

Replacing concentrate with pea (*Pisum sativum*) straw in sheep feeding: Effect on rumen fermentation, microbial nitrogen synthesis and nutrient utilization

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ABSTRACT

A feeding trial was conducted in ewes for a period of 60 days during extreme summer months to envisage the effect of partial replacement of concentrate with pea straw on rumen fermentation, microbial nitrogen synthesis and nutrient utilization. Thirty adult ewes of Malpura breed were randomly divided in three groups of 10 each on the basis of body weight. Ewes were fed *ad libitum* chaffed cenchrus hay, mixture of chaffed cenchrus hay plus pea straw (1:1 ratio) and exclusively pea straw, in group 1 (Control), 2 (Test 1) and 3 (Test 2), respectively. Ewes in control group were supplemented with concentrate mixture @ 300 g/ewe/day, whereas quantity of concentrate mixture was reduced to 250 and 200 g /ewe/day in Test 1 and Test 2 group, respectively. Total dry matter intake was similar in all the groups whereas digestible crude protein and ME intake was higher ($P < 0.05$) in Test 1 and Test 2 than Control which was due to higher ($P < 0.05$) digestibility of DM, OM and CP in these groups. Nitrogen balance as percent of intake was highest in Test 2 followed by Test 1 and Control. Higher ($P < 0.05$) concentration of total nitrogen and TVFA along with higher propionic acid proportion in Test 2 group revealed favorable rumen fermentation with pea straw however, microbial nitrogen synthesis was lowered in this group. It may be concluded that adding pea straw, either alone or in conjunction with cenchrus grass hay, can replace concentrate feeding by 1/3rd in adult ewes fed at maintenance level.

Keywords: Cenchrus hay, Microbial nitrogen, Nutrient utilization, Pea straw, Rumen fermentation

Sheep husbandry is a sustainable resource base for people residing in dryland regions of India and Asia Pacific (Lal *et al.* 2022). Sheep production in these regions primarily relies on community rangelands and post-harvest stubble grazing and crop residues, with supplemental top feeds provided during the lean season to meet nutritional demands. The biomass yield of the community rangelands is low while stocking density is high in general (Sankhyan *et al.* 1999) and grazing on this land are unable to meet their nutritional needs for maximum period of the year. Additionally, the majority of sheep producers in semi-arid zones do not practice concentrate supplementation, even during critical physiological stages such as late gestation or lactation. In a semi-intensive feeding system, supplementing ewes with concentrates at 1% of body weight in addition to unrestricted grazing on community rangelands significantly improves their production performance (Chaturvedi *et al.* 2001). Under stall feeding system, concentrate supplementation @ 300-450 g/Sheep /day besides *ad libitum* Cenchrus (*Cenchrus ciliaris*), fodder suffices their maintenance requirements (Bhatt *et*

al. 2024). Legume straws have generally better nutritional quality which are degraded rapidly in the rumen and have higher dry matter digestibility (Lopez *et al.* 2005). *Pisum sativum* is widely cultivated for both vegetable and pulse production, generating pea straw as a major post-harvest by-product. Owing to its leguminous nature, pea straw possesses considerable potential as an alternative feed resource for livestock nutrition. The present study was therefore, undertaken to evaluate the feeding value of pea straw as a partial replacement for concentrate in sheep diets, with specific emphasis on its effects on rumen fermentation characteristics, microbial nitrogen synthesis, and overall nutrient utilization.

MATERIALS AND METHODS

The study was conducted at ICAR-Central Sheep and Wool Research Institute, Avikanagar, Rajasthan, India (75°28'E longitude, 26°17'N latitude and 320 m above sea level) having hot semi-arid climatic conditions with erratic distribution of rainfall throughout the year. The minimum and maximum temperatures ranged from 2.5-23.0°C and 19.0-39.0°C, respectively with relative humidity varied from 58.0 to 93.0% in the study area. Animal handling and sampling techniques were approved by the Committee for the Purpose of Control and Supervision of Experiment on

