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Effect of Functional Nutrient Supplements on Milk Production Performance and Hormonal Profile in Crossbred (Holstein-Fresian X) Cows Fed on Finger Millet Straw (*Eleuicine Coracana*) Based Rations

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ABSTRACT

A lactation trial of four months duration was conducted to study the effect of feeding functional nutrient supplements on milk production performance and hormonal profile of crossbred cows under field condition. Twenty-eight crossbred cows were randomly divided into four comparable groups (control and experimental) of seven each based on lactation number, milk yield and stage of lactation. The cows in control group (G-I) were fed finger millet straw (FMS) with supplements such as groundnut meal (GNC) and wheat bran (WB) as practiced by the farmers. Animals in experimental groups (G-II, G-III, and G-IV) were fed finger millet straw (FMS) with GNC, wheat bran and supplements 1 (Sup I), Sup II and Sup III, respectively @ 200g /day/animal by replacing the double the quantity of GNC in the experimental groups. These supplements (Sup I, Sup II and Sup III) were prepared with 65% of locally available different bypass rich protein/amino acid supplements and 30% of the bypass fat and 5% of area specific mineral mixture. Milk yield was recorded daily. Blood was collected and separated for plasma to determine hormonal status by radio immune assay (RIA). Microbial protein synthesis was estimated by analysing urinary purine derivatives. Total DM, OM, CP and TDN intakes recorded among experimental animals were not significantly different (P>0.05%). The 4% fat–corrected milk (FCM) recorded was comparable among groups G-II, G-III, and G-IV, but higher (P<0.05) than G-I. Further, the milk composition in terms of fat, total solids and SNF showed a significant (P<0.05) improvement in experimental groups over the control group on FMS based diets. The results showed the trend of 16, 12 and 9 % increase in FCM yield in animals of G-II, G-III and G-III, fed with limiting nutrient supplements Sup I, Sup II and Sup III, respectively when compared to G-I animals. Plasma hormonal profiles of growth hormone (GH), prolactin (PRL), insulin like growth factor1 (IGF1), estradiol-17â (E2â), progesterone (P4), triiodothyronine (T3) and thyroxine hormone (T4) were positively correlated with (r=0.69, P<0.05) milk yield in cows fed with functional nutrient supplements compared to controls. The microbial protein synthesis and nitrogen capture efficiency (NCE) was comparable among the experimental groups (G-II, G-III, and G-IV) but higher than the control group (G-I). The results indicated that, feeding of functional nutrient supplements, reduced CP intake by 8 to 12%, increased FCM yield from 9 to 16% without compromising the microbial protein synthesis, reduced feed cost by Rs 4 to 9 and increased overall income of the farmers by Rs 24 to 38 /animal /day in medium yielding cows fed on FMS based diets..

KEYWORDS: Functional nutrient supplements, Milk yield, Hormonal profiles, Crossbred cows

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INTRODUCTION

The major source of feeding for dairy animals in India is crop residues, low quality feeds, mixed grasses / grazing. These feeds are utilized very poorly because of the unbalanced nutrients present in them leading to low productivity in animals. Novel feed technologies such as protected bypass protein, fat, supplementation of limiting amino acids in the dairy cattle ration have significantly contributed to improve the efficiency and profitability of milk production (Chandrasekharaiah et al., 2008; Sirohi et al., 2010; Amrutkar et al., 2014). However, under field conditions, the farmers do not feed their livestock as per their production potential (Chandrasekharaiah et al., 2004). They feed different supplements routinely without looking into nutritive

value and cost factor. Solvent-extracted soybean meal (SBM), an alternate protein of choice is being used for replacing the GNC in livestock feeding. SBM is as good a source as GNC or even better in its nitrogen and amino acid content (Chandrasekharaiah et al., 2003; Chandrasekharaiah et al., 2012) and its use in appropriate levels in livestock feeding is necessary for optimum microbial protein synthesis, which otherwise might result in wastage of large quantities of nutrients, particularly nitrogen, which might add to cost of production and ultimately lead to environmental pollution.Research conducted on demonstration of effect of functional nutrient supplements on milk production at field level with simple scientific intervention is very scanty. Therefore, effort was made in this study to improve the FCM in dairy animals by feeding functional nutrient supplements while maintaining high level of milk production with optimum levels of endogenous hormone secretions and to reduce the feed cost in the villages. Mechanisms mediating the effects of nutrition and hormones on lactation, however, have not yet been fully elucidated. A better understanding of the mechanisms involved in the sensitivity of cows to nutritional challenges was warranted. Our objective, therefore, was to assess the effect of feeding functional nutrient supplements on milk production performance and hormonal profile of crossbred cows under field condition.

