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Effect of Different Levels of Protein in Total Mixed Ration on Growth Performance, Digestibility and Microbial Protein Production in Vechur Cattle

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ABSTRACT

The effect of different levels of protein in total mixed ration was evaluated based on the growth performance, digestibility, and microbial protein production in Vechur cattle. Fifteen Vechur cattle of six to ten months of age were randomly assigned to one of the three treatments. T1 - Total mixed ration containing 16 % CP and 60% TDN, T2 – Total mixed ration containing 14% CP and 60% TDN, and T3 - Total mixed ration containing 12 % CP and 60% TDN. The results indicated that total dry matter intake (DMI), fortnightly average dry matter intake, total body weight gain, average daily gain (ADG), and feed conversion efficiency (FCE) were similar ($P > 0.05$) among the three treatment groups. No variations were observed for the apparent digestibility of nutrients. The purine derivatives excretion, urinary creatinine excretion, microbial protein production, the duodenal flow of microbial nitrogen, PDC index, and the efficiency of microbial nitrogen production showed no significant ($P > 0.05$) distinction among the dietary treatments. These findings suggest that a total mixed ration containing 12% CP may be adequate to meet the nutritional requirements of growing Vechur cattle without compromising performance and digestibility, thereby offering a potentially cost-effective feeding strategy.

KEYWORDS: Digestibility, Microbial protein, Total mixed ration, Vechur cattle

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INTRODUCTION

Livestock rearing is an integral part of the agriculture system in tropical countries, which have the highest share in the livestock population of the world (FAO 2013). According to 20th livestock census (2019) of India, the total livestock population is 535.78 million of which the cattle population is 192.49 million. India has an indigenous/nondescript cattle population of 142.11 million.

The preservation of indigenous cattle breeds is important because of their unique traits. Vechur cattle are the sole recognized indigenous breed in Kerala and are classified as a critically maintained breed category by the Food and Agriculture Organisation.

The protein requirement of ruminants encompasses the requirement of the rumen microorganisms to maintain optimum growth and proliferation in the rumen and the requirement of the host animals for various physiological functions in the body. In ruminants, 50 to 100% of their total protein requirements are met from ruminal microbial synthesis. More specifically for the ruminant, an adequate protein level in the diet is needed for maximal growth and activity of ruminal microorganisms, thus producing desired microbial crude protein amounts and maximizing ruminal fermentation. Microbial protein (MP) plays a pivotal role in ruminant nutrition because ruminants get most of their protein from microbial cells formed in the rumen as a result of feed digested under anaerobic conditions. This microbial protein provides 60 to 85 % of amino acids (AA) reaching the animal's small intestine (Fujihara and Shem, 2011). The amino acid profile of MP is better than several dietary protein sources. Increasing the efficiency of its production would subsequently improve cattle productivity. Protein supplementation is costly and can result in excess nitrogen (N) excretion (Zhang et al., 2017).

Proper determination of the protein requirement of animals is crucial for maximizing production and minimizing N input in dairy production systems. The reports on the nutrient requirements of Vechur cattle are scanty. They are fed as per the requirements of crossbred cattle. Additionally, there haven't been many studies done on the optimum dietary levels of crude protein in the Total mixed ration (TMR) for feeding Vechur cattle. Hence, the present research project is undertaken to study the effect of different levels of protein in the TMR on growth performance and digestibility of nutrients in Vechur cattle, and to assess the efficiency of rumen microbial protein production.

MATERIALS AND METHODS

This experiment was conducted at the Vechur Cattle Conservation Unit, Centre for Advanced Studies in Animal Genetics and Breeding (CASAGB), Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala, India, for 120 days in Vechur cattle.

