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Effect of Exogenous Fibrolytic Enzymes on Milk and Components Yield as Well as on Feed Efficiency and Body Weight in Holstein Friesian x Kankrej Crossbred Cows After Peak Lactation.

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ABSTRACT

An experiment was conducted to evaluate effect of supplementation of exogenous fibrolytic enzymes in diet on milk yield, milk yield efficiency and birth weight of calf after peak lactation in Holstein Friesian x Kankrej (HFK) crossbred cows. An on-Station study of 126 days duration was conducted with cellulase and xylanase enzymes (3000 and 200000 IU/g, respectively) @ 3000+12000 IU/kg diet, respectively as revealed optimum under in vitro experiment. Sixteen HFK crossbred cows were allotted to two dietary treatments *viz.* T_1 (control) and T_2 (Enzymes) of eight cows in each group on the basis of days in milk (57.44± 9.69), 4% FCM yield (12.66±0.45 kg) and body weight (418.00±19.08 kg). Cows were individually fed concentrate, green hybrid Napier (Coimbture-3) and sorghum hay to meet the crude protein (CP) and total digestible nutrient (TDN) requirements. Bi-weekly milk samples were collected in proportion to morning and evening milk yield to measure gross milk composition. The intake of dry matter (DM), CP and TDN on daily, per cent body weight and metabolic weight basis were non-significant between the groups. The yield of milk, 4% fat corrected milk (FCM) and milk components yield like fat, protein, lactose, solid not fat (SNF) and total solid (TS) were also non-significant between groups. Cows of enzyme group had consumed numerically less DM (1.19 vs. 1.13 kg) and TDN (670.11 vs. 640.01 g) to yield one kg milk. Efficiency of 4% FCM yield also followed same the trend of DM (1.20 vs. 1.14 kg) and TDN (674.46 vs. 645.82 g) consumption. However, the efficiency of CP utilization for milk (123.96 vs. 123.08 g/kg milk) and FCM (124.74 vs. 124.19 g/kg FCM) yield was similar in both the groups. Body weight gain (+12.38 vs. +18.55 kg) of crossbred cows was significantly higher on feeding fibrolytic enzymes. Birth weight of calf (29.50 vs. 32.00 kg) was numerically higher in fibrolytic enzymes group, even though female to male ratio (5:3 vs. 3:5) was higher in fibrolytic enzyme supplemented group and pregnancy period (280.50 vs. 280.13 days) was similar between the groups. Exogenous fibrolytic enzymes had released more energy owing to action on fibrous fodder, resulted in higher body weight gain in enzyme group which converted to higher body weight of calf even though having more female (5:3 female to male ratio) than control group (3:5 female to male ratio). Feeding of exogenous fibrolytic enzymes (Cellulase + Xylanase @ 3000+12000 IU/kg diet) to Holstein Friesian X Kankrej crossbred cows after peak lactation resulted higher body weight gain of cows, which resulted in improved calf weight (+2.50 kg) even though numbers of female were more (female to male ratio 5:3 vs. 3:5) in EFE supplemented group.

Key words *crossbred cows, exogenous fibrolytic enzyme, milk yield, feed conversion efficiency, body weight of cows and calf*

Ruminant animals may be considered as the foundation of animal agriculture because they have served mankind many millennia (Weimer *et al.,* 2009). The ruminant production systems are dependant worldwide on forage as the main nutritional components (Wilkins, 2000). The biggest problem faced by many developing countries in animal production sector is the wide gap between animal's requirements and availability of feeds. In India the majority of livestock subsist on low quality native grasses, crop residues, and agro-industrial by-products. Feeding agricultural by products (lignocellulosic materials) directly to farm animals in general are low protein content, high crude fiber and low digestibility. Thus, to increase the digestibility of these agricultural crop residues, it is important to break down the linkage between cellulose, hemicellulose and lignin. Plant cell walls typically consist of about 35-50% cellulose, 20-35% hemicellulose and 10-25% lignin in the dry mass (Gemeda *et al.*, 2014). The various methods such as physical processing, physico-chemical processing, biological treatment and use of feed additives have been tried. For many years, researchers were discouraged from using enzymes to enhance the fibrous diets utilization because enzymes would be ineffective due to ruminal proteolysis. These concerns have been disproved by several recent studies that have demonstrated that EFE supplementation enhanced the productivity of livestock (Miachieo and Thakur, 2007; Bandla *et al.,* 2008), and several fibrolytic enzyme products are currently commercially available.

The meta-analysis revealed variable response of EFE on milk yield and milk composition needs reconsideration (Ortiz-Rodea *et al.*, 2013). Strategic approach like *in vitro* testing for dose optimization before *in vivo* application and use of cows in early lactation (21±5 DIM) are needed for accurate response on milk yield and milk composition (Adesogan *et al.*, 2014). This study aimed to evaluate effect of EFE supplementation on milk and components yield as well as feed conversion efficiency and birth weight of cows and calf after peak lactation in Holstein Friesian x Kankrej crossbred cows.

