Effect of Different Degradable Protein and Starch Sources on the Blood Metabolites and Rumen Biochemical Profile of Early Weaned Crossbred Calves

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ABSTRACT : Thirty new born crossbred (*Bos taurus*×*Bos indicus*) calves, divided randomly in a 3×2 factorial design, were fed calf starters containing one of three protein sources i.e., groundnut cake (GN), cottonseed meal (CS) and meat and bone meal (MB) along with either raw (M) or gelatinized maize (MG) for 90d. Milk was fed upto 56d of age. Green oats and respective calf starters were offered from 14d of age onwards *ad lib.* Clinical profile of serum suggested significantly ($p<0.05$) higher albumin and lower alanine aminotransferase activity due to CS feeding. Alklaine phosphatase activity varied significantly ($p<0.05$) among dietary treatments showing interaction between protein and starch sources. Inclusion of gelatinized maize resulted in significantly higher concentration of serum globulin ($p<0.05$) and alkaline phosphatase activity ($p<0.01$). Reduced ($p<0.05$) ruminal pH was accompanied by a significant decrease ($p<0.01$) in NH_3-N concentration in the strained rumen liquor (SRL) of MG fed calves. Ruminal amylase activity was lower ($p<0.05$) on MG diets. Alanine aminotransferase activity in the rumen exhibited a significant ($p<0.01$) interaction between protein and starch sources. While feeding of CS significantly ($p<0.01$) reduced alanine aminotransferase activity, inclusion of thermally processed maize reduced ($p<0.01$) both aspartate and alanine aminotransferase activities in the rumen. The overall blood picture was similar among treatments, whereas rumen metabolites especially enzyme activities, seems to be altered with source of degradable protein and starch. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 5 : 728-734*)

Key Words : Protein, Starch, Degradability, Blood Metabolites, Rumen Enzymes, Calves

INTRODUCTION

Intensive livestock production systems in a developing country like India have to rely upon the limited availability of feed and fodder resources which, in turn, escalates the cost of production. To overcome this, dietary manipulations for more efficient utilization of nutrients seems to be one of the best available options. The ruminal microbial fermentation is capable of supplying 70-85 and 70-100 percent of a ruminant's need for energy and protein, respectively (AFRC, 1992; Dewhurst et al., 1986). Hence, feeding for optimization of microbial fermentation by co-ordinated supply of both energy (starch) and protein sources of matching ruminal degradabilities would be a much better proposition than considering the degradability of protein alone. Responses of ruminants to varying protein degradabilities can be altered by the rate of starch breakdown in the rumen (Herrera-Saldana and Huber, 1989), which could be achieved by manipulating the grain source or its processing. Based on these observations the present study was taken up to assess the effects of sources of protein, methods of processing of grain (in order to achieve varied ruminal degradability of grain starch) and their combinations, on the blood and rumen biochemical and enzymatic profile of crossbred calves in the early post weaning phase of life.

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MATERIALS AND METHODS

Feeds and feeding

Thirty new born crossbred (*Bos taurus*×*Bos indicus*) male calves, after 24h colostrum feeding, were distributed in a 3×2 factorial arrangement. The six different calf starters contained three sources of protein, viz. highly degradable groundnut cake (GN), medium degradable cottonseed meal (CS) and low degradable meat and bone meal (MB) in combinations with either raw (M, low degradable) or gelatinized (pressure cooked) maize (MG, highly degradable). The ingredient and chemical composition of the calf starters are given in table 1. The calves were fed milk as per standard schedule upto 56d of age. The respective calf starters and green oats were introduced to the calves *ad lib.* from 14d of age onwards individually, by keeping them in separate pens. The calves had an easy access to potable water throughout.

Blood collection and analysis

After 90d of experimental feeding, blood samples were collected in serum separation tubes by jugular venipuncture of the calves (four from each group) and allowed to clot at room temperature for 30 min. Serum was separated by centrifugation at 3,000 rpm for 15 min, decanted into plastic vials and stored at -20℃ till analyzed.

