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Effect of fibrolytic enzymes treated soybean straw based total mixed rations on haemato-biochemical parameters in red Kandhari calves

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Abstract

This study evaluated the effect of fibrolytic enzyme-treated soybean straw (SBS) on hematobiochemical parameters in Red Kandhari calves when included in total mixed rations (TMR). Eighteen 8±2 months old calves, weighing 62±2 kg BW, were divided into three groups (S₀, S₁, S₂; n = 6 each) using a completely randomized design over 90 days. The TMRs were isocaloric and isonitrogenous: S₀ included 50% jowar kadbi (dry roughage), 20% green fodder (DHN-6), and 30% balanced concentrate mixture. S₁ replaced part of the dry roughage with 37.5% untreated SBS, S₂ contained 37.5% Exogenous fibrolytic enzyme (EFE) treated soybean straw (SBS) (exoglucanase 80U/g, endoglucanase 190U/g, xylanase 70U/g; applied at dose of 300g/tonne). Blood samples were collected at the end of the experimental trial to analyze hemoglobin (Hb), packed cell volume (PCV), glucose, total protein, albumin, globulin, albumin: globulin (A: G) ratio, and blood urea nitrogen (BUN). There were no significant differences ($p>0.05$) were found among groups for any parameter, and values stayed within normal physiological ranges: Hb: 9.72-10.63 g/dL; PCV: 32.89-35.36%; glucose: 66.80-74.08 mg/dL; total protein: 6.47-6.75 g/dL; albumin: 2.79-2.95 g/dL; globulin: 3.68-3.83 g/dL; A:G ratio: 0.75-0.79; BUN: 20.03-20.50 mg/dL. These results showed that including EFE-treated SBS up to 37.5% as dry roughage in TMR maintains hematobiochemical balance, indicating it can be safely used as a sustainable feed resource without negative health effects.

Keywords: Soybean straw, exoglucanase, endoglucanase, xylanase, Jowar Kadbi

Introduction

The livestock sector is vital to India's agricultural economy. It supports the livelihoods of over 80 million rural households and contributes about 5.5% to the national Gross Value Added (GVA) and 31% to agricultural GVA (BAHS, 2025) ^[1]. However, the sector still faces a growing gap between the demand and supply of quality feed and fodder. Recent estimates show a deficit of 11% to 32% in green fodder and 23% in dry fodder, especially in the western zone, including Maharashtra, where dry fodder availability is short by up to 43.5% (Roy *et al.* 2019) ^[2]. Dry fodder in this area is mainly derived from crop residues of food grains, supplemented by forest resources, byproducts such as groundnut and sugarcane residues, including kitchen, horticultural, or farm waste.

Soybean (*Glycine max* L.) is one of the fastest-growing oilseed crops in India. It covered more than 13.5 million hectares in the 2024-25 season, with Maharashtra alone accounting for almost 39% of the national acreage (USDA-Foreign Agricultural Service, 2024) ^[3]. After harvesting the seeds, this crop leaves behind a large amount of straw, which is an abundant, low-cost agricultural by-product. Although soybean straw is easily available, it has limited nutritional value for ruminants. It is coarse and fibrous. The plant cell walls usually contain 35% to 50% cellulose, 20% to 35% hemicellulose, and 10% to 25% lignin based on dry matter (Sticklen, 2008) ^[4]. The strong bond between cellulose, hemicellulose, and lignin restricts microbial access and enzymatic breakdown in the rumen. This results in low voluntary intake, poor digestibility, and suboptimal animal performance.

The ongoing fodder deficit and environmental problems from stubble burning create an urgent need for viable alternative feed resources. These should be locally available, cost-effective, and nutritionally improved.

Soybean straw meets this need, provided its limitations can be addressed through effective pre-treatment. Among the various methods studied, supplementation with exogenous fibrolytic enzymes (EFE) has proven to be a safe, environmentally friendly, and cost-effective approach. EFE includes cellulase (endoglucanase, exoglucanase) and xylanase activities, which partially break down structural polysaccharides before ingestion. This process increases the surface area for microbial colonization, improves fiber degradability, and boosts the release of fermentable carbohydrates in the rumen (Beauchemin *et al.* 2003; Yang *et al.* 2024) [5, 6].

