



Effect of Different Feeding Regimes on Biochemical and Hormonal Profile of Holstein Friesian × Kankrej Crossbred Cows

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ABSTRACT

An experiment was conducted on 18 crossbred cows to study the effect of different feeding regimes on biochemical and hormonal profiles. Animals of T₁ (Farmers' feeding) group (n=6) were maintained as per the feeding regime, followed by small and marginal farmers. Animals in T₂ (Modified feeding) group (n=6) comprised feeding with scientific interventions. Animals of T₃ (Farm feeding) group (n=6) were fed as per standard feeding followed at Livestock Research Station. Average plasma glucose did not differ significantly from each other while plasma phosphorus was significantly (p<0.05) higher in T₃ as compared to T₁ but the value of T₂ group was at par with the other two groups. Average plasma protein was significantly (p<0.05) higher in T₂ and T₃ when compared with T₁ group. Average plasma creatinine did not differ significantly among treatment groups. Average plasma progesterone (ng/ml) was below 1 ng/ml on the day of parturition, which increased to more than one ng/ml on the 28th, 21st, and 21st d post-partum in T₁, T₂, and T₃ groups, respectively indicating the onset of cyclicity was earlier in animal of T₂ and T₃ group as compared to T₁ group. It may be concluded from the present study that plasma glucose and creatinine were not influenced by the feeding regime. However, the feeding regime had a significant effect on plasma protein and phosphorus. Further, plasma progesterone levels in animals of modified and farm feeding groups indicated resumption of cyclicity earlier as compared to animals of farmer's feeding group.

HIGHLIGHTS

- ⦿ Plasma glucose and creatinine were not influenced by the feeding regime.
- ⦿ The feeding regime had a significant effect on plasma protein and phosphorus.
- ⦿ Resumption of cyclicity was earlier in modified and farm feeding group.

Keywords: Feeding Regime, Biochemical, Hormonal, Crossbred Cows

In developing countries like India, more than 70 % of expenditure on dairy farming is on the feeding of animals. Most of the animals are underfed in field conditions, as the poor and illiterate farmers are not aware of the benefits of quality feeding and the non-availability of the required quantity of feed. The success of livestock farming greatly

depends on the continuous supply of good quality balanced feed (Suharyono *et al.*, 2018).

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The biochemical profile of blood is a good indicator of the health of the cows (Coroian *et al.*, 2017). Not solely, but in combination with clinical examination or other diagnostic procedures, biochemical tests are highly informative as a diagnostic tool in bovine medicine (Roland *et al.*, 2014). Nutrition is the main technological factor that can produce profound changes in the metabolic profile of animals. Profound changes in the metabolism of lactating cows take place due to a deficiency of minerals, proteins, and vitamins in the diet. Mineral supplementation greatly enhances the blood biochemical parameters in dairy animals (Sahoo *et al.*, 2017b). The biochemical profile within normal physiological limits reflects a good health status and is highly correlated with milk production (Coroian *et al.*, 2017). The time of resumption of post-partum ovarian activity is one of the major factors related to subsequent fertility in dairy cattle (Melendez *et al.*, 2018). Delayed resumption of ovarian activity leads to subsequent impaired fertility. The concentration of progesterone hormone during the post-partum period is a good indicator of the resumption of ovarian activity (Dhami *et al.*, 2017).

MATERIALS AND METHODS

Location and duration of the experiment

The present experiment was conducted at Livestock Research Station (LRS), College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India. The study was conducted on 18 dry pregnant HF × Kankrej (50:50) crossbred cows distributed into three treatment groups comprising 6 animals in each. Experimental animals were randomly selected based on first lactation milk (300 d) yield, calving sequence (parity), and body weight (kg) in chronology. The experiment was conducted from 45 d before calving (advance pregnancy) to 300 d post-partum.

Animal grouping and treatments

Animals of T₁ (Farmer's feeding) group were maintained as per the feeding regime, followed by small and marginal farmers. Concentrate feed was not given during pre-partum period and fed @ 50% of milk production in the lactation period. Animals were devoid of mineral mixture

and fed with paddy/wheat straw on *ad lib.* basis and 10 kg/animal/d cereal green fodder. Animals of T₂ (Modified feeding) group comprised of feeding with scientific interventions with resources available with farmers. Animals were provided concentrate feed @ 1 kg/animal/d during pre-partum and @ 50% of milk production in the lactation period. The mineral mixture was provided to animals @ 30 g/animal/d. Legume straw and paddy/wheat straw (50:50 ratio) were given to animals on *ad-lib.* basis and 10 kg/animal/d cereal green fodder were given to the animals. Animals of T₃ (Farm feeding) group were fed as per standard feeding followed at LRS. Concentrate feed was given for steaming up in pre-partum period, starting from 500 g/animal/d in the first week of the experiment and increasing by 500 g every week, reaching 3.5 4.0 kg/animal/d till parturition. During the lactation period, concentrate feed was given @ 40% of milk production plus 1 kg maintenance/animal/d. Animals were fed with a 50 g/animal/d mineral mixture. Jowar hay was fed to animals on *ad-lib.* basis and 10 kg/animal/d cereal green fodder were fed to the animals.

