown an increment in all other major milk constituents in enzyme supplemented group in comparison to control group, while statistically the difference was found non-significant. The average DM intake (kg/d, kg/100 kg body wt. and g/kg $W^{0.75}$) remained static in all treatment groups while the CP intake (g/d, g/100 kg BW and g/kg $W^{0.75}$) was significantly higher in cellulase supplemented buffaloes. The TDN intake (kg/d, kg/100kg BW and g/kg $W^{0.75}$) was significantly higher in enzyme supplemented groups. Similarly the digestibility coefficient of CF, NDF and ADF were higher in buffaloes of T_3 and T_4 over control (T_1) group. So, it is concluded from the present study that the milk production and milk composition improves in the combine use of xylanase and cellulase enzyme supplemented groups of buffaloes due to enhanced protein and energy intake and fibre digestibility.

Keywords: Buffaloes, Enzyme, Milk production, Murrah, Nutrient, Summer

Buffalo (*Bubalus bubalis*) is an important livestock resource in many agricultural production systems providing milk, meat and work power (Gasparrini 2002). Buffalo are an excellent alternative for low-input production systems due to their low maintenance requirements and high feed conversion efficiency (Paul *et al*. 2002). India has the greatest bovine population (302.79 million) in the world. According to 20th livestock census, India has 109.85 million buffaloes, with Uttar Pradesh alone accounting for 33.0 million. (BAHS 2019). According to the breed survey report on breeds total number of Murrah buffaloes in India was 47 million, accounting for 42.8% of the entire buffalo population, with 17.3 million Murrah buffaloes in Uttar Pradesh. (Breed Report 2022). The total area under cultivated fodders in India is 8.4 mha on individual crop basis. India is maintaining about 15% of total livestock population of the world in 2.29% of the global land area. At present, the country faces a net deficit of 35.6% green fodder, 10.95% dry crop residues and 44% concentrate feeds. The deficit may further rise due to consistent growth of livestock population at the rate of 1.23% in near future. Therefore it is imperative that forage production and its quality must be augmented to improve productivity of livestock. The region's regularly changing agroclimatic conditions affect dairy animal productivity and reproductive ability, especially the pioneer breed Murrah buffaloes. Climate is one of the primary influences on animal productivity (Marai and Haeeb 2010). The low productivity of Indian dairy animals in comparison to other nations has always posed a significant issue for all relevant breeders and scientists in the country. The environment, especially dietary management, has a significant impact on an animal's genetic production capacity. Enzymes can improve the nutritional digestibility of buffalo. This could be because the complex carbohydrates and proteins in feed are broken down into smaller molecules that are easier to digest in the animal GI tract. How fibre is broken down

Present address: [1]College of Veterinary and Animal Science, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh. ✉Corresponding author email: drvetanuj@gmail.com

depends on the number of bacteria in the rumen and the type of feed consumed by ruminants. Rumen bacteria produce enzymes that degrade cellulose, hemicelluloses and lignin although tropical plant fibres are more resistant to this process (Van Soest *et al.* 1978). The benefits of oral enzyme supplementation include reduced particle size, improved digestion and rumen transit rate of ingesta, reduced viscosity of ingested feed material, greater absorption, and improved feed efficiency at a lower feeding cost of production. Enzyme preparations with specific activity are known for their capacity to activate specific metabolic and digestive processes in the gastrointestinal system. This can boost natural digestion processes, resulting in better nutritional availability and higher feed intake (Dawson and Tricarico 1999; McAllister *et al.* 2002, Colombatto *et al*. 2003). Commercial enzyme preparations typically contain many active enzymes and are designed particularly to improve the digestion of dietary fibre. These products are made up of mixes with varying amounts of xylanases, glucanases, cellulases, amylases, proteases, and lipases. Multiple studies on enzyme blends have demonstrated that they improve yield in cattle (Yang *et al*. 1999, Kung *et al.* 2000) but evidence on their impact on buffalo productivity is limited.

MATERIALS AND METHODS

Ethical approval: 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 (IAEC/SVPUAT/2022/127).

Experimental animals and diets: Twenty four lactating Murrah buffaloes were selected for the research trail from Livestock Research Centre of university. All the selected lactating buffaloes were randomly allocated into four treatment groups, viz. T_1, T_2, T_3 and T_4 having six animals in each group nearer to their average body weight, lactation length and milk yield.

