www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(10): 1873-1876

www.thepharmajournal.com
Received: 17-07-2023
Accepted: 21-08-2023

Sagar M
Department of Veterinary Physiology, Veterinary College, Hebbal, Bangalore, Karnataka, India

Narayana Swamy M
Department of Veterinary Physiology, Veterinary College, Hebbal, Bangalore, Karnataka, India

GP Kalmath
Department of Veterinary Physiology, Veterinary College, Hebbal, Bangalore, Karnataka, India

MP Veena
Department of Veterinary Physiology, Veterinary College, Hebbal, Bangalore, Karnataka, India

Hemanth Gowda K
Department of Veterinary Physiology, Veterinary College, Hebbal, Bangalore, Karnataka, India

Rashmi R
Department of Veterinary Physiology, Veterinary College, Hebbal, Bangalore, Karnataka, India

Corresponding Author:
Sagar M
Department of Veterinary Physiology, Veterinary College, Hebbal, Bangalore, Karnataka, India

Effect of supplementation of horse gram based bypass protein on serum and milk biochemical components in Holstein Friesian cows

Sagar M, Narayana Swamy M, GP Kalmath, MP Veena, Hemanth Gowda K and Rashmi R

Abstract
The present study was undertaken to ascertain the effect of supplementation of horse gram based bypass protein on certain serum and milk biochemical components in Holstein Friesian crossbred cows. A total of 24 animals in their 4th lactation with low SNF syndrome were selected for the study and were grouped in to four groups. Group I animals that were fed with only basal diet served as control group. Group II, Group III and Group IV animals were fed with soaked and grinded horse gram, heat treated and powdered horse gram and commercially available bypass protein, respectively, in addition to the basal diet. The blood and milk samples were collected from all the animals when they are in their 4th and 8th week of lactation. The blood samples were immediately utilized for blood glucose determination. Serum obtained from blood samples were utilized for β-hydroxybutyrate (BHB), total protein, albumin and urea nitrogen. The milk samples were screened for protein, lactose, SNF and milk urea nitrogen. Results indicated that the blood glucose levels were significantly higher in supplemented groups animals compared to control group animals. Serum total protein and serum albumin values were significantly higher in group IV animals compared to Group I animals at 4th week of lactation however, the values were significantly ($p<0.05$) higher in all the supplemented group animals compared to control animals at 8th week of lactation. Milk protein levels were significantly higher in animals fed with commercial bypass protein compared to control group animals at 4th and 8th week of 4th lactation. It was concluded that the supplementation of horse gram based bypass protein to HF crossbred animals with low SNF at their 4th lactation improved the levels of blood glucose, serum total protein, albumin and milk protein. However, it did not influence the other components significantly.

Keywords: Horse gram based bypass protein, serum, milk biochemical, Holstein Friesian cows

Introduction
The intensive cross-breeding of low-yielding local cattle and non-descript cattle with Holstein Friesian semen might have caused inbreeding effect. The intensive crossbreeding programme might have increased milk production but, has resulted in low solids-not-fat (SNF) syndrome. Various studies involving the chemical analysis of milk have revealed that the lower SNF levels was due to drop in milk protein content, but not due to the lactose content of milk (Murthy, 2014) [7]. As per the survey conducted in Karnataka around 28 to 50 percent of crossbred cows were yielding milk low SNF ranging from 8.00 to 8.5 percent. The animals that give milk with SNF less than 8.3 percent is treated as low SNF syndrome cows (Food Safety and Standards Authority of India, 2011).

Variations in the values of biochemical tests for different metabolic parameters may reveal details about the nutrition and health of the herd (Grunwaldt *et al*., 2005) [4]. The most prominent metabolites used to evaluate the energy status of cattle are blood glucose, beta hydroxy butyrate, and non-esterified fatty acids (Ndlovu *et al*., 2007) [9].

