EFFECT OF SUPPLEMENTATION OF PLANT PROTEIN SOURCES WITH LYSINE AND METHIONINE ON DRY MATTER INTAKE AND NUTRIENT DIGESTIBILITY IN CROSS BRED CALVES*

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Abstract

A feeding trial was conducted with fourteen crossbred calves below one month of age for 145 days to study the effect of replacement of unsalted dried fish in the calf starter with a plant protein source supplemented with lysine and methionine on dry matter intake, digestibility of nutrients and haematological parameters. Calves were divided into two groups as uniformly as possible and allotted randomly to two dietary treatments. The control group was fed with a calf starter containing unsalted dried fish. The treatment group was fed a calf starter with only plant protein sources formulated to be isonitrogenous and isocaloric with control calf starter and equated by supplementation with lysine and methionine plus cystine. Dry matter intake and haematological parameters such as haemoglobin, serum phosphorus, serum calcium, plasma glucose, serum total protein and blood urea nitrogen were similar in calves that received the two dietary treatments. The digestibility coefficients of nutrients observed were 66.37 and 65.84 for dry matter, 72.21 and 72.36 for crude protein, 64.28 and 61.85 for ether extract, 62.34 and 60.71 for neutral detergent fibre and 51.95 and 52.25 for acid detergent fibre for the control and treatment diets respectively. It could be inferred from the study that unsalted fish in a calf starter can be replaced by plant protein sources supplemented for existing deficiencies in lysine and methionine plus cystine to give a similar digestion performance in cross bred pre-ruminant calves.

Key Words : *Dry matter intake, pre-ruminant calves, calf starter*

Intake of colostrum by neonatal calves and early transition to calf starter are two important management factors in a successful calf-rearing programme (Franklin *et al.*, 2003). Fish protein is an important animal protein source incorporated in calf starter. Hot and humid climate in tropical and sub tropical regions results in putrefaction of fish before processing, transport and storage. The high ratio of less digestible stroma protein such as scales, viscera, cartilage and bone and non protein nitrogenous substances such as peptides, free amino acids, amines, betaines and trimethylamine oxide make the Indian fish protein markedly inferior. The quality is further deteriorated by processing at sea shore where contamination by sand particles is inevitable (Zombade *et al.*, 1980). Unsalted dry fish is prone to autolytic, lipolytic and microbial spoilage and may lodge harmful pathogens dangerous for the newborn calf (Hussein and Jordan, 1991). Because of these reasons replacement of unsalted dry

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fish with alternative sources of protein mainly plant protein sources in the diet of pre-ruminant calves thus has considerable field application. Lysine is often found limiting in diets high in corn protein while soyabean protein is known to be deficient in methionine (Abe *et al.*, 1998). In such a scenario this study was undertaken to assess the effect of replacing unsalted dry fish in the calf starter by plant protein sources equated with regard to energy, protein and amino acids- lysine and methionine plus cystine on nutrient digestibility in crossbred calves.

Materials and Methods

Fourteen crossbred calves below one month of age, were randomly selected for the experiment after colostrum feeding and they were grouped into two as uniformly as possible with regard to the breed, age, sex, and body weight. The animals of each group were housed in individual pens, fed individually and were maintained under identical feeding and managemental conditions for a period of 140 days. The experimental diets consisted of grass (hybrid napier- *Pennisetum para typhoides purpureum)* and two isonitrogenous and isocaloric concentrate mixtures, the control with unsalted dried fish and the treatment with all plant protein sources, both formulated as per BIS(1983) (Table 1). The lysine and methionine plus cystine content of the treatment diet were equated with that of control diet by supplementing it with synthetic lysine and methionine. Milk feeding of the calves was carried out for the first three

Table1. Ingredient composition of the two concentrate mixtures given to the experimental animals (kg)

Ingredient	Control	Treatment
Maize	33.0	31.5
Wheat bran	22.5	22.0
Soya bean meal	34.0	44.0
Unsalted dry fish	8.5	Nil
Shell grit	1.5	2.0
Salt	0.5	0.5
L-Lysine	Nil	0.23
DL-Methionine	Nil	0.12

To every 100 kg calf starter, 100 g of Ultra-TM* and 8 g of Indomix-AB_2D_3K** were added.