MATERIALS AND METHODS

Selection of villages for on-farm trials

Two villages i.e. Anagalapura and Menesi in Doddaballapura taluk of Bangalore rural district of Eastern dry zone of Karnataka, India were selected for on-farm trials.

Supplements

Three functional nutrient supplements were prepared with locally available bypass rich protein/ amino acid supplements (such as soya extraction, maize gluten meal (MGM), cottonseed extraction and their combinations were used to supply limiting amino acids) and bypass fat to supply energy and area specific mineral mixture (ASMM) to supply essential minerals in the supplement. The bypass fat and ASMM preparation was added @ 30% and 5%, respectively in all the supplements whereas remaining 65% was added with bypass rich proteins/ amino acid supplements.

Preparation of by-pass rich protein/amino acid supplements

Protected soybean extraction (0.9% Formaldehyde treated; PS) was added as a source of bypass rich protein/amino acid supplement in Supplement 1, PS and MGM (60:40) in Supplement 2, and , PS, MGM and CSC (70:15:15) in Supplement 3

Preparation of bypass fat

Rice bran acid oil (RBO) was used as a source of bypass fat to supply energy in strategic nutrient supplements. Concentrate sulphuric acid (120 ml) was added to 500 ml of tap water and mixed in 4 kg hot RBO. After few minutes (when effervesces almost subsided), 1.6 kg technical grade calcium hydroxide dissolved in 10 lit. water, was added to it and boiled for 30 minutes without cover on medium heat When the product became granular and non-sticky, it was filtered through a cloth with repeated washings under running tap water and was sun dried. The bypass fat was kept in air tight container in a cool place after mixing with butylated hydroxy toluene (BHT) @ 0.05% as an antioxidant (Naik et al., 2007).

Area specific mineral mixture (ASMM)

The ASMM (NIANP-ASSM technology commercialized) was prepared with most limiting macro and micro minerals for dairy animals in the region for this research purpose.

Functional nutrient supplements

The final compositions of functional nutrient supplements are:

Supplement 1. 65% of PS + 30% bypass fat and 5% ASMM.

Supplement 2. 65% of PS and MGM (60:40) + 30% bypass fat and 5% ASMM

Supplement 3. 65% of PS, MGM and CSC (70:15:15) + 30% bypass fat and 5% ASMM

These strategic limiting nutrient supplements were used @ 200g/day/animal in experimental groups by replacing the double the quantity (about 400g/ day/animal on as such basis) protein supplement (GNC) in the control group.

On-farm trial- treatments

One On-farm lactation trial of 4 months duration was conducted in Anagalpura and Menesi villages to study the effect of feeding functional nutrient supplements on the milk production performance of crossbred cows. 28 crossbred cows were randomly divided into 4 comparable groups (control and experimental) of 7 each based on lactation number, milk yield and stage of lactation. In both the villages, the cows in control group were fed FMS with supplements such as GNC and wheat bran as practiced by the farmers. Animals in experimental groups were fed FMS with GNC, wheat bran and supplements 1, 2, and 3, respectively @ 200g/day/ animal by replacing double the quantity (about 400g/ day/animal on as such basis) of GNC in the experimental groups (Table 3). All the animals in both the villages were maintained under identical managemental conditions throughout the period of study which lasted for 4 months in both the villages.

Data recording

Daily records of the amount and type of supplement offered to each animal, feed intake and daily milk yield (Two times a day) were maintained.

Chemical analysis

Feeds and functional nutrient supplements (FNS)

The feeds used in the experiment were analysed for proximate principles (AOAC, 2010) and fibre fractions (Goering and Van Soest, 1970). The rumen degradable content of functional nutrient supplements was determined by estimating the dry matter (DM) and crude protein (CP) disappearing from samples in nylon bags (pore size 40µm) of size 100mm×170mm, incubated in the rumen of three crossbred fistulated steers for 3, 6, 9, 12 and 24 h (Mehrez and Orskov, 1977). The effective protein degradability for the different protein supplements were calculated assuming an outflow rate of 5% (Orskov and McDonald, 1979). Three crossbred steers (HFX) of about 350 kg body weight fitted with large rumen canula were used to estimate the degradability of feedstuffs. Animals were fed concentrate mixture (*Zea mays* grain 30%, Arachis hypogaea cake 25%, wheat bran 42%, mineral mixture 2% and salt 1%), green grass (*Brachiara mutica*) and *Eleuicine coracana* (finger millet straw -FMS) to meet the nutrient requirement (ICAR, 2013). The rumen degradable nitrogen (RDN) provided by straw was not taken into account for calculating the total RDN of the diet.