Hematobiochemical parameters serve as critical indicators of animal health, nutritional status, and metabolic function in ruminants. Haemoglobin (Hb) and packed cell volume (PCV) reflect oxygen-carrying capacity and hydration status, while serum glucose, total protein, albumin, globulin, and albumin: globulin (A: G) ratio provide insights into energy metabolism, protein synthesis, and immune competence. Blood urea nitrogen (BUN) indicates nitrogen balance and renal function. In growing calves, deviations in these parameters can signal nutritional imbalances, particularly from high-fiber diets like crop residues, which may limit nutrient absorption if not properly processed. This study aimed to assess the effects of EFE-treated soybean straw (replacing 75% of cereal fodder) in total mixed rations on blood biochemical parameters in growing Red Kandhari calves.

Materials and Methods

Experimental Design and Animals

Eighteen healthy growing Red Kandhari calves (initial BW 62±2kg; age 8±2 months) were selected from the Livestock Farm Complex, College of Veterinary and Animal Sciences (COVAS), Parbhani, Maharashtra Animal and Fishery Sciences University (MAFSU), Nagpur, India. Calves were randomly assigned to three groups (n = 6 per group; male:female ratio 1:1) in a completely randomized design (CRD). The experiment lasted 90 days, with a 15-day adaptation period.

Housing and Management

Calves were housed in well-ventilated sheds under hygienic conditions and stall-fed throughout the trial. They were dewormed with albendazole and ivermectin before the trial. Fresh water was provided ad libitum.

Experimental Diets and Feeding

Total mixed ration (approximately 12.3% CP; roughage: concentrate ratio 70:30 on DM basis) was formulated to meet ICAR (2013) nutrient requirements. The control group (S₀) received TMR with 50% dry fodder (Jowar kadbi), 20% green fodder (DHN-6), and 30% balanced concentrate mixture. In S₁, 75% of Jowar kadbi was replaced with untreated SBS, 20% green fodder (DHN-6), and 30% balanced concentrate mixture. In S₂, 75% of Jowar kadbi was replaced with treated EFE (exoglucanase 80 U/g, endoglucanase 190 U/g, xylanase 70 U/g) at 300 g/tonne SBS on a dry matter basis, 20% green fodder (DHN-6), and 30% balanced concentrate mixture. Fibrolytic enzymes procured from Department of Animal Nutrition, Nagpur Veterinary College, Nagpur. TMR was offered twice daily (10:00 AM and 6:00 PM). Daily feed intake was recorded by weighing refusals.

Table 1: Percent ingredient composition of concentrate mixture

Feed Ingredients	Percentage
Gram remnants	20
Jowar remnants	15
Maize grain	29.6
Soya Doc	31.6
Mineral mixture	2
Salt	1
DCP	0.8

Enzyme treatment of soybean straw

The enzymatic treatment of soybean straw was carried out by using a fibrolytic enzyme cocktail in powdered form consisting of cellulase and xylanase. The enzymes were applied @ 300 g per ton of soybean straw (equivalent activity: Exoglucanase 80 U/g, Endoglucanase 190 U/g, and Xylanase 70 U/g). The required quantity of powdered enzyme was weighed accurately and mixed thoroughly with soybean straw during the preparation of the total mixed ration (TMR), ensuring uniform distribution of the enzymes.

Hematobiochemical Parameters

At the end of the experiment, blood samples were taken from each calf, totaling six calves per treatment. The samples were collected from the jugular vein in tubes without anticoagulant; they were allowed to clot for 3 hours. Serum was separated through centrifugation at 3000 rpm for 5 minutes and stored at -20 °C until analysis.

Haemato-biochemical parameters were measured using commercial diagnostic kits and a clinical analyzer:

- **Hemoglobin:** Acid-hematin method (Jain, 1986) [7].
- **Packed Cell Volume:** Wintrobe microhaematocrit method (Jain, 1986) [7].
- **Serum Total Protein:** Biuret method (Kaneko, 1980) [8].
- **Serum Albumin:** BCG (bromocresol green) end point method (Dumas *et al.* 1971) [9].
- **Serum Globulin:** Serum globulin was calculated by subtracting serum albumin from total protein.
- **A: G Ratio:** Albumin/Globulin
- **Blood glucose:** GOD-POD method (Trinder, 1969) [10].
- **Blood Urea Nitrogen:** Berthelot method (Kaneko *et al.* 2008) [11].