* Trace mineral mixture supplied by Neospark Drugs and Chemicals Pvt Ltd., Unit-2, 64-B, I.D.A, Jeedimetla, Hyderabad-500055, having in every 5 kg, Manganese-270 g, Zinc-260 g, Iron-100 g, Iodine-10 g, Copper-10 g, Cobalt -5 g.

** Vitamin premix supplied by Nicholas Piramal India Ltd, 100, Centrepoint, Dr. Ambedkar road, Parel, Mumbai-400012, having in every gram, Vitamin A-82500 I.U, Vitamin D_3-12000 I.U, Vitamin B_2-50 mg, Vitamin K-10 mg.

months @ 1/10th, 1/12th and 1/15th of body weight respectively. The quantity of calf starter fed to animals of each group was fixed as per standards (NRC, 1989). A digestibility trial was conducted at the end of experiment with a five-day collection period. Proximate analysis of the experimental calf starters, dung, green grass and milk was carried out as per standard procedure (AOAC, 1990). Crude protein in dung was estimated using fresh samples, rest of the analysis was done with dry samples. The acid detergent fiber (ADF) was estimated by the method suggested by Van Soest (1963) and neutral detergent fiber (NDF) by the method suggested by Van Soest and Whine (1967). The calcium content in feed and dung was found out by Atomic Absorption Spectrophotometer using hollow calcium cathode tubes. Phosphorus content in the feed and dung was determined by Vanado-Molybdate method (AOAC, 1990). Blood samples from experimental animals were collected at the first, third and fifth month of the feeding trial. Blood samples were used to determine

Haemoglobin (cyanmethae-moglobin method), plasma glucose (GOD-PAP method), serum inorganic phosphorus (phosphomolybdate method), serum total protein (direct biuret method) using the kits supplied by Agappe diagnostics. Serum urea nitrogen was estimated by DAM method using kit supplied by Qualigens diagnostics and serum calcium by Atomic Absorption Spectrophotometer using hollow calcium cathode tubes. All the results were compared statistically using students't' test (Snedecor and Cochran, 1985).

Results and Discussion

Chemical composition of the calf starters and grass given to the calves during the experimental period is given as Table 2. The

Table 2. Chemical composition of calf starters and grass fed to experimental calves (%)

Parameter	Calf starter		Grass
	Control	Treatment	
Dry matter	91.10 ± 0.01	91.04 ± 0.02	22.06± 2.149
Crude protein	24.64 ± 0.15	24.55 ± 0.27	6.55 ± 0.39
Ether extract	1.96 ± 0.06	1.80 ± 0.04	0.53 ± 0.03
Crude fibre	7.87 ± 0.21	7.97 ± 0.19	30.14 ± 0.78
Total ash	10.45 ± 0.08	9.65 ± 0.07	13.34 ± 0.22
Nitrogen free extract	55.08 ± 0.34	56.03 ± 0.49	49.44 ± 0.20
Acid insoluble ash	4.18 ± 0.60	3.55 ± 0.01	7.46 ± 0.68
Neutral detergent fibre	46.93 ± 0.68	45.53 ± 0.23	73.57 ± 0.21
Acid detergent fibre	21.54 ± 0.17	15.25 ± 0.15	40.40 ± 0.04
Acid detergent lignin	5.44 ± 0.07	4.29 ± 0.15	7.54 ± 0.01
Calcium	0.90 ± .002	0.82 ± 0.002	0.38 ± .0005
Phosphorus	0.42 ± 0.06	0.40 ± 0.05	0.28 ± 0.01