Collection of blood samples

Considering the gestation period of 277 d and the day of calving as day zero in crossbred cattle maintained at Livestock Research Station, the following pattern was followed for blood collection-

-42, -28, -14, -7, -3, 0, +3, +7, +14, +21, +28, +35 d
likewise at every 14 d interval till 180 days of lactation
and at every 30 days from 180 to 300 days of lactation.

Five to six ml of blood was collected from the jugular vein of each experimental animal in a vacutainer containing K₃ EDTA. Plasma was separated by centrifugation at 3000 rpm for 15 minutes and stored in deep freeze at -20 °C until analysis of various biochemical and hormonal profiles.

Analysis of serum samples

Biochemical profiles viz., Glucose, Total Protein, Phosphorus, and Creatinine were analyzed using standard diagnostic kits in a chemistry analyzer (BS-120, Mindray). Plasma progesterone concentrations were estimated by the standard Radio-Immuno-Assay (RIA) technique using a standard diagnostic kit at the RIA laboratory. Coat-a-count

RIA kits were supplied by Immunotech-S.A.S. © 2016 Beckman Coulter, Inc. Marseille, France.

STATISTICAL ANALYSIS

Observations of various biochemical and hormonal parameters recorded during the experimental period were statistically analyzed by Completely Randomized Design (Factorial) using SAS software 9.3 version.

RESULTS AND DISCUSSION

Feed and nutrient intake

Results of feed and nutrient intake are presented in Table 1. Average fortnightly DMI either kg/animal/d or kg/100 kg b.wt. was significantly ($p < 0.05$) higher in T_2 (12.45 ± 0.28 and 2.74 ± 0.06) and T_3 (12.62 ± 0.22 and 2.81 ± 0.06) as compared to T_1 (9.71 ± 0.20 and 2.24 ± 0.05) group. Average fortnightly DCPI (g/animal/d) was significantly ($p < 0.05$) high in T_2 (801.87 ± 22.30) and T_3 (839.53 ± 16.63) as compared to T_1 (543.83 ± 14.83) group. The average DCPI (g/100 kg b.wt.) was 128.49 ± 4.51 , 177.40 ± 5.00 , and 187.84 ± 4.51 g in T_1 , T_2 , and T_3 groups, respectively, which differed significantly ($p < 0.05$) among each other. Average fortnightly TDNI either kg/animal/d or kg/100 kg b.wt. was significantly ($p < 0.05$) more in T_2 (7.13 ± 0.16 and 1.57 ± 0.04) and T_3 (7.30 ± 0.13 and 1.63 ± 0.04) when compared with T_1 (4.82 ± 0.10 and 1.11 ± 0.03) group.

Biochemical profile

Results of biochemical and hormonal profiles are presented in Tables 2 and 3, respectively.

Glucose

Average plasma glucose found at the beginning of the experiment was 57.08 ± 1.24 , 55.93 ± 0.77 , and 55.96 ± 1.23 mg/dL, which increased to 78.95 ± 8.09 , 78.98 ± 8.44 , and 66.80 ± 6.38 mg/dL on the day of parturition in T_1 , T_2 , and T_3 groups, respectively. After parturition, there was a sudden decline in plasma glucose on 3rd d post-partum, and thereafter the level was maintained at pre-calving level throughout the lactation period in all treatment groups. Irrespective of periods, overall plasma glucose was 58.62 ± 0.67 , 58.41 ± 0.78 , and 58.78 ± 0.56 mg/dL, respectively in T_1 , T_2 , and T_3 groups which did not differ significantly from each other.

In the present study, irrespective of treatments, average glucose concentration varied between 54 and 61 mg/dL before calving, increased to 75 mg/dL on the day of calving and decreased following calving. Similar results were obtained by Garverik *et al.* (2013) who reported that the average concentration of glucose varied between 61 and 64 mg/dL before calving, increased to over 70 mg/dL on the day of calving, and decreased following calving. No effect of concentrate feed amount (group fed with either high or low concentrate) on plasma glucose concentration was seen by Lawrence *et al.* (2015), which again supports the present findings. Contradictory to the present study, Sahoo *et al.* (2017a) observed that serum glucose (mg/dL) was significantly ($p < 0.05$) more in mineral supplemented group (56.00 ± 3.50) as compared to the control group (43.80 ± 3.23) after 60 days of treatment being at par at the beginning of the study.