Serum glucose was estimated using o-toluidine method (Hultman, 1959) and total protein by biuret reaction (Hiller and Van, 1927). Procedure of

Table 1. Ingredient and chemical composition of calf starters*

	M-GN	MG-GN	M-CS	MG-CS	M-MB	MG-MB
Ingredient composition (per cent as fed basis)						
Maize, ground	50.0	-	50.0	-	50.0	-
Maize, gelatinized	-	50.0	-	50.0	-	50.0
Wheat bran	12.0	12.0	-	-	19.0	19.0
Groundnut cake	35.0	35.0	25.0	25.0	19.0	19.0
Cottonseed meal	-	-	22.0	22.0	-	-
Meat and bone meal	-	-	-	-	10.0	10.0
Mineral mixture	2.0	2.0	2.0	2.0	1.0	1.0
Common salt	1.0	1.0	1.0	1.0	1.0	1.0
Chemical composition (per cent in DM or kcal/g)						
Crude protein	22.35	21.57	22.91	22.35	23.50	23.23
Ether extract	3.04	2.38	4.22	3.22	4.84	3.75
Total carbohydrates	65.60	68.36	64.06	67.67	63.44	66.04
Total ash	9.01	7.69	8.81	6.76	8.22	6.98
Gross energy	4.32	4.47	4.46	4.61	4.47	4.75

* Added with 20g vitablend (vitamin AD_3) per 100 kg of calf starter, which contained vitamin A 50,000 IU and D_3 5,000 IU per gram.

M: raw maize, MG: gelatinized maize, GN: groundnut cake, CS: cottonseed meal, MB: meat and bone meal.

Gustafsson (1976) was adopted for estimating albumin, and globulin was determined by difference. The samples were also analyzed for urea (Rahmatullah and Boyde, 1980), creatinine (Bones and Taussky, 1945), aspartate and alanine aminotransferases (AST and ALT; Reitman and Frankel, 1957). Alkaline phosphatase (ALP) activity and cholesterol were determined by methods of King and Armstong and Liberman-Burchard, respectively as described by Oser (1976).

Collection and analysis of rumen liquor

Rumen liquor was collected from three calves from each group for two consecutive days at 4h post-feeding during 14th week of age with the help of a stomach tube by creating negative pressure with a plastic syringe attached at the end. The pH was recorded immediately using digital pH meter. The strained rumen liquor (SRL) samples, obtained by filtering through four layers of muslin cloth, were preserved at -20℃ for further analysis. The total volatile fatty acids (TVFA) was determined as per Barnett and Reid (1957). The total, ammonia and trichloroacetic acid preciptiable (TCAP-) nitrogen were determined by standard procedures using Kjeltec auto analyser (M/s Tecator, Sweden).

Rumen enzymes

About 15 ml of SRL was ultrasonicated followed by ultracentrifugation at 24,000×g for 20 min. The supernatant, thus obtained, was used far estimating the activities of various enzymes viz. amylase and carboxymethyl (CM-) cellulase (Miller, 1959), protease (Lowry et al., 1951), AST and ALT (Reitman and

Table 2. Blood metabolites and enzymatic profile of calves as influenced by diets

