



Effect of protected protein and protected fat on production, reproduction performance and blood metabolites in crossbred cattle

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ABSTRACT

Feeding trial of 4 months was conducted on 24 crossbred cattle, which were subjected to treatment T₀ (control), T₁ (protected protein), T₂ (protected fat) and T₃ (protected protein and fat). Crossbred cows in second to fourth lactation with most probable production ability (MPPA) of average 2,300 litre milk production per lactation for each group were selected. All animals were fed with 2/3 DM through roughages (2/3 from dry and 1/3 from green) + 1/3 DM from concentrate mixture. In T₀ and T₂ groups, untreated groundnut cake was given in ration whereas, in T₁ and T₃ groups, groundnut cake was treated with formaldehyde (FA) @ 1.0 g FA/100g CP. Also bypass fat (99%) was supplemented in T₂ and T₃ groups @ 10 g/ liter milk production. The DMI was significantly higher in T₃ followed by T₁, T₀ and T₂. However, the DMI/100 kg body weight was nonsignificant among all treatment. The average digestible crude protein intake (DCPI) and total digestible nutrient intake (TDN) was significantly higher in T₃ followed by T₂, T₁ and T₀. However, DCPI and TDNI /100 kg body weight did not differ among all groups. The average milk production was 16.49% higher in T₁, 13.93% higher in T₂ and 20.99% in T₃ over T₀. Similar trend was observed in 4% FCM and ECM yield in all treatment groups. Reproductive performance of animal was significantly improved by feeding protected fat groups. By supplementation of protected fat, blood cholesterol and triglycerides level were significantly increased and BUN level decreased significantly by feeding protected protein.

Key words: Blood metabolites, Milk production, Protected fat, Protected protein, Reproduction

Nutrition management has an important role in increasing production and reproduction parameters in a dairy cow farm. Certain biochemical constituents in blood serum during transition period were found associated with the fertility status of cows and their reproductive behaviour. Maintaining production and reproductive performance of lactating dairy cattle is a challenge for dairy producers because poor fertility reduces productivity and profit. Decline in fertility reflect associations with intensification of production and higher levels of milk production. Gradual decline in dry matter intake (DMI) that starts 2–3 week prepartum, an abrupt increase in nutrient demand with initiation of lactation results in negative energy balance (NEBAL) and extensive mobilization of body fat reserves as non-esterified fatty acids (NEFA). Good nutritional management during the transition period, in particular nutritional strategies, i.e. protected nutrients technology, can reduce the effects of this metabolic stress and improve

production and reproductive performance of animals. Protected fat and protein in metabolism open new opportunities for improving production, health, and reproduction in animals. Inclusion of protected fats and protein in the diet during this transition period has improved the production and reproductive performance (Shelke *et al.* 2011).

Keeping the above in view, the present study was undertaken to see the effect of protected protein and protected fat on production, reproduction and blood metabolites in crossbred cattle.

MATERIALS AND METHODS

Crossbred cows (24) in second to fourth lactation with most probable production ability (MPPA) of average 2300 litre milk production per lactation for each group were selected from the herd maintained at university. The experimental animals were randomly divided into 4 treatment groups (6 in each group) which were subjected to treatment T₀ (control), T₁ (protected protein), T₂ (protected fat) and T₃ (protected protein and protected fat). The duration of experiment was considered from 30 days prepartum to 90 days postpartum. The animals were fed according to the nutrient requirement of livestock and poultry laid down by ICAR, 1998 i.e @ 3% DM of their

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body weight. The groundnut cake used in T₁ and T₃ groups was treated with formalin (40% formaldehyde) @ 1g formaldehyde/100g CP of cake (Chatterjee and Walli 2003). Also bypass fat (99%) was supplemented in T₂ and T₃ groups @ 10 g/ litre milk production.

