



Effect of strategic supplementation of limiting nutrients on milk production performance in crossbred cows under field conditions

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ABSTRACT

An on-farm lactation trial of 4 months duration was conducted to study the effect of feeding strategic limiting nutrient supplements on milk production performance and hormonal profile of crossbred cows under field condition. Crossbred cows (36) were equally divided into 6 groups (control and experimental) based on lactation number, milk yield and stage of lactation. Strategic nutrient supplements (1, 2, 3, 4 and 5) were prepared with 65% of locally available bypass rich protein/amino acid supplements and 30% of the bypass fat and 5% of area specific mineral mixture (ASMM), respectively in all the 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 maize gluten meal (MGM) (60: 40) in supplement 2, cottonseed cake (CSC) and MGM (60:40) in supplement 3, PS, MGM and CSC (70:15:15) in supplement 4 and PS, MGM and CSC (40:40: 20) in supplement 5. The cows in control group were fed local mixed grass with supplements such as groundnut cake (GNC) and wheat bran as practiced by the farmers. Animals in experimental groups (G 1, 2, 3, 4 and 5) were fed local mixed grass with GNC, wheat bran and supplements 1, 2, 3, 4 and 5, respectively @ 200g/day/animal by replacing the double the quantity of GNC in the experimental groups. There was an increase of 16, 14, 9, 10 and 2 % in 4% fat-corrected milk (FCM) yield in animals fed with limiting nutrient supplements 1, 2, 3, 4 and 5, respectively when compared to control. Plasma hormonal profiles of growth hormone, insulin like growth factor 1, estradiol-17 β , progesterone, triiodothyronine and thyroxine hormone were positively correlated ($r=0.69$; $P<0.05$) with milk yield in cows fed with strategic nutrient supplements (SNS). The study showed that feeding of supplements 1 and 2 in experimental groups-1 and 2 significantly ($P<0.05$) increased FCM yield and reduced the feed cost in medium yielding cows on local mixed grass based diets and increased the overall income of the farmers.

Key words: Crossbred cows, Hormonal profiles, Milk yield, Strategic limiting nutrient supplements

The major source of roughage 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. Feed and 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 (Sampath *et al.* 1997, Chatterjee and Walli 2003, Tyagi and Thakur 2007, Chandrasekharaiah *et al.* 2008, Sirohi *et al.* 2010, Amrutkar *et al.* 2014). As a practice, farmers do not feed their livestock as per their production potential in villages (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 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.

The information on strategic limiting nutrient supplements that can be used at field level with simple scientific intervention is very limited. Therefore, an attempt has been made in this study to improve the FCM in dairy animals by feeding strategic limiting 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 is warranted. Our objective therefore was to assess the effect of feeding strategic limiting nutrient supplements on milk production performance and hormonal profile of crossbred cows under field condition.

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MATERIALS AND METHODS

Selection of villages for on-farm trials: 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: Five limiting 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, cottonseed cake (CSC) and MGM (60:40) in supplement 3, PS, MGM and CSC (70: 15:15) in supplement 4 and PS, MGM and CSC (40:40: 20) in supplement 5.

The cows in control group were fed local mixed grass with supplements such as groundnut cake (GNC) and wheat bran as practiced by the farmers. Animals in experimental groups (G 1, 2, 3, 4 and 5) were fed local mixed grass with GNC, wheat bran and supplements 1, 2, 3, 4 and 5, respectively @ 200g/day/animal by replacing the double the quantity of GNC in the experimental groups.

Preparation of bypass fat: Rice bran acid oil (RBO) was used as a source of bypass fat to supply energy in strategic nutrient supplements. Concentrated 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 l 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 to KMF) was prepared with most limiting macro and micro minerals for dairy animals in the region such as calcium (19.2%), phosphorus (9.6%), magnesium (1.36%), zinc (1.62%), copper (0.25%), with common salt (iodized) as a base.

