RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 44 Issue 2: 299-305 (April 2025)

Enhancing Milk Fat Synthesis in Murrah Buffalo with Inclusion of Fodder Oat Through-Induced Volatile Fatty Acid Production (VFA)

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10.18805/ajdfr.DR-2273

ABSTRACT

Background: Forage quality plays a critical role in the productivity and health of dairy buffalos, directly influencing milk yield, nutrient utilization and overall animal performance. As livestock diets evolve, there is growing interest in optimizing feed composition to improve digestibility and fermentation efficiency. Fodder varieties like OL 10, dual-purpose rabi cereal, multi-cut nature, rapid regeneration capacity, quick growing habit, high palatable, succulent and nutritious fodder characteristics it as more suitable for animal sustainability.

Methods: The study assessed the effect of substituting concentrate mixture with fodder oats on milk yield and composition in Murrah buffalo during the *rabi* season of 2023-2024, using a 50-days feeding trial with six buffalo divided into Group 1: Control group (CG) and Group 2: Treatment group (TG)

Result: The study evaluated the nutritional impact of OL 10 forage on dairy buffalo performance compared to a concentrate mixture. OL 10 showed a crude protein digestibility of 76.49% and dry matter digestibility of 61.30%. Its nutrient profile included 46.88% ADF, 65.28% NDF and 12.67% non-fibre carbohydrates. Milk yield in buffalo fed OL 10 increased by 12.0% (5.34 kg/day) compared to those on the concentre mixture (4.77 kg/day), with a 5.2% rise in milk fat content (7.12% vs 6.77%). The feed conversion ratio (FCR) improved, with dry matter efficiency increasing by 52.1% and crude protein conversion improving by 47.6%. Rumen fermentation was enhanced, showing a 6.2% rise in total volatile fatty acid (VFA) production, particularly acetate (11.12 mmol/L). Additionally, buffalo on the OL 10 diet gained 12.4 kg in body weight, 22.4% higher than the control group. Overall, OL 10 is an efficient forage that improves productivity, nutrient utilization and animal health.

Key words: Crude protein degradability, Lactation production efficiency, Nutrient utilization efficiency, OL 10 cultivar, Ruminant performance.

INTRODUCTION

The livestock production and agricultural sector are intrinsically linked, mutually dependent and along essential for ensuring global food security. The livestock sector is a substantial sub-sector of agriculture in the Indian economy. An average annual growth rate (CAGR) of 13.36% was recorded in the Livestock Sector during the year 2014-15 and 2021-22. Between 2014-15 and 2021-22, the livestock sector share of the total Gross Value Added (GVA) of agriculture and its associated business increased from 24.38% to 30.19%. As per 20th Livestock Census in the nation recoded over 303.76 million bovines (cattle, buffalo, Mithun and yak) (Ministry of Fisheries, Animals Husbandry and Dairying, 2023). India ranks 1st worldwide in terms of total milk production, annual milk production in the country for the year 2022-23 is 230.58 million tons (FAOSTAT, 2022), with almost 50% of total milk production the country (DAHD, 2017-2018). Moreover, India represents almost 70% of the global buffalo milk production. Therefore, buffalo are the primary dairy animals in India, whereas buffalo provide the large share of the global milk supply. In Punjab and Haryana, Murrah is often referred to as “Black Gold”, emphasizing the significance of this breed for farmers. The Murrah buffalo is characterised by its tightly curled small horns.

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How to cite this article: Sai, E.J., Rajeev, Kumari, M.S. and Devi, N.D. (2025). Enhancing Milk Fat Synthesis in Murrah Buffalo with Inclusion of Fodder Oat Through-Induced Volatile Fatty Acid Production (VFA). Asian Journal of Dairy and Food Research. 44(2): 299-305. doi: 10.18805/ajdfr.DR-2273.