Fifteen Vechur cattle of six to ten months old age (Average body weight 50 kg) were used in this study. The Vechur cattle were randomly assigned into three treatment groups of five animals each in a completely randomized design and were allotted randomly to one of the three dietary treatments T1, T2 and T3. All the experimental animals were fed with total mixed ration (TMR) with a concentrate: roughage ratio of 70:30. Three experimental rations were formulated as follows. T1 - TMR containing 16 % CP and 60 % TDN T2 - TMR containing 14 % CP and 60 % TDN, T3 - TMR containing 12 % CP and 60 % TDN. The paddy straw-based TMRs were prepared in the School of Animal Nutrition and Feed Technology (SANFT), Mannuthy, Thrissur. The animals were maintained under a uniform system of feeding and management throughout the experimental period. The feeding trial will be conducted for a period of four months. All the calves will be fed as per ICAR standards (ICAR, 2013). All the animals were dewormed for controlling endoparasites. All the experimental cattle were housed in the experimental shed with individual feeding and watering facilities. Clean fresh drinking water was offered to all the animals *ad libitum*. Individual data on quantities of feed offered daily were recorded. Weighed quantities of TMR were fed individually to the animals of the three experimental groups based on their requirement, and the leftover feed in the manger was collected manually and weighed, twice a day, in the morning and afternoon at 9 AM and 2 PM, respectively. Samples of the left-over portions of the feed were taken daily for analyzing the moisture content and the daily dry matter intake was calculated. Daily dry matter intake data was recorded during the entire experimental period. Based on the body weight, feed allowances were reviewed fortnightly. Animals were weighed at the beginning of the experiment and thereafter at fortnightly. The ingredient and chemical composition of the total mixed rations used for this study is presented in Table 1.

A digestibility trial for five days duration was carried out towards the end of the feeding trial by total collection method. The feed and dung samples were analyzed for proximate principles following the methods outlined by the Association of Official Analytical Chemists (AOAC, 2016). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were measured using the procedures of Van Soest et al. (1991). Additionally, calcium and phosphorus content in both feed and faeces were determined using standard AOAC methods.

Total urine collected during the digestion trial were used for the estimation of the purine derivatives such as allantoin and uric acid and thus for the estimation of microbial protein production (IAEA-TECDOC-945, 1997; Cetinkaya et al., 2006; George, 2012). Urine samples were centrifuged, diluted (1: 10), filtered using (0.22µm) Millipore filter and analyzed (George et al., 2006). Urinary uric acid was determined by the uricase method and creatinine by Modified Jaffe's method using standard kits. Urinary purine derivative excretion is the sum of urinary allantoin and uric acid excretion in mM/L. The microbial Nitrogen (g N/day) from the microbial purine derivatives (X, mMol/day) was calculated as described by Chen and Gomes (1992). The efficiency of microbial nitrogen production was expressed as g N/kg of organic matter digested in the rumen (DOMR) by multiplying digestible OM by 0.65. (Chen and Gomes, 1992).

Statistical analysis of the experimental data was conducted using one-way ANOVA with SPSS 24.0, and means were compared through Duncan's range test, as described by Snedecor and Cochran (1994)

RESULTS AND DISCUSSION

Chemical composition of ration

The chemical composition of the total mixed

rations used for the feeding trial is given in Table 2. Purushothaman (2018) prepared TMR for Vechur heifers containing 15.20 and 15.02 % of CP which was within the range of CP content of the TMR used in the present study. Nair (2020) also used similar levels of protein and energy in TMRs (12, 14, 16 and 18 % CP with 60 % TDN) in the study using lactating crossbred cows.

Yousefinejad et al. (2021) also formulated the ration for Brahman bulls with a total mixed ration containing 12.2 and 14.1 % CP which was similar to the formulation in this study.