The ratio of rumen degradable protein (RDP) to undegradable protein (UDP); lysine, methionine and essential amino acid (EAA) content (%) in metabolizable protein (MP) in the bypass rich protein supplements used in preparation of functional limiting nutrient supplement 1, 2, and 3 was calculated (Sampath et al., 2002; 2003; Chandrasekharaiah et al., 2003; Schwab et al., 2005).

Samples

Milk samples were drawn at fortnightly intervals for further analysis (ISI, 1961; ISI, 1977). Fat corrected milk at 4% (4% fat-corrected milk, FCM) was calculated (Tyrell and Reid, 1965).

Estimation of hormones by radio immunoassay (RIA)

Blood samples were collected from all the animals at two week intervals from the jugular vein in a heparinised vial and plasma was separated by centrifugation at 5000 rpm for 10 minutes. Plasma levels of growth hormone (GH), prolactin (PRL), insulin like growth factor1 (IGF1), estradiol-17â (E2â), progesterone (P4), triiodothyronine (T3) and thyroxine hormone (T4) were estimated by RIA kits obtained from Immune Tech France. Bovine GH and PRL pure hormone, iodination grade and antisera were obtained from John. A. Proudman, USDA as a gratis from USA. Hormones were estimated in duplicate. The intra assay variance of E2â (9.2%/),

P4 (8.13%), IGF1(6.39%), T3 (8.91%), T4 (7.43%), GH (8.44%) and PRL (8.21%) and inter assay variance of E2â (12.78%/), P4 (11.69%), IGF1(10.23%), T3 (11.22%), T4 (12.16%), GH (12.11) and PRL (10.11%) was calculated.

Determination of microbial protein synthesis

The urine samples excreted by animals in experimental groups were mixed, collected, measured and a fixed sample (10-15 ml based on volume collected during the period), was immediately transferred into plastic vials each containing sufficient quantity of 10 % H2SO4 to maintain pH below 3. The urine samples were diluted with distilled water in such a way that the concentration in the final samples would fall within the range of standards (5-50 mg/lit) used in the assays for estimation of purine derivatives. These diluted samples were centrifuged and filtered through surgical gauze and stored at -20^0 C.

Microbial protein synthesis was determined based on urinary purine derivatives (PD) and Creatinine as markers in the urine (Chen et al., 1995). Spectrophotometric determination of allantoin and uric acid in urine sample was carried out as per the method suggested by IAEA, 2002.

The amount of microbial purines absorbed (**X** mM/ day) corresponding to the PD excreted (**Y** mM/day) was calculated based on relationship derived by Chen and Gomes (1992).

The quantitative relationship between absorption of PD and excretion of PD was determined by the following equation

$$Y = 0.85X + (0.385\ W^{0.75})$$

Where, W0.75 represents the metabolic body weight (kg). The slope of 0.85 in the equation represents the recovery of purines as PD. Thus, the daily purine absorption was calculated back as

$$X = (Y - 0.385 \times W^{0.75}) \div 0.85$$

Intestinal supply of microbial N was calculated as

$$\text{Microbial N (g N/d)} = 70\ \mathbf{X} / (0.116 \times 0.83 \times 1000) = 0.727\ \mathbf{X}$$

Where,

0.83 is assumed to be the digestibility of microbial purines, 70 mg N/ mmol is the N content of purines,

0.116 represents the ratio of purine: total N in mixed rumen microbes.

The Nitrogen Conversion Efficiency (NCE) were calculated in experimental groups according to method of Chen et al. (1999).

Statistical analysis and cost economics

The statistical analysis of data was carried out in accordance with Snedecor and Cochran (1989). The data were analyzed by PROC GLM procedure using the statistical software SAS for Windows version 9.2 (SAS Institute Inc., 2009). The cost of milk production was calculated taking into account the cost of the feeds, milk yield etc.

RESULTS AND DISCUSSION

Functional nutrient supplements

The chemical composition of the feeds and functional nutrient supplements used in the experiment was recorded (Table 1 and 2).

Table 1. Chemical composition feeds (%DM basis)

Parameters	Wheat bran	Groundnut cake	Finger millet straw	Soyabean meal	MGM	CSC	Protected Soya
Dry matter	94.3	96.8	97.1	95.3	96.4	94.5	95.6
Organic matter	93.7	91.4	90.4	91.5	97.0	93.4	91.7
Crude protein	14.1	44.1	4.60	48.0	63.2	35.7	48.8
Ether extract	1.98	0.72	0.85	1.26	0.60	0.37	1.29
Total ash	6.26	8.53	9.6	8.43	2.96	6.51	8.28
Neutral detergent fibre	41.1	27.8	74.1	27.8	20.8	36.8	28.6
Acid detergent fibre	11.9	19.2	48.2	19.4	9.34	26.4	19.4

MGM, maize gluten meal; CSC, cottonseed cake

The CP content (%) was 48.1, 54.5, and 49.0, the calculated values for ratio of rumen degradable protein (RDP) to undegradable protein (UDP) was 24:76, 23:77, and 25:75 ; lysine content (%) in metabolizable protein (MP) was 4.06, 2.74 and 3.54 ; methionine content (%) in MP was 2.35, 1.73 and 0, 1.86 ; and essential amino acid (EAA) content (%) in MP was 43.93, 33.06 and 34.97 , respectively in bypass rich protein/amino acid supplements used in preparation of functional nutrient supplement 1, 2, and 3 (Chandrasekharaiah et al., 2003; Sampath et al., 2002; 2003; Schwab et al., 2005).