	M-GN	MG-GN	M-CS	MG-CS	M-MB	MG-MB
Glucose, mg/dl	45.1±3.16	56.5±4.66	53.1±2.45	60.6±3.53	56.1±5.22	52.1±4.20
Total protein, g/dl	6.3±0.34	6.4±0.47	6.5±0.20	7.3±0.23	6.5±0.18	7.1±0.28
Albumin, g/dl	3.6±0.22	3.7±0.27	4.1±0.09	4.2±0.06	3.9±0.14	3.8±0.17
Globulin, g/dl	2.7±0.29	2.7±0.35	2.4±0.11	3.1±0.27	2.5±0.10	3.3±0.18
Albumin:Globulin	1.4±0.17	1.4±0.20	1.7±0.05	1.4±0.14	1.6±0.08	1.2±0.07
Cholesterol, mg/dl	83.8±9.77	94.4±4.24	106.5±1.08	96.9±5.21	81.5±9.97	94.1±7.93
Urea, mg/dl	27.1±4.44	29.0±4.60	29.5±2.98	30.9±1.78	27.9±4.53	34.0±4.30
Creatinine, mg/dl	1.3±0.25	1.4±0.23	1.1±0.12	1.4±0.20	1.3±0.14	1.4±0.25
AST, U/L	21.8±3.95	24.2±2.03	20.7±1.37	21.5±3.14	21.0±0.81	24.2±4.17
ALT, U/L	40.3±8.69	27.3±2.84	22.2±1.73	21.7±2.66	38.4±3.75	29.7±5.85
ALP, U/L*	84.5 ± 7.81^{b}	181.8 ± 18.74^{a}	105.8 ± 12.64^{b}	149.8 ± 25.28^{a}	149.1 ± 5.89^{a}	150.5 ± 4.83^{a}

^{a,b} Means with different superscripts in a row differ significantly; * $p<0.05$.

Table 3. Blood metabolites and enzymatic profile of calves as influenced by sources of protein and starch

	Protein source			Starch Source	
	GN	CS	MB	MG	M
Glucose, mg/dl	50.8±3.38	56.9±2.44	54.1±3.19	56.4±2.41	51.4±2.43
Total protein, g/dl	6.4±0.27	6.9±0.20	6.8±0.19	6.9±0.21	6.4±0.13
Albumin*, g/dl	3.7±0.16^{b}	4.2±0.05^{a}	3.9±0.11^{ab}	3.9±0.11	3.9±0.10
Globulin*, g/dl	2.7±0.21	2.7±0.19	2.9±0.17	3.0±0.16^{a}	2.5±0.11^{b}
A: G ratio*	1.4±0.12	1.6±0.10	1.4±0.09	1.3±0.09^{b}	1.6±0.07^{a}
Cholesterol, mg/dl	89.1±5.33	101.7±3.06	87.8±6.37	95.2±3.16	90.6±5.42
Urea, mg/dl	28.0±2.98	30.2±1.63	30.9±3.11	31.3±2.07	28.2±2.14
Creatinine, mg/dl	1.3±0.16	1.3±0.12	1.3±0.13	1.4±0.12	1.2±0.10
AST, U/L	23.0±2.10	21.1±1.59	22.6±2.06	23.3±1.73	21.2±1.29
ALT*, U/L	33.8±4.89^{a}	21.9±1.47^{b}	34.1±3.62^{a}	26.2±2.34	33.6±3.80
ALP**, U/L	133.5±20.6^{b}	127.8±15.55	149.8±3.55	160.5±10.65^{a}	112.9±9.37^{b}

^{a,b} Means bearing different superscripts within protein or starch sources in a row vary significantly, * $p<0.05$; ** $p<0.01$.

Frankel, 1957).

Statistical analysis

The data were analysed using standard procedures laid down by Snedecor and Cochran (1967) to assess the effects of the protein source, starch source and protein × starch interaction.

RESULTS AND DISCUSSION

The data pertaining to the serum biochemistry as influenced by dietary variations and sources of protein and starch are presented in table 2 and table 3, respectively.

Blood metabolites

Mean serum glucose concentrations were within bovine reference range, without any significant ($p>0.05$) variations among diets. However, serum glucose concentration was 25, 14 and -7 per cent greater ($p>0.05$) when MG replaced M in the diets based on GN, CS and MB, respectively, showing a definite trend of diminishing effect of MG supplementation as escape protein content of the diet increased. When compared between starch sources, MG fed calves tended to have relatively greater ($p>0.05$) glucose levels as compared to M which is similar to the observations of Kirilov et al. (1987). This could be due to the fact that processing of grains increases propionate production in the rumen (Ørskov et al., 1974; Viera and Macleod, 1980) which, along with valerate, may provide around 70% of the exogenous glucose precurssors in ruminants (Baldwin and Smith, 1979). The glucose level relative to sources of protein were similar but the comparatively higher value observed in CS fed calves agree well with the results of Nagalakshmi (1996) and Peterson et al. (1992) who showed that serum glucose increased with CS feeding.