Statistical Analysis

Data were analyzed using completely randomized design (CRD) with treatment as the factor using SPSS software package (version 30.0). Means were compared using Duncan's multiple range test. Differences were considered significant at $p < 0.05$ and highly significant at $p < 0.01$.

Results

Haemato-biochemical Parameters

Haemoglobin concentration did not vary significantly among treatments ($P = 0.175$). Values recorded were 10.63±0.32 g/dL in S₀, 9.72±0.22 g/dL in S₁ and 10.23±0.42 g/dL in S₂. Packed cell volume also showed no significant differences across groups ($P = 0.111$). The recorded values were 32.89±0.87% in S₀, 33.66±0.98% in S₁ and 35.36±0.39% in S₂.

Table 2: Haemato-biochemical parameters in Red Kandhari Calves

Parameter	S0	S1	S2
Haemoglobin (g/dL)	10.63±0.32	9.72±0.22	10.23±0.42
PCV (%)	32.89±0.87	33.66±0.98	35.36±0.39
Glucose (mg/dL)	74.08±3.82	72.44±5.78	66.80±2.94
Total Protein (g/dL)	6.47±0.06	6.75±0.14	6.67±0.10
Albumin (g/dL)	2.79±0.02	2.95±0.07	2.83±0.05
Globulin (g/dL)	3.68±0.06	3.80±0.17	3.83±0.15
A: G Ratio	0.76±0.02	0.79±0.05	0.75±0.04
BUN (mg/dL)	20.50±1.30	20.03±2.11	20.35±1.00

Values are means±SEM. Means superscripted with different superscripts within a row differ significantly from each other ($p < 0.05$).

Serum glucose levels were statistically similar among the three treatments ($P = 0.479$). Mean concentrations were 74.08±3.82 mg/dL in S₀, 72.44±5.78 mg/dL in S₁ and 66.80±2.94 mg/dL in S₂, indicating stable blood glucose homeostasis. Total serum protein concentration remained unaffected by dietary treatments ($P = 0.205$). The values were 6.47±0.06 g/dL in S₀, 6.75±0.14 g/dL in S₁ and 6.67±0.10 g/dL in S₂. Albumin levels did not differ significantly ($P = 0.126$). The observed values were 2.79±0.02 g/dL in S₀, 2.95±0.07 g/dL in S₁ and 2.83±0.05 g/dL in S₂. Globulin concentration was statistically comparable across groups ($P = 0.690$), with means of 3.68±0.06 g/dL in S₀, 3.80±0.17 g/dL in S₁ and 3.83±0.15 g/dL in S₂. Albumin: Globulin (A: G) ratio showed no significant variation ($P = 0.767$). The ratios recorded were 0.76±0.02 in S₀, 0.79±0.05 in S₁ and 0.75±0.04 in S₂. Blood urea nitrogen (BUN) levels were also statistically similar among treatments ($P = 0.975$). The values were 20.50±1.30 mg/dL in S₀, 20.03±2.11 mg/dL in S₁ and 20.35±1.00 mg/dL in S₂. All recorded values stayed well within the normal physiological ranges for growing red kandhari calves.

Discussion

Haemato-biochemical Parameters

The results of this study demonstrated that supplementation of exogenous fibrolytic enzymes (EFE) in soybean straw-based total mixed rations (TMR) exerted no adverse effect on haemato-biochemical parameters in growing Red Kandhari calves. Inclusion of 37.5% EFE-treated soybean straw (S₂) maintained all haematological and biochemical indices within normal physiological ranges and statistically similar to the control (S₀) and untreated soybean straw (S₁) groups, indicating that the enzyme treatment did not induce metabolic stress, anaemia, hepatic dysfunction or renal impairment.