The ether extract content was comparable and the protein content was in the range of 32 to 36% in the strategic limiting nutrient supplements (SLNS) prepared. The ratio of rumen degradable protein (RDP) to undegradable protein (UDP) was 22:78, 16: 84 and 23:77, respectively in sup 1,2 and 3 (Table 2). The effective degradability of bypass fat was 86% indicating the availability of fat/energy for absorption at the lower tract.

Table 2. Chemical composition and degradability (%) of strategic nutrient supplements (% DM basis)

Parameters	Sup I	Sup II	Sup III	Bypass fat*
Dry matter	95.4	94.4	94.4	97.0
Organic matter	88.3	89.2	87.1	-
Crude protein	32.3	36.5	32.7	-
Ether extract	19.8	19.0	19.0	56.8
Total ash	11.6	10.7	12.8	29.2
Neutral detergent fibre	19.62	17.56	19.65	-
Acid detergent fibre	12.76	10.12	12.46	-
Degradability **	22.3	16.1	23	14.1

** Degradability of protein for strategic supplements I to V and degradability of fat of bypass fat supplement
* Prepared in the laboratory

Soybean extraction is a good source of essential amino acids; hence, it was treated with formaldehyde @ 0.9% to protect protein as well as essential and limiting amino acids (lysine and methionine). The CSC and MGM are also good sources of naturally protected proteins (Chandrasekharaiah et al., 2001; 2002) and their bypass protein is a good source of one or the other limiting amino acids such as lysine and methionine (Chandrasekharaiah et al., 2003;). Hence, PS, MGM and CSC were used as a source of bypass rich protein/amino acids in the preparation of Functional nutrient supplements in the study (Table 1 and 2). In all the bypass rich protein/amino acid supplements, the RDP and UDP content was comparable, whereas the limiting amino acid (LAA) and EAA in MP differed and played a significant role in the process of milk production. The LAA and EAA in MP are comparatively higher in bypass rich protein/amino acid supplements 1, compared to 2 and 3, which contributed for higher milk production there by correlated well with hormones also.

Intake of nutrients

Body weights of the animals used in the study ranged from 418 to 458 kg and was not significantly ($P > 0.05$) different (Table 3). Total DM, OM, CP and TDN intakes recorded among experimental animals were not significantly different ($P>0.05$%; Table 3).

Table 3. Effect of incorporation of functional nutrient supplements on intake of nutrients in control and experimental groups on FMS based diet in crossbred cows