Strategic limiting nutrient supplements: The final composition of strategic nutrient supplements was:

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 CSC and MGM (60:40) + 30%

bypass fat and 5% ASMM

Supplement 4: 65% of PS, MGM and CSC (70:15: 15) + 30% bypass fat and 5% ASMM

Supplement 5: 65% of PS, MGM and CSC (40:40:20) + 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 400 g on fresh basis) of the protein supplement (GNC) that was used in the control group, so that, all the experimental groups (G 1, 2, 3, 4 and 5) contained lesser amount of GNC (about 400 g on fresh basis).

On-farm trial-treatments: One on-farm lactation trial of 4 months duration was conducted in Anagalapura and Menesi villages to study the effect of feeding strategic nutrient supplements on the milk production performance of crossbred cows. Crossbred cows (36; weight 400 ± 9.36 kg) were divided into 6 comparable groups (control and experimental) of 6 each based on lactation number, milk yield and stage of lactation. Shaeffer's formula as described by Sastry *et al.* (1983) was used to calculate the body weight of the animal. According to formula, $W = (C^2 \times L) / 300$, where 'W', live weight of animal in pounds; 'C', heart girth (in inches); and 'L', length from the point of shoulder to the point of pin bone (in inches). The body weight was converted from pound to kilogram.

In both the villages, the cows in control group were fed local mixed grass with supplements such as groundnut cake (GNC) and wheat bran as practiced by the farmers. Animals in experimental groups were fed local mixed grass with GNC, wheat bran and supplements 1, 2, 3, 4 and 5, respectively @ 200g/day/animal by replacing double the quantity of GNC that was used in the control group, so that, all the experimental groups (G 1, 2, 3, 4 and 5) contain lesser amount of GNC (about 400 g on fresh basis). All the animals in both the villages were maintained under identical managerial 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 and daily milk yield were maintained. The information on various parameters, viz. feed intake, date of heat symptom, date of insemination etc., was collected daily in the respective data sheets from the farmers. Perception of participating farmers regarding feasibility of using strategic nutrient supplements in the diet of lactating cows was also recorded.

Chemical analysis

Feeds and strategic limiting nutrient supplements (SLNS): The feeds used in the experiment were analysed for proximate principles (AOAC 1990) and fibre fractions (Goering and Van Soest 1970). The rumen degradable content of strategic nutrient supplements was determined by estimating the dry matter (DM) and crude protein (CP) disappearing from samples in nylon bags (pore size $40 \mu\text{m}$) of size 100 mm \times 170 mm, incubated in the rumen of crossbred fistulated steers for 3, 6, 9, 12 and 24 h (Mehrez

and Orskov 1977). The effective protein degradabilities for the different protein supplements were calculated assuming an outflow rate of 5% (Orskov and McDonald 1979). Crossbred steers (3) of about 350 kg body weight fitted with large rumen canula were used to estimate the degradability of feedstuffs. Animals were fed concentrate mixture (maize 30%, groundnut cake 25%, wheat bran 42%, mineral mixture 2% and salt 1%), green grass (para grass) and finger millet straw (FMS) to meet the nutrient requirement for maintenance (ICAR 2013).

Milk 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 2 week intervals from the jugular vein in a heparinised vial and plasma was separated by centrifugation at 5,000 rpm for 10 min. Plasma levels of GH, PRL, IGF-1, E₂β, P₄, T₃ and T₄ were estimated in all the groups by RIA. IGF-1, E₂β, P₄, T₃ and T₄ were estimated by RIA kits obtained from Immune Tech France. Bovine - bib10GH and PRL pure hormone, iodination grade and antisera were obtained from John. A. Proudman, USDA as a gratis from USA.

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

Strategic nutrient supplements: The chemical composition of the feeds and strategic nutrient supplements used in on-farm trials is presented in Tables 1 and 2. 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, Sampath *et al.* 2002, 2003). Hence, PS, MGM and CSC were used as a source of bypass rich protein/amino acids in the preparation of strategic limiting nutrient supplements in the study.