Table 1. Composition of feeds and fodder (% on DM basis)

Particular	CP	EE	CF	Ash	NFE	Ca	P
Concentrate	18.92	3.76	8.70	14.89	53.73	1.62	1.02
NB Var. CO3	9.21	3.18	34.32	8.94	47.35	1.01	0.43
Sorghum hay	5.84	2.51	30.2	9.20	52.25	0.52	0.41

Note: NB=hybrid napier, CP= crude protein, EE=ether extract, NFE=nitrogen free extract, Ca= calcium, P= phosphorus

MATERIALS AND METHODS

An experiment was conducted as On-Station study of 126 days duration on 16 Holstein Friesian x Kankrej (HFK) crossbred cows at Livestock Research Station, College of Veterinary Science & Animal Husbandry, Anand Agricultural University, Anand. The cellulase and xylanase enzymes were procured from M/s Aumgene Bioscience Pvt. Ltd., Surat, Gujarat, India and contained 3000 and 200000 IU/g, respectively as per manufacturer's labeling. An *in vitro* study was carried out to arrive at optimum dose of cellulase and xylanase for on station study. Cellulase tested was 1000, 2000, 3000, 4000, 5000 and 6000 IU/kg diet, with combination of xylanase @ 2, 3 and 4 time of cellulase, making 18 treatment and control (total 19 treatments). *In vitro* optimum dose of cellulase and xylanase was found to be 3000+12000 IU/kg diet, respectively and was tested on station study.

Sixteen HFK crossbred cows were selected and allotted to two dietary treatments *viz.* T_1 (control) and T_2 (Enzymes) of eight cows in each group, following Completely Randomized Design on the basis of days in milk (57.44± 9.69), 4% FCM yield (12.66±0.45 kg) and body weight (418.00±19.08 kg). The cows were individually fed concentrate, green hybrid Napier (Coimbture-3) and sorghum hay to meet the CP and TDN requirements (NRC, 2001). Bi-weekly samples of ingredients offered were collected and stored for chemical analysis (AOAC, 1995 and Van Soest *et al*., 1991). An individual feeding of cows carried out in well ventilated and hygienic stall. Cows let loose for two hours each in the morning and evening for exercise. All cows had free access to fresh, clean and wholesome drinking water during they were let loose. De-worming of all the lactating cows was carried out using broad spectrum anthelmintic before initiation of the experiment. Cows were machine milked twice daily (5:30 a.m. and 5:30 p.m.), recorded milk yield and weekly average was calculated. The milk samples were drawn at bi-weekly interval in proportion to morning and evening milk yield from individual animals. Milk samples were analyzed for milk fat, solids-not-fat (SNF), milk protein and lactose content as per BIS (1981) using Milkoscan. The 4% FCM production was calculated using formula 4% FCM (kg) = (0.4*Milk yield kg)+(15*fat yield kg). The amount of dry matter (kg), crude protein (g) and total digestible nutrient (kg) required to produce one kg of whole milk or 4% FCM was calculated for feed conversion efficiency. After completion of 18 weeks experiments cows were fed in group to meet nutrients requirements (NRC, 2001) till calving. Gestation period of cow; birth weight and sex of calf recorded to ascertain effect of body weight of cows on birth weight of calf on account of feeding EFE supplemented diet. The data generated were subjected to t test analysis (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Dry Matter (DM) and Nutrients in intake

Ingredient compositions of feed and fodders are given in Table 1. Dry matter (DM) and nutrients intakes of cows

Table 2. Dry matter and nutrients intake of crossbred cows

Particulars/Treatment	Control	Enzyme
DMI (kg/day)	13.83 ± 0.84	13.43 ± 0.86
% DMI (kg/day)	3.29 ± 0.16	3.26 ± 0.16
DMI (g/kg $W^{0.75}$)	148.48 ± 5.95	146.59 ± 6.78
CPI (kg/day)	1.46 ± 0.12	1.47 ± 0.11
TDNI (kg/day)	7.80 ± 0.49	7.59 ± 0.48
%CPI (kg/day)	0.347± 0.02	0.357 ± 0.02
%TDNI (kg/day)	1.86 ± 0.09	1.84 ± 0.09

Note: DMI=dry matter intake, CPI=crude protein intake, TDNI= total digestible nutrient intake

The means without superscripts in a row differ non-significantly (P>0.05)