As per the available literature, the low SNF levels in the milk is attributed to decreased milk protein levels rather than the lactose levels. There is a hypothesis that, the problem of low SNF milk due to low protein supplementation could be overcome by increasing the amino acids pool required for milk protein synthesis which could be achieved by providing the bypass protein. Bypass protein is a protein that escapes rumen degradation and is prepared by the treatment of dietary protein with heat, formaldehyde and tannic acid (Reddy, 2015) [13].

The mammary gland of the lactating dairy cows may extract up to 85 % of the total available glucose from all the sources, and glucose entry rate and calorie intake are strongly connected (Bickerstaffe *et al*., 1974) [1].

The protein level could be used as a reliable parameter of nutritional status of the cattle (Ndlovu *et al*., 2007) [9].

Materials and Methods

Twenty four Holstein Friesian crossbred cows with low SNF syndrome (4th lactation) and reared by farmers belonging to different milk societies of Chikkaballapur District, Karnataka, were selected for the present study. Selected animals were divided into four groups with six animals in each group. Selected animals were maintained in the semi - intensive management system and fed with green forage, hay and Nandini gold feed. Animals were divided into four groups with six animals in each group. The approval for the study was obtained from the Institutional Animal Ethics Committee (IAEC), vide No. VCH/IAEC/2022/18 date: 08.06.2022.

The procured horse gram (Agricultural Produce Market Committee yard, Chikkaballapur) was utilized for further processing to use it as two sources of bypass protein. One portion of horse gram was soaked overnight and grinded to the particle size ranging from 20 to 45 micro meter which was determined and confirmed by micrometry method (Tan *et al*., 2019) [15] and dissolved in a 15 liter of water in a bucket and fed to Group II animals (@ 30 g per liter milk produced) on daily basis for about two months. This preparation of horse gram served as the source of bypass protein since the particle size is smaller and it escapes rumen fermentation as described by Reddy (2015) [13].

The other portion of the horse gram was roasted in the pan for about 15 minutes at approximate temperature of 160 °C in low flame (Ojha *et al*., 2020) [10] and then grinded in to the particle size of 60 to 80 micro meter which was determined and confirmed by micrometry method (Tan *et al*., 2019) [15] and the pulverized horse gram was fed to the Group III animals (@ 30 g per liter milk produced) on daily basis for about two months. Nandini bypass feed was procured from the milk cooperative society of Varlakonda village, Gudibande taluk, Chikkaballapura district was fed to the Group IV animals (@ 30 g per liter milk produced) on daily basis for about two months.

At 4th and 8th weeks of 4th lactation, the blood samples and the milk samples were collected from each of the study animal. At the time of collection of the blood and milk samples one or two drops of blood was utilized for the determination of the blood glucose levels using the glucometer. The collected blood samples were transported to the laboratory at a refrigerated temperature (4 °C) and then centrifuged at 1398 RCF for 20 minutes to separate the serum, which was then kept at -20 °C until they were utilized for determination of various biochemical components. Milk samples were stored at refrigerated temperature until they are used for determination of various biochemical parameters. Serum biochemical components were determined with help of STAR-20 clinical chemistry analyzer (Rapid Diagnostic Group of Companies Pvt. Ltd., Bengaluru) by utilizing the reagent kits.

The data of the present study were statistically analyzed using two-way ANOVA with application of Bonferroni's multiple comparison post-test using the computer programme, GraphPad Prism version 8.4.3. founded in San Diego, California.

Results and Discussion

The results of the present study are presented in Table 1. The significantly increased blood glucose levels in bypass protein supplemented groups in the present study were in accordance with the findings of Nair *et al*. (2021) [8] who also reported significant increase in the serum glucose concentration in dairy cows fed with four different combinations of dietary regimens. The results were also in agreement with the report of normal blood glucose level in HF crossbred cows with normal SNF compared to the cows with low SNF at 4th week and 8th week of 3rd and 5th lactation (Maneesha *et al*., 2019) [6]. The feeding of horse gram based bypass protein and commercial bypass protein having a good quantity of RDP may optimize the blood glucose levels which serve as a source for lactose synthesis that might contribute significantly to the SNF percentage. Increased blood glucose level might also indicates the nutritional status of lactating cows.