Feeding of experimental animals: The daily nutrient requirements of lactating Murrah buffaloes in terms of all essential nutrients were provided as per the feeding standards of the Indian Council of Agricultural Research, New Delhi. To meet the daily nutrient requirement of trial animal's requirements, a total mixed ration was prepared by mixing roughage and concentrating at the ratio of 60:40 after grinding/chaffing. The roughage part consisted of available during kharif season green fodder and wheat straw available at the research centre. The feeding trial was planned in 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. A commercially prepared enzyme in powder form was procured from the market, i.e. Xylanase has 400000 IU and Cellulase has 100000 IU activity. The enzyme was supplemented to each treatment individually at the time of feeding by preparing a premix with concentrate with the required quantity of enzyme. An adjustment period of 10 days was given to the experimental animals. Feeding material concentrate ingredients, wheat straw, green fodder, left over feed and faecal matter were ground and analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), crude fiber (CF) and total ash (AOAC 2005). Fiber fractions of feed, fodder and fecal samples were analyzed using the detergent method of fiber estimation (Van Soest *et al.* 1991). The chemical composition (on % DM basis) of feed ingredients used during the trial in the summer season is given in Table 1. Buffaloes were milked manually and the milk yield of buffaloes were recorded individually with an added amount of milk suckled by the calf weighing before and after each milking on test day. Milk samples were collected from each buffalo and pooled in proportion to the milk yield of individual buffalo and analyzed for its composition through a milk analyzer (Lasctoscan). The monthly average temperature (°C) and humidity (%) in the animal shed were recorded by a digital thermometer hygrometer temperature sensor humidity meter, which is presented in Table 2. The temperature humidity index (THI) was calculated through an equation given by Kendall and Webster in 2009. The formula is as follows.

Table 1. Chemical composition (on % DM basis) of feed ingredients used during the lactation trial during summer season

Item	Wheat straw	Green fodder	Concentrate	Total mixed ration
Dry matter	90.41	23.55	86.56	73.54
Organic matter	88.54	87.65	92.36	88.96
Total ash	13.16	11.14	8.74	10.45
Crude protein	3.19	9.40	20.65	14.02
Ether extract	0.59	3.34	3.66	3.21
Crude fibre	36.95	25.12	8.85	20.16
Nitrogen free extract	44.15	51.00	58.10	52.16
Neutral detergent fibre	85.96	61.47	36.45	52.13
Acid detergent fibre	52.19	49.51	14.91	33.88
Hemicellulose	33.77	11.96	21.54	18.25
Acid detergent lignin	3.89	2.33	1.28	2.19
Cellulose	48.30	47.18	13.63	31.69

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

Month	Temperature (°C)			Mean RH%	Mean THI
	Avg.	Max.	Min.		
May	26.12	32.90	19.35	14.12	69.12
June	27.83	32.54	23.12	29.71	72.74
July	24.88	28.10	21.66	55.83	72.24
Overall	26.29	31.21	21.38	32.97	71.47

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

Statistical analysis: Statistical Package for the Social Sciences (S.P.S.S. 2011) 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

Nutrient utilization and digestibility coefficient: Effect of enzyme supplementation on nutrient utilization and digestibility coefficient in lactating Murrah buffaloes during summer season is shown in Table 3. Average body weights were 494.01, 517.21, 533.66 and 534.64 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 104.77, 108.36, 110.95 and 111.17, in control (T_1), T_2, T_3 and T4 groups, respectively. Total DMI as kg/d, and kg/100 kg weight was 14.62 and 2.79 in control group (T_1), 14.64 and 2.74 in T_2 group, 14.70 and 2.82 in T_3 and, 14.76 and 2.86 in T_4 group. CP intake as kg/d and g/kg body weight was 1.49 and 265.87 in control group (T_1), 1.53 and 267.81 in T_2 group, 1.55 and 271.51 in T_3 and 1.61 and 276.39 in T_4 groups, respectively. During the summer season, enzyme supplementation significantly improved the digestibility of CF, NDF and ADF in buffaloes ($P<0.05$). Fibre digestion is improved by the synergistic effect of ruminal and exogenous enzymes. The addition of enzyme to the ruminant feed promotes microbial colonisation (Rode *et al.* 1999, Beauchemin *et al.* 2000).