Daily dry matter intake was observed by recording the daily feed offered and leftover. Daily intake of nutrients of each cow was recorded throughout the experimental period. Animals were milked twice a day. Daily milk yield for individual animals were recorded by making the sum of both time (morning and evening) milking. Reproduction parameters, viz. first estrous after calving, service period, number of AI/conception, conception rate were recorded. Blood samples were collected at fortnightly interval in prepartum (30 days before calving) and at monthly interval in postpartum (90 days after calving) period of individual animals. The blood samples from individual animals were collected by puncturing the jugular vein. The serum were separated by centrifugation of the blood samples at 5,000 RPM for 5 min and stored in deep freeze for subsequent analysis by using fully automatic blood analyzer for albumin (BCG dye method-end point), total protein (Biuret method-end point), cholesterol (Chod-pod method end point), triglycerides (Gpo-pod method end point), uric acid (uricase-pod method end point), creatinine (modified Jaffe's method), glucose (God-Pod method end point), NEFA (colorimetric method), blood urea nitrogen UV (Gldh/UV – kinetic method).

The data generated during experimental period were subjected to statistical analysis, which was done by Factorial Randomized Block Design with SAS, (2011) 9.3.1 version by following standard method of analysis of variance (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

Chemical composition: The chemical composition of feed and fodder is presented in Table 1. Also, concentration composition and chemical composition are given in Table 2. The protected fat contains 85.66 % saturated fat, 13.28% mono unsaturated fat and 1.03% poly unsaturated fat.

Nutrients intake: Average dry matter and nutrient intake in different treatment groups is presented in Table 3. The DMI was significantly (P<0.05) higher in T₃ followed by T₁, T₀ and T₂. It might be due to higher body weight in T₃, T₁ and T₀ than T₂. The DMI/100 kg body weight did not differ among all groups. The results confirmed the finding that in crossbreds, the dry matter requirement is @ 3% of

the body weight of animal. Ramteke *et al.* (2014), Singh *et al.* (2014) and Sontakke *et al.* (2014) reported that the DM intake of dairy animals was not altered on supplementation of bypass fat. Sai *et al.* (2014) observed nonsignificant effect of supplementing bypass protein on DMI. Our results indicated that there was no adverse effect of rumen protected fat and protein supplementation on DMI of lactating crossbreed cattle whereas Amrutkar *et al.* (2014) reported significant increase in DMI by feeding bypass protein. Vahora *et al.* (2013) reported significant increase in DMI by feeding bypass fat and protein.

The average digestible crude protein intake was significantly higher (P<0.01) in T₃, T₂ and T₁ than T₀ due to higher DMI (Table 3). However, DCPI/100 kg body weight was not different among all the groups. Similar, trend was observed in TDN intake. Singh *et al.* (2014) and Ramteke *et al.* (2014) reported increase in CP intake on supplementation with bypass fat. Ramteke *et al.* (2014) reported increased TDN intake on supplementation of bypass fat in the diet of the dairy animals. Also Sai *et al.* (2014) reported nonsignificant difference on CP and TDN intake, whereas Amrutkar *et al.* (2014) reported increased CP and TDN intake by supplementing rumen protected methionine and lysine. Gajera *et al.* (2013) reported increased in CP and TDN intake by supplementing bypass fat and protein.

Milk production: Milk yield in treatment group fed protected protein and fat in combination (T₃) had highest milk yield (Table 3) followed by treatment group fed protected protein alone (T₁) or fat alone (T₂) over the control group (T₀). The improved supply of amino acids in the presence of sufficient energy might have also improved the protein-energy balance and created a better balance of precursors for milk synthesis, resulting in increased milk production. Therefore, the milk yield in treatment group fed protected protein and fat in combination (T₃) had highest milk yield followed by treatment group fed protected protein (T₁) and fat alone (T₂) over the control group (T₀). The significant improvement in milk production on supplementation of rumen protected fat and protein was in line with the findings of many researchers who reported significantly increased milk yield by feeding bypass fat (Ramteke *et al.* 2014, Sontakke *et al.* 2014, Kirovski *et al.* 2015, Yadav *et al.* 2015). Positive response on milk production performance as a result of feeding protected proteins and amino acids have been observed by Amrutkar *et al.* (2014) and Kumar *et al.* (2015) who reported