Significantly higher total serum protein levels found in Group II, III and IV, the bypass protein supplemented groups compared to the control group animals were in conformity with the findings of Ndlovu *et al*. (2007) [9] who reported significantly higher total protein level in lactating cows compared to pregnant and non-lactating cows. The results were in conformity with the findings of Maneesha *et al*. (2019) [6] who reported higher levels of serum total protein in Holstein Friesian crossbred cows with normal SNF compared to low SNF HF crossbred cows in 8th week of 3rd and 5th lactation. However, Gallego *et al*. (2017) [3] reported decreased levels of serum total protein at the initial stages of lactation in cows which could be due to utilization of glucogenic amino acids to overcome the negative energy balance during that period. The significantly higher mean serum total protein levels in animals supplemented with horse gram bypass protein and commercial bypass protein could be due to increased protein synthesis as a result of increased availability of the amino acids pool in the blood. The significant increase in the serum albumin levels in the supplemented group in the present study were in concurrence with the findings of Coroian *et al*. (2017) [2] who also reported significant increase in the serum albumin levels from 3rd lactation to 6th lactation in Holstein cows and with the findings of Nair *et al*. (2021) [8] who found increased serum albumin levels in cows supplemented with the diets containing the crude protein (12 to 14 %) compared to the animals that received diets with varying crude protein percentages.

However, there are reports that indicate non varying levels of serum albumin in HF crossbred cows at 4 weeks of lactation (Kupczynski and Chudoba-Drozdowsk, 2002) [5], and decrease in serum albumin level at 12th week of lactation compared to 4th week of lactation in HF crossbred cows (Praveen *et al*., 2020) [12]. It is concluded that the bypass protein supplementation could significantly contribute to higher serum albumin levels at 8th week of lactation by way of providing amino acids for albumin synthesis. The increased albumin levels is an indirect indication of positive nitrogen balance that could also help in increased synthesis of milk protein by alveolar epithelial cells of mammary gland by providing the essential amino acids to increase the SNF content of the milk.

The significantly higher milk protein levels in animals supplemented with bypass protein in the present study was in accordance with findings of Sherasia *et al*. (2016) [14] who observed significant increment in the milk protein percentage before and after feeding with balanced rations in their early days of second lactation in crossbred cows. The results are in conformity with the findings of Thapa *et al*. (2019) [16] who have reported increased milk protein percentage in soya cake bypass protein supplemented group animals compared to control group and same was attributed to increased absorption of essential amino acids for the intestine and their increased availability for milk protein synthesis in the alveolar epithelial cells (Park and Lindberg, 2005) [11]. It is concluded that the increase in milk protein values in Holstein Friesian crossbred cows when fed with commercial bypass protein could be useful for milk protein synthesis by way of supplementation of amino acids.

Table 1: Mean ± SE values of different biochemical components of serum and milk in different groups of HF crossbred cows at 4th lactation

(n = 6)

Parameters / Groups	Weeks of Lactation	Group I (n=6)	Group II (n=6)	Group III (n=6)	Group IV (n=6)
Blood Glucose (mg/dL)	4th Week	35.17±2.51a	51.83±1.11b	55.00±1.32b	54.17±1.30b
	8th Week	38.83±2.89a	53.00±1.06b	55.67±1.02b	55.00±1.15b
Serum BHB (mmol/L)	4th Week	0.95±0.15	0.82±0.09	0.82±0.07	0.73±0.09
	8th Week	0.74±0.08	0.69±0.04	0.77±0.07	0.62±0.07
Serum Total Protein (g/dL)	4th Week	7.95±0.22a	8.52±0.22ab	8.70±0.20ab	8.74±0.19b
	8th Week	7.53±0.22a	8.37±0.17b	8.48±0.19b	8.55±0.17b
Serum Albumin (g/dL)	4th Week	3.72±0.11a	3.99±0.10ab	4.07±0.10ab	4.09±0.09b
	8th Week	3.52±0.10a	3.92±0.18b	3.97±0.09b	4.00±0.08b
Serum Urea Nitrogen (mg/dL)	4th Week	22.44±0.54	23.28±0.63	24.04±0.69	23.60±0.70
	8th Week	22.02±0.78	22.42±0.71	22.88±0.72	22.52±0.68
Milk Protein (%)	4th Week	2.95±0.05a	3.58±0.22ab	3.33±0.22ab	3.82±0.31b
	8th Week	3.12±0.06	3.80±0.22	3.62±0.17	3.92±0.33
Milk Lactose (%)	4th Week	4.09±0.20	4.37±0.15	4.27±0.12	4.42±0.09
	8th Week	4.29±0.15	4.60±0.10	4.53±0.11	4.44±0.10
Milk SNF (%)	4th Week	8.05±0.09	8.95±0.46	8.03±0.28	8.68±0.28
	8th Week	8.08±0.08	8.65±0.14	8.40±0.22	8.55±0.25
Milk Urea Nitrogen (mg/dL)	4th Week	13.23±0.44	12.21±0.34	12.25±0.17	11.91±0.50
	8th Week	13.88±0.28	12.97±0.24	12.79±0.21	12.92±0.38

