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RESEARCH ARTICLE

Effect of exogenous fibrolytic enzymes addition in Pearl millet (*Pennisetum glaucum)* stovers based complete pellet on intake, milk production, composition and fatty acid profile in lactating Barbari goats

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Abstract: Effect of fibrolytic enzyme supplementation on pearl millet stovers based complete pellet feeding was studied in lactating Barbari goats. Two type of complete pellet feed (TMR) was prepared having concentrate mixture and pearl millet stovers in 50:50 ratio. In control pellet (MS) no enzyme was added while in treatment pellet (MSE) 20 gm commercial fibrolytic enzymes/ quintal feed was added. Twelve lactating Barbari goats (Avg. BW 31.81 kg, in 3-5th lactation) were divided into two groups (CON and SUPP) of six each. CON group of goats was fed MS pellet while SUPP group of goats were fed with MSE pellet *ad libitum* to meet their nutrient requirement. The duration of experimental feeding was 90 days and body weight changes, dry matter intake, milk yield and composition was studied. There was no statistically significant difference in body weight at different fortnight in both the groups. Dry matter intake (kg/d) was better in first two fortnights in SUPP group but later on the intake was similar. The average milk yield (g/day) during lactation trial was 888.26 and 811.34 for Gr CON and SUPP respectively. Milk pH was slightly acidic in both the group and no significant difference was recorded in the milk composition (total solids, total protein, Solid-not fat, Ash) Milk fat was lower in enzyme supplemented group. There was no significant effect of addition of enzymes in proportions of individual fatty acids in milk fat. Per cent of t-Vaccenic acid (a precursor of conjugated linoleic acid) in total fatty acids was 1.61 and 1.83 in Gr CON and Gr SUPP respectively being higher in enzyme supplemented goats. A lower proportion of saturated fatty acids and higher proportion of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and Unsaturated fatty acids (UFA) were reported after addition of enzymes in the diet. Fatty acid indexes were better in milk from goat fed with MSE (enzyme supplemented complete feed). Present study concluded that adding of fibrolytic enzyme in the pearl millet based stovers had no significant effect on intake, milk production and milk constituents but tend to shift the milk fatty acid towards healthier fatty acids in lactating Barbari goats.

Key words: Complete feed, Fibrolytic enzymes, Fatty acids, Lactating goats, Milk, Pearl millet

Introduction

Pearl millet (*Pennisetum glaucum)* is a dual purpose crop which can provide grain for humans and green fodder, stovers for animal consumption. Pearl millet stovers is the part of the plant that remains after grain harvest and is a fibrous by-product characterized by low nutritive value due to high concentrations of structural carbohydrates and lignin. However, dual purpose (grain and forage) varieties with higher forage quality are being developed in South and West Asia (Alexander et al. 2007; Blümmel et al. 2003). These millets are generally grown in arid and semi-arid region of country where rainfall is low and goat populations are substantial. Supplementation of millet stovers with other nutritive ingredients can help in better utilization of these feed resources to optimize the productivity of animals. Since major component of these stovers are fibre, addition of exogenous fibrolytic enzymes (EFE) can help in better utilization of these forage resources and improvement in their productivity. EFE like Cellulases and xylanases addition in the ruminant diet can increase fiber digestibility and production efficiency. Supplementing the ruminant diet with EFE can increase the availability of energy in fibrous feed by improving ruminal fermentation, fiber and DM degradability and microbial protein synthesis (Selzer et al. 2021). The livestock sector in India is currently facing major constraints such as limited availability of fodder crops, high cost and lower nutritional quality of available feed resources. Some studies showed that enzyme addition increases nutrient digestibility and enhanced productive animal performance of ruminants (Gado et al. 2014, Tirado-González et al. 2018 while others observed minimal effects (Bueno et al. 2013). In recent years, the use of EFE as feed