Haematological Parameters

Haemoglobin (Hb) and packed cell volume (PCV) remained unaffected across treatments ($p > 0.05$). Values ranged from 9.72 to 10.63 g/dL for Hb and 32.89 to 35.36% for PCV, staying well within the normal physiological ranges for growing calves. These findings are consistent with Yusuf *et al.* (2016) [12], who reported no changes in Hb and PCV in calves fed grass-or hay-based diets supplemented with fibrolytic enzymes at varying levels. Similarly, Harani *et al.* (2021) [13] and Jamra *et al.* (2025) [14] observed non-significant shifts in Hb and PCV in crossbred calves and Murrah buffalo calves receiving probiotic-EFE combinations and exogenous fibrolytic enzymes with gram straw respectively. The stability of these parameters

suggests that improved fibre degradation and nutrient availability from EFE treatment did not alter erythropoiesis or hydration status. In contrast, Sheikh *et al.* (2017) [15] observed increase in haemoglobin levels in Corriedale sheep supplemented with urea-molasses treated rice straw with fibrolytic enzymes. Mousa *et al.* (2022) [16] reported significantly higher Hb in lambs under enzyme (CalfoCare®) supplementation, possibly due to more severe basal dietary limitations or higher enzyme doses than those applied in the present study.

Biochemical Parameters

Serum glucose levels were statistically comparable among groups ($P = 0.479$), reflecting stable energy homeostasis. This aligns with Jihene *et al.* (2022) [17], Harani *et al.* (2021) [13], Kumar *et al.* (2023) [18] and Marwan *et al.* (2019) [19], who also found no alterations in glucose despite enhanced rumen fermentation in enzyme-supplemented lambs, crossbred calves, kids and buffalo calves respectively. Total serum protein, albumin, globulin and albumin: globulin (A:G) ratio remained unaltered ($p > 0.05$), indicating balanced protein synthesis, hepatic function and immune competence. These observations corroborate Harani *et al.* (2021) [13], Kumar *et al.* (2023) [18] and Mohammed *et al.* (2025) [20], who documented normal protein profiles in crossbred calves, Black Bengal kids and Awassi lambs fed EFE-supplemented diets. Contrary Kholif *et al.* (2017) [21], Gado *et al.* (2007b) [22] and Marwan *et al.* (2019) [19] noted modest increases in total protein or albumin under certain conditions, the absence of such changes here is attributable to the moderate fibre replacement level (37.5%) and the isonitrogenous nature of the TMRs. Blood urea nitrogen (BUN) concentrations were also statistically similar ($P = 0.975$), confirming efficient nitrogen utilisation and renal health. This matches with Mohammed *et al.* (2025) [20], who reported stable BUN in lambs fed enzyme-treated ration. Overall, the lack of treatment effects on haemato-biochemical parameters demonstrates that EFE-treated soybean straw at 300 g/tonne maintains metabolic equilibrium in growing Red Kandhari calves. The stability of all indices supports the safety and suitability of 37.5% inclusion of EFE-treated soybean straw in TMR as a sustainable, cost-effective roughage source without compromising animal health. Variations reported in the literature underscore the importance of enzyme dosage, substrate quality and physiological stage in modulating blood responses.

Conclusion

Supplementation of exogenous fibrolytic enzymes (@ 300 g/tonne) in soybean straw-based total mixed rations (TMR) exerted no adverse effect on haemato-biochemical parameters in growing Red Kandhari calves. Inclusion of 37.5% EFE-treated soybean straw as dry roughage-maintained haemoglobin, packed cell volume, serum glucose, total protein, albumin, globulin, albumin: globulin ratio and blood urea nitrogen at levels statistically similar to the control and untreated soybean straw groups while keeping all values well within normal physiological ranges. The results indicate that EFE treatment does not induce metabolic stress, anaemia, hepatic dysfunction or renal impairment. These findings demonstrate that EFE-treated soybean straw can be safely incorporated up to 37.5% of TMR dry matter as a sustainable, cost-effective roughage

source without compromising the metabolic health or haematological status of growing calves, offering practical implications for fodder-scarce regions and economical calf rearing strategies.