Serum total protein (TP) did not differ among diets or protein meals, and were within bovine reference range (6.0-7.0 mg/dl; Kaneko, 1980) corroborating the earlier reports of Heinrichs and Garman (1992) who also did not observe any effect of varying dietary protein degradabilities. Feeding of MG tended to enhance the serum TP, although non-significantly ($p>0.05$), in comparison to raw maize feeding mainly because of the disproportionate increase in the globulin levels.

Serum concentration of albumin was comparable ($p>0.05$) among the diets and were well within normal range. However, feeding of CS significantly ($p<0.05$) elevated the serum albumin level as compared to GN feeding on contrary to the comparable values reported in GN and CS fed lambs by Nagalakshmi (1996) and that of CS fed heifers by Colin-Negrate et al. (1996). The source of starch had no effect on serum albumin levels. Serum globulin concentrations were similar ($p>0.05$) on different sources of protein. However, it was significantly ($p<0.05$) higher on MG as compared to M diets despite similar albumin values. It was reflected significantly ($p<0.05$) through wider albumin: globulin (A:G) ratio in M than MG fed calves. While the dietary variations had no significant effect on the A:G ratios, CS as the source of protein induced marginally wider ratio as compared to the other two sources, mainly because of higher albumin concentration. This was in contrast to the observations of Lindsey et al. (1980) who reported hyperproteinemia in CS fed cows characterised by a disproportionate increase in the globulin fraction.

Serum cholesterol levels among diets were within the reported normal range. Feeding of CS diets tended to enhance serum cholesterol as compared to the feeding of GN or MB diets, similar to the findings of Colin-Negrete et al. (1996) and Williams et al. (1989). The CS supplementation might have increased the rate

Table 4. Rumen biochemical profile of calves fed various protein and starch sources

	pH	TVFA (mEq/dl)	NH_3-N	Total N	TCAP-N
			(mg/dl)		
Diets:					
M-GN	6.3±0.28	12.8±1.53	29.7±2.83	179.5±28.19	109.2±33.26
MG-GN	5.9±0.32	14.9±5.08	16.4±1.99	199.7±49.37	135.9±10.38
M-CS	6.0±0.43	14.1±0.76	25.4±3.77	128.3±11.52	70.6±7.47
MG-CS	5.4±0.17	13.9±1.37	24.1±0.77	179.5±33.07	133.9±27.28
M-MB	6.0±0.28	11.5±0.06	30.2±0.19	181.4±22.13	107.3±25.46
MG-MB	5.3±0.20	15.7±3.19	25.8±3.18	186.6±41.40	124.8±38.89
Degradable protein:					
High (GN)	6.1±0.20	13.8±2.42	23.0±3.36	189.6±25.82	122.5±16.69
Medium (CS)	5.7±0.24	14.0±0.70	24.8±1.74	153.9±19.39	102.2±19.00
Low (MB)	5.6±0.22	13.6±1.71	28.0±1.73	184.0±21.02	116.1±21.15
Degradable starch:					
High (MG)	5.5±0.16^{b}*	14.8±1.79	22.1±1.82^{b}**	188.6±21.12	131.5±14.14
Low (M)	6.1±0.17^{a}	12.8±0.62	28.4±1.56^{a}	163.1±13.92	95.7±13.80

^{a,b} Means bearing different superscripts in a column within a particular criterion differ significantly, * $p<0.05$; ** $p<0.01$.

of cholesterol synthesis with a possible involvement of hepato-cellular functioning as suggested by Kaneko, (1980) which could be substantiated by the observed lower concentration of aminotransferases (table 3) in CS fed calves due to the presence of increminating factor, gossypol. But source of starch had no effect on the cholesterol levels.