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 were comparatively higher in bypass rich protein/amino acid supplements 1, 2 and 4, which contributed for higher milk production thereby correlated well with hormones also.

The ether extract content was similar and the protein content was in the range of 32 to 35% in the strategic limiting nutrient supplements (SLNS) prepared. The

Table 1. Chemical composition feeds (% DM basis)

Parameter	Wheat bran	Groundnut cake	Mixed grass	Soya	MGM	CSC	Protected soya
Dry matter	92.1	95.25	30.1	95.36	96.45	94.56	95.61
Organic matter	93.99	91.29	84.5	91.57	97.04	93.49	91.72
Crude protein	13.80	43.26	6.25	48.08	63.2	35.74	48.82
Ether extract	2.31	0.698	1.8	1.26	0.607	0.377	1.29
Total ash	6.01	8.71	15.5	8.43	2.96	6.51	8.28
Neutral detergent fibre	40.01	28.1	70.1	27.87	20.83	36.82	28.64
Acid detergent fibre	12.01	19.61	38.08	19.43	9.34	26.45	19.49

MGM, maize gluten meal; CSC, cottonseed cake.

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

Parameter	Sup 1	Sup 2	Sup 3	Sup 4	Sup 5	Bypass fat
Dry matter	95.42	94.41	94.75	94.46	94.67	97.03
Organic matter	88.39	89.23	89.75	87.14	90.86	-
Crude protein	32.37	36.53	32.08	32.71	34.62	-
Ether extract	19.8	19.08	19.21	19.06	18.98	56.84
Total ash	11.61	10.77	10.25	12.86	9.14	29.23
Neutral detergent fibre	19.62	17.56	20.76	19.65	18.62	-
Acid detergent fibre	12.76	10.12	12.85	12.46	11.04	-
<i>Degradability*</i>						
Protein	22.3	16.1	26.3	23	27.4	
Fat	-	-	-	-	-	14.1

*Degradability of protein for strategic supplements I to 5 and degradability of fat of bypass fat supplement.

effective degradability of bypass fat was around 86% indicating the availability of fat/energy for absorption at the lower tract.

Intake of nutrients: Intakes of DM and OM/kgW^{0.75} were not significantly different among experimental groups (Table 3). The mean DM intake values ranged from 126 to 149 g/kgW^{0.75} which were comparable to the value suggested by ICAR (2013). The total dry matter and CP intakes and the per cent reduction in intake of CP over control, indicated that these strategic nutrient supplements can 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.

The intakes of DM, OM, CP and TDN from GNC decreased at a decreasing rate (quadratic: P<0.001) in experimental groups, since the strategic limiting nutrients in the experimental groups were offered @ 200g /day/ animal by replacing the double the quantity 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 about 5 kg, which was still lower than the recommended level of 6.55 kg (ICAR 2013) for the animals yielding 8 to 10 litres of milk/animal/day indicating the scope for further increasing the energy density in the strategic limiting nutrient supplements. The similar trend was reported by Chandrasekharaiah *et al.* (2003) while assessing the nutritional status of the animals in Anagalapura and Menesi villages of Doddaballapura taluk, Bengaluru district. The protein requirement was almost met with but 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.