Submitted: 21-09-2024 **Accepted:** 29-11-2024 **Online:**31-12-2024

The primary breeding areas of Murrah are in the districts of Hisar, Rohtak, Gurgaon, and Jind in Haryana and Delhi. Nowadays, this animals breed is being raised in almost all region of the nation. The milk yield of Murrah ranging from 1,000 to 2,000 kg (avg 1,700) each lactation, with an average fat content of 6.9 to 8.3% (avg 7.3%) (Yadav *et al*., 2022).

Oats (*Avena sativa* L.) area significant and nutritious fodder crop cultivated in India during the rabi season (Dangi Swotantra, 2020). This crop can be grown throughout the

northern, central and western parts of the country. In worldwide cereal production, the oat crop ranks 6th, behind wheat, maize, rice, barley, and sorghum. The cultivation of fodder oat in India covers an estimated total area of over 10.0 lakh hectares. Uttar Pradesh has the largest area at 34%, followed by Punjab at 20%, Haryana at 9% and Madhya Pradesh at 6% (Chauhan *et al*., 2016). The nutritional components of oat green fodder include fat, crude protein (9-10%), vitamin B, Phosphorus and Iron. An Oat kernel is composed of 66% carbs, 11% dietary fibre, 4% beta-glucan, 7% fat and 16-17% protein. Furthermore, they are rich in phosphorus, potassium, magnesium and the B complex vitamins. The generation after the selective harvesting and the establishment of superior-grade herbage (Kamangadazi *et al*., 2016). Due to its higher palatability and texture in comparison to wheat and barley, all animals quickly ingest its green feed (Hameed *et al*., 2014).

The fat content of milk is a fundamental component of milk and is a crucial measure for assessing the quality of milk. The content of milk fat usually ranges from 3%-5%, Triglycerides, which constitute about 98% of milk fat, are the main primary constitute. The fatty acids (FAs) are essential components of milk fay and are associated with a variety of potential benefits and risks to human health. Patel (2023) revealed rumen microbiological activity and external absorption as the primary sources for milk fatty acid production. The rumen epithelial cells receive and convert butyric acid produced by microbial fermentation into β-hydroxybutyric acid. Acetic acid and β-hydroxybutyric acid are then transferred to the mammary gland for the synthesis of short and medium chain fatty acids. Although dietary lipids adipose tissues are the primary sources of long chain fatty acids (Buitenhuis *et al*., 2019). Several factors affect the milk fat content in milk, such as the rumen microbiome, food feeding management, health condition, session and the stage of lactation in dairy cattle's (Buitenhuis *et al*., 2019). Bacteria present in the rumen of dairy buffalos carry out fermentation to break down plant fibres into volatile fatty acids (VFAs) (Patil *et al.,* 2018). Research show that rumen bacteria significantly influence rumen fermentation, as well as the variability of milk production and milk composition (Bainbridge *et al.,* 2016). Hassan *et al.* (2020) shown that the alternation of diet might increase the relative abundance of Firmicutes and reduce the relative abundance of Bacteroidetes, resulting to an enhancement in milk fat synthesis. Thus, the main objective of this study was to determine (i) To enhance the milk synthesis in Murrah buffalo during winter by optimizing dietary fibre intake to boot VFA production. (ii) To enhance rumen health and fermentation efficiency by incorporating specific dietary fibres.