Beauchemin *et al.* (2000) discovered that exogenous fibrolytic enzymes may help to expose additional cell wall sites for bacterial adhesion, allowing for more complete digestion of the meal. Knowlton *et al.* (2002), Bowman *et al.* (2002) and Gado *et al.* (2009) all reported comparable findings. Gaffar *et al.* (2010) found that lactating buffaloes treated with cellulase and xylanase had significantly increased digestibility of crude fibre (CF) ($P<0.05$). The current study's findings are consistent with those of Arriola *et al.* (2011) who supplemented lactating cows diets with fibrolytic enzyme at low and high concentrate proportions and found that supplemented high concentrate groups had higher digestibility than non-supplemented groups. Titi and Tabbaa (2004) discovered that NDF digestibility increased considerably with fibrolytic enzyme supplementation. El-Bordeny *et al.* (2017) fed EFE to barkey lambs and found that enzyme addition significantly improved the digestibility of DM, OM, CP, CF, NFE, NDF, ADF, cellulose and hemicellulose. According to Shekhar *et al.* (2010) rumen microbes higher digestion of CF, NDF

Table 3. Effect of enzyme supplementation on nutrient utilization and digestibility coefficient in lactating Murrah buffaloes during summer season

Parameter	Treatment groups				SEM	P-Value
	T_1	T_2	T_3	T_4		
Average body weight (kg)	494.01	517.21	533.66	534.64	20.277	0.479
Metabolic body weight ($W^{0.75}$)	104.77	108.36	111.17	110.95	3.202	0.785
DMI (kg/d)	14.62	14.64	14.70	14.76	0.279	0.984
DMI, kg/100 kg BW	2.79	2.74	2.82	2.86	0.123	0.966
CP intake (kg/d)	1.49^{a}	1.53^{b}	1.55^{b}	1.61^{c}	0.014	<0.001
CP intake (g/kg BW)	265.87^{a}	267.81^{a}	271.51^{ab}	276.39^{ab}	0.895	<0.001
TDN intake (kg/d)	6.90	7.23	7.49	7.56	0.152	0.147
TDN intake (kg/100 kg BW)	1.05	1.15	1.20	1.21	0.049	0.127
TDN intake per kg$W^{0.75}$ (g/d)	494.01	517.21	533.66	534.64	20.277	0.930
Digestibility coefficient						
DM	53.94	55.35	56.46	58.64	2.603	0.684
OM	57.75	58.63	59.83	60.72	2.354	0.819
CP	58.32	64.11	62.90	68.01	2.511	0.359
EE	74.95	73.04	76.41	78.02	2.672	0.494
CF	47.90^{a}	54.36^{ab}	55.90^{b}	56.62^{b}	2.193	0.031
NFE	64.08	63.98	64.67	68.73	2.321	0.737
NDF	53.09^{a}	54.03^{a}	56.77^{ab}	61.75^{b}	2.526	0.015
ADF	50.55^{a}	49.92^{a}	54.73^{b}	52.46^{c}	0.273	<0.001

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

and ADF (P<0.05) could be attributed to the synergistic action of exogenous fibrolytic enzymes with ruminal enzymes produced by the microbes themselves. According to Azam *et al.* (2017) increasing the quantity of enzyme in the feed of lactating Nili Ravi buffaloes improves the digestibility of crude protein and NDF.