Table 1. Ingredient and chemical composition of total mixed ration offered to cattle maintained on three dietary treatments

Ingredient (%)	% composition of total mixed ration		
	T1	T2	T3
Maize	26	24.5	26
Rice Polish	6.5	7.5	8
De-oiled Rice Bran	5.5	5.5	6.5
Alfalfa pellet	15	9	5.5
Black Gram Husk	1.5	6	8
Corn gluten fibre	8	9.5	7
Coconut Oil Cake	3.5	4	5
Paddy Straw	30	30	30
Calcite	1.5	1.5	1.5
Salt	0.5	0.5	0.5
Mineral mixture	2	2	2
Total	100	100	100
Vitamin AB2D3K g/100 kg	20	20	20
Nutrients	% Chemical composition of total mixed ration		
Dry matter	91.88 ±0.18	91.66 ± 0.13	91.50 ± 0.23
Organic matter	90.03 ± 0.16	90.41 ± 0.05	90.57 ± 0.09
Crude protein	16.31 ± 0.31	14.12 ± 0.12	12.55 ± 0.19
Ether Extract	3.40 ± 0.07	3.54 ± 0.09	3.57 ± 0.05
Crude fibre	13.76 ± 0.04	13.55 ± 0.03	13.72 ± 0.04
Total Ash	9.97 ± 0.21	9.59 ± 0.08	9.43 ± 0.10

[1]Values from the second row onwards are expressed on DM basis, an average of six values.

Growth Performance

The average body weight of experimental Vechur cattle maintained on different treatments, documented at fortnightly intervals. Statistical analysis of the data showed that there was no significant (P>0.05) variation in the average body weight of Vechur cattle fed on different dietary treatments. Summarized data on total weight gain and ADG for the Vechur cattle maintained on three treatments are given in Table 2. The ADG of Vechur cattle fed on three dietary treatments T1, T2 and T3 were 350, 340 and 360 g and total weight gain was 41.63, 41.51, and 43.36 kg. Statistical analysis of the data revealed that there was no significant variation (*P*>0.05) in ADG and total weight gain of Vechur

cattle fed on various treatments. In agreement with the results, Ozkaya and Toker (2012) found no significant difference (P>0.05) in body weight of Holstein calves fed with a starter diet containing 22 % CP and 18 % CP. Gowda (2019) also observed no significant difference in the average body weight of crossbred heifer calves fed different TMR. The present ADG values were comparable to Lohakare et al. (2006), who got values in the range of 337 to 367 g in crossbred calves fed different protein levels (100, 75, 125 % of protein requirement). In disagreement with the results of this study, Bhadane et al. (2004) discovered improved body weight gain in goats fed with complete pelleted feed containing 12 % CP, 65.81 % TDN and 14 % CP, 67.44 % TDN of 75.7 and 72.9 g/day. Similarly, Girdhar and Balaraman (2005) also detected better body weight gain in lactating cross bred cows fed on berseem based TMRs containing higher levels of protein and energy (12 % CP and 60 % TDN, 14 % CP and 65 % TDN) than those fed on a lower level of protein and energy (10 % CP and 55 % TDN).

Table 2. Growth performance of Vechur cattle fed on TMR with varying levels of protein in total mixed ration

Parameters	T1	T2	T3	p- value
Initial body weight (kg)	50.17 ± 8.19	50.21 ± 8.54	51.74 ± 6.62	0.987^{ns}
Final body weight (kg)	91.80 ± 15.75	91.72 ±10.57	95.10 ± 8.01	0.974 ^{ns}
Total weight gain (kg)	41.63 ± 7.81	41.51 ± 3.06	43.36 ± 2.84	0.960 ^{ns}
Average daily gain (kg)	0.35 ± 0.07	0.34 ± 0.03	0.36± 0.02	0.953 ^{ns}
Total dry matter intake (kg/animal)	235.31 ± 35.21	237.17 ± 29.08	242.67± 23.23	0.983 ^{ns}
Average daily dry matter intake (kg/animal/day)	1.96 ± 0.29	1.98 ± 0.24	2.02 ± 0.19	0.982 ^{ns}

[1]Mean values are based on five replicates with SE
ns- non-significant

Dry matter intake and Feed conversion ratio (FCR)

Statistically, there was no significant variation (P>0.05) found in the daily DMI of Vechur cattle maintained in the three treatment groups. In accordance with the results in the present study, Lohakare et al. (2006) reported that the dry matter intake was similar in crossbred calves fed different protein levels (100, 75, and 125 % of protein requirement) and it ranged from 2,053.66 to 2,279.22g. Similar results were reported by Chantiratikul et al. (2009), Queiroz et al. (2012), Kumar et al. (2013) and Javaid et al. (2015). They found that the dry matter intake was not affected by the protein content of the diet. However, Yuangklang et al. (2010) reported that feed intake (kg DM/day) was 7.37, 7.26, 7.15 and 6.96 respectively for 8, 10, 12 and 14 % CP in Brahmin bulls which indicated that feed intake was linearly decreased with increasing the protein levels in the diet.