Table 1: Average feed and nutrient intake of crossbred cows during the experiment

Parameters	Treatments		
	T_1	T_2	T_3
DMI (kg/animal/d)	$9.71^a \pm 0.20$	$12.45^b \pm 0.28$	$12.62^b \pm 0.22$
DMI (kg/100 kg b.wt.)	$2.24^a \pm 0.05$	$2.74^b \pm 0.06$	$2.81^b \pm 0.06$
DCPI (g/animal/d)	$543.83^a \pm 14.83$	$801.87^b \pm 22.30$	$839.53^b \pm 16.63$
DCPI (g/100 kg b.wt.)	$128.49^a \pm 4.51$	$177.40^b \pm 5.00$	$187.84^c \pm 4.51$
TDNI (kg/animal/d)	$4.82^a \pm 0.10$	$7.13^b \pm 0.16$	$7.30^b \pm 0.13$
TDNI (kg/100 kg b.wt.)	$1.11^a \pm 0.03$	$1.57^b \pm 0.04$	$1.63^b \pm 0.04$

Means with dissimilar superscripts in a row differed significantly ($p < 0.05$).

Table 2: Average hematological profile of crossbred cows during the experiment

Parameters	Treatments		
	T ₁	T ₂	T ₃
Plasma glucose (mg/dL)	58.62 ± 0.67	58.41 ± 0.78	58.78 ± 0.56
Plasma phosphorus (mg/dL)	6.69 ^a ± 0.08	6.81 ^{ab} ± 0.09	7.04 ^b ± 0.07
Plasma protein (g/dL)	7.51 ^a ± 0.06	7.85 ^b ± 0.06	7.94 ^b ± 0.05
Plasma creatinine (mg/dL)	1.49 ± 0.04	1.43 ± 0.03	1.45 ± 0.02

Means with dissimilar superscripts in a row differed significantly ($p < 0.05$).

Table 3: Plasma progesterone (ng/ml) in crossbred cows during the experimental period

Stage	Days	Treatments			Overall
		T ₁	T ₂	T ₃	
Day of calving	0	0.77 ± 0.15	0.92 ± 0.10	0.95 ± 0.08	0.88^a ± 0.07
	7	0.37 ± 0.09	0.65 ± 0.10	0.68 ± 0.03	0.57^a ± 0.06
	14	0.38 ± 0.10	0.78 ± 0.15	0.73 ± 0.11	0.63^a ± 0.08
	21	0.97 ± 0.65	3.07 ± 2.19	1.97 ± 1.29	2.01^a ± 0.85
	28	2.05 ± 0.83	1.08 ± 0.36	2.28 ± 0.70	1.80^a ± 0.38
Post calving	35	5.99 ± 3.15	6.05 ± 2.69	6.08 ± 2.06	6.04^{bc} ± 1.45
	49	2.57 ± 1.22	5.03 ± 2.18	6.94 ± 2.39	4.85^b ± 1.17
	63	4.90 ± 2.12	3.08 ± 1.39	7.82 ± 2.35	5.27^{bc} ± 1.18
	77	6.67 ± 2.32	5.20 ± 1.96	11.22 ± 2.57	7.70^c ± 1.39
	91	7.65 ± 2.47	7.09 ± 1.50	8.17 ± 2.60	7.64^c ± 1.22
	Overall	3.23 ± 0.60	3.30 ± 0.55	4.68 ± 0.70	3.74 ± 0.36

Means with dissimilar superscripts in a column differed significantly ($p < 0.05$).

Phosphorus

Average plasma phosphorus at the beginning of the experiment was 7.02 ± 0.25 , 7.07 ± 0.47 , and 7.14 ± 0.36 mg/dL in T₁, T₂, and T₃ groups, respectively. In T₁ and T₂ groups, plasma phosphorus levels decreased to 6.35 ± 0.44 and 6.80 ± 0.60 mg/dL, respectively, on the day of parturition, while in the case of the T₃ group, phosphorus levels increased to 7.26 ± 0.71 mg/dL. Plasma phosphorus level in all the treatment groups was maintained at the same level similar to the day of parturition during early and mid-lactation while increasing during the late lactation period. Irrespective of periods, average plasma phosphorus (mg/dL) was significantly ($p < 0.05$) higher in T₃ (7.04 ± 0.07) as compared to T₁ (6.69 ± 0.08), while the value of T₂ (6.81 ± 0.09) was at par with the other two groups. However, there was no significant difference in plasma phosphorus of crossbred cows during various periods of the experiment