weekly average daily dry matter intake (DMI), by the animals of both the groups is presented in Table 3. The average daily DMI was 1.29 ± 0.07 kg in the control group and 1.34 ± 0.05 kg in the treatment group. Total dry matter consumed by the animals in the trial was 181.65 ± 9.36 kg and 188.20 ± 7.53 kg in the control and treatment group respectively. Dry matter consumption /100 kg body weight was 3.06 ± 0 .07 kg and 3.14 ± 0.11kg for the calves in the control and treatment diets, respectively (Table 4). The dry matter intake was similar between the two dietary treatments and presented no significant difference when analyzed statistically. Bhagavat and Srivastava (1991) reported a DMI of 3.14 kg per 100 kg body weight by crossbred calves fed on concentrate mixtures with 24.7 per cent crude protein (CP). Lack of influence of the source of protein in the diet on DMI was also reported by Abu-Ghazaleh *et al.* (2001) and Davenport *et al.* (1990).

Digestibility coefficient (Table 5) for dry matter determined were 66.37 ± 0.59 per cent and 65.84 ± 0.65 per cent for the control and treatment diets respectively, the value being in accordance with Pandey and Agrawal (2001) while Bhagavat and Srivastava (1991) and Asitha (2002) reported a dry matter digestibility of 71 per cent. Dry matter digestibility was not significantly different between treatments, which correlates well with the observations of Mandal *et al.* (2002) and Khan *et al.* (2002). Digestibility coefficient of crude protein was 72.21 ± 0.59 per cent and 72.36 ± 0.59 per cent for the control and treatment diets respectively and there was no significant difference between the two dietary treatments for crude protein digestibility (Khan *et al.*, 2002; Mandal *et al.*, 2002). Kanjana pruthipong (1998) reported a significant improvement in ileal nitrogen digestibility from 66.7 to 68.4 per cent when it was supplemented with amino acids lysine, methionine and threonine in calves fed soy protein based milk replacer. Digestibility

Table 3. Weekly average of daily dry matter intake of calves maintained on two experimental rations (kg)

Week	Control	Treatment	P value
1	0.35 ± 0.009	0.35 ± .009	0.8338
2	0.42 ± 0.034	0.42 ± .027	0.9499
3	0.54 ± 0.047	0.55 ± 0.041	0.9111
4	0.65 ± 0.074	0.68 ± 0.063	0.7875
5	0.72 ± 0.095	0.77 ± 0.080	0.7054
6	0.82 ± 0.095	0.85 ± 0.073	0.8610
7	0.88 ± 0.103	0.95 ± 0.850	0.5802
8	1.07 ± 0.097	1.14 ± 0.077	0.5758
9	1.21 ± 0.074	1.29 ± 0.068	0.5006
10	1.28 ± 0.074	1.34 ± 0.056	0.4894
11	1.34 ± 0.057	1.39 ± 0.051	0.7814
12	1.42 ± 0.059	1.49 ± 0.048	0.3428
13	1.55 ± 0.050	1.58 ± 0.053	0.6060
14	1.63 ± 0.069	1.63 ± 0.072	1.00
15	1.67 ± 0.086	1.73 ± 0.069	0.6146
16	1.71 ± 0.089	1.81 ± 0.075	0.3865
17	2.00 ± 0.050	2.08 ± 0.068	0.4134
18	2.15 ± 0.074	2.21 ± 0.077	0.5924
19	2.28 ± 0.066	2.32 ± 0.076	0.7502
20	2.28 ± 0.065	2.34 ± 0.057	0.4820

coefficient for ether extract observed in the trial was 64.28 ± 1.64 in the control diet and 61.85 ± 1.46 for the treatment diet. (Bhagavat and Srivastava, 1991; Singh *et al.*, 2000). Digestibility coefficient for neutral detergent fibre (NDF) was 62.34 ± 0.80 and 60.71 ± 0.74 and that of acid detergent fibre (ADF) being 51.95 ± 1.27 and 52.25 ± 1.79 in the control and treatment diets respectively.

The haematological parameters estimated at first, third and the fifth month in the present study are given in Table 6. There was no statistical difference between the two groups regarding the various parameters estimated in any of these collections. Serum total protein values recorded in the trial for the three collections were 6.09, 5.67 and 5.73 g/dl in control and 6.10, 5.70 and 5.71 g/dl in treatment diets respectively. The values obtained were with in the range (4.11 to 6.4 g/dl) reported by Lammers *et al.* (1998) and Wilson *et al.* (1994).