Parameters	G I	G II	G III	G IV	SEM	P-value
Body weight (kg)	434.7	417.7	422.8	457.5	15.96	0.685
Metabolic body weight (kg)	94.9	92.0	92.9	98.7	2.58	0.679
DM intake (kg)						
FMS	7.63	7.45	7.32	7.00	0.11	0.256
GNC	0.97 ^{b}	0.59 ^{a}	0.59 ^{a}	0.59 ^{a}	0.03	0.001
WB	2.40	2.57	2.29	2.70	0.07	0.176
Supplements	0.00^{a}	0.19 ^{b}	0.19 ^{b}	0.19 ^{b}	0.02	0.001
Total	11.00	10.80	10.39	10.48	0.14	0.431
OM intake (kg)						
FMS	6.90	6.75	6.62	6.32	0.10	0.256
GNC	0.89 ^{b}	0.54 ^{a}	0.54 ^{a}	0.54 ^{a}	0.03	0.001
WB	2.26	2.42	2.15	2.53	0.06	0.175
Supplements	0.00 ^{a}	0.17 ^{b}	0.17 ^{b}	0.17 ^{b}	0.01	0.001
Total	10.05	9.90	9.51	9.59	0.13	0.430
TDMI (Kg/100 kg B.W)	2.62	2.70	2.56	2.34	0.10	0.469
TOMI (Kg/100 kg B.W)	2.39	2.47	2.33	2.13	0.09	0.470
TDMI (g/kgW0.75)	118.19	120.46	114.44	107.34	3.54	0.427
TOMI (g/kgW0.75)	107.82	109.88	104.34	97.96	3.23	0.427
CP intake (kg)						
FMS	0.35	0.34	0.34	0.32	0.01	0.256
GNC	0.43 ^{b}	0.26 ^{a}	0.26 ^{a}	0.26 ^{a}	0.01	0.000
WB	0.34	0.36	0.32	0.38	0.01	0.176
Supplements	0.00 ^{a}	0.06 ^{b}	0.06 ^{b}	0.06 ^{b}	0.01	0.000
Total	1.12 ^{b}	1.03 ^{a}	0.99 ^{a}	1.03 ^{a}	0.01	0.012
TDN intake (kg)						
FMS	3.93	3.84	3.77	3.60	0.06	0.256
GNC	0.68 ^{b}	0.41 ^{a}	0.41 ^{a}	0.41 ^{a}	0.02	0.000
WB	1.44	1.54	1.37	1.62	0.04	0.175
Supplements	0.00 ^{a}	0.15 ^{b}	0.15 ^{b}	0.15 ^{b}	0.01	0.001
Total	6.05	5.95	5.71	5.78	0.08	0.427
RDN intake (g)						
FMS						
GNC	58.7 ^{b}	36.1 ^{a}	36.0 ^{a}	36.1 ^{a}	1.90	0.001
WB	124.0	132.7	118.2	130.6	2.96	0.331
Supplements	0.00 ^{a}	2.21 ^{c}	1.79 ^{b}	2.30 ^{d}	0.18	0.000
Total	182.7^{b}	171.0^{ab}	156.0^{a}	169.0 ^{ab}	3.29	.034
RDN intake (g/kg OMI)	18.2 ^{b}	17.2 ^{ab}	16.4 ^{a}	17.7 ^{ab}	0.22	.023

$^{a, b}$ means with different superscripts in the row differ significantly (P<0.05)

SEM: standard error of mean; DM: dry matter; FMS: finger millet straw; OM: organic matter; TDMI: total dry matter intake; B.W: body weight; TOMI: total organic matter intake;

The total dry matter intake was in the range of 10.4 to 11.0 kg /animal/day among the experimental groups. Intakes of DM and OM/kgW$^{0.75}$ were not significantly different among experimental groups. The mean DM intake values ranged from 112 to 117 g/kgW$^{0.75}$ which were comparable to the value suggested by ICAR (2013). The CP intake was in the range of 1.0 to 1.12 kg/ animal /day and the percent reduction in intake of CP over control was 8.30, 11.70 and 8.04, respectively in G1, GII and

GIII. The RDN intake from GNC was significantly($P<0.001$) different among the experimental animals. The RDN intake was in the range of 16 to 18 g/ kg OM intake and the percent reduction in intake of RDN over control was 5, 10 and 3, respectively in groups G1, GII and GIII.

The intakes of DM, OM, CP, TDN and RDN from GNC decreased at a decreasing rate ($P<0.001$) in experimental groups, since the functional nutrients in the experimental groups were offered @ 200g/ day/animal by replacing the double the quantity (about 400g/ day/animal on as such basis) of GNC that was used in control group. However, variation in intake of all the nutrients from other feedstuffs among the experimental groups was not significant. The TDN intake was 5.71 to 6.05 kg which was still lower than the recommended level of 6.24 for a 450kg cow yielding 8 litres of milk with 4% fat (ICAR, 2013) indicating the scope for further increasing the energy density in the functional nutrient supplements and also the protein intake needs to be balanced with bypass rich protein/amino acid supplements. Similar to the present study, Chandrasekharaiah et al.(2003) earlier observed that the protein requirement was almost met with but the total digestible nutrients (TDN) was deficient by 2.61 kg and 3.12 kg in Anagalapura and Menesi villages, respectively and needs to be supplied more energy for increased milk production and also the protein intake needs to be balanced with bypass rich protein/ amino acid supplements.

The reduced CP and RDN intakes in experimental groups when compared to control group indicated that these functional nutrient supplements could be used in future studies for precise feeding of high quality proteins thereby making the best use of expensive protein supplements at the same time to reduce the nitrogen loss as urea in urine, since the inefficient use of nitrogen indicates wastage of large quantity of nutrients, particularly nitrogen, adding to the cost of production and ultimately leading to environmental pollution.

Milk production performance

The average FCM yield recorded was significantly ($P<0.05$) different among the experimental groups (Table 4). FCM recorded was comparable among groups GII, GIII and GIV, but higher ($P<0.05$) than GI, indicating that the groups (II, III and IV) fed with e" 60% PS in their FNS, showed better production performance. Further, the average percentage of milk composition i.e. fat, total solids and SNF showed a significant ($P<0.05$) improvement in experimental groups over the control group on FMS based diets. The feed conversion efficiency was also improved in groups II III and IV, when compared to control group (Table 4).