irrespective of treatments. A significantly ($p < 0.05$) higher level of phosphorus in T₃ as compared to T₁ group was observed, which also might be due to mineral mixture supplementation. Plasma phosphorus values irrespective of treatments and periods were within the normal range of 5.6 to 8.0 mg/dL. Phosphorus has been most commonly associated with decreased reproductive performance in dairy cows. Inactive ovaries, delayed sexual maturity, and low conception rates have been reported when phosphorus intakes are low. In a field study, when heifers received only 70-80% of their phosphorus requirements and serum phosphorus levels were low, fertility was impaired (3.7 services per conception). Services per conception were reduced to 1.3 after adequate phosphorus was supplemented (Yasothai, 2014).

As per the study of Sahoo *et al.* (2017a), serum phosphorus was significantly ($p < 0.05$) more in mineral supplemented

group (5.37 ± 0.33 mg/dL) as compared to the control group (4.14 ± 0.26 mg/dL) after 60 days of treatment being at par at the beginning of the study. Similarly, in the present study also overall phosphorus was significantly ($p < 0.05$) higher (7.04 ± 0.07 mg/dL) in the group supplemented with a 50 g/animal/d mineral mixture as compared to the group without supplementation (6.69 ± 0.08 mg/dL); however, the values were lower as compared to present study. In the present study, during the post-partum period lowest average value of phosphorus (mg/dL) was seen on 3rd d (6.59 ± 0.29 mg/dL). Similar results were obtained by Coroian *et al.* (2017), who observed the lowest average values on 3rd d post-partum (4.66 mg/dL); however, the value was higher in the present study.

Total Protein

Average plasma total protein was 8.71 ± 0.39 , 8.88 ± 0.31 , and 8.96 ± 0.26 g/dL at the beginning of the experiment, which decreased to 7.56 ± 0.32 , 8.18 ± 0.34 , and 8.40 ± 0.09 g/dL on the day of calving in T₁, T₂, and T₃ groups, respectively. Plasma protein decreased gradually during the post-partum period and reached 7.05 ± 0.85 , 7.79 ± 0.31 , and 7.38 ± 0.00 g/dL at the end of the experiment in T₁, T₂, and T₃ groups, respectively. Overlooking periods, average plasma protein (g/dL) was significantly ($p < 0.05$) higher in T₂ (7.85 ± 0.06) and T₃ (7.94 ± 0.05) as compared to T₁ (7.51 ± 0.06) group. If treatments are ignored, average plasma protein was 8.85 ± 0.18 g/dL at the beginning of the experiment, which decreased significantly ($p < 0.05$) and reached 8.05 ± 0.17 g/dL on the day of parturition. During the post-partum period, plasma protein level further decreased up to 35th d post-partum and then increased and maintained throughout the lactation period. Significantly ($p < 0.05$), higher plasma protein level was seen in T₂ and T₃ as compared to T₁, which might be due to the effect of feed. Plasma total protein values irrespective of treatments and periods were either on the higher side or slightly above the normal range, which is 6.7 to 7.5 g/dL.

Similar results were obtained by Ashmawy (2015), who observed that plasma total protein value in Egyptian buffalo was higher during pregnancy, i.e., 60th d before calving (7.04 ± 0.12 g/dL) as compared to 10th d of lactation (6.20 ± 0.27 g/dL). Serum total protein was significantly ($p < 0.05$) more in mineral supplemented group (6.77 ± 0.66 g/dL) as compared to the control group (5.78 ± 0.62 g/dL) after 60 d of treatment being at par at the beginning of the

study as reported by Sahoo *et al.* (2017a) which supports the present findings. Contrary to the present study, Taskin (2013) observed that an increase of 0.1 kg in concentrate feed per kg of milk was not effective on serum protein. Serum protein concentration was 7.1, and 7.5 g/dL in low and high concentrate fed groups, respectively.