MATERIALS AND METHODS

The study was conducted during the rabi season of 2023-2024 at the Research Farm of Lovely Professional University in Jalandhar, Punjab. LPU is situated at 31°24′39″N latitude, 75°69′54″E longitude, at an elevation of 245 meters above sea level. The fodder oats were sown in different plots every 15 days to ensure they remained at the harvesting stage that occurs 45 days following seeding. The study used six Murrah buffalo, with an average bodyweight weight of 320.80 kg, a milk output of 4.71 Liters per day, a lactation number of 3.5 and an average lactation duration of 113 days. These buffalo were divided into two groups: control group (CG) and treatment group (TG) each consisting of three animals. The selection was based on body weight, milk yield, lactation days. All the standard protocols of deworming and vaccination were followed. At milking time, the concentrate mixture was provided in two quantities according to the thumb rule approach, which involves feeding 1 kilogram of maintenance food long with 1 kg of concentrate for a milk production of 2.5 kg. The concentrate mixture's ingredient composition was as follows: crushed maize (Makki Dala) (35%), wheat bran (Patri/choker) (20%), rice polish (Bugdu) (3%), cotton seed cake (Binolla Khal) (32%), gram husk (Chilka) (5%), gram husk with grain parts (Fandar/Khanda) (2%) and salt (1%). Animals in the control group were offered daily 4 kg of crude meal (CM) and 1 kilogram of green fodder oats daily. In the treatment group, half of the CM provided in the control group was substituted with 20 kg of green fodder oats daily. Therefore, animals in the treatment group were given 2 kg of CM along with 20 kg of green fodder oats. The nutritional needs of the animals in both groups were fulfilled (Ranjhan, 1998) and a 50-day feeding research was carried out. The essential nutritional needs of animal groups were fulfilled. Equally split dosages of the daily ration were provided twice daily, while clen drinking water was made accessible three times daily. The body weight (BW) was measured every two days for two consecutive days before feeding food and water in the morning. The average results were considered as the true BW. Two times a day, at 7 AM and 4 PM, the animals were milked manually. The milk production was recorded daily at each milking using a digital balance. Weekly, milk samples were obtained from each buffalo following meticulous mixing of the milk for a thorough analysis. The soil in the experimental field had a pH of 7.25, an electrical conductivity of 0.321 ds/m, organic carbon content of 0.61%, 212.25 kg/ha o available nitrogen, 28.45 kg/ha of available phosphorous and 198.24 kg/ha of available potassium. Recommended dose of FYM was applied @ 10.0 t/ha at the time of sowing (as per respective treatment). The recommended dosages of nitrogen (N), phosphorous (P_2O_5) and potassium (K_2O) were applied at rates of 120 kg ha^{-1}, 40 kg ha^{-1} and 40 kg ha^{-1} respectively, using urea, SSP and MOP. An initial dose of one third of nitrogen (N) and the whole dosage of P_2O_5 and K_2O have been given as a basal. The remaining two thirds of nitrogen were supplied in two equal amounts at 32 days after sowing (DAS) and three days after the first cut. A seed rate of 70-80 kg ha^{-1} was applied and treated with Bavistin 50% WP at a rate of 2 g per kg seeds, followed by PGPR at a rate of 120 ml ha^{-1} of seeds.

Statistical analysis

Analysed for proximate principles were the feeds and fodder provided (AOAC, 2000). Analysis of milk composition, including fat and solid not fat (SNF), was conducted using milk analyser. The formula recommended by NRC (1989) was used to compute the 4% fat corrected milk (FCM) yield. The feed conversion ratio was determined by considering the weight of intake per kilogram of FCM produced each day. The data were statistically evaluated for the significance test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Quality parameters of OL 10

A metabolic study was undertaken by the Department of Animals Nutrition at GADVASU, Ludhiana to assess the quality characteristics of the OL 10 and check forms (Table 1). The present study provides an analysis of the quality characteristics of the fodder variety OL 10, with a specific emphasis on its nutritional content and digestibility. The analysis reveals that OL10 is a forage rich in nutrients, including a well-balanced combination of fibres, namely acid detergent fibre (46.88%), neutral detergent fibre (65.28) and cellulose (31.98%), along with carbohydrates, comprising non-fibre carbohydrates (12.67%) and total carbohydrate, (80.12%). Furthermore, it has a significant crude protein digestibility of 76.49% and dry matter Digestibility of 61.30%, revealing that a significant amount of the nutrients in the forage by its ash level of 8.54% and organic matter content of 90.33%. These parameters suggest that OL 10 is a valuable feed option, capable of supporting the nutritional needs of animals and promoting their overall health and productivity.