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additives in ruminants has drawn significant interest (Lourenco et al. 2020, Carrillo-Díaz et al. 2022). The majority of research on supplementation of EFE in ruminants are restricted to large ruminants (Reddy et al. 2016) and in growing animals. Zhou et al. (2023) reported that feeding with compound enzyme preparation for 56 days significantly improved the body weight gain but did not affect the antioxidant capacity and immunity of goats. Limited studies have been undertaken to study the impact of enzyme addition in lactating animals particularly in goats. So, the present study was undertaken to evaluate the effect of supplementing exogenous fibrolytic enzymes (EFE) in a pearl millet-based complete pellet on voluntary dry matter intake, milk yield, milk composition, and milk fatty acid profile in lactating Barbari goats.

Materials and Methods

Animal feeding and management

Feeding cum lactation trial was conducted at Animal Nutrition experimental animal farm, ICAR-Central Institute for Research on Goats, Makhdoom, Farah, Mathura India. The institute is located in the semiarid region of India and pearl millet is cultivated in nearby locality in plenty. Two type of complete pellet feed (TMR) was prepared at experimental feed unit of the Institute. Both the complete pellet was having concentrate mixture and pearl millet stovers in 50:50 ratio. In control pellet no enzyme was added (MS) while in treatment pellet (MSE) 20 gm/ quintal of enzymes was added. The weighed quantity of enzyme powder was uniformly mixed with about 1 kg of grounded maize and this one kg grounded maize was again mixed with 9 kg of grounded maize. Later on this 10 kg of premix was uniformly mixed with mineral mixture and salts. This mixture was uniformly distributed and mixed properly during preparation of complete pellet feed. The enzyme was blend of fibrolytic enzymes xylanase, cellulase, hemicellulase and beta-glucanase produced by Solid State Fermentation (SSF) techniques from selected strains of Trichoderma species. Enzyme was purchased from Aum Enzymes, Anand. Twelve lactating Barbari goats (Avg. BW 31.81 kg, Avg. milk yield 1.10 kg in 3-5th lactation) were divided into two groups (CON and SUPP) of six each. Goats were randomly divided on the basis of body weight, milk yield and parity. Feeds were formulated to meet the nutrient requirements of lactating goats as per NRC (2007) and were offered *ad libitum*. CON group of goats was fed MS pellet while SUPP group of goats were fed with MSE pellet. Lactating goats were housed in well ventilated shed and followed standard management practice. The duration of experimental feeding was 90 days (3rdDecember 2024 to 4thMarch 2025). Free water was provided throughout the lactation trial. Goats were provided with measured amount of complete pellet in the morning and refusals were recorded on next day. The dry matter of offered pellet and refusal was estimated and daily dry matter intake was calculated. Body weight of the animals was recorded using digital weighing balance at fortnightly interval to estimate body weight changes.

Chemical analysis of feed

The representative samples of both type of pearl millet stovers based complete pellet were analysed for proximate composition as per AOAC (2006).Organic matter (OM) was determined by ashing at 550æ%C for 5 h. Crude protein (CP) content (N × 6.25) was measured as per the method described by AOAC (2006), method 984.13 (A–D). Ether extract (EE) was estimated using AOAC (2006) method 920.39 (A).Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were discerned by the methods of Van Soest et al. (1991). All the samples were analyzed in triplicates and values are presented as mean with standard error.