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 1, 2, and 4, but higher (P<0.05) than control, groups 3 and 5 indicating that the groups (1, 2

Table 3. Effect of incorporation of strategic nutrient supplements on intake of nutrients in control and experimental groups on local mixed grass based diet in crossbred cows

Parameter	Control	G 1	G 2	G 3	G 4	G 5	SEM	P-value
Body weight (kg)	411.0	399.0	385.0	462.0	356.0	371.0	13.43	0.270
Metabolic body weight (W ^{0.75} kg)	90.74	89.02	86.87	99.37	81.82	84.04	2.24	0.280
<i>DM intake (kg)</i>								
Mixed grass	7.68	8.13	7.98	8.08	8.13	7.89	0.07	0.380
Groundnut cake	0.95 ^b	0.57 ^a	0.57 ^a	0.57 ^a	0.57 ^a	0.57 ^a	0.02	<0.001
Wheat bran	2.84	2.91	2.65	3.32	2.76	2.67	0.10	0.530
Supplements	-	0.19	0.19	0.19	0.19	0.19		
Total DM intake (kg)	11.47	11.80	11.39	12.16	12.21	11.32	0.14	0.490
<i>OM intake (kg)</i>								
Mixed grass	6.49	6.87	6.74	6.83	6.87	6.67	0.06	3.80
Groundnut cake	0.87 ^b	0.52 ^a	0.52 ^a	0.52 ^a	0.52 ^a	0.52 ^a	0.02	<0.001
Wheat bran	2.67	2.74	2.49	3.12	2.59	2.51	0.10	0.510
Supplements		0.17	0.17	0.17	0.17	0.17		
Total OM intake (kg)	10.03	10.29	9.92	10.64	10.15	9.87	0.12	0.490
TDMI (g/100 kg BW)	2.79	2.96	2.96	2.63	3.43	3.05	0.11	0.700
TOMI (g/100 kg BW)	2.44	2.58	2.58	2.30	2.85	2.66	0.10	0.720
TDMI (g/kgW ^{0.75})	126.41	132.55	131.12	122.37	149.23	134.70	3.70	0.790
TOMI (g/kgW ^{0.75})	110.49	115.63	114.24	107.06	124.05	117.44	3.25	0.820
<i>CP intake (kg)</i>								
Mixed grass	0.48	0.50	0.50	0.50	0.50	0.49	0.02	0.380
Groundnut cake	0.43 ^b	0.26 ^a	0.26 ^a	0.26 ^a	0.26 ^a	0.26 ^a	0.01	<0.001
Wheat bran	0.43	0.44	0.40	0.50	0.41	0.40	0.01	0.510
Supplements		0.06	0.07	0.06	0.06	0.08		
Total CP intake (kg)	1.34	1.26	1.25	1.32	1.23	1.23	0.02	0.260
<i>TDN intake (kg)</i>								
Mixed grass	2.55	2.70	2.65	2.68	2.70	2.62	0.02	0.380
Groundnut cake	0.70 ^b	0.42 ^a	0.42 ^a	0.42 ^a	0.42 ^a	0.42 ^a	0.02	<0.001
Wheat bran	1.85	1.90	1.73	2.16	1.80	1.80	0.06	0.510
Supplements		0.15	0.15	0.15	0.15	0.15		
Total TDN intake (kg)	5.10	5.22	4.95	5.41	5.07	4.99	0.07	0.490

^{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; BW, body weight; TOMI, total organic matter intake.

and 4) fed with $\geq 60\%$ PS in their SLNS, showed better production performance. This may probably be due to presence of higher bypass LAA and EAA content in the respective SLNS. However, the average percentage of milk composition i.e. fat, total solids and SNF showed a non significant variation among the experimental groups. The milk yield was increased by 0.23 to 1.81 litre/cow/day, feed cost was reduced by ₹ 0.71 to 2.63/litre and the overall income of the farmers was increased by ₹ 14 to 50 /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) and Sampath *et al.* (2008) earlier on finger millet straw and local mixed grass based diets supplemented with bypass nutrient supplements 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 maize grain along with *ad lib.* berseem fodder. Type of carbohydrate and nitrogen included and nitrogen to energy ratio of ration influence milk production and feed efficiency (Clark and Davis 1980). The differences in the overall cost benefits among the experimental groups can be attributed to the differences in quantity of basal feed offered to the animals by the farmers in these 2 villages (green grass and wheat bran).