Table 4. Effect of incorporation of functional nutrient supplements on milk yield, composition, feed cost and overall income in control and experimental groups on FMS based diet in crossbred cows

Particulars	Control	G I	G II	G III	SEM	P- value
Milk yield(lit) (4 % FCM)	8.38 ^{a}	9.72 ^{b}	9.38^{b}	9.10^{b}	0.40	0.04
% increased FCM	-	16.0	12.0	9.00		
Fat(%)	3.53^{a}	3.69^{b}	3.68^{b}	4.17 ^{c}	0.05	0.001
SNF(%)	8.38^{a}	8.46^{a}	8.54^{ab}	8.73^{b}	0.08	0.003
Total solids(%)	11.91^{a}	12.15^{b}	12.22 ^{b}	12.89^{c}	0.11	0.001
DM intake (kg)	11.00	10.80	10.39	10.48		
Total feed cost (Rs)	138.06	136.67	129.43	132.93		
Feed conversion efficiency (kg DMI/kg FCM)	1.31	1.11	1.11	1.15		
Total milk cost (Rs) (revenue)	217.88	252.72	243.88	236.63		
Farmers income (Rs)	79.82	118.05	114.45	103.67		

Further, the average percentage of milk composition i.e. fat, total solids and SNF showed a significant ($P<0.05$) improvement in experimental groups over the control group on FMS based diets. This was probably be due to presence of higher bypass LAA and EAA content in the respective SLNS. The milk yield was increased by 0.72 to 1.34 L/cow/day, feed cost was reduced by Rs. 1.86 to 2.67/litre and the overall income of the farmers was increased by Rs 24 to 38 /cow/day in experimental groups by the refinement of existing feeding practices followed by the farmers.

The similar findings were reported by Chandrasekharaiah et al. (2004, 2008, 2017) and Sampath et al. (2008) earlier on finger millet straw and local mixed grass-based diets supplemented with different energy and bypass protein ingredients such as maize grain and cottonseed cake. The animals also came to heat at stipulated time, conceived and no reproductive problems were observed in these two villages. Pathak and Panday (1995) also reported higher milk yield in crossbred cows fed with higher levels of energy supplements along with *ad libitum* Egyptian clover (*Trifolium alexandrinum*) fodder. Type of carbohydrate and nitrogen included and nitrogen to energy ratio of ration influence milk production and feed efficiency (Clark and Davis, 1980).

Further, GNC is a highly degradable feed ingredient (Sampath et al., 1999, Chandrasekharaiah et al. 2012) hence, the inclusion of slowly degradable strategic nutrient supplements would have provided matching energy and protein requirement to the rumen microbes for better digestibility of local mixed grass based diet. As such also replacement of GNC with functional nutrient supplements enhanced the energy and bypass protein level of ration which probably contributed for higher level of milk production. Chandrasekharaiah et al. (2001) also reported lower rate of degradability of energy supplements in the rumen of crossbred cows. There is a tendency in increase in milk fat with increasing intake of energy (Morely, 1970) even with high roughage diets (El-Gallard et al., 1988), accompanied by increase in milk yield (Sivaiah and Mudgal, 1983). In this study FNS were prepared with bypass fat as energy source, protected proteins as a source of limiting amino acids and area specific mineral mixtures as a source of most bio-available minerals. Hence, the escape of limiting nutrients such as protein and energy of FNS from rumen fermentation, which will be available in the lower tract for direct absorption, would have caused increased milk production in the experimental group of cows. Mackle et al. (1999) also reported increased milk yield in cows grazing ryegrass-white clover pastures when supplemented with maize grain and or silage. Dahiya et al. (1991) observed a significant increase in milk yield (2-3 kg per day) in buffaloes in early lactation fed on urea treated straw supplemented with 1.5 and 3.0 kg cottonseed cake (bypass protein source).

The better production response observed in the present study in the experimental groups was attributed to the presence of higher bypass LAA and EAA content in the respective FNS. Sirohi et al. (2013) reported that rumen bypassing of protein fractions (formaldehyde treated mustard cake) showed beneficial effect on milk production without affecting the composition in medium producing crossbred cows fed wheat straw-based diets. Similar results were reported by various workers by feeding bypass protein feeds in lactating animals which might be due to increased pool of amino acids at tissue level for utilization (Kaim et al., 1987; Hamilton et al., 1992; Gulati et al., 2002; Mishra et al., 2006). Feeding formaldehyde protected protein at higher levels in the ration of crossbred cattle (Sampath et al., 1997; Shelke and Thakur, 2011) and buffaloes (Chatterjee and Walli, 2003) improved the milk yield by 16-20%. The increase in milk production reported on feeding protected protein in the present study could also be due to more availability of protein for digestion in small intestine, thereby increasing supply of precursors of milk production (Forster et al., 1983). Santos et al. (1998) after a thorough review, reported varying trend with milk fat also. Positive effect was reported by Voss

et al. (1988), whereas other workers had reported no effect on milk fat percentage (Mohamed et al., 1988; Mishra et al., 2006; Fathi Nasri et al., 2007). Milk lactose and SNF contents were not affected on feeding different levels of rumen un-degradable protein or fat (Santos et al., 1998; Fathi Nasri et al., 2007).