Table 3. Milk and components yield of crossbred cows

Particulars/Treatment	Control	Enzyme
Milk yield (kg/day)	11.93 ± 1.15	12.08 ± 0.98
4% FCM yield (kg/day)	11.86 ± 1.15	11.97 ± 0.97
Milk fat %	3.66 ± 0.13	3.84 ± 0.10
Milk SNF %	8.02 ± 0.04	8.01 ± 0.06
Milk TS%	11.68 ± 0.15	11.85 ± 0.09
Milk lactose%	4.27 ± 0.02	4.24 ± 0.03
Milk protein%	3.16 ± 0.01	3.15 ± 0.02
Milk fat yield (kg/day)	0.44 ± 0.05	0.47 ± 0.04
Milk SNF yield (kg/day)	0.96 ± 0.09	0.97 ± 0.08
Milk TS yield (kg/day)	1.40 ± 0.14	1.43 ± 0.12
Milk lactose yield (kg/day)	0.51 ± 0.05	0.51 ± 0.04
Milk protein yield (kg/day)	0.38 ± 0.04	0.38 ± 0.03

Note: FCM= fat corrected milk, SNF= solid not fat, TS=total solid

The means without superscripts in a row differ non-significantly (P>0.05)

are given in Table 2. Daily dry matter intake on total (13.83 vs. 13.43 kg), per cent of body weight (3.29 vs. 3.26 kg) and metabolic body weight (148.48 vs. 146.59 g) basis of cows was numerically lower in fibrolytic enzymes supplemented groups. Total and per cent intake of crude protein (1.46 vs. 1.47 kg and 0.347 vs. 0.357 kg, respectively) and total digestible nutrients (7.80 vs. 7.59 kg and 1.86 vs. 1.84 kg, respectively) were also non-significant between the groups.

DM and nutrients intake were statically not influenced on supplementation of fibrolytic enzyme (blend of active xylanase and cellulase) @ 15 g/animal/day in TMR in Holstein cows as reported by El-Bordeny *et al.* (2015). Similarly non-significant effect of fibrolytic enzymes supplementation on DM and nutrients intake were observed when supplemented @ 15 g/cow in Holstein dairy cows (Shadmanesh, 2014; Mohamed *et al.,* 2013) and @ 50 mg/kg DM in Holstein Friesian crossbred cows (Khanh *et al*., 2012). However, linear (P<0.05) effect of fibrolytic enzyme supplementation (@ 0, 8, 16 and 24 g/cow/day) was observed by da Silva *et al.* (2015) on DM and NDF intakes in mid-lactating Holstein cows.

Milk yield and composition

Daily milk (11.93 vs. 12.08 kg) and fat corrected yield (FCM; 11.86 vs. 11.97 kg) yield was numerically higher in fibrolytic enzyme supplemented group of cows in comparison to control (Table 3). Milk fat (3.66 vs. 3.84%) and milk TS (11.68 vs. 11.85) content also followed same trend, whereas milk SNF (8.02 vs. 8.01%), milk lactose (4.27 vs. 4.24) and milk protein (3.16 vs. 3.15%) was slightly lower in EFE supplemented group. Daily yield of milk fat (0.44 vs. 0.47 kg), milk SNF (0.96 vs. 0.97 kg) milk TS (1.40 vs. 1.43 kg) was numerically higher in enzyme supplemented cows in comparison to control, whereas milk lactose (0.51%) and milk protein (0.38 kg) was similar in both group.

da Silva *et al.* (2015) had observed no beneficial effect of fibrolytic enzymes @ 0, 8, 16 and 24 g/cow/day supplementation on milk yield and composition in mid-lactating Holstein cows. Similarly, Chung *et al.* (2012) also observed non-significant effect of exogenous fibrolytic enzyme (0, 0.5 and 1.0 ml/kg DM of TMR) on milk yield in Holstein dairy cows. However, Mohamed *et al.* (2013) had observed significant effect of exogenous fibrolytic enzymes on milk, SNF and energy corrected milk yield; whereas non-significant effect on milk fat, protein, lactose and solid not fat (SNF) content in early lactating dairy cows. Similarly El-Bordeny *et al.* (2015) had also reported significant increased in milk, FCM and ECM yield as well as milk fat contents, while milk protein, lactose, total solid and solid not fat contents were non-significantly improved on enzymes supplementation to Holstein cow's rations. Higher (P<0.05) daily milk and FCM yield; and numerical improvement in milk total solids and fat content while numerical reduction in milk protein content was reported by Miachieo and Thakur (2007) on feeding cows TMR supplemented with fibrolytic enzymes.