Milk yield, composition and fatty acid analysis

Milk yield of each of the goats was recorded for two consecutive days at 0, 30, 60 and 90 days of experimental feeding by individual milking. Digital weighing balance was used to weigh the milk produced in morning (6AM) and evening (4PM). Total of morning and evening milk produced was regarded as daily milk produced. Representative of morning and evening pooled milk samples were analysed for different milk constituents using automatic milk scanner. For analysis of individual fatty acids in milk, fatty acid methyl esters (FAME) of milk samples were prepared as per O'Fallon et al. (2007). For FAME preparation, One ml milk sample was taken in 50ml centrifuge tubes and 0.7 ml of 10N KOH in water and 5.3ml of methanol (Sigma Aldrich) were added. These tubes containing mixture were incubated in a 55 °C water bath for 1.5 hour with vigorous shaking for 5 sec after every 20min. After cooling to room temperature in tap water, 0.58 ml of 24N H_2SO_4 was added. These tubes having mixtures were again incubated at 55 °C for 90 min in a water bath with shaking for 5 sec every 20 min. The tubes were cooled in tap water. Three milliliter of hexane (Sigma Aldrich) was added and the tubes were vortexed for 5 min on a multi-tube vortex. The tubes were centrifuged for 10 min at 2000g and the hexane layer was taken out and placed into Eppendorf tubes stored at -20p C till analysis in the GC-MS/MS analysis. The fatty acid composition of the FAME was determined using GC-MS triple quadrupole (GC-MS TQ8030, Shimadzu Corp., Japan) attached with RestekStabilwax®-MS capillary column (30m× 0.25mm ID × 0.25 μm) and flame ionization detector (FID). Helium was used as a carrier gas at a flow rate of 1ml/min. Both the injector and detector were set at 260 °C. The initial oven temperature was 120p C, held for 5 min, subsequently increased to 240 °C at a rate of 2 ºC min-^{1} and held for 60 min. The split ratio was 30:1. GC-Real time Analysis software was used to run the sample for analysis. Fatty acid methyl standard (Supelco 37) was used for comparing the samples peaks and different fatty acids in milk were identified by comparing their retention time with the standards. They were expressed as a percentage of total fatty acids.

Statistical analysis

The data obtained during the feeding cum lactation trial and analysis of different milk samples were statistically analyzed for

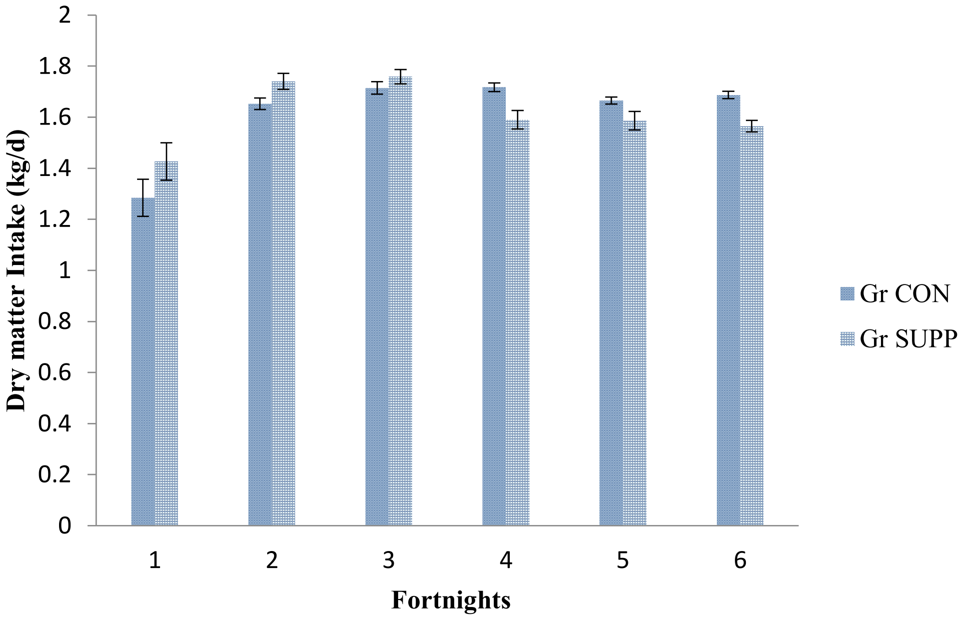

Fig. 1. Daily dry matter intake (kg/d) in different group of lactating goats.

their significance by independent sample t-test (Snedecor and Cochran, 1989). The data has been presented as mean ± standard error. The difference of mean was observed at 5% significance level (p< 0.05). The data was analysed using Statistical Package for the Social Sciences (SPSS) (2012).