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References

1. BAHS. Basic Animal Husbandry Statistics. Department of Animal Husbandry & Dairying, Ministry of Fisheries, Animal Husbandry & Dairying, Government of India; 2025.
2. Roy AK, Agrawal RK, Bhardwaj NR, Mishra AK, Mahanta SK. Revisiting national forage demand and availability scenario. Indian fodder scenario: redefining state wise status. 2019;1-21.
3. USDA Foreign Agricultural Service. India: Soybean Production Data. Washington DC: USDA-FAS; 2024.
4. Sticklen MB. Plant genetic engineering for biofuel production: towards affordable cellulosic ethanol. Nat Rev Genet. 2008;9(6):433-443.
5. Beauchemin KA, Colombatto D, Morgavi DP, Yang WZ. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. Journal of animal science. 2003 Feb 1;81(14_suppl_2):E37-47.
6. Yang J, Zhao S, Lin B. Effect of commercial fibrolytic enzymes application to normal-and slightly lower energy diets on lactational performance, digestibility and plasma nutrients in high-producing dairy cows. Front Vet Sci. 2024 May ;11:1302034.
7. Jain NC. Schalm's Veterinary Pathology. Philadelphia: Lea and Febiger; 1986.
8. Kaneko JJ. Clinical biochemistry of domestic animals. 3rd ed. Florida: Academic Press Inc; 1980.
9. Dumas BT, Watson WA, Briggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chim Acta. 1971;31:87-96.
10. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of clinical Biochemistry. 1969 Jan;6(1):24-7.
11. Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals. 6th ed. San Diego: Academic Press; 2008.
12. Yusuf KO, Isah OA, Aderinboye RY, Fasae OA. Effects of enzyme additive on haematological and serum biochemical parameters of calves fed fresh grass and hay-based diets. Bull Anim Health Prod Afr. 2016; 64:187-196.
13. Harani M, Suryanarayana MVAN, Devasena B, Reddy YR. Effect of probiotic and exogenous fibrolytic enzymes on blood and faecal consistency score. Pharma Innov J. 2021;10(5):476-479.
14. Jamra MS, Nanavati S, Jain RK, Yadav DS, Mahour SS, Jamra N, *et al.* Influence of non-leguminous and leguminous straw based total mixed ration supplemented with fibrolytic enzymes in blood parameters of growing Murrah buffalo calves. Int J. 2025;9(2S):229-234.
15. Sheikh GG, Ganai AM, Sheikh FA, Bhat SA, Masood D, Mir S, Ahmad I, Bhat MA. Effect of feeding urea molasses treated rice straw along with fibrolytic enzymes on the performance of Corriedale Sheep. Journal of entomology and zoology studies. 2017;5(6):2626-30.
16. Mousa GA, Allak MA, Shehata MG, Hashem NM, Hassan OG. Dietary supplementation with a combination of fibrolytic enzymes and probiotics improves digestibility, growth performance, blood metabolites, and economics of fattening lambs. Animals. 2022;12(4):476.
17. Jihene J, Khalil A, Samia BS, Hela Y, Atef M, Jamel R, *et al.* Effect of fibrolytic enzyme supplementation of urea-treated wheat straw on nutrient intake, digestion, growth performance, and blood parameters of growing lambs. Small Rumin Res. 2022 Dec; 217:106840.
18. Kumar M, Chatterjee A, Dutta TK, Reena Y, Mohammad A, Bhakat C, *et al.* Effect of exogenous fibrolytic enzymes supplementation on voluntary intake, availability of nutrients and growth performance in Black Bengal kids (*Capra hircus*). Small Rumin Res. 2023 Mar; 220:106912.
19. Marwan AA, Mousa SA, Singer AM. Impact of feeding exogenous fibrolytic enzymes (EFE) on digestibility, rumen fermentation, haemobiochemical profile and productive performance in buffalo calves. Int J Vet Sci. 2019;8(3):127-133.
20. Mohammed AM, Saleh FH, Noaman AI. Effect of using treated and untreated wild reed with fibrolytic enzymes on some blood parameters in Awassi lambs. Euphrates J Agric Sci. 2025;17(3):67-77.
21. Kholif AE, Elghandour MMY, Rodríguez GB, Olafadehan OA, Salem AZM. Anaerobic ensiling of raw agricultural waste with a fibrolytic enzyme cocktail as a cleaner and sustainable biological product. J Clean Prod. 2017;142:2649-2655.
22. Gado HM, Sohair A, Nasr BKM, Mahrous AA. Effect of biological treatments on the nutritive value of rice straw. Egypt J Nutr Feeds. 2007;9(2):207-219.