Because of adequate dietary protein supply, as expected, the serum urea nitrogen was found comparable in calves on different diets. A non-significant effect was observed on urea nitrogen levels due to protein sources of varied degradabilities in contrast to the findings of Tiwari (1995) and Abe et al. (1985). Similarly, the source of starch did not exert any significant effect on the serum urea nitrogen level of the calves contrary to the observations of decreased values obtained in cows fed thermally processed barley (Guglya and Safonov, 1985; Kirilov et al., 1987).

The concentrations of creatinine were almost similar on different protein and starch sources and, in turn, among the diets which were well within the normal range (1.0-2.0 mg/dl) reported for bovines (Kaneko, 1980). This was, though in contrary to the observations of Warren et al. (1988), similar to the findings of Barraza et al. (1991).

Serum enzymes

Neither source of protein nor starch, and in turn diet, had any effect on the AST concentrations (table 2 & 3). But feeding of CS significantly ($p<0.05$) depressed serum ALT activity, probably due to hepatic damage exerted by gossypol as the lowered ALT activity is a sensitive and specific indicator of liver damage and hepatopathy (Kaneko, 1980). Contrary to the present and Nagalakshmi's (1996) findings Colin-Negrete et al. (1996), otherwise observed a linear increase in ALT activity with time and level of CS feeding in growing heifers.

The ALP activity varied significantly ($p<0.05$) among calves fed different diets (table 2) with a significant interaction between protein and starch sources. Feeding of raw maize in combination with GN or CS (i.e., M-GN and M-CS diets) reduced the serum ALP activity significantly ($p<0.05$) when compared with all other combinations. Besides a possible involvement in membrane phospholipid synthesis, ALP activity is also believed to be associated with the process of calcification (Kaneko, 1980) and, therefore, could be correlated with growth. This is, in fact, supported by the observed higher growth rate in MB and MG fed calves (Pattanaik, 1997).

Rumen metabolites

The ruminal pH were similar among the diets (table 4). But calves fed MG diets exhibited significantly ($p<0.05$) lower pH as compared to those on M diets similar to the observations of Ørskov et al. (1974) and Epifanov et al. (1988). Ruminal pH below 6.0 may have a detrimental effect on microbial growth and cellulolysis (Stewart, 1977). But when the reductions are cyclic and of short duration, the depression in cellulolysis may be moderate (Hoover, 1986). When comparison was made between protein sources, diets with low protein degradability showed diminishing tendency in ruminal pH, coinciding with the observation of McAllan (1991) who noticed lowered pH in steers fed fish meal against highly degradable urea. The relatively higher ruminal TVFA concentration, with a concommitant decrease in pH of MG fed calves, was in line with observations of

Table 5. Rumen enzymatic profile of calves fed various protein and starch sources

	Amylase[1]	CM-cellulase[1]	Protease[2]	AST[3]	ALT[3]
Diets:					
M-GN	60.0±6.36	6.6±1.44	6.6±0.85	10.9±2.86	12.6±1.82^{b}**
MG-GN	41.8±10.06	4.4±0.82	6.2±1.32	2.8±0.07	7.8±1.18^{b}
M-CS	50.0±3.76	7.5±1.57	4.7±1.76	9.8±2.36	11.8±2.75^{b}
MG-CS	36.5±12.12	7.3±0.75	4.1±1.48	2.7±0.89	6.2±1.42^{b}
M-MB	58.1±1.39	6.6±0.54	6.6±0.38	14.0±1.27	31.1±5.40^{a}
MG-MB	39.9±1.24	3.7±0.64	4.8±0.32	6.1±0.54	5.3±1.71^{b}
Degradable protein:					
High (GN)	50.9±6.70	5.5±0.89	6.4±0.71	6.8±2.21	10.2±1.45^{a}**
Medium (CS)	43.3±6.42	7.4±0.78	4.4±1.08	6.3±1.94	9.0±18.7^{b}
Low (MB)	49.0±4.15	5.2±0.76	5.7±0.45	10.1±1.88	18.2±6.30^{a}
Degradable starch:					
High (MG)	39.4±4.62^{b}*	5.2±0.67	5.0±0.62	3.9±0.63^{b}**	6.4±0.81^{b}**
Low (M)	56.0±2.66^{a}	6.9±0.65	6.0±0.65	11.6±1.29^{a}	18.5±3.65^{a}