Unlike the observations in the current study, Shahzad et al. (2011) found significant difference (P<0.05) in feed intake in Nili- Ravi buffalo calves fed with different dietary protein levels (CP; 10.5, 12.20, 13.80 and 15.55 %) and energy levels (1.72, 2.11 and 2.5 Mcal/kg of metabolizable energy). They observed higher feed intake for buffalo calves fed 12.20 CP and 2.11 Mcal/kg ME. On the contrary, Tauqir et al. (2011) concluded from their experiment in 36 growing male Nili Ravi buffalo calves that dry matter intake was reduced in calves that were fed a ration containing high CP (16.5 %) than those fed low (11.85 %) or medium (14.2 %) CP. Paengkoum et al. (2019) also detected an increase in dry matter intake linearly with increasing undegradable intake protein (UIP) levels in growing Thai indigenous beef cattle receiving different levels of crude protein (10 and 12 % of dry matter (DM) and undegradable intake protein UIP (15, 25 and 35 % of CP).

Data on cumulative FCR of Vechur cattle maintained on three different treatments is presented

in Table 2. Statistically, no significant difference was observed in the cumulative feed conversion ratio of Vechur cattle maintained on various treatments. Similarly, Sharma et al. (2010) observed that there was no significant difference in feed conversion efficiency in crossbred calves provided with complete feed in block (7.59), mash (7.47), or conventional feed form (7.93). In accordance with the results, Kumar et al. (2015) also observed no significant difference in FCE in Murrah buffaloes under the traditional feeding system and feeding of total mixed ration. Kavya et al. (2025) also observed similar FCR in Punganur calves fed with concentrate mixtures containing varying levels of CP.

Digestibility of nutrients

The digestibility coefficients of nutrients such as dry matter, crude protein, crude fibre, ether extract, nitrogen-free extract, neutral detergent fibre and acid detergent fibre are shown in Table 3. The data revealed non-significant effect ($P>0.05$) on the digestibility of nutrients with TMR comprising varying levels of crude protein. Similar to this study, several researchers reported that dietary crude protein levels did not change the digestibility of nutrients (Malik et al., 1998; Mehra et al., 2001; Chantriatikul et al., 2009; Verma et al., 2009; Tatsapong et al., 2010 and Kavya et al., 2025)

Lohakare et al. (2006) evaluated the effect of different protein levels (normal protein (NP) - 100 %, low protein (LP) - 75 and high protein (HP) - 125 % of protein requirement) in 30, three to five months old male crossbred calves on digestibility and reported that the dry matter digestibility was higher in the HP fed animals. The digestibility of CP, CF, OM and NFE was significantly higher on HP diets compared to LP or NP diets.

Table 3. Digestibility coefficients of nutrients[1] in TMR with varying levels of protein fed to Vechur cattle, %