Vahora Safimahmad et al. (2013) reported that feeding bypass protein alone or in combination with bypass fat resulted in improvement in dry matter intake, yield of whole milk, 6% FCM, and return over feed cost from buffaloes during their early lactation. Garg et al. (2002) also observed an increase in the net average daily income of Rs. 9.61/- on feeding of 1kg bypass protein (protected sunflower meal) in the ration of lactating cows. Moallem et al. (1997), Tyagi and Thakur (2007) and Sirohi et al. (2010) reported increase in milk and FCM yield in dairy animals fed with bypass fat.

The differences in the overall cost benefits among the experimental groups could be attributed to the differences in quantity of basal feed offered to the animals by the farmers in these two villages (FMS and wheat bran). The results of 4 months on-farm lactation trail showed the trend of 16, 12 and 9 % increase in FCM yield in animals fed under G-II, III and G-IV group when compared to G-I along with improvements in feed conversion efficiency.

Hormonal profiles

Blood samples were collected from all the animals at two weekly intervals to correlate the concentration of hormones with milk yield. Plasma hormonal profiles of GH, IGF-1, E2â, P4, T3 and T4 were positively correlated with ($r=0.69$; $P<0.05$) milk yield in cows fed with strategic nutrient supplements compared to controls. However, PRL levels were statistically similar among the different treatment groups (Table. 5). In this study, animals fed with SLNS increased the plasma concentrations of GH, IGF-1, T3, T4, E2â and progesterone compared to controls. However, PRL levels were low in treated cows

Table 5. Endocrine parameters in control and experimental groups on FMS based diet

Particulars	Control	G-I	G-II	G-III	SEM	P-value
PRL (ng/ml)	237.3^{a}	178.1^{b}	188.2^{b}	185.2^{b}	40.81	0.04
GH (ng/ml)	2.7^{a}	3.5^{b}	3.9^{b}	4.1^{b}	0.42	0.03
IGF-1(ng/ml)	28.6^{a}	38.7^{b}	44.5^{b}	47.2^{b}	11.95	0.03
Estradiol-E2β (pg/ml)	16.2^{a}	18.1^{b}	19.1^{b}	19.3^{b}	0.95	0.05
Progesterone (ng/ml)	0.99^{a}	1.8^{b}	1.6^{b}	1.9^{b}	0.30	0.02
Tri iodothyronin (T3) (ng/ml)	0.30^{a}	1.19^{b}	1.24^{b}	1.34^{b}	0.03	0.03
Thyroxine (T4) (ng/ml)	31.19^{a}	39.14^{b}	41.34^{b}	40.27^{b}	1.16	0.03

a, b Means with different superscripts in the row differ significantly ($P<0.05$)

Once lactation is initiated various hormones such as somatotrophic (GH, IGF-1) (Baumrucker and Erondu, 2000), PRL, thyroid (T3, T4) and steroid hormones (E2â, progesterone) play a key role in maintaining it. Adequate nutrition, maintenance of health and various hormones play a role on lactation in ruminants (Wilde and Knight, 1989). In this study, animals fed with FNS increased the plasma concentrations of GH, IGF-1, T3, T4, E2â and progesterone compared to controls. However, PRL levels were low in treated cows. It is known that, once lactation is established PRL levels may drop without affecting lactation adversely and we observed similar PRL pattern in the treated group. Furthermore, PRL is involved more in initiation rather than maintenance of lactation (Tucker, 1994).

Thyroid hormones are not essential for galactopoises, but thyroidectomy decreased milk production and shortened lactation length probably

by decreasing basal metabolic rate (BMR). T3 if given during the declining phase of lactation it increases milk yield but the fall would be abrupt after withdrawal of T3. Administration of T3 increased feed consumption by the animal and therefore such increase in milk yield might not be so economical (Lasren and Berry, 1995). Steroid hormones induce growth of mammary gland (Bachman 1982). Estradiol-17 2â and progesterone induce alveol-lobular development and enhance milk yield (Tucker, 1994; Akers, 1990). Results obtained in this study were also supported by increase in concentrations of E2â and progesterone in treated cows and explained the reason that, why hormones were low in controls. Endocrine data supported the notion that ensuring adequate nutrients reserves is essential for cattle to produce milk and also to keep hormonal levels adequately required for quality milk production, which otherwise, they run the risk of abnormal/low endocrine profiles required for milk production during different stages of lactation as observed in the controls. Further they might develop metabolic problems and even problems with milk yield (Wilde and Hurley, 1996).