^{a,b} Means bearing different superscripts in a column within a particular criterion differ significantly, * $p<0.05$; ** $p<0.01$.
[1] mole glucose produced/ml/h; [2] mg casein hydrolysed/ml/h; [3] mmol pyruvate produced/l/min.

Ørskov et al. (1974) and Epifanov et al. (1988) probably due to high degradability of maize starch after gelatinization. The sources of protein as well as diets failed to have any significant effect on ruminal TVFA, similar to the reports of Lines and Weiss (1996) and Chiou et al. (1995).

A significantly ($p<0.01$) lowered ruminal NH_3-N concentration was observed when thermally processed maize replaced raw maize in diets of calves. Kirilov et al. (1987) also noticed reduced NH_3-N concentration upon inclusion of thermally processed barley. No significant difference could be observed when protein sources of varying degradabilities or diets were compared, in agreement with Chiou et al. (1995).

The source of protein or starch as such, and hence dietary variation, did not significantly influence the concentration of total- as well as TCAP-N, perhaps due to large individual variation as indicated by high SE values (table 4) . But relatively lower total- and TCAP-N concentrations on CS and M diets was indicative of insufficient energy supply for efficient utilization of NH_3-N for microbial protein synthesis. This was substantiated by the observed lower ME in the above said diets (Pattanaik, 1997). Similar relationship was also observed by Walker and Nader (1970).

Rumen enzymes

The dietary varations failed to exert any significant influence on the activity of various ruminal enzymes except for that of ALT (table 5). No significant effect of sources of protein could be observed on the activities of amylase and CM-cellulase. Among starch sources, gelatinized maize significantly ($p<0.05$) lowered amlyase activity with relatively reduced CM-cellulase activity. The latter was perhaps due to the lower ruminal pH observed on the said diet (table 4). Ben-Ghedalia et al. (1989) have also reported that lower pH, as in the present study, reduced the digestion of proteins, cellulose, hemicellulose and pectins but not starch. But no plausible explanation could be offered for the lower amylase activity in spite of the lower ruminal pH.

Both sources of protein and starch had no significant effect on the protease activity in the ruminal fluid but the relatively lower value observed in calves fed low degradable protein meals (CS and MB) and highly degradable starch (MG) could be attributed to the lower ruminal pH (below 6.0) observed on these diets corroborating the results of Ben-Ghedalia et al. (1989).

The inclusion of thermally processed maize significantly ($p<0.01$) reduced the activities of both the transaminases of SRL which could be due to the lower NH_3-N concentration (table 4) as they are reported to be positively correlated (Bhatia et al., 1979). It is also reflected in significantly ($p<0.01$) higher activity of ALT in SRL of calves fed M-MB diet as compared to those fed other diets, showing a significant ($p<0.01$) interaction between protein and starch sources.

CONCLUSION

Feeding of different combinations of protein and starch sources of varying degradabilities exerted no significant effect on overall serum biochemical profile in young calves. Rumen biochemical and enzymatic profiles, though varied and possibly of physiological importance, were not statistically significant, probably because of relatively high variability among animals. Moreover, feeding of cottonseed meal to young calves

seems to affect ruminal nitrogen metabolism which needs further confirmation through long-term studies.

REFERENCES

Abe, M., T. Iriki, T. Kimoto and M. Tokuma. 1985. Significance of rumen-bypass protein: growth of calves in 3-6 months of age. Jap. J. Zootech. Sci. 56:103-107.