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Animal and feeding management: Thirty adult (3-5 years of age) ewes of Malpura breed (average body weight of 34.8 ± 0.79 kg) were randomly divided into three groups of ten ewes in each group on the basis of body weight. Ewes were stall fed and reared in well-ventilated sheds with pre-coated galvanized iron sheet roofing. These animals were kept in the open corral for two hours (7.00-8.00 AM) in the morning and evening (5.00-6.00 PM). Ewes were offered *ad libitum* roughage and in group 1 (Control) it was chaffed cencrus (*Cenchrus ciliaris*) hay, in group 2 (Test 1) mixture of chaffed cencrus hay plus pea straw (1:1) and in group 3 (Test 2) pea (*Pisum sativum*) straw only. Additionally, ewes in control, group 2 (Test 1) and group 3 (Test 2) were supplemented 300, 250 and 200 concentrate per ewe per day, respectively. The concentrate mixture was a mixture of maize, barley, solvent extracted groundnut cake, solvent extracted mustard cake, mineral mixture and common salt mixed in a proportion of 45, 45, 4, 3, 2 and 1 percent, respectively. Animals were group fed at 09:00 AM in the morning and water was available during the whole day. Feeding trial was conducted for a period of 60 days during the summer months (April to May, 2023). Daily records of feed offered and leftover were maintained and animals were weighed at fortnightly interval.

Nutrient utilization, rumen metabolites and microbial nitrogen synthesis: On 45 day of experimental feeding a metabolism trial was conducted on six ewes in each group. The metabolism trial lasted for 10 d (4 d adaptation followed by 6 d sample collection) during which daily feed offered, residue left, faeces and urine excreted were collected and recorded as per standard procedure. The dry matter (DM) of feed, faeces and residue samples was determined by drying to a constant weight in a hot air oven at 70°C. Sample of each six days, after drying, were pooled, ground and filtered through 1 mm sieve and kept for further analysis as per AOAC (2000). Feed, orts and feces were analyzed for nitrogen (984.13; AOAC, 2000), ash (942.05; AOAC, 2000) and ether extract (920.39; AOAC, 2000). Fiber fractions namely, NDF (Neutral detergent fiber), ADF (acid detergent fiber), ADL (acid detergent lignin) in feed, residue and feces were analyzed as per the standard methods (Van Soest *et al.* 1991). Approximately 20 mL of urine samples were stored at -20°C for estimation of purine derivatives (PD) by high performance liquid chromatography to determine microbial protein synthesis as described by Bhatt *et al.* (2024). Metabolizable energy intake (MEI) was calculated by prediction equation using digestible organic matter intake (Bhatt *et al.* 2021). At 56th day of the experiment, rumen liquor sample (50 ml) from each ewe was collected at 4 h post feeding using a stomach tube. pH was determined using a portable pH meter within 4-5 min of sampling. Then the rumen fluid was strained with four layers of muslin cloth under anaerobic condition and stored at -20°C for further analysis. The strained rumen liquor (SRL) samples were analyzed for ammonia nitrogen ($\text{NH}_3\text{-N}$; Weatherburn 1967) and total and tri-chloroacetic

acid perceptible N (AOAC, 2000). The samples for volatile fatty acids (VFA) analysis were centrifuged at 10,000g for 5 min at 4°C in a micro centrifuge and the supernatant (1.0 μL) was fractioned as per Sahoo *et al.* (2011) with gas chromatograph (DANI, Modell 1000, Series 011124002, Cologno, Monzese, Italy) using a flame ionizing detector, programmable temperature vaporize injector and packed column (length 2 m x 1/8th inch diameter, packed with 10% SP-1000). The analytical conditions for fractionation of VFA included injection port temperature 250°C, column temperature step up from 70°C to 150°C in 16 min and detector temperature 250°C with flow rate of N gas 30 mL/min; hydrogen 35 mL/min and air 300 mL/min. Ewes were weighed at the beginning and end of the experiment.

Statistical analysis: The data were subjected to statistical analysis using SPSS (2005), Base 14.0 (SPSS Software products, Marketing Department, SPSS, Chicago, IL, USA). Mean values of various treatments were compared using Tukey's-b test, considering 5% level of significance ($P < 0.05$).

RESULTS AND DISCUSSION

Chemical composition, nutrient intake and utilization: Proximate composition and fiber fractions of roughages and concentrate is exhibited in Table 1. The crude protein content of pea straw, cencrus hay, cencrus hay plus pea straw (1:1) and concentrate mixture was 8.8, 6.4, 7.8 and 10.7%, respectively. Lignin content was higher in pea straw as compared to cencrus hay. The higher protein content in pea straw can be substantiated with the findings of Lopez *et al.* (2005) who reported that due to higher nitrogen and lower fiber contents legume straws are generally of better nutritional quality than cereal straws. Similar to the present results, Jane *et al.* (2017) reported a CP content ranging from 7.3 to 10.2 percent with similar amount of fiber fractions in different varieties of field pea straws. The composition of cencrus hay was at par with the results

Table 1. Chemical composition of concentrate mixture and roughage used in the experiment (% on dry matter basis)

Items	Pea straw	Cencrus hay	Cencrus hay + pea straw	Concentrate mixture
Dry matter	91.3	95.1	94.6	96.2
Organic matter	94.1	94.3	93.1	92.1
Crude protein	8.8	6.4	7.8	10.7
Ether extract	1.8	2.7	2.2	2.0
Neutral detergent fiber	68.7	73.2	67.1	17.7
Acid detergent fiber	52.0	47.3	44.2	5.5
Lignin	10.1	7.96	8.06	1.57
Hemicellulose	16.7	25.9	22.9	12.3
Cellulose	41.9	39.4	36.2	3.9

reported by Singh *et al.* (2024) for five different genotypes cultivated in India.