Parameter	T1	T2	T3	p- value
Dry matter	62.32 ± 0.96	62.48 ± 1.62	61.8 ± 1.78	0.945^{ns}
Organic matter	66.86 ± 0.73	66.83 ± 1.56	65.57 ± 1.68	0.760 ^{ns}
Crude protein	60.49 ± 1.30	60.65 ± 1.50	60.65 ± 1.40	0.995 ^{ns}
Crude fibre	55.35 ± 1.44	55.41 ± 1.77	56.33 ± 2.22	0.915 ^{ns}
Ether extract	82.06 ± 1.14	81.43 ± 1.07	82.36 ± 1.18	0.838 ^{ns}
Nitrogen free extract	72.11 ± 0.55	71.65 ± 1.46	69.16 ± 1.72	0.285 ^{ns}
Neutral detergent fibre	52.53 ± 1.28	52.94 ± 2.11	52.24 ± 2.51	0.971 ^{ns}
Acid detergent fibre	36.73 ± 1.19	36.42 ± 2.45	39.15 ± 2.65	0.639 ^{ns}
CPI (kg)	0.41 ± 0.066	0.35 ± 0.047	0.32 ± 0.031	0.463^{ns}
DCPI (kg)	0.25 ± 0.039	0.21 ± 0.026	0.19 ± 0.018	0.441^{ns}
TDNI (kg)	1.63 ± 0.265	1.61 ± 0.211	1.63 ± 0.148	0.998^{ns}
CPI (kg/kg W0.75)	0.014^{a}±0.001	0.012^{b}± 0.001	0.011^{b} ± 0.001	0.006*
DCPI (kg/kg W0.75)	0.008^{a} ± .000	0.007^{b} ± .000	0.006^{b} ± .000	0.007*
TDNI (kg/kg W0.75)	0.055 ± 0.002	0.054 ± 0.003	0.054 ± 0.002	0.983^{ns}

*Means bearing different superscripts in a row differ significantly ($p < 0.05$)

Tauqir et al. (2011) concluded from their experiment in 36 growing male Nili Ravi buffalo calves that digestibility of NDF and CP were similar among treatment groups (six experimental diets with three levels of crude protein (CP; 11.85, 14.20 and 16.50 %) each with two levels of metabolisable energy (ME; 1.86 and 2.23 Mcal/kg) whereas digestibility of DM was significantly higher in groups supplemented with higher protein levels. Purushothaman (2018) noticed that the digestibility of DM, CP, CF and EE were similar among the two rations (TMR containing soya sauce waste (TMR 1 CP-15.2 %) and TMR containing tapioca starch waste (TMR 2 with CP-14.7 %), whereas the digestibility of OM and NFE were increased significantly in TMR 1 than in TMR 2. The nutrient intake such as CP, DCP and TDN were found to be similar among the groups, whereas the CP intake and DCP intake per kg metabolic body size were found to be significantly ($P<0.05$) lower in T2 and T3 compared to T1.

Urinary purine derivatives and microbial protein production

The urinary allantoin, urinary uric acid and total purine derivative excreted by experimental Vechur cattle retained on T1, T2 and T3 are presented in Table 4. Statistically, there was no significant difference ($P>0.05$) observed in these parameters among the three treatment groups. The values are comparable to those of Purushothaman (2018), who reported total purine derivatives - 11.66 mM/L in Vechur heifers fed on grass based TMR and Jasmine (2021) who reported 12.28 mM/L in cross bred cows fed on straw based TMR. Similarly, Chacko (2015) observed no significant difference in the excretion of urinary total purine derivatives in crossbred cattle fed with complete feed containing varying levels of neutral detergent fibre (25, 30 and 35 % NDF).

Table 4. Urinary purine derivative excretion[1], microbial protein production[1] and PDC index[1] of Vechur cattle fed on TMR with varying levels of protein

Parameter	T1	T2	T3	p-value
Allantoin, mMol/L	11.13 ± 0.38	10.85 ± 0.55	10.81 ± 0.44	0.873^{ns}
Uric acid, mMol/L	0.76 ± 0.03	0.81 ± 0.04	0.85 ± 0.02	0.215^{ns}
Purine derivative excretion, mMol/L	11.89 ± 0.35	11.66 ± 0.53	11.66 ± 0.43	0.916^{ns}
Creatinine (mMol/L)	3.52 ± 0.21	3.42 ± 0.29	3.39 ± 0.45	0.960^{ns}
Purine derivative: Creatinine Index	97.75 ± 4.99	100.79 ± 5.95	106.96 ± 7.89	0.597^{ns}
Duodenal flow of Microbial Nitrogen, g N/day	32.60 ± 4.18	31.40 ± 2.58	33.27±2.5	0.915^{ns}
Microbial Protein production, g/day	203.71 ± 26.11	196.22 ± 16.09	207.95 ± 15.64	0.915^{ns}
Efficiency of Microbial nitrogen production, g N/kg DOMI	33.8 ± 1.65	33.23 ± 2.29	34.31 ± 1.85	0.927^{ns}