Table 1: Quality parameters of fodder oats OL10.

Trait (%)	OL 10
Ash content	8.54
Acid detergent fibre-ADF	46.88
Cellulose	31.98
Crude protein digestibility	76.49
Dry matter digestibility	61.30
Hemi cellulose	18.49
Neutral detergent fibre-NDF	65.28
Non-fibre carbohydrate	12.67
Organic matter	90.33
Total carbohydrate	80.12

Table 2: Chemical composition (on % DM basis) of feeds and fodder.

Parameters	Concentrate mixture	Fodder oats
Dry matter	89.54	31.88
Crude protein	23.21	9.02
Ether extract	5.45	2.86
Crude fibre	7.79	27.89
Nitrogen free extract	54.39	56.44
Total ash	6.28	10.21
Acid insoluble ash	1.21	1.63

Comparative analysis of the chemical composition of concentrate mixture and fodder oats on a dry matter basis

The data in (Table 2) indicate significant differences in the chemical composition of the concentrate mixture and fodder oats, which have a direct effect on their nutritional value in Murrah buffalo diets. The concentrate mixture had a more substantial dry matter (DM) content of 89.54% compared to fodder oats (31.88%), which is typical given the higher moisture content in green forages like oats. This lower dry matter (DM) percentage in fodder oats indicates thar larger intake quantities are necessary to fulfil the same nutritional needs as concentrate mixture, which are denser in dry matter. Regrading crude protein (CP), the concentrate mixture showed a significant greater value of 23.21% in comparison to fodder oats (9.02%), indicating a 61.1% decrease in protein in the fodder oats. This is according to past studies indicating that concentrate, traditionally composed of protein-rich components such as oilseed meals, had greater crude protein levels compared to forages (Mc Donald *et al.,* 2011). The lower protein content in fodder oats suggest the needs for protein supplementation when oats are the primary forage in the diet, especially for lactating buffalos with high protein demands. The ether extract (EE), which represents the fat content, was also higher in the concentre mixture (5.45%) compared to fodder oats (2.86%), a 47.5% difference. This is significant because fats provide a concentrated source of energy. While fodder oats offer less energy from fats, their fibre content plays a critical role in maintaining rumen health. A major difference was observed in crude fibre (CF) content, with fodder oats having 27.89% fibre, compared to just 7.79% in the concentrate mixture. The observed rise of 257.9% in fibre content in fodder oats highlights its significance as bulk feed abundant in structural carbohydrates that contribute to the overall functionally and fermentation of the rumen. High fibre content in forage such as fodder oats is advantageous for sustaining the acetate synthesis required for milk fat formation (Van Soest, 1994). The nitrogen-free extract (NFE), which consists of carbohydrates excluded from fibre, was slightly higher in fodder oats (56.44%) compared to the concentrate mixture (54.39%). Although this difference is small (around 3.8%), it suggests that fodder oats contain a same amount of easily metabolised carbohydrates, which helps maintain the overall energy balance in the diet. The total ash content, which represents the minerals content of the feed, was significant greater in fodder oats (10.21%) than in the concentrate mixture (6.28%), representing a 38.5% rise. This finding implies that fodder oat may provide a higher concentration of intrinsic minerals, therefore enhancing the total minerals value of the diet. Nevertheless, it is necessary to do more comprehensive analysis of certain

minerals to ascertain the need of supplementing. Furthermore, the acid-insoluble ash (AIA), which represents the indigestible portion of the ash (such as silica), was slightly higher in fodder oats (1.63%) compared to the concentre mixture (1.21%).