Table 1. Feed composition and chemical analysis of fodder and feed ingredients

Parameter	Maize	Lucerne	S. cane	Dry jowar	Rice bran	Wheat bran	Tur chuni	GNC
DM	17.76	23.73	33.93	60.68	90.72	93.36	92.08	92.06
CP	09.85	21.54	05.24	04.94	15.44	15.07	10.46	38.01
CF	31.90	25.29	31.66	33.74	12.37	04.26	19.99	15.24
EE	01.68	01.85	0.68	0.96	01.13	01.85	01.69	07.21
TA	08.94	11.26	08.18	11.84	12.61	01.89	09.99	02.31
NFE	47.63	40.06	54.24	48.52	58.45	76.93	57.87	37.23

Table 2. Feed composition and chemical analysis of concentrate mixture

Ingredients composition	T ₀	T ₁	T ₂	T ₃
Rice bran	35	35	35	35
Wheat bran	15	15	15	15
Groundnut cake (untreated)	20	-	20	-
Groundnut cake (treated with FA)	-	20	-	20
<i>Tur chuni</i>	27	27	27	27
Mineral mixture	2	2	2	2
Common salt	1	1	1	1
<i>Chemical composition</i>				
DM	88.15	88.1	88.00	88.2
CP	18.61	18.65	18.40	18.55
CF	13.41	13.45	13.65	13.71
EE	2.57	2.55	2.62	2.61
TA	7.85	7.85	7.86	7.86
NFE	57.56	57.5	57.47	57.27

significantly increased milk yield by feeding bypass protein. Similarly, Grewal *et al.* (2014) and Nam *et al.* (2014) had also reported significantly increased milk yield by feeding bypass fat and protein.

Significantly higher FCM (Table 3) was observed in T₃ over all the treatment groups but T₁ and T₂ were at par whereas T₀ was significantly lower among treatment groups. Ramteke *et al.* (2014), Sontakke *et al.* (2014) and Yadav *et al.* (2015) reported significantly increased FCM yield by feeding bypass fat whereas Amrutkar *et al.* (2014) reported significantly increased FCM yield by feeding bypass protein. Also, Nam *et al.* (2014) reported significantly increased FCM yield by feeding bypass fat and protein.

The daily energy corrected milk yield (Table 3) was 24.61% significantly higher in T₃, 14.82% higher in T₂ and

13.89% in T₁ than T₀ whereas treatment T₂ and T₁ were at par with each other, and significantly lower ECM was found in T₀. Ramteke *et al.* (2014) reported significant increase in ECM yield by feeding bypass fat and by feeding bypass protein (Amrutkar *et al.* 2014).

Reproductive performance: The reproductive performance of cows fed different treatments is shown in Table 3. The number of hours required for expulsion of placenta was statistically ($P < 0.05$) lower (2.30 h) in bypass fat supplemented group (T₂) than other groups. This might be due to better energy status of the animals, which is strongly associated with reproductive performance. Our results are in accordance with that of Tyagi *et al.* (2010). The number of days required for involution of uterus decreased statistically (by 5.83 days) in bypass protein and fat group (T₃) over other groups. Prostaglandins play an important role in reestablishing estrous cycle both immediately after parturition and thereafter, until conception occurs, prostaglandins F₂ α is responsible for uterine involution after parturition. This might be due to a positive effect of protected fat supplementation on uterine functions. Reduction of incidence of RFM and less time taken for involution of uterus would facilitate the early and successful conception of the cow. Our results are in accordance with that of Tyagi *et al.* (2010) who reported decrease in time required for involution of uterus in bypass fat supplemented cows. The number of days required for appearance of first post partum estrus was significantly ($P \leq 0.05$) reduced by 26 days in bypass protein and fat supplemented (T₃) group. The feeding of additional energy in the form of bypass fat reduces the cow's negative energy status so that cow returns to estrus earlier after calving and therefore conceives sooner. Our results are in accordance with Tyagi *et al.* (2010) and Gowda *et al.* (2013) who fed bypass fat whereas, Shelke *et al.* (2012) reported similar

Table 3. Nutrient intake, production and reproduction performance of cattle fed with or without protected fat and protein