Table 4. Data on the dry matter intake of calves maintained on two experimental rations,(kg)

Parameter	Control	Treatment
Total dry matter intake in 140 days, (kg/calf)	181.65 ± 9.36	188.20 ± 7.53
Average dry matter intake, (kg/day)	1.29 ± 0.07	1.34 ± 0.05
Dry matter intake per100 kg body weight (kg)	3.06 ± 0 .07	3.14 ± 0.11

Blood Urea Nitrogen (BUN) values recorded in the first, third and fifth month of the trial were 9.84, 11.36 and 12.58 mg per cent for control and 10.21, 11.86 and 13.22 mg per cent for the treatment diets respectively. The data presented no significant difference between the treatments when analyzed statistically. The BUN values were in the range of 8.52 to 13.3 mg per cent reported earlier by Lammers *et al.* (1998) and Kay *et al.* (1976).

It could be concluded from the study that unsalted fish in a calf starter can be replaced by plant protein sources supplemented for existing deficiencies in lysine and methionine plus cystine to give a similar digestion performance in cross bred pre-ruminant calves.

Table 5. Digestibility coefficient of nutrients of the two experimental rations (%)

Nutrients	Control	Treatment	P value
Dry Matter	66.37 ± 0.59	65.84 ± 0.65	0.55
Crude Protein	72.21 ± 0.59	72.36 ± 0.59	0.9
Ether Extract	64.28 ± 1.64	61.85 ± 1.46	0.29
Nitrogen Free Extract	63.84 ± 0.80	64.86 ± 0.74	0.46
Neutral detergent Fibre	62.34 ± 0.80	60.71 ± 0.74	0.16
Acid detergent Fibre	51.95 ± 1.27	52.25 ± 1.79	0.9

Table 6. Haematological parameters of calves maintained on the two experimental rations

Parameter	Diets	Month		
		First	Third	Fifth
Blood haemoglobin (g/dl)	Control	13.45 ± 0.93	12.58 ± 0.92	12.21 ± 0.59
	Treatment	13.35 ± 0.83	12.00 ± 0.93	12.12 ± 0.42
Serum calcium (mg %)	Control	10.11 ± 0.61	11.88 ± 0.68	11.47 ± 0.50
	Treatment	11.04 ± 0.70	10.76 ± 0.77	12.19 ± 0.55
Serum phosphorus(mg %)	Control	6.76 ± 0.29	6.98 ± 0.19	6.94 ± 0.21
	Treatment	7.09 ± 0.12	6.86 ± 0.21	7.05 ± 0.14
Plasma glucose (mg/dl)	Control	97.74 ± 12.79	71.35 ± 3.00	59.12 ± 1.23
	Treatment	91.30 ± 5.67	71.15 ± 3.91	62.22 ± 2.48
Serum total protein (g/dl)	Control	6.09 ± 0.17	5.67 ± 0.19	5.73 ± 0.08
	Treatment	6.10 ± 0.23	5.70 ± 0.13	5.71 ± 0.09
Blood urea nitrogen (mg %)	Control	9.84 ± 0.44	11.36 ± 0.24	12.58 ± 0.24
	Treatment	10.21 ± 0.53	11.86 ± 0.26	13.22 ± 0.0

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References

Abe, M., Iriki, T., Funaba, M. and Onda, S. 1998. Limiting amino acids for a corn and soybean meal diet in weaned calves less than three months of age. *J. Anim. Sci.*, **76**: 628-636

Abu-Ghazaleh, A.A., Schingoethe, D.J. and Hippen, A.R. 2001. Blood amino acids and milk composition from cows fed soybean meal, fish meal or both. *J. Dairy. Sci.*, **84**: 1174-1181

Asitha, T.V. 2002. Effect of supplementation of dried yeast on growth performance of pre-ruminant

calves. *M.V.Sc. thesis.* Kerala Agricultural University, Thrissur, 64 p

AOAC. 1990. *Official methods of analysis.* 15th ed., Association of Official Analytical Chemists, Washington D.C., 587 p.