Assessment of rumen fermentation dynamics in concentrate mixture and fodder oats: A comparative study of volatile fatty acids and nitrogen parameters

Table 3 presents the rumen fermentation parameters observed in the two dietary groups concentrate mixture and fodder oats. The results indicate that the diet containing fodder oats led to a marginal increase in rumen volatile fatty acid (VFA) production compared to the control concentrate mixture diet. Specifically, acetate concentrate mixture were 5.9% higher in the fodder oats group (11.12 mmol/L) compared to the concentrate mixture (10.50 mmol/L), aligning with findings from previous studies that suggest forage-based diets promote acetate production in the rumen (McDonald *et al.,* 2011). Butyrate concentrations were 5.0% higher in the fodder oats group (5.04 mmol/L) compared to the concentrate mixture (4.80 mmol/L). In comparison to the concentrate mixture (4.20 mmol/L), the fodder oats group showed an 8.1% rise in propionic acid concentrates, attaining 4.54 mmol/L. This reflects the potential of forage diets to enhance VFA production as a function of increase fibre fermentation (Gabler and Heinrichs, 2003). The total VFA concentration was 6.2% higher in the fodder oats group (21.31 mmol/L) compared to the control concentrate mixture group (20.05 mmol/L), which is consistent with reports suggesting that higher in the fodder oats diet (2.71) compared to concentrate mixture diet (2.50), indicating an obvious progression towards higher acetate production which is a reliable indicator of enhanced fibre fermentation. The significance of this ratio is due to its correlation with the economic efficiency of energy use in ruminants (Van Soest, 1994). Furthermore, the pH levels in the concentrate mixture group (6.6) were slightly lower than those in the fodder oats group (6.7), both being within the ideal range for preserving rumen function. The concentrate of ammonia nitrogen (NH_3-N) was comparable in both groups, including slightly higher values in the fodder oats group (10.71mg/dL), which represents 2.0% increases compared to the concentrate mixture group (10.50 mg/dL). Concentrations of NH_3-N serve as a reliable measure of protein breakdown and use in the rumen, indicating that there is no significant variation in nitrogen metabolism between the two diets (Erdman *et al*., 1986).

Impact of concentrate mixture and fodder oats on milk yield, composition, feed efficiency, and body weight changes in dairy buffalo

Table 4 presents a comparison of the impacts of two concentrate combination diets and fodder oats on milk production, milk composition, feed conversion ratio and changes in body weight in dairy buffalos. The integration of fodder oats into the diet led to significant improvements in both milk production and composition. The daily milk production of Murrah buffalo on fodder oats was 5.34 kg, indicating a 12.0% increase compared to the 4.77 kg/day produced by buffalo fed on the concentrate mixture. Additionally, the fat-corrected milk (FCM) yield was 10.2% higher in the fodder oats group (4.87 kg/day) compared to the control (4.42 kg/day). The observation increases in milk yield agree with previous research that have shown that diets abundant in fermentation fibre, such as fodder oats, can improve the energy availability for lactating buffalos, leading to higher milk production (McDonald *et al.,* 2011). In terms of milk composition, the fat percentage in the group fed with fodder oats was significantly greater (7.12%) tan in the group fed with a concentrate mixture (6.77%), suggesting a 5.2% rise in at content. This increase in milk fat is likely linked to the higher acetate production seen in the fodder oats diet, as acetate plays a crucial role as a precursor for the synthesis of new fat in the mammary

Table 3: Composition of rumen fermentation parameters in fodder oats percentage group.

Parameters	Concentrate mixture	Fodder oats
	VFA concentration, mmol/L	
Acetate	10.50	11.12
Butyrate	4.80	5.04
Isobutyric acid	0.55	0.61
Propionic acid	4.20	4.54
Acetate to propionate ratio	2.50	2.71
Total VFA	20.05	21.31
pH	6.6	6.7
NH_3-N, mg/dL	10.50	10.71
	Molar proportion, %	
Acetate	0.50	0.52
Butyrate	0.22	0.23
Isobutyric acid	0.015	0.02
Propionic acid0.20	0.21	