[1]Mean values are based on five replicates with SE. ns- non significant

Urinary creatinine excretion values were similar (P>0.05). The concentration of creatinine remained unaffected by the different protein levels in the diets. The excretion of creatinine was constant per metabolic body weight and was proportional to muscle mass, but the season affected its excretion (Whittet et al., 2004). Ashwin (2015) and Srinivas and Ramesha (2017) noted the urinary creatinine ranged from 4.2 to 4.7 mMol/L in Malnad Gidda cows and 3 to 5 mMol/L in Deoni cows, respectively. The PDC index was 97.75, 100.79 and 106.96 for the three treatments T1, T2 and T3, respectively, and the values were similar (P>0.05) statistically. The findings indicated that the calculated PDC index for the treatments showed no significant (P>0.05) response to varying protein levels in the diet. The PDC index values obtained in the present study are comparable to those of George (2012) found a PDC index in the range of 115.05 to 162.21 in crossbred calves of nine months of age fed with concentrate mixtures (incorporating with and without urea and slow-release urea) and green grass. Ashwin (2015) observed a PDC index of 223.56 in Deoni cows weighing 370 kg fed ragi straw and concentrate. Cetinkaya et al. (2006) found that the purine derivatives-creatinine index differed significantly across treatment groups (37.6, 51.2, 59.3 and 75.0 respectively) when given a diet containing 125g/kg DM crude protein at four different levels of the voluntary feed intake (40, 60, 80 and 95 %) in Yerli Kara crossbred cattle.

Microbial protein production was 203.71, 196.22 and 207.95 g/day for the three treatments T1, T2 and T3 respectively. The efficiency of microbial protein production for the treatment T1, T2 and T3 were 33.80, 33.23 and 34.31 gN/ kg digestible organic matter intake, respectively. Statistically, there was no significant difference (P>0.05) observed in these parameters among the three treatment groups. Similarly, Chacko (2015) conducted experiments in crossbred cattle fed with complete feed containing varying levels of neutral detergent fibre (25, 30 and 35 % NDF) and noticed microbial protein synthesis was similar among three dietary treatments. Purushothaman (2018) conducted studies in Vechur heifers fed with different total mixed rations (TMR) containing soya sauce waste (TMR 1 CP- 15.2 %) and tapioca starch waste (TMR 2 with CP-14.7 %) and observed higher concentration of microbial protein production in animals fed with TMR 2 (364.59 g/day) than TMR 1 (227.77g/day). The microbial protein production (MPP) values obtained in the present study are comparable to those of George (2012) found MPP values in the range of 61.51 to 196.30 g/day in crossbred calves fed with concentrate mixtures (incorporating with and without urea) and green grass.

In the current study, the observed microbial protein efficiency was slightly above the recommended optimal efficiency of 30 gN/ kg of digestible organic matter intake, as advised by ARC (1980). Srinivas and Ramesha (2017) observed that the efficiency of microbial protein production ranged from 27.96 to 49.83 g/kg DOMI in the dwarf cattle breed, Malnad Gidda, maintained on different feeding systems. Similarly, Ashwin (2015) also observed the efficiency of microbial protein production of 33.57 gN/kg DOMI for Deoni cows fed ragi straw and concentrate.

CONCLUSION

From the results obtained on the present study, it could be observed that cattle in T2 and T3 had similar growth performance as that of T1. Microbial protein production was also similar among the treatment groups. These findings suggest that a total mixed ration containing 12% CP might be adequate to meet the nutritional requirements of growing Vechur cattle without compromising performance and digestibility, thereby offering a potentially economical feeding strategy.

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