GNC is a highly degradable feed ingredient (Sampath *et al.* 1999) 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 strategic nutrient supplements enhanced the energy and bypass protein level of ration which could have 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, SLNS 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 strategic nutrient supplements 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) also reported a significant increase in milk yield (2–3 kg/day) in buffaloes in early lactation fed on urea treated straw supplemented with 1.5 and 3.0 kg cottonseed cake (bypass protein source).

In the present study, higher FCM was recorded in groups 1, 2, and 4, where, SLNS were prepared with $\geq 60\%$ protected soybean extraction (PS) as a source of bypass rich protein/amino acids. The better production response observed in the present study in the experimental group may also be attributed to presence of higher bypass LAA and EAA content in the respective SLNS. 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 supplements in lactating animals which may 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

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

Particular	Control	G 1	G 2	G 3	G 4	G 5	SEM	P-value
Milk yield (4% FCM)	9.43 ^a	11.24 ^b	10.73 ^b	10.25 ^a	10.33 ^{ab}	9.66 ^a	0.18	0.050
% increased FCM	-	19.19	13.79	8.70	9.54	2.44		
Fat %	3.83	4.00	4.28	4.24	4.03	4.43	0.09	0.440
SNF %	8.50	8.52	8.53	8.51	8.50	8.57	0.12	0.710
Total solids %	12.33	12.52	12.81	12.58	12.53	12.72	0.02	0.770
DM intake (kg)	11.47	11.80	11.39	12.16	12.21	11.32	0.14	0.490
Total feed cost (₹/day/animal)*	142.94	140.78	136.26	148.52	138.10	135.70		
Feed conversion efficiency	1.22	1.05	1.06	1.19	1.18	1.17		
Total milk cost (₹/animal/day)* (revenue)	254.61	303.48	289.71	276.75	278.91	260.82		
Farmers income (₹/day/animal)*	111.00	163.00	153.00	128.00	141.00	125.00		

^{a,b}Means with different superscripts in the row differ significantly (P<0.05). *Calculated on the basis of market rate of milk ₹ 27/litre, local mixed grass ₹ 50/30kg (on fresh basis), Wheat bran ₹ 18/kg, GNC ₹45/kg, Supplements 1, 2, 3, 4 and 5 (₹/200g) were 11.90, 13.25, 12.00, 12.10 and 12.83, respectively.

in small intestine, thereby increasing supply of precursors of milk production (Forster *et al.* 1983). Santos *et al.* (1998) after thorough review reported varying trend with milk fat also. Positive effect was reported by Voss *et al.* (1988), whereas others 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 content also have not been affected on feeding different levels of rumen undegradable protein or fat (Santos *et al.* 1998, Fathi Nasri *et al.* 2007).

Supplementing rumen protected methionine and lysine in the ration of high yielding lactating cows during early lactation was cost effective (Amrutkar *et al.* 2014). Increase in milk yield on supplementing rumen protected methionine and /or lysine was reported by several research workers (Wu *et al.* 1997, Dinn *et al.* 1998, Bach *et al.* 2000, Lara *et al.* 2006, Socha *et al.* 2008, Broderick *et al.* 2009, Yang *et al.* 2010). However, reports on effects of methionine and lysine supplementation on milk composition have been inconsistent. 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 ₹ 9.61 on feeding of 1 kg 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 bypass fat.