glands (Fig1) (Van Soest, 1994). However, the solids-not-fat (SNF) percentage showed a slight decrease in the fodder oats group (9.01%) compared to the control group (9.24%). A slight decrease in SNF may be attributed to changes in protein and lactose production, while the overall effect on milk quality is negligible. The feed conversion ratio (FCR) showed significant improvements in the fodder oats group. The crude protein (CP) conversion ratio decreased by 47.6%, from 1.24 kg feed/FCM in the concentrate mixture group to 0.65 kg feed/FCM in the fodder oats group, indicating more efficient protein utilization. Furthermore, the conversion ratio of digestible crude protein (DCP) declined by 34.3% (0.99 vs. 0.65 kg feed/FCM). The conversion ratio of total digestible nutrients

Table 4: Effect on milk yield, milk composition and feed conversion ratio.

Parameters	Concentrate mixture	Fodder oats
	Milk yield	
Milk yield (kg/day)	4.77	5.34
4.0% Fat corrected milk (kg/day)	4.42	4.87
	Milk composition	
Fat (%)	6.77	7.12
SNF (%)	9.24	9.01
	Feed conversion ratio (kg feed/4% FCM kg milk yield)	
CP	1.24	0.65
DCP	0.99	0.65
TDN	4.00	4.66
DM	4.78	2.29
	Body weight changes (kg)	
Initial body weight	314.65	330.14
Final body weight	324.78	342.54
Net gain body weight	10.13	12.4