The digestibility of DM, OM and CP followed a similar trend being significantly higher ($P < 0.05$) in Test 2 followed by Test 1 and Control (Table 2). The significantly higher ($P < 0.05$) digestibility of DM, OM and CP in pea straw fed groups (Test 2 and Test 1) and a linear increase from Control to Test 2 was coupled with feeding of better quality of leguminous (Lopez *et al.* 2005) pea straw-based diet in treatment groups as compared to ewes in control group which were fed cenchrus hay being slightly lower in quality. Apparent crude protein (CP) digestibility largely depends on the protein content of the diet, as metabolic fecal nitrogen imposes a constant loss on dietary nitrogen. In ruminants, this loss is approximately 30 g CP per kg of dry matter intake (McDonald *et al.* 1981). The digestibility of NDF, ADF, hemicellulose, and cellulose was comparable across treatments; however, feeding pea straw appeared to enhance these values, although the differences were not statistically significant. During the metabolic trial nitrogen intake was almost similar in all the treatments (Table 2). Although, fecal nitrogen excretion was lowered with pea straw feeding however, differences were non-significant due to larger group variations. Nitrogen balance as percent of intake was different ($P < 0.05$) among the treatments and improved with pea straw feeding being higher ($P < 0.05$) in Test 2 compared to Test 1 and Control. The nitrogen balance (g/day) and nitrogen balance as percent of intake ($P < 0.05$) revealed a linear increase from Control to Test 1

Table 2. Nutrient digestibility (g/100g dry matter) and nitrogen balance of ewes in different treatments

Items	Control	Test 1	Test 2	SEM	p-value
Nutrient digestibility (%)					
Dry matter	50.9 ^c	54.8 ^b	57.9 ^a	0.86	<0.001
Organic matter	53.4 ^c	57.4 ^b	60.8 ^a	0.87	<0.001
Crude protein	44.3 ^c	51.6 ^b	60.3 ^a	2.45	0.014
Neutral detergent fiber	40.9	44.4	42.4	1.06	0.434
Acid detergent fiber	39.5	39.3	39.5	1.24	0.998
Hemicellulose	43.3	52.7	48.4	1.65	0.055
Cellulose	44.9	46.5	49.5	1.12	0.252
Nitrogen balance (g/day)					
Intake	11.7	12.3	11.0	0.52	0.658
Fecal excretion	6.6	6.1	4.4	0.45	0.100
Urinary excretion	1.9 ^c	2.6 ^a	2.3 ^b	0.10	0.015
Absorbed	5.2	6.2	6.7	0.31	0.118
Balance	3.2	3.6	4.3	0.24	0.174
Balance % of intake	27.8 ^b	30.0 ^b	39.1 ^a	1.91	0.022

Value bearing different superscripts in a row differ significantly ($P < 0.05$). SEM: standard error of mean

and Test 2 group and was positively correlated with the CP and digestible CP intake in these diets. It is well established that nitrogen loss in feces remains relatively constant and is primarily influenced by dry matter intake (DMI). Therefore, higher CPI, when accompanied by similar DMI and fecal nitrogen loss, results in greater DCPI, as observed in the present findings.

Rumen fermentation characteristics and microbial nitrogen synthesis: Data in Table 3 revealed similar rumen pH and $\text{NH}_3\text{-N}$ in different groups whereas total nitrogen (Total-N) differed significantly ($P < 0.05$) between the treatments being higher ($P < 0.05$) in Test 2 followed by Test 1 and Control. Although, the TCA-ppt-N showed the similar trend to that of Total-N but the differences were non-significant among the treatments. These findings were corroborated in view of the buffering capacity of rumen

Table 3. Rumen fermentation characteristics and microbial nitrogen synthesis of ewes in different treatments