Creatinine

Average plasma creatinine at the beginning of the experiment was 1.75 ± 0.14 , 1.76 ± 0.07 , and 1.72 ± 0.05 mg/dL, which increased to 2.29 ± 0.14 , 2.03 ± 0.12 , and 1.91 ± 0.14 mg/dL on the day of parturition in T₁, T₂, and T₃ groups, respectively. A maximum increase in creatinine level from the beginning of the experiment to the day of calving was seen in T₁ group, followed by T₂ and T₃ groups. During the post-partum period, a decreasing trend was seen in all the treatment groups throughout the lactation period. Irrespective of periods, average plasma creatinine was 1.49 ± 0.04 , 1.43 ± 0.03 , and 1.45 ± 0.02 mg/dL, respectively, which did not differ significantly among the treatment groups. If treatments are ignored, average plasma creatinine was 1.74 ± 0.05 mg/dL at the beginning of the experiment, which increased significantly ($p < 0.05$) and reached 2.07 ± 0.08 mg/dL on the day of parturition. During the post-partum period, a significant ($p < 0.05$) decline in plasma creatinine was seen on 3rd and 7th d post-partum as compared to the day of parturition. Thereafter also the creatinine level was seen in a declining trend throughout the lactation period. Significantly ($p < 0.05$) higher level of plasma creatinine was observed in all three groups on the day of calving as compared to the pre and post-partum period, which was due to increased muscular contraction during calving. Plasma creatinine values irrespective of treatments and periods were within the normal range of 0.5 to 2.2 mg/dL.

Results of the present study are corroborated with the results of Wadhwa *et al.* (2012), who observed no significant difference in blood creatinine level of the control group (1.13 mg/dL) and bypass fat supplemented group (1.16 mg/dL), although the value was higher in later one. As per the report of Garg *et al.* (2016), there was no effect of ration balancing on the serum creatinine level of cows. Serum creatinine was found non-significant in cows before (1.23 ± 0.03 mg/dL) and after (1.34 ± 0.03 mg/dL) ration balancing, which supports the present findings. Contrary

to the present findings, Ashmawy (2015) observed that the concentration of plasma creatinine in Egyptian buffalo was higher during the 10th d of the lactation period (1.49 ± 0.12 mg/dL) than in pregnancy i.e., 60th d (1.22 ± 0.11 mg/dL) before calving. Present study showed smaller value at 7 d postpartum (1.55 ± 0.06 mg/dL) as compared to 42 d pre-partum (1.74 ± 0.05 mg/dL).

Hormonal profile

Average plasma progesterone was 0.77 ± 0.15 , 0.92 ± 0.10 , and 0.95 ± 0.08 ng/ml on the day of parturition, which increased to more than one ng/ml on the 28th (2.05 ± 0.83 ng/ml), 21st (3.07 ± 2.19 ng/ml), and 21st (1.97 ± 1.29 ng/ml) d post-partum in T₁, T₂, and T₃ groups, respectively. The corresponding value of plasma progesterone was 7.65 ± 2.47 , 7.09 ± 1.50 , and 8.17 ± 2.60 ng/ml on 91st d post-partum. Irrespective of periods, the average plasma progesterone was 3.23 ± 0.60 , 3.30 ± 0.55 , and 4.68 ± 0.70 ng/ml T₁, T₂, and T₃ groups, respectively, which did not differ significantly. Irrespective of treatments, the average plasma progesterone was 0.88 ± 0.07 ng/ml on the day of parturition, which reached beyond one ng/ml on the 21st d post-partum (2.01 ± 0.85 ng/ml). On 91st d post-partum, plasma progesterone was 7.64 ± 1.22 ng/ml. Animals returned to their cyclicity as indicated by plasma progesterone level was earliest in T₂ group followed by T₃ and T₁ groups which were due to the quality of feed provided to the animals of different groups. Plasma progesterone values irrespective of treatments and periods were within the normal range.

Patel (2014) reported that overall mean serum progesterone concentrations in crossbred HF cows on 7 d pre-partum and 3, 10, 20, and 30 d post-partum were 5.47 ± 0.23 , 0.64 ± 0.03 , 0.74 ± 0.03 , 1.24 ± 0.08 and 1.38 ± 0.10 ng/ml, respectively. Similarly, in the present study also the progesterone levels were found in the same fashion, which goes beyond one ng/ml at 21 d post-partum and indicates the beginning of cyclicity in animals. Results of the present study are in agreement with Dhama *et al.* (2017) as they reported that the plasma progesterone reached the basal levels (<1 ng/ml) on the day of calving, remained basal till day 14, and thereafter showed a rising trend on 28 and 42 d post-partum.

CONCLUSION

It may be concluded from the present study that plasma glucose and creatinine were not influenced by the feeding regime. However, the feeding regime had a significant effect on plasma protein and phosphorus. Further, early rise in post-partum plasma progesterone levels in animals of modified and farm feeding groups indicated resumption of cyclicity earlier as compared to animals of farmer's feeding group.

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