AFRC. 1992. Agricultural and Food Research Council. Technical Committee on Responses to Nutrients No. 9. Nutritive requirements of ruminant animals. Protein. Nutr. Abstr. Rev. (B) 62: 787-818.

Baldwin, R. L. and N. E. Smith. 1979. Regulation of energy metabolism in ruminants. In H. H. Draper (Editor), Advance in Nutritional Research, Vol. 2, Plenum Press, New York pp.1-27.

Barnett, A. J. and R. L. Reid. 1957. Studies on the production of volatile fatty acids from grass by rumen liquor in an artificial rumen. The volatile acid production from fresh grass. J. Agric. Sci. 48:315.

Barraza, M. L., C. E. Coppock, K. N. Brooks, D. L. Wilks, R. G. Saunders and G. W. Latiner Jr. 1991. Iron sulfate and feed pelleting to detoxify free gossypol in cottonseed diets for dairy cattle. J. Dairy Sci. 74:3457-3467.

Ben-Ghedalia, D. E., E. Yosef, J. Miron and Y. Est. 1989. The effects of starch-and pectin-rich diets on the quantitative aspects of digestion in sheep. Anim. Feed. Sci. Technol. 24:289.

Bhatia, S. K., K. Pradhan and R. Singh. 1979. Microbial transaminase activities and their relationship with bovine rumen metabolites. J. Dairy Sci. 62:441-446.

Bones, R. W. and H. H. Taussky. 1945. On the colorimetric determination of creatinine by Jaffee reaction. J. Biol. Chem. 158:581-591.

Chiou, P. W., K. Chen, K. Kuo, J. Hsu and B. Yu. 1995. Studies on the application of an undegradable protein system to high yielding dairy cattle in Taiwan. Anim. Feed. Sci. Technol. 54:93-102.

Colin-Negrete, J., H. E. Kiesling, T. T. Ross and J. F. Smith. 1996. Effect of whole cottonseed on serum constituents, fragility of erythrocyte cells, and reproduction of growing holstein heifers. J. Dairy Sci. 79:2016-2023.

Dewhurst, R. J., A. J. F. Webster, F. W. Wainman and P. J. S. Dewey. 1986. Prediction of the true metabolizable energy concentration in forages for ruminants. Anim. Prod. 43:183-194.

Epifanov, G. V., A. F. Zakachurin, Z. A. Netecha and A. E. Zaikin. 1988. Digestibility of nutrients and productivity of young cattle on diets containing varying amounts of sugar and sulphur. Doklady Vsesoyuznoi Akademi Sel'skokho Zyaistvennykh Nauk. 2:27-29.

Guglya, V. G. and V. S. Safonov. 1985. Utilization of extruded barley for feeding of cows. Zhirotnovodstvo. 9:51-52.

Gustaffson, E. C. J. 1976. Improved specificity of serum albumin determination and estimation of 'acute phase reactants' by use of bromocresol green reduction. Clin. Chem. 22:616-622.

Grubic, G. 1988. Nutritional value of heat processed maize grain when feeding calves. Archiv za Poljoprivredne Nauke, 49:11-36.

Heinrichs, A. J. and C. L. Garman. 1992. Effects of varying protein undegradability in dairy calf diets on growth, feed efficiency and specific blood metabolites. J. Dairy Sci. 75:(Suppl. 1):128(Abstr).

Herrera-Saldana, R. and J. T. Huber. 1989. Influence of varying protein and starch degradabilities on performance of lactating cows. J. Dairy Sci. 72:1477-1483.

Hiller, M. and S. Van. 1927. Colorimetric determination of proteins. J. Clin. Invest. 4:235-242.

Hoover, W. H. 1986. Chemical factors involved in ruminal fibre digestion. J. Dairy Sci. 69:2755.

Hultman, E. 1959. Rapid specific method for determination of aldosaccharides in body fluids. Nature. 189:109-110.