Items	Control	Test 1	Test 2	SEM	p-value
pH	6.48	6.50	6.57	0.035	0.626
Ammonia (mg/dL)	13.3	12.1	13.0	0.97	0.888
Total nitrogen (mg/dL)	63.0 ^c	80.5 ^b	99.6 ^a	5.34	0.01
TCA-ppt-N (mg/dL)	32.1	43.0	63.1	5.74	0.073
VFA production (mEq/L)					
Acetic acid	30.5 ^b	31.2 ^b	39.5 ^a	1.72	0.040
Propionic acid	8.2 ^c	11.1 ^b	14.1 ^a	0.96	0.025
Butyric acid	9.1	8.9	8.8	0.54	0.231
Total	49.2 ^b	52.2 ^b	64.0 ^a	2.02	0.041
Proportion (%) of different fatty acids					
Acetic acid	62.1	62.6	62.1	0.66	0.951
Propionic acid	16.7 ^b	21.9 ^a	21.9 ^a	0.84	0.007
Butyric acid	18.5 ^a	13.6 ^b	13.6 ^b	0.73	0.001
Microbial nitrogen synthesis					
PD Excretion (meq/day)	13.2 ^a	11.2 ^b	8.2 ^c	0.70	0.004
Microbial nitrogen (MN) synthesis (g/day)	18.2 ^a	15.4 ^b	11.3 ^c	0.98	0.004
MN Synthesis (g/kg DMI)	19.5	16.7	16.0	1.16	0.451
MN Synthesis (g/Kg DOMI)	39.1	31.4	28.2	2.34	0.153
MCP/100g DMI	12.2 ^a	10.5 ^b	10.0 ^b	0.52	0.045
MCP/100g DOMI	24.4 ^a	19.6 ^b	17.7 ^c	0.46	0.015

Value bearing different superscripts in a row differ significantly ($P < 0.05$). SEM: standard error of mean; TCA: tri-chloroacetic acid; VFA: volatile fatty acids; PD: purine derivatives; MN: microbial nitrogen; DMI: dry matter intake; DOMI: digestible organic matter intake; MCP: microbial crude protein

regulated by recycling of saliva in the rumen along with rapid absorption of the rumen volatile fatty acids (VFAs) as well as ammonia. Rumen pH is maintained at 6.2-6.8 under normal conditions (Mao and Wang, 2025) and the rumen pH values observed in present experiment were in close agreement to this value. The increasing rumen total N ($P<0.05$) and trichloroacetic acid perceptible-N (TCA-ppt-N) concentration from control to Test 2 group in the present experiment followed a similar trend to that of CP and DCP intake. Concentration of acetic and propionic acid increased with pea straw feeding and, as a result, the total VFA production was also higher ($P<0.05$) in Test 2 compared to Test 1 and control group lambs. The increased volatile fatty acid production in Test 2 than Test 1, and in Test 1 than Control revealed better rumen degradability and resulted in higher digestibility of nutrients viz. DM, OM, CP and Cellulose. Higher volatile fatty acid production resulted in higher energy availability to the animal, which is a confirmation of higher ME intake in ewes of the Test 1 and Test 2 groups than that of control. The values of Total VFA and its fractions viz. acetate, propionate, butyrate are at par to those reported by Bhatt *et al.* (2025). The proportion (%) of different fatty acids production indicated higher ($P<0.05$) proportion of propionic and lower ($P<0.05$) that of butyric acid with pea straw feeding in Test 2 and Test 1 compared to control. These values were similar in Test 1 and Test 2 group. Generally, the proportion of volatile fatty acids i.e. acetate and propionate depend on the proportion of roughage and concentrate in diet i.e. higher proportion of concentrate in diet yields higher propionate proportion and reverse is true with roughage feeding and acetate proportion (McDonald *et al.* 1981). However, in the present experiment acetate proportion remained unchanged

even after increasing the roughage proportion from 78.0 percent in control to 84.0 per cent in T2. This might be due to feeding of good quality leguminous pea straw either alone (Test 1) or in conjunction with cencrus hay in test group. Purine derivatives excretion (mEq/day) in urine and daily microbial nitrogen (MN) synthesis which was also expressed as microbial crude protein (MCP) synthesis per 100g digestible organic matter intake were lowered ($P<0.05$) in pea straw fed group and the values were lower ($P<0.05$) in Test 2 followed by Test 1 compared to control group lambs. This might be attributed to the higher amount of readily available carbohydrates supplied through the concentrate in the control group compared to Test 1 and Test 2. The increased carbohydrate availability in the control group enhanced the incorporation of ammonia into microbial protein. Further, the rumen microbes exert a "levelling" effect on the protein supply in ruminants. They enhance both the quantity and quality of protein derived from low-quality roughages by microbial protein synthesis. However, in the case of protein-rich concentrates, the extensive microbial degradation can lead to a net reduction in the availability of high-quality dietary protein to the host animal (McDonald *et al.* 1981). The present values of PD excretion, MN synthesis and MCP synthesis (g/100g DOMI) are almost similar to those reported by Bhatt *et al.* (2025).