Body weight and nutrient intake: Overall body weight (BW) and metabolic body weight remained similar (P>0.05) in different treatment groups (Table 4). Average body weight was 475.02, 504.69, 523.11 and 539.11 kg in T_1, T_2, T_3 and T_4 groups, respectively. The findings are consistent with previous reports in buffaloes (Shekhar *et al.* 2010) and cattle (Kung *et al*. 2000, Knowlton *et al*. 2007, Lopuszanka and Bilik 2011, Barbadikar *et al.* 2012, Dean *et al*. 2013). Lewis *et al.* (1999) find that enzyme therapy provided more nutrients to cows for energy deposition to body reserves beyond those needed for milk production. Yang *et al.* (2000) found no effect on body weight changes in cows fed a control diet, enzyme-treated TMR or enzyme-treated barley-based concentrate. Similarly, Shojaeian and Thakur (2007) found no influence on body weight variations caused by enzyme supplementation to urea-treated wheat straw-based TMR fed to dairy cows. Knowlton *et al.* (2002) discovered a numerical increase in body weight in early lactation cows fed diets including enzyme supplements. However, because the buffaloes employed in this study were in both early and mid-lactation, no apparent influence on body weight was detected. Several research workers Rode *et al*. (1999), Beauchemin *et al*. (2000), and Titi (2003). reported similar findings. Beauchemin *et al.* (2000) found that utilising enzyme supplementation enhanced feed intake and digestible nutrient intake to a larger level in cows administered enzyme supplements. The overall average DMI in four treatment groups was 13.95, 14.99, 15.53 and 16.01 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 other three treatment groups throughout the experimental period. The average DM intake (kg/100 kg body weight) was 2.90, 2.92, 2.93 and 2.94 kg for the T_1, T_2, T_3 and T_4 groups, respectively, which was statistically similar. The difference in dry matter intake among treatment groups was found non-significant and almost similar in all four groups. These findings are in line with other studies on cattle (Mohamed *et al*. 2013, Dean *et al*. 2013, Dunda 2015, El-Bordeny *et al*. 2015). In the case of buffalo, Shekhar *et al.* (2010) reported no rise in DMI, however Gaafar *et al.* (2010) revealed 4% higher DMI in the enzyme supplemented group of buffaloes. The cumulative average of CP intake g/day and g/100kg body weight due to the supplementation of the enzyme xylanase and cellulase in four treatment groups was 1455.91, 1472.13, 1476.22 and 1480.61: 255.83, 264.16, 269.86 and 273.77, respectively. CP intake g/day and g/100kg was found to be significantly higher in cellulase supplemented group of buffaloes. The results are found in agreement with Romero *et al.* (2016) who observed that supplementation of xylanase plus @ 1 mL/kg DM of TMR (T_1) significantly (P<0.001) improved the DCP intake (kg/d) in Holstein cows. Average total TDN intakes was 7.42, 7.79, 7.91 and 8.02 kg/day in control (T_1), T_2, T_3 and T_4 groups respectively, average TDN intake, kg/100 kg BW was 1.27, 1.39, 1.42 and 1.46 in control (T_1), T_2, T_3 and T_4 groups respectively. The average TDN intakes g/kg $W^{0.75}$ were 69.52, 72.77, 74.32 and 75.02 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 in treatment groups supplemented with xylanase and cellulase in lactating Murrah buffaloes. According to Shojaeian and Thakur (2007) enzyme supplementation in Sahiwal cows fed urea-treated wheat straw resulted in increase in average TDN intake compared to the control group. Shekhar *et al.* (2010) found that total digestible nutrient intake (kg/day) was significantly more in the enzyme supplemented group.

Milk yield and composition: The average milk yield (kg/d) was 5.47, 5.59, 5.88 and 5.93 in T_1, T_2, T_3 and T_4 groups, respectively (Table 5). The average milk yield (5.93 litre/day) was significantly higher in cellulase supplemented groups in buffaloes. Titi (2003) discovered that cows fed an enzyme-treated diet produced more milk, which was most likely due to more accessible nutrients for

Table 4. Effect of enzyme supplementation on body weights and nutrient intakes in lactating Murrah buffaloes during summer season

Parameter	Treatment groups				SEM	P-value
	T_1	T_2	T_3	T_4		
Body weight (kg)	475.02	504.69	523.11	539.11	16.746	0.073
Metabolic body weight ($W^{0.75}$)	101.70	106.39	109.33	111.87	2.658	0.073
Dry matter intake, kg/day	13.95	14.99	15.53	16.01	0.729	0.253
Dry matter intake, kg/100 kg BW	2.90	2.92	2.93	2.94	0.009	0.065
Dry matter intake (g/kg $W^{0.75}$)	137.09	140.72	141.88	143.08	1.177	0.145
Crude protein intake, g/day	1455.91^{a}	1472.13^{b}	1476.22^{b}	1480.61^{b}	5.471	0.025
Crude protein intake g/100 kg BW	255.83^{a}	264.16^{ab}	269.86^{b}	273.77^{b}	3.547	0.011
CP intake, g/$W^{0.75}$	13.24^{a}	13.57^{b}	13.89^{b}	14.05^{b}	0.165	0.041
Total digestible nutrient intake, kg/day	7.42^{a}	7.79^{b}	7.91^{ab}	8.02^{b}	0.055	<0.001
Total digestible nutrient intake/100 kg BW	1.27^{a}	1.39^{b}	1.42^{b}	1.46^{b}	0.024	<0.001
Total digestible nutrient intake, g/kg $W^{0.75}$	69.52^{a}	72.77^{b}	74.32^{b}	75.02^{b}	1.880	0.041