Effect of different FNS on nitrogen use efficiency, microbial protein synthesis in crossbred cattle

The mean values for allantoin, uric acid and total purine derivatives (TPD) excretion per day were significantly different ($P<0.05$) among the experimental groups (Table 6).

Table 6. Effect of different functional nutrient supplements on purine derivatives excretion, microbial nitrogen supply (MNS) and nitrogen capture efficiency (NCE) in crossbred cattle fed on finger millet straw based diet.

Particulars	Control	GI	GII	GIII	SEM	P-Value
Purine derivatives(mmol/day)						
Allantoin	117.7 ±5.99^{a}	162.0 ± 13.24^{b}	150.1 ± 16.91^{ab}	169.2 ± 13.54 ^{b}	6.97	0.03
Uric acid	13.1 ± 0.90^{a}	15.6 ± 1.18 ^{ab}	17.7 ± 1.05^{b}	15.1 ± 1.08 ^{ab}	0.52	0.05
TPD	130.8 ± 5.26^{a}	177.5 ± 13.67 ^{b}	167.6 ± 17.49 ^{b}	184.4 ± 6.75^{b}	7.23	0.03
Microbial purine absorbed (mmoles/day)	110.4 ±6.23^{a}	170.3 ± 17.00^{b}	155.1 ± 19.25 ^{b}	173.7 ± 7.79 ^{b}	8.56	0.02
MNS (g/d)	80.3 ±4.53^{a}	123.8 ± 12.36^{b}	112.7 ± 13.90 ^{b}	126.3 ± 5.594 ^{b}	6.23	0.02
NCE	0.45 ± 0.03^{a}	0.76 ± 0.11^{b}	0.71 ± 0.17 ^{b}	0.76 ± 0.11 ^{b}	0.04	0.001

The allantoin, uric acid and total purine derivatives (TPD) concentration was comparable among FNS supplemented groups (G II, III and IV) but significantly ($P<0.01$) higher than the control group (G-I). Similar trend was followed for microbial purine absorption and estimated microbial nitrogen supply (MNS). The estimated microbial purine absorption, microbial nitrogen supply (MN) was significantly ($P<0.03$) lower in control group, however it was comparable among the supplemented groups. The mean nitrogen capture efficiency (NCE) values were significantly ($P<0.001$) different among experimental groups. The control group (G-I) recorded lower NCE while, higher NCE values were observed among G II, III and IV groups. Lower excretion of purine derivatives, microbial purine absorption and estimated microbial nitrogen supply in G-I when compared to G II, III and IV, indicated that optimum RDN from the supplement 1, 2 and 3 might have been available for microbial fermentation, thereby providing matching nitrogen energy requirement to rumen microbes which provided desired rumen environment for increased microbial protein synthesis in G I, II and III groups.

Very limited literature is available with regards to NCE of ruminant diets. It is suggested that NCE can potentially be used as an indicator of the efficiency with which degradable dietary nitrogen is converted to microbial protein in the rumen (Chen et al., 1999). The excretion of PD in urine provides

an estimation of the intestinal flow of microbial protein, and therefore, if a dietary regimen has higher nitrogen conversion efficiency proportionally more RDN is converted to microbial protein and less nitrogen is excreted in urine (Chen et al., 1999, Chandrasekharaiah et al., 2012).Therefore, in the present study, higher MNS and NCE values observed in G-II, III and IV groups indicated higher N use efficiency and the RDN was utilized more efficiently for optimum microbial protein synthesis thereby less nitrogen was excreted in urine of cattle fed on finger millet straw based diets.

CONCLUSION

The results of the present study indicated that FCM yield was increased from 9 to 16%, feed cost was reduced by of Rs 4 to 9, CP intake by 8 to 12% and RDN intake by 3 to 10% and increased NCE from 0.45 to 0.76, without compromising the productivity, and overall income of the farmers was increased by Rs 24 to 38 /animal /day by feeding these functional nutrient supplements in medium yielding cows fed on FMS based diets. Plasma hormonal profiles were not affected in cows supplemented with functional nutrient supplements. Therefore, the functional nutrient supplements developed in the present study could be included in the precision feeding and management of dairy animals, reducing the protein intake without compromising the productivity and microbial protein synthesis, thereby reducing the nitrogen excretion in to the environment.

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