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, E2 β , P₄, T3 and T4 were positively correlated with milk yield ($r=0.69$; $P<0.05$) in cows fed with strategic nutrient supplements compared to controls. However, PRL levels were statistically similar among the different treatment groups (Table 5). Once lactation has been initiated various hormones such as somatotrophic (GH, IGF-1) (Baumrucker and Erondu 2000), PRL, thyroid (T3, T4) and steroid hormones (E2 β , progesterone) play a 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 SLNS increased the plasma

concentrations of GH, IGF-1, T3, T4, E2 β and progesterone compared to controls. However, PRL levels were low in treated cows. It is known that, once lactation is established, PRL levels may be dropped 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 decreases milk production and shortens lactation length probably by decreasing basal metabolic rate (BMR). T3 if given during the declining phase of lactation increases milk yield but the fall would be abrupt after withdrawal of T3. Administration of T3 increases feed consumption by the animal and therefore such increase in milk yield may not be so economical (Lasren and Berry 1995). Steroid hormones induce growth of mammary gland (Bachman 1982). Estradiol-17 β and progesterone induces alveol-lobular development and enhances milk yield (Tucker 1994, Akers 1990). Results obtained in this study were also supported by increase in concentrations of E2 β and progesterone in treated cows and explained the reason why hormones were low in controls. Endocrine data support 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 develop metabolic problems and may have problems with milk yield (Wilde and Hurley 1996).

Therefore, the results of 4 months lactation trail showed 16, 14, 9, 10 and 2% increase in FCM yield in animals fed with limiting nutrient supplements 1, 2, 3, 4 and 5 respectively, when compared to control without affecting the quality of the milk.

Farmers perception: Since the method of feeding involved only minor changes in the existing feeding practices followed by the farmers, the farmers realized the beneficial effect of supplementing strategic nutrient supplements and appreciated the positive beneficial effects of supplementation on general health and production. Using SLNS is one way of reducing the normally high feed costs. However, lack of knowledge of their nutritional and economic value limits their use. Although the reduction of costs may be significant, they have to be used carefully

Table 5. Endocrine parameters in cows of control and experimental groups fed on local mixed grass based diet

Particular	Control	G 1	G 2	G 3	G 4	G 5	SEM	P-value
PRL (ng/ml)	259.4 ^a	188.5 ^b	179.2 ^b	246.1 ^{ab}	192.4 ^b	239.7 ^b	42.87	0.030
GH (ng/ml)	2.7 ^a	4.7 ^b	4.5 ^b	3.5 ^b	4.6 ^b	4.0 ^b	0.54	0.029
IGF-1 (ng/ml)	30.7 ^a	47.2 ^b	49.7 ^b	38.5 ^b	45.1 ^b	36.6 ^b	12.49	0.024
Estradiol-E2 β (pg/ml)	15.3 ^a	20.4 ^b	21.3 ^b	17.0 ^a	20.5 ^b	18.1 ^b	1.21	0.040
Progesterone (ng/ml)	1.0 ^a	2.2 ^b	2.0 ^b	2.1 ^b	2.0 ^b	2.4 ^b	0.26	0.030
Triiodothyronin (T3) (ng/ml)	0.48 ^a	1.29 ^b	1.34 ^b	1.10 ^b	1.41 ^b	1.29 ^b	0.04	0.022
Thyroxine (T4) (ng/ml)	35.89 ^a	41.28 ^b	44.21 ^b	39.18 ^b	42.36 ^b	38.44 ^b	1.36	0.023

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

because animal may have negative reactions to radical changes in feeds. Such a change must then be made slowly and with the proper follow up. If there would have been drastic changes in the entire feeding practices of the farmers, they would not have accepted the technology, since it is only the refinement and with little addition of nutrients through strategic supplementation, the farmers accepted the technology very well. Hence with minimum alteration in farmers feeding practices under village conditions, milk production thereby farmer's income can be increased.

The results of on-farm trial indicated that FCM yield was increased from 2 to 16%, feed cost was reduced by ₹ 2 to 7, reduced CP intake by 1.5 to 8.2% and overall income of the farmers was increased by ₹ 14 to 50/animal/day by feeding these strategic limiting nutrient supplements in medium yielding cows fed on grass based diets. Strategic limiting nutrient supplements prepared with $\geq 60\%$ protected soybean extraction (PS) as a source of bypass rich protein/amino acids showed better production performance when compared to other supplements used in the study.

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