Plane on nutrition, nutritive value and body weight change of ewes: Plane of nutrition data (Table 4) revealed similar roughage intake in all the treatments. However, differences existed in concentrate intake among the treatments which influenced the total dry matter intake, as a result of which the total dry matter intake was higher ($P<0.05$) in control and Test 1 compared to Test 2 group. In spite of lower DMI, the intake of crude protein, digestible

Table 4. Plane of nutrition, nutritive value and body weight change of ewes in different treatments

Items	Control	Test 1	Test 2	SEM	p-value
Dry matter intake ((g/day)					
Roughage intake (g)	1023	1052	1021	8.49	0.252
Concentrate intake (g)	289 ^a	240 ^b	192 ^c	9.52	<0.001
Total Dry matter intake (g)	1312 ^b	1293 ^b	1214 ^a	12.9	0.025
Crude protein intake (g/day)	96.4 ^b	107.8 ^a	110.5 ^a	1.60	<0.001
Digestible crude protein intake (g/day)	42.7 ^c	55.7 ^b	66.6 ^a	2.39	<0.001
ME Intake (MJ/day)	9.8 ^b	10.3 ^a	10.3 ^a	0.08	0.015
Nutritive value in different feeding regimes					
Crude protein (g/kg DM)	73.5 ^a	83.4 ^b	91.0 ^c	1.74	<0.001
Digestible crude protein (g/kg DM)	32.5 ^a	43.0 ^b	54.9 ^c	2.21	<0.001
Metabolizable energy (Kcal/kg DM)	7.51 ^a	7.99 ^b	8.44 ^c	0.09	<0.001
Body weight change of ewes					
Initial weight (kg)	34.8	34.8	34.8	0.79	0.996
Final weight (kg)	36.8	37.3	37.5	0.89	0.946
Gain in weight (kg)	2.0	2.5	2.7	0.25	0.507

Value bearing different superscripts in a row differ significantly ($P<0.05$). SEM: standard error of mean; ME: metabolizable energy, MJ: mega joule

crude protein and ME intake was significantly ($P < 0.05$) higher in Test 1 and Test 2 than Control. The almost similar intake (g/day) of roughage in control and treatment (Test 1 and Test 2) groups indicated that all the roughage fed to ewes were optimally palatable in accordance to acceptability of the experimental ewes. The higher ($P < 0.05$) intake of CP in Test 1 and Test 2 than control was owing to feeding of comparatively higher CP containing pea straw either alone or in conjunction with cenchrus hay. Similarly, due to higher ($P < 0.05$) intake of CP and its digestibility, the intake of DCP was also higher in these groups. The higher intake of ME in Test 1 and Test 2 than Control can be explained due to higher OM digestibility as ME intake is positively related to digestibility of organic matter. Metabolizable energy is the portion of gross energy of feed which is available to the animal after deducting the losses in feces, urine, and gases produced during digestion. The evaluation of nutritive value under different feeding protocols revealed higher digestible crude protein (DCP) and metabolizable energy (ME) contents in diets containing pea straw compared to those containing cenchrus hay. This improvement might be attributed to the higher crude protein (CP) content of pea straw and its superior organic matter and crude protein digestibility.

The final body weight and body weight change of ewes at the end of the experiment did not differ significantly among the treatment groups, although numerically higher values were observed in Test 2, followed by Test 1 and the Control group (Table 4). This trend might be attributed to improved utilization of dry matter and crude protein, resulting in enhanced energy and protein intake despite the proportional reduction in concentrate supplementation in the test groups. Rumen fermentation patterns also indicated a shift towards increased propionate production, which could have improved energy efficiency. However, the reduced microbial nitrogen synthesis might have offset these beneficial effects, thereby limiting the improvement in body weight gain and resulting in non-significant differences among the treatment groups.

The study revealed that pea straw can be a valuable leguminous feed resource for livestock, particularly for sheep and goats. In sheep at maintenance feeding levels, pea straw could be included up to 70–75% of the ration and can replace 30–40% of concentrate feed, without adversely affecting animal performance. However, the applicability of these findings under higher nutritional demands, such as in growing lambs, lactating ewes, or large ruminants, is yet to be known. Therefore, further studies are required to validate its feeding value and performance under such production conditions.

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