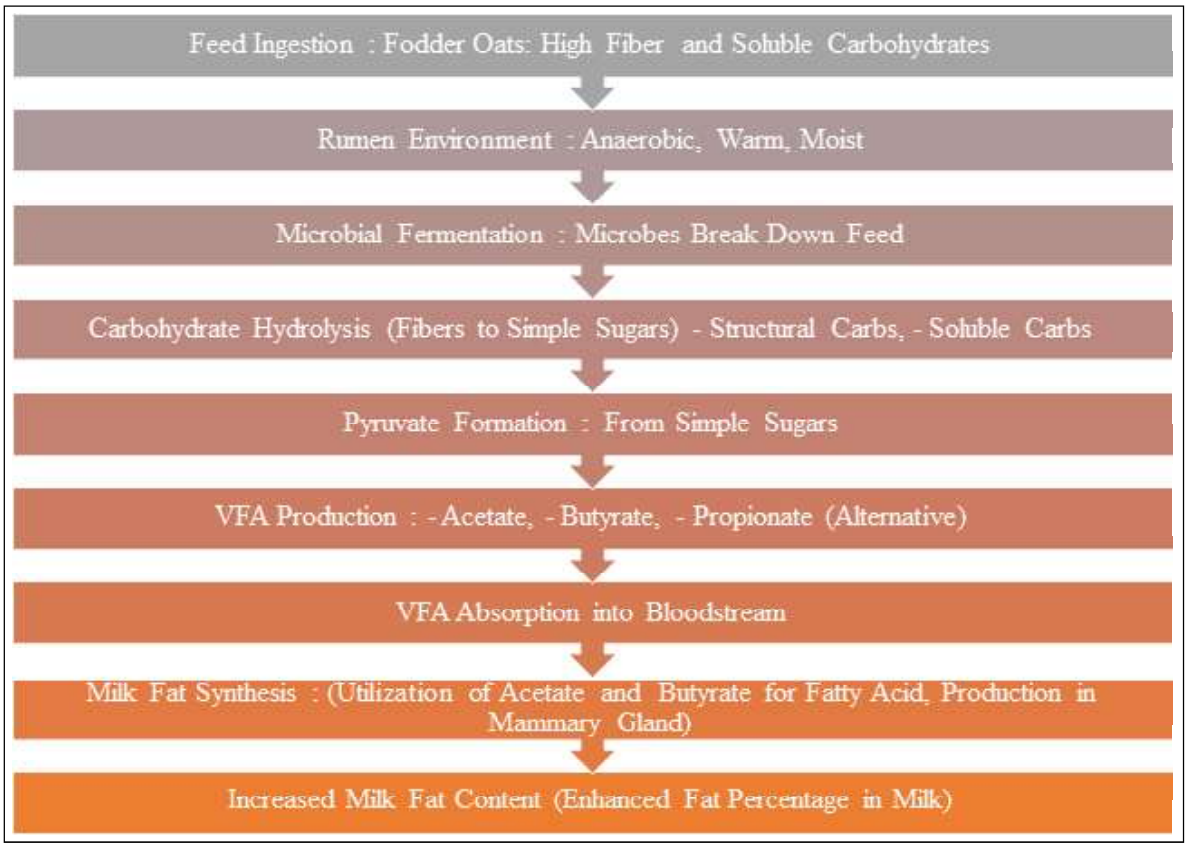


Fig 1: Flow chart detailing the process of increasing milk fat content through the inclusion of fodder oats.

(TDN) was 16.5% higher in the group fed with fodder oats (4.66 kg feed/FCM) compared to the concentrate mixture (4.00 kg feed/FCM). These findings indicate that while more nutrients were necessary to sustain increased milk production, the rise in milk yield rationalizes the increased intake of TDN. Notably, the conversion of dry matter (DM) was much more effective in the group using fodder oats. Specifically, there was a decrease of 52.1%, from 4.78 kg feed/FCM in the concentrate group to 2.29 kg feed/FCM in the concentrate group to 2.29 kg feed/FCM. This significant increase in dry matter efficiency emphasizes the improved digestibility of the fodder oats diet and its capacity to sustain lactation with a reduced amount of total feed. Regarding body weight changes, Murrah buffalo in the fodder oats group, where buffalo gained 10.13 kg (314.65 kg to 324.78 kg), this indicates a weight gain that is 22.4% more. The improved body weight gain in the fodder oats group reflects better nutrient utilization and energy balance, likely due to the enhanced fermentation of the fibrous components in fodder oats, which provide a consistent supply of energy over time (Gabler and Heinrichs, 2003).

CONCLUSION

In conclusion, the metabolic research carried out on OL 10 forage provides evidence of its superior nutritional value and efficacy as a feed alternative for dairy cattle's. Superior nutritional content of OL 10 was observed, characterized by a well-balanced fibre profile (ADF, NDF and cellulose) and a high level of crude protein digestibility (76.49%). The consumption of the fodder greatly enhanced the digestibility of dry matter (61.30%), resulting in more effective uses of nutrient by cattle. Relative to concentrate mixes, OL 10 improved rumen fermentation, namely by increasing volatile fatty acid synthesis, leading to a significant rise in acetate, butyrate and propionic acid concentration. This change in rumen fermentation resulted in a 12.0% rise of milk production and a 5.2% expansion in milk fat content after correcting for fat. Moreover, the improved feed conversion ratio (FCR) and body weight gain in buffalo fed OL 10 underline its potential as a highly efficient dietary option. Overall, OL 10 presents a nutritionally advantageous and economically viable forage for enhancing dairy buffalo productivity.

ACKNOWLEDGEMENT

The author greatly acknowledges Lovely Professional University (LPU), Jalandhar, Punjab, for providing facilities to conduct research during Ph.D. programme at Division of Agronomy, and School of Agriculture (SOA).

Disclaimers

The opinions and conclusion outlined in this article reflect those of the author and should not be considered as representing the perspectives of their affiliated institutions. While the authors have ensured the accuracy and completeness of the information presented, they disclaim any responsibility for direct or indirect damages that may result from the application of this content.

Informed consent

All experimental procedures involving animals were conducted in accordance with protocols approved by the University's Animals Care Committee, ensuring compliance with ethical standards for animal's care and handling.

Conflict of interest

The authors declare no conflict of interest.

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