Parameters	Treatment				SEM \pm
	T ₀	T ₁	T ₂	T ₃	
<i>Nutrient intake</i>					
DMI (kg/day)	12.47 ^a	12.59 ^a	12.23 ^b	12.72 ^a	12.5
DMI(%BW)	3.05	3.01	3.13	2.81	3
DCPI (kg/day)	1.15 ^b	1.21 ^a	1.18 ^a	1.23 ^a	1.19
DCPI(%BW)	0.28	0.29	0.3	0.27	0.28
TDNI(kg/day)	7.11 ^c	7.86 ^b	7.93 ^b	8.32 ^a	7.8
TDNI(%BW)	1.74	1.88	2.03	1.84	1.87
<i>Production performance</i>					
Milk yield	9.82 ^b	11.76 ^a	11.41 ^a	12.43 ^a	0.403
FCM yield	9.66 ^c	11.40 ^b	11.56 ^b	13.37 ^a	0.455
ECM yield	9.54 ^c	11.08 ^b	11.20 ^b	12.68 ^a	0.449
<i>Reproduction performance</i>					
Expulsion of fetal membranes (h)	5.81 ^a	4.11 ^b	3.51 ^b	3.93 ^b	0.55
Involution of uterus (days)	28.33 ^a	27.50 ^a	25.17 ^{ab}	22.50 ^b	1.13
Open period (days)	87.83 ^a	76.00 ^{ab}	66.00 ^b	61.83 ^b	6.36
Service period (days)	141.33 ^a	130.67 ^{ab}	82.00 ^c	99.67 ^{bc}	13.7
AI/ conception	3.00 ^a	2.50 ^{ab}	1.83 ^b	2.00 ^b	0.29
Conception rate (%)	33.34	40	66.67	54.55	

Table 4 Blood analysis of experimental animal

Parameters	T ₀	T ₁	T ₂	T ₃	SEM ±
Albumin	3.28	3.21	3.21	3.24	0.048
Globulin	4.06	4.26	3.99	3.97	0.155
BUN	16.57 ^a	13.69 ^b	15.98 ^a	14.48 ^b	0.376
Cholesterol	112.83 ^c	137.20 ^b	147.68 ^{ab}	164.02 ^a	6.008
Glucose	65.09 ^b	67.76 ^{ab}	67.22 ^{ab}	70.65 ^a	1.653
NEFA	1.22	1.24	1.02	1.12	0.09
Total blood protein	7.35	7.46	7.21	7.21	0.166
Triglyceride	12.65 ^b	12.71 ^b	15.26 ^a	17.01 ^a	0.759
Uric acid	2.29	2.32	2.31	2.35	0.164
Creatinine	0.94	0.92	0.98	0.97	0.032

findings by feeding bypass fat and protein. The service period was statistically reduced by 59.33 and 41.66 days in T₂ and T₃ groups than control. Similarly, number of AI/conceptions was statistically lower in bypass fat supplemented group (T₂). The service period and AI/conception was shorter in bypass fat group (T₂) than the other groups, indicating that lesser time was required for the cows in T₂ for conception. This might be due to slight increase in the blood progesterone concentration in cows supplemented with bypass fat. Bypass fat increases concentration of circulating cholesterol, the precursor of progesterone, which then secreted by the corpus luteum helps in preparing the uterus for implantation of the embryo and further maintenance of pregnancy. Tyagi *et al.* (2010), Gowda *et al.* (2013), Shelke *et al.* (2012) and Grewal *et al.* (2014) reported similar findings. Highest conception rate was found in T₂ treatment group followed by T₃, and T₁ and lowest in T₀. A similar result was reported by Tyagi *et al.* (2010) on supplementing rumen inert fat in the diet of high yielding lactating dairy cows. Gowda *et al.* (2013) also reported better reproductive performance in cows fed indigenously prepared bypass fat. The supplementation of protected fat and protein during early lactation improved the reproductive performance in Murrah buffaloes along with increase in the milk production and its persistency (Shelke *et al.* 2012 and Grewal *et al.* 2014).

Blood metabolites: The albumin and globulin level remained within normal range and no difference was observed at any stage within treatment groups during the experimental period (Table 4). These findings are in accordance with Kirovski *et al.* (2015) who reported that bypass fat supplementation had no effect on the albumin. Moveliya *et al.* (2013) reported no effect and Sai *et al.* (2014) reported significantly increased trend of albumin level by feeding bypass protein whereas, Shelke (2010) reported no effect on albumin level by feeding bypass fat and protein.