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 during summer season

Parameter	Treatment groups				SEM	P-value
	T_1	T_2	T_3	T_4		
Milk yield (kg/d)	5.47[a]	5.59[a]	5.88[b]	5.93[b]	0.070	0.001
Fat (%)	7.55[a]	7.71[b]	7.72[b]	7.79[b]	0.040	0.003
Lactose (%)	5.23	5.36	5.39	5.41	0.141	0.797
SNF (%)	10.40	10.43	10.52	10.50	0.132	0.906
Protein (%)	3.46	3.50	3.65	3.68	0.164	0.729
*p*H	6.48	6.51	6.59	6.54	0.069	0.709
Total solids (%)	18.00	18.10	18.21	18.23	0.148	0.680

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

milk production. Shojaeian and Thakur (2007) observed increase in milk yield for Sahiwal cows fed with xylanase and cellulase in a urea-treated wheat straw-based feed. Gado *et al.* (2009) studied the effect of supplementing TMR with a mixture of exogenous enzymes at a rate of 40 g/cow/day on milk production in Brown Swiss cows. He found that the enzyme-supplemented group produced more milk (15.70 kg/day) than the control group (12.75 g/day) (P=0.046). According to Ortiz-Rodea *et al*. (2013) improved nutrient utilisation and digestibility can lead to an increase in milk yield. El-Bordeny *et al.* (2015) observed that feeding Holstein cows TMR supplemented with fibrolytic enzyme (a combination of active xylanase and cellulase) at 15 g/animal/day resulted in a considerable increase in milk yield. The effect of enzyme supplementation on milk composition of lactating Murrah buffaloes throughout the summer season was found to be non-significant, except fat content. Milk fat percentages were significantly increased in all enzyme supplement groups compared to the control group. Lewis *et al.* (1999) and Yang *et al.* (1999) discovered that introducing fibrolytic enzymes raised milk fat levels. The rise in fat percentage could be attributed to an increase in available energy and fatty acids for fat formation. Miller *et al.* (2008) found that adding varied doses of liquid fibrolytic enzyme to lactating cows meals boosted milk protein and milk fat content quadratically and linearly, respectively. Gaffar *et al.* (2010) investigated the effect of fibrolytic enzyme supplementation and fibre content in total mixed feed on lactating buffaloes productive efficiency and discovered a considerable increase in milk fat. Shekhar *et al.* (2010) discovered no significant increase in milk fat content in Murrah buffaloes fed enzyme supplements. According to Dean *et al*. (2013) alterations in the acetate: propionate ratio in the rumen may result in an increase in milk fat content. El-Bordeny *et al.* (2015) found a large increase in actual milk production as well as a significant rise in fat percentage in buffaloes. Morsy *et al*. (2016) found that adding cellulase enzyme may have resulted in an increase in fat content and yield because the rumen breaks down a large amount of fibre, making more acetate accessible for fatty acid synthesis. According to Mohamed *et al.* (2013) and El-Bordeny *et al.* (2015) enzyme supplementation in buffaloes had no effect on lactose, total solids, milk protein, or SNF% levels. Azam *et al.* (2017) discovered that milk protein, solids not fat and lactose were not changed (P>0.05). Liu *et al.* (2022) investigated the effects of a combination of fibrolytic and amylolytic enzymes on milk production in dairy cows and discovered no significant impacts on milk composition. The variation in responses between research may be attributable to the range of enzyme products and experimental conditions.

It is concluded from the present study that the milk production and milk fat content improves in the combine use of xylanase @800000 IU and cellulase enzyme @240000 IU supplemented groups of Murrah buffaloes due to improved protein and energy intake during hot summer months.

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