Results and Discussion

Chemical composition of feed

Chemical composition (proximate and fibre content) of MS and MSE is presented in Table 1. No significant difference was reported in chemical composition between MS and MSE pellet. However, a lower value of different fibre fractions were reported. The content (%) of Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) was 10.68 and 14.43 lower in complete pellet with enzyme (MSE) as compared to control complete pellet (MS). A lower value of cellulose and hemicelluloses was also reported in MSE pellet.

The mean body weight (kg) of lactating goats at the start of feeding trial was 31.30 and 32.57 which changed to 34.31 and 32.92 in Gr CON and SUPP respectively after 90 days of feeding. There was no (P>0.05) statistically significant difference in body weight at different fortnight in both the groups showing that feed was optimum to meet the nutrient requirement of the lactating goats. Dry matter intake (kg/d) was better in first two fortnights after enzyme supplementation but later on the intake was similar or slightly lower in enzyme supplemented group (Gr SUPP) (Fig. 1).

The average milk yield (g/day) during lactation trial was 888.26 and 811.34for Gr CON and SUPP respectively. The milk production (g/day) at different fortnights (15, 30, 45, 60, 75 and 90 days of experimental feeding) was statistically similar among the groups. However, Kholif et al. (2015) reported increased milk

Table 1: Chemical composition of Pearl millet stovers based complete pellet feed (on dry matter basis)

Attributes (%)	Pearl millet stovers complete pellet (MS)	Pearl millet stovers complete pellet with enzyme (MSE)
Organic matter	90.94±0.06	90.29±0.15
Crude protein	11.11± 0.06	12.20± 0.01
Ether extract	1.36±0.031	0.50±0.031
Total ash	9.06± 0.06	9.71± 0.15
Neutral detergent fibre	43.45±3.88	38.813±4.39
Acid detergent fibre	25.36±3.98	21.70±4.82
Lignin	4.59±0.36	3.71±0.42
Cellulose	23.94±0.39	20.39±0.48
Hemicellulose	18.09±0.37	17.10±0.755

Body weight changes, Feed intake, and milk yield

Table 2: Milk yield and composition in different group of goats at 60 days of experimental feeding

Attributes	Gr CON	Gr SUPP	Sig
Milk Yield (g/day)	872.50±161.48	948.33±64.73	0.246
pH	6.40±0.07	6.28±0.03	0.265
Moisture (%)	84.18±0.43	85.74±0.35	0.449
Total solids (%)	15.82±0.43	14.26±0.35	0.449
Milk fat (%)	6.20±0.39	4.45±0.38	0.857
Milk total Protein (%)	3.77±0.08	3.53±0.18	0.307
Solid Not Fat (%)	10.40±0.21	10.13±0.09	0.214
Ash (%)	0.78±0.05	0.64±0.17	0.081

Values are presented as mean ± SE (n = 6). Differences were considered significant at $p < 0.05$

Table 3: Milk fatty acid profile (%of total fatty acid) in different group of goats at 60 days of experimental feeding