Feed conversion efficiency

Feed conversion efficiencies for milk and FCM yield of crossbred cows are given in Table 4. Cows of enzyme group had consumed numerically less DM (1.19 vs. 1.13

Table 4. Feed conversion efficiency of crossbred cows

Particulars/Treatment	Control	Enzyme
DMI (kg/kg milk)	1.19 ± 0.06	1.13 ± 0.04
DMI (kg/kg FCM)	1.20 ± 0.06	1.14 ± 0.04
CPI (g/kg milk)	123.96 ± 3.71	123.08 ± 3.49
CPI (g/kg FCM)	124.74 ± 3.58	124.19 ± 3.52
TDNI (g/kg milk)	670.11± 30.46	640.01 ± 26.54
TDNI (g/kg FCM)	674.46 ±30.46	645.82 ± 26.78

Note: DMI=dry matter intake, FCM= fat corrected milk, CPI=crude protein intake, TDNI=total digestible nutrient intake

The means without superscripts in a row differ non-significantly (P>0.05)

kg) and TDN (670.11 vs. 640.01 g) to yield one kg milk. Efficiency of 4% FCM yield also followed same the trend of DM (1.20 vs. 1.14 kg) and TDN (674.46 vs. 645.82 g) consumption. However, the efficiency of CP utilization for milk (123.96 vs. 123.08 g/kg milk) and FCM (124.74 vs. 124.19 g/kg FCM) yield was similar in both the groups.

Miachieo and Thakur (2007) have reported non-significant effect of fibrolytic enzymes on feed conversion efficiency in dairy cows. Dean *et al*. (2013) had shown non-significant effect of fibrolytic enzyme on feed conversion efficiency in mid-lactating Holstein cows. Similarly, £opuszañska-Rusek and Bilik (2011) and Bernard *et al.* (2010) also reported non-significant effect of fibrolytic enzymes on feed conversion efficiency in Polish Red-and-White Holstein-Friesian cows and Holstein cows, respectively. Whereas, El-Bordeny *et al.* (2015) and Mohamed *et al.* (2013) reported significant improvement in feed conversion efficiency on feeding fibrolytic enzyme in Holstein cows.

Body weight of cows and calf

Body weight change (+12.38 vs. +18.55 kg) of crossbred cows was significantly higher on feeding fibrolytic enzymes. Birth weight of calf (29.50 vs. 32.00 kg) was numerically higher in fibrolytic enzymes group, even though female to male ratio (5:3 vs. 3:5) was higher in fibrolytic enzyme supplemented group and pregnancy period (280.50 vs. 280.13 days) was similar between the groups. Exogenous fibrolytic enzymes had released more energy owing to action on fibrous fodder, resulted in higher body weight gain in enzyme group which converted to higher body weight of calf even though having more female (5:3 female to male ratio) than control group (3:5 female to male ratio).

An investigation of Knowlton *et al.* (2002) had shown more weight gained (P<0.02) on supplementation of fibrolytic enzyme (15,000 units cellulase/g) @ 204 g/tonne DM of TMR in Holstein cows for the period of 28 days. Whereas, Dean *et al*. (2013) reported that fibrolytic enzyme supplementation had only numerically increased (P>0.05) body weight gain in lactating Holstein cows during mid lactation. However, contradictory findings on body weight changes were reported by Miachieo and Thakur (2007) and the findings revealed that supplementation of fibrolytic enzymes resulted in numerically higher loss in body weight (10.97 kg) than control diet (6.15 kg) of cows. Milk yield can increased up to six week after calving and after lactation peak (6-8 weeks), decrease due to decrease in the number of secreting cells (Chilliard, 1991), more energy released owing to higher digestibility on EFE feeding (Lunagariya, 2016) will stored as body fat resulting in higher body weight gain. Higher body weight of dam will result in higher birth weight of calf (+2.50 kg) evethough having higher female to male ratio (5:3) in comparison to control group where female to male ratio was 3:5.

CONCLUSION

Feeding of exogenous fibrolytic enzymes (Cellulase + Xylanase @ 3000+12000 IU/kg diet) to Holstein Friesian X Kankrej crossbred cows after peak lactation resulted in non-significant improvement in dry matter and nutrients intake; milk and component yield; and feed conversion efficiency. However, more energy released on account of EFE feeding reflected in higher body weight gain of cows at the end of experiments. Higher body weight gain of cows resulted in improved calf weight (+2.50 kg) even though numbers of female were more (female to male ratio 5:3 vs. 3:5) in EFE supplemented group.

Table 5. Body weight and pregnancy period of crossbred cows; and calf weight

Particulars/Treatment	Control	Enzyme
Body weight (kg)		
Initial	418.38± 29.51	417.25 ± 26.18
Final	430.75± 31.04	435.80 ± 25.05
Difference	12.38^{a} ± 2.20	18.55^{b} ± 1.60
Reproduction		
Pregnancy period (days)	280.13 ± 2.77	280.50 ± 3.12
Birth weight of calf (kg)	29.50 ± 1.41	32.00 ± 1.20
Sex ratio (Female to male)	3:5	5:3

The means with different superscripts in a row differ significantly (P<0.05)

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