Kaneko, J. J. 1980. Clinical Biochemistry of Domestic Animals (3rd edn.). Academic Press. New York.

Kirilov, M. P., V. F. Tokarev and V. D. Li. 1987. Metabolism in high yielding cows fed on extruded or flaked grain. Sel'skokhozyaistvennaya Biol. 8:94-99.

Lindsey, T. O., G. E. Hawkins and L. E. Guthrie. 1980. Physiological response of lactating cows to gossypol from cottonseed meal rations. J. Dairy Sci. 63:562-573.

Lines, L. W. and W. P. Weiss. 1996. Use of nitrogen from ammoniated alfalfa hay, urea, soybean meal, and animal protein meal by lactating cows. J. Dairy Sci. 79:1992-1999.

Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. C. Randall. 1951. Protein measurement with Folin-phenol reagent. J. Biol. Chem. 183:265.

McAllan, A. B. 1991. Carbohydrate and nitrogen metabolism in the forestomach of steers given untreated or ammonia treated barley straw diets supplemented with urea or urea plus fishmeal. Anim. Feed. Sci. Technol. 33:195-208.

Miller, G. L. 1959. Modified DNS method for reducing sugars. Anal. Chem. 31:426.

Nagalakshmi, D. 1996. Utilization of undecorticated cottonseed (*Gossypium*) meal in the diets of growing lambs. Ph. D. Thesis, Deemed Univ. IVRI, Izatnagar, India.

Ørskov, E. R., C. Fraster and J. G. Gorden. 1974. Effect of processing of cereals on rumen fermentation, digestibility, rumination time and firmness of subcutaneous fat in lambs. Br. J. Nutr. 32:59.

Oser, B. 1976. Hawks' Physiological Chemistry (14th Ed.). Tata McGraw Hills Publ., New Delhi, India.

Pattanaik, A. K. 1997. Effect of synchronization of degradable protein and starch on the performance of crossbred calves. Ph. D. Thesis. Deemed Univ. IVRI, Izatnagar, India.

Peterson, M. K., D. M. Hallford, D. Dhuyreter, D. Gambil, M. Ward, J. Campbel, E. Perzeguia, J. Rubio and D. Wallace. 1992. The effect of supplemental feathers, blood and/or cottoseed meal on metabolic hormones and serum metabolites in ewe lambs fed blue grama hay. Proc. West. Sect. Am. Soc. Anim. Sci. July 8-10, 1992.

Rahmatullah, M. and T. R. C. Boyde. 1980. Improvement in determination of urea using diacetyl monoxime with or without deproteinization. Clin. Chem. Acta. 107:3-9.

Reitman, R. and S. Frankel. 1957. A colorimetric method for

determination of serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase. Am. J. Clin. Path. 20:56-59.

Snedecor, G. W. and W. G. Cochran. 1967. Staitstical Methods (6th Ed). Iowa State Univ. Press, Ames, Iowa.

Tiwari, C. M. 1995. Evaluation of fish meal supplementation to ammoniated wheat straw based rations in buffalo calf using respiration calorimetry technique. Ph. D. Thesis. Deemed Univ. IVRI, Izatnagar, India.

Viera, D. M. and G. K. Mcleod. 1980. Effect of physical form corn and urea supplementation on the performance of male holstein calves. Can. J. Anim. Sci. 60:931-936.

Walker, D. J. and C. J. Nader. 1970. Rumen microbial protein synthesis in relation to energy supply : Diurnal variation with once-daily feeding. Aust. J. Agric. Res. 21:747-754.

Warren, H. M., S. A. Neutze, J. M. Morrison and P. J. Nicholis. 1988. The value of whole cottonseed in a wheat based maintenance ration for sheep. Aust. J. Expt. Agric. 28:453-458.

Williams, G. L., A. R. Morgan and M. E. Wehrman. 1989. Effect of feeding high lipid supplement via whole cottonseed on reproduction in cow and heifer. Texas. Agric. Exp. Stn. Info. Rep. No. 89:1, College Station.