Milk fatty acids	Gr CON	Gr SUPP	Significance
Butyrate (C4:0)	0.29±0.05	0.32±0.03	0.235
Hexanoate (C6:0)	0.89±0.12	0.91±0.05	0.060
Octanoate (C8:0)	1.86±0.38	1.89±0.15	0.192
Decanoate (C10:0)	9.55±0.82	9.51±0.62	0.517
Laurate (C12:0)	5.70±0.36	5.16±0.55	0.284
Myristate (C14:0)	13.59±1.84	11.59±0.34	0.083
Myristoleate (C14:1)	0.26±0.06	0.20±0.02	0.127
Pentadecanoate (C15:0)	1.09±0.10	1.13±0.06	0.401
CIS-10 Pentadecenoate (C15:1)	0.23±0.02	0.28±0.03	0.570
Palmitate (C16:0)	31.65±0.49	30.97±0.10	0.338
Palmitoleate (C16:1)	0.77±0.15	0.68±0.10	0.556
Heptadecanoate (C17:0)	0.89±0.02	0.95±0.02	1.000
Heptadecenoate (C17:1)	0.22±0.02	0.20±0.00	0.269
Stearate (C18:0)	7.12±0.97	7.51±0.36	0.146
t-Vaccenic acid (TVA)	1.61±0.19	1.83±0.14	0.423
CIS-9 Oleate (C18:1c)	19.85±0.79	21.54±0.76	0.299
Linoleate (C18:2Cn6)	2.92±0.19	3.82±0.22	0.949
Linolelaidate (C18:2Tn6)	0.03±0.01	0.05±0.04	0.119
Arachidiate (C20:0)	0.19±0.03	0.20±0.01	0.139
Cis-11-Eicosanoate (C20:1)	0.10±0.01	0.00±0.00	0.059
Linolenate (C18:3n3)	0.13±0.01	0.18±0.01	0.620
Heneicosanoate (C21:0)	0.56±0.05	0.67±0.08	0.474
Gamma-Linolenate (C18:3n6)	0.06±0.00	0.05±0.04	0.000
Behenate (C22:0)	0.12±0.02	0.05±0.04	0.370
Cis-8, 11, 14-Eicosatrienoate C20:3n6)	0.03±0.01	0.01±0.01	0.168
Cis-5, 8, 11,14-Eicosatetraenoate (C20:4n6)	0.33±0.03	0.36±0.01	0.244
Medium chain triglycerides (MCT)	18.28±0.81	17.78±0.67	0.676
Saturated fatty acids (SFA)	73.49±0.87	70.83±0.55	0.444
Monounsaturated fatty acids (MUFA)	23.03±0.80	24.71±0.38	0.304
Polyunsaturated fatty acids (PUFA)	3.49±0.23	4.46±0.22	0.782
Unsaturated fatty acids (UFA)	26.52±0.87	29.17±0.55	0.444
N-6 PUFA	3.36±0.22	4.28±0.21	0.818
N-3 PUFA	0.13±0.01	0.18±0.01	0.620
PUFA/SFA	0.05±0.00	0.06±0.00	1.000
Index of atherogenicity	3.48±0.23	2.83±0.09	0.357
Hypocholesterolemic / Hypercholesterolemic ratio	0.46±0.03	0.55±0.02	0.565
Health- Promoting index	0.29±0.02	0.36±0.02	0.530

Values are presented as mean ± SE (n = 6). Differences were considered significant at $p < 0.05$

yield (g/d) (P < 0.05) with commercial enzyme addition in goats. Similar results were obtained by Titi and Lubbadeh (2004) when the enzymes were fed to goats and sheep. Gado et al. (2009) reported a 7-15% higher in milk production of dairy cows fed fibrolytic enzymes. They explained the improvement as being due to increased nutrient digestibility and microbial protein synthesis. Alsersy et al. (2015) reported the use of enzyme additives improved efficiency of microbial protein synthesis is a result of enzyme action on the forage structural polysaccharides, altering the rate of ruminal degradation of structural carbohydrates and the provision of a suitable ruminally degradable nitrogen source. Moreover, Valdes et al. (2015) reported that the supplementation of exogenous enzymes (10 g/day), improved their DM and OM digestibility with a positive impact on the use of N and microbial protein synthesis.