Bhagawat, S.R. and Srivastava, A. 1991 Comparative study on digestibility of nutrients in crossbred calves fed soyabean cake treated with heat, formaldehyde and tannic acid. *Indian J. Anim. Nutr.* , **8**: 305-307.

BIS. 1983. Bureau of Indian standards. Specification for compounded feeds for young stock: IS: 5560-1970.Manak Bhavan, 9, Bhahadur Shah Zafar Marg, New Delhi, 15 p.

Davenport, G.M., Boling, J.A. and Gay, N. 1990. Performance and plasma amino acids of growing calves fed corn silage supplemented with ground soyabeans, fish meal, and rumen protected lysine. *J. Anim. Sci.,* **68:** 3773-3779

Hussein, H.S. and Jordan, R.M. 1991. Fish meal as a protein supplement in ruminant diets: A review. *J. Anim. Sci.*, **69**: 2147-2156

Kanjanapruthipong, J. 1998. Supplementation of milk replacers containing soy protein with threonine, methionine, and lysine in the diets of calves. *J. Dairy Sci.*, **81**: 2912-2915

Kay, R.M., Little, W. and Kitchenham, B.A.1976. A comparison of growth performance and blood composition of twin and singleton calves. *Anim. Prod.*, **22**: 19-25

Khan, A.G., Atiya, A. and Mirza, I.H. 2002. Response of feeding amino acids supplemented cotton-seed meal on growth, performance, and digestibility of early weaned cow calves. *Asian-Aust. J. Anim. Sci.*, **15**: 184-187

Lammers, B.P., Heinrichs, A.J. and Aydin, A. 1998. The effect of whey protein concentrate or dried skim milk in milk replacer on calf performance and blood metabolites. *J. Dairy. Sci.,* **81**: 1940-1945

Mandal, G.P., Kurar, C.K. and Neelam, K. 2002. Effect of methionine and lysine supplementation on dry matter intake, nutrient utilization and growth in buffalo calves. *Anim. Nutr. Feed. Sci. Technol.*, **2**: 39-47

NRC. (1989) *Nutrient requirement of dairy cattle.* 6th ed. National Academy of Science, Washington, DC, 159 p.

Pandey, P. and Agrawal, I.S. 2001. Nutrient utilization and growth response in cross bred calves fed antibiotic and probiotic supplemented diets. *Indian J. Anim. Nutr.,* **18**:15-18.

Singh, S.P., Verma, D.N. and Lal, S.N.2000. Digestibility of nutrients and growth rate in crossbred cow calves on rations of different levels of crude protein content. *Inter. J. Anim. Sci.*, **15**: 209-212

Snedecor, G.W. and Cochran, W.G. 1985. *Statistical Methods.* 8th ed., The Iowa State University Press, Iowa, USA, 313 p

Van Soest, P.J. 1963. Uses of detergents in the analysis of fibrous feeds. II. A rapid method for the determination of fibre and lignin. *J. Ass. off. analyt. Chem.*, **46:** 829-835

Van Soest, P.J. and Whine, R.H. 1967. Use of detergents in the analysis of fibrous feeds. IV. The determination of plant cell wall constituents. *J. Ass. off. analyt. Chem.,* **50**: 50-54

Wessels, R.H. and Titgemeyer, E.C. 1997. Protein requirements of growing steers limit-fed corn-based diets. *J. Anim. Sci.*, **75**: 3278-3286

Wilson, L.L., Egan, C.L. and Drake, T.R. 1994. Blood, growth, and other characteristics of special-fed veal calves in private cooperator herds. *J. Dairy. Sci.*, **77**: 2477-2477

Zombade, S.S., Lodhi, G.N. and Ichhponani, J.S. 1980. Evaluation of protein quality of poultry feeding stuffs - Fish Meal. *J. agric. Sci.*, **94**: 111-116

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