The average BUN level was significantly lower ($P < 0.01$) in T₁ and T₃ compared to T₀ and T₂ treatment groups. This may be due to protection of groundnut cake from microbial degradation which bypassed the rumen as intact protein. The higher value of blood urea level in T₀ and T₂ is

indicative of less efficient utilization of dietary nitrogen for microbial protein synthesis due to higher ammonia level. Yadav *et al.* (2015) reported significantly decreased BUN by feeding prilled fat to crossbred cows. Nonsignificant difference in BUN on supplementing bypass fat was reported by Wadhwa *et al.* (2012) and Kirovski *et al.* (2015). Similar results were reported by Sherasia *et al.* (2012), Moveliya *et al.* (2013) and Sai *et al.* (2014) by feeding bypass protein supplements. Shelke (2010) and Grewal *et al.* (2014) reported significantly decreased level of BUN by feeding bypass fat and protein whereas, Garg *et al.* (2012) reported non-significant difference in BUN on supplementing bypass fat and protein.

The cholesterol level was significantly higher ($P < 0.05$) in groups T₃ and T₂ as compared to T₀ and T₁. The cholesterol level was significantly lower in control group (T₀) over all the treatment groups. This higher level of cholesterol may have favourable effect on the synthesis of reproductive hormones like progesterone level (Wadhwa *et al.* 2012), which might be responsible for better reproductive performance of protected fat group. Similar observation was reported by Singh *et al.* (2014) and Kirovski *et al.* (2015) on supplementing bypass fat. Yadav *et al.* (2015) reported significantly decreased cholesterol level by feeding prilled fat to cows. However, by feeding bypass fat and protein, significant improvement in cholesterol level was observed by Grewal *et al.* (2014) whereas, nonsignificant difference was observed by Garg *et al.* (2012) and Shelke (2010).

The blood glucose level remained significantly higher in treatment groups over control, whereas T₃, T₁ and T₂ were at par over control and T₀, T₁ and T₂ were at par over T₃. Similar results were reported by Singh *et al.* (2014) on supplementing bypass fat. This increase in glucose may have a positive effect on LH release. No difference was observed by Shelke (2010) by feeding bypass fat and protein.

The overall mean of NEFA did not differ statistically in treatment groups which may be due to less mobilization of body fat by supplementing protected nutrients. Singh *et al.* (2014) reported decreased NEFA trend by feeding bypass fat. Sai *et al.* (2014) observed no difference in NEFA level by feeding protected amino acids. Shelke (2010) reported no difference in NEFA level by feeding bypass fat and protein to buffaloes.

Highest total blood protein was observed in T₁ followed by T₀, T₃ and T₂. However, there was no significant difference between the groups. The period significantly affected the average total protein level of the animals. Similar nonsignificant observations were reported by Moveliya *et al.* (2013) by feeding bypass protein, Grewal *et al.* (2014) and Shelke (2010) by feeding bypass fat and protein, whereas, Sai *et al.* (2014) reported significant increase in total protein level by feeding bypass protein.

The triglyceride level was significantly higher in bypass fat supplementing group T₂ and T₃ which was due to enriched intake of dietary fatty acids through protected fat.

Singh *et al.* (2014) and Kirovski *et al.* (2015) observed nonsignificant difference by feeding bypass fat. Sai *et al.* (2014) reported that triglyceride level significantly increased by feeding bypass protein. Also Grewal *et al.* (2014) and Shelke (2010) observed nonsignificant effect on triglyceride by feeding bypass fat and protein.

The higher uric acid value was nonsignificantly different among the groups. Wadhwa *et al.* (2012) reported increase ($P < 0.05$) in blood uric acid levels in animals fed bypass fat, whereas, Shelke (2010) reported no difference in treatment by feeding bypass fat and protein.

The average creatinine was nonsignificantly different among the groups. These results are in agreement with the findings of Wadhwa *et al.* (2012) who reported that bypass fat supplementation had no effect on the creatinine. Similarly, Grewade *et al.* (2014) and Shelke (2010) also reported no difference by feeding bypass fat and protein.

Hence, supplementation of protected protein and fat before parturition might reduce the detrimental effects of negative energy balance which might increase both lactation, reproductive as well as metabolic performance.

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