Milk composition

Effect of adding enzyme in the pearl millet based complete pellet feed on milk yield and composition at sixty days of experimental feeding is presented in Table 2. Milk pH was slightly acidic in both the group and no significant difference was recorded in the milk composition (total solids, total protein, Solid-not fat, Ash) Milk fat was lower in enzyme supplemented group. Kholif et al. (2015) also reported no significant difference in goat milk composition (%) after cellulase enzyme supplementation (P > 0.05). Titi and Lubbadeh, (2004) also reported no effect of feeding enzyme to dairy goats on the concentration of all milk components. The lower fat content of milk from enzyme supplemented group might be due to increased soluble sugar due to fibrolytic enzymes which may have increased more propionic acid in rumen fermentation. Milk fat percentage is related positively to rumen molar percentages of acetic and butyric acids and negatively to that of propionic acid (NRC, 1988). A positive relationship exists between the molar ratio of acetate to propionate and milk fat percentage. Thus, diets that increase propionate production have the greatest effect on milk fat percentage.

Milk fatty acid profile

In the present study the effect of enzyme addition on Pearl millet based complete pellet feed was evaluated. There was no significant effect of addition of enzymes in proportions of individual fatty acids in milk fat. t-Vaccenic acid (TVA) a precursor of conjugated linoleic acid was 1.61 and 1.83 in Gr CON and Gr SUPP respectively being higher in enzyme supplemented goats. A lower proportion of saturated fatty acids and higher proportion of Monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and Unsaturated fatty acids (UFA) were reported after addition of enzymes in the diet. An increase in the poly- and mono-unsaturated FA content and a decrease in saturated FA content may be due to the availability of more soluble carbohydrates and propionic acid production for fatty acids synthesis. There may be shift in short chain fatty acids proportions which could increase precursor availability for fatty acids synthesis. Fatty acid related nutritional indices like N-6 PUFA, N-3 PUFA, PUFA/SFA, Index of atherogenicity (IA), Hypo/Hyper cholesteremic ratio (HH) and Health promoting index (HPI) was better with milk of goats supplemented with enzymes. Kholif et al. (2012) also reported that conjugated linoleic acid (C18:2trans-10,cis12), n-3 fatty acid (C18.3N3) and total unsaturated fatty acids were higher in milk fat of buffaloes supplemented with enzymes. They also reported that Buffaloes fed enzymes (Zad1 and Zad2) had lower proportions of total saturated fatty acids in milk fat compared with control. Gado et al. (2009) suggest that enzyme supplemented diet modified ruminal acetate, propionate and butyrate proportions. Also, enzyme supplemented diet stimulated lactic acid-utilizing bacteria which also produce propionate. Beauchemin et al. (2003) reported that supplementing the diet of feedlot cattle with lactic acid bacteria increased the proportion of propionate and, consequently, decreased the proportion of butyrate in rumen fluid compared with the control. This shift in short chain fatty acids proportions could increase precursor availability for fatty acids synthesis, particularly for dairy cows in early lactation when nutrient intake lags nutrient demand (Eun et al. 2007). Milk fatty acid composition is mostly affected by the type of forage, forage to concentrate ratio, lipids supplementation and starch level, together with their interactions (Lopez et al. 2019). Dietary fatty acids had a great impact in human nutrition due to their role in health and disease conditions so any change in fatty acid profile towards healthier side add the value to milk and its product. Kumar et al (2024) reported that on Moringa based complete pellet feeding to lactating goats there is improvement in milk fatty acid profile towards higher MUFA and better health index. However, supplementation of green azolla has no significant (P<0.05) effect on milk fatty acid profile of goats (Kumar et al. 2022). There was no significant effect of azolla supplementation on different fatty acids, short chain fatty acids (SCFA), saturated fatty acids, mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA) in lactating goats. Milk fatty acids are modified due to change in rumen fermentation pattern which lead to change in precursors of milk fatty acids (Table 3).

Conclusion

Present study concluded that adding of exogenous fibrolytic enzymes in the pearl millet stover-based complete pellet diet had no significant effect on intake, milk production and milk constituents but tend to shift the milk fatty acid towards healthier fatty acids in lactating Barbari goats.

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