The values within the column and rows with different superscripts differ significantly ($p<0.05$).

Group I: HF crossbred cows with SNF < 8.3 in 4th lactation

Group II: HF crossbred cows with SNF < 8.3 in 4th lactation fed with soaked and grinded horse gram

Group III: HF crossbred cows with SNF < 8.3 in 4th lactation fed with heated and grinded horse gram

Group IV: HF crossbred cows with SNF < 8.3 in 4th lactation fed with commercial bypass protein: Nandini bypass protein

Conclusions

In the present study, though the supplementation of horse gram bypass protein and commercial bypass protein significantly improved the blood glucose levels, serum levels of protein and albumin it did not influence the levels of serum BHB and serum urea nitrogen in HF crossbred cows with low SNF syndrome. With respect to the milk components, supplementation horse gram bypass protein helped in increasing the milk protein levels but, other components of the milk remained uninfluenced in low solids-not-fat Holstein Friesian crossbred cows during 4th lactation. It was concluded that the protein supplementation during production stage in animals put them in a state of positive nitrogen balance and energy during that period to cope up with the increased demand of milk production. Hence, adding horse gram based bypass protein could be recommended in feeding trails in dairy cattle to improve productivity and to improve the farmers' income.

References

1. Bickerstaffe R, Annison EF, Linzell JL. The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. The Journal of Agricultural Science. 1974;82(1):71-85.
2. Coroian CO, Miresan V, Coroian A, Raducu C, Andronie L, Marchis Z, Terhes S, *et al*. Biochemical and haematological blood parameters at different stages of lactation in cows. Bulltein UASVM Animal Science and Biotechnologies. 2017;74(1):31-36.
3. Gallego C, Penalver A, Garcia A, Mendez M, Souza MIL, Oba E, *et al*. Influence of the physiological state on the energy, protein and endocrine metabolism, in Siboney de Cuba cows. Cuban Journal of Agricultural Sciences. 2017;51(4):427-432.
4. Grunwaldt EG, Guevara JC, Estevez OR, Vicente A, Rousselle H, Alcuten N, *et al*. Biochemical and haematological measurements in beef cattle in Mendoza plain rangelands (Argentina). Tropical Animal Health and Production. 2005;37(6):527-540.
5. Kupczynski R, Chudoba-Drozdowsk B. Values of selected biochemical parameters of cow's blood during their drying-off and the beginning of lactation. Electronic Journal of Polish Agricultural Universities. 2002;55:225-231.
6. Maneesha HS, Swamy MN, Kalmath GP, Ranganath L, Ramesh PT, Sudha G, *et al*. Serum Enzymatic and Lipid Profile in Holstein Friesian Cross Bred Cows with Low Solids-Not-Fat Syndrome. International Journal of Current Microbiology and Applied Sciences. 2019;8(02):3422-3430.
7. Murthy N. Prevalence of low solids not fat (SNF) in milks of crossbred and Holstein Friesian cows. J Vet. Sci. Technol. 2014;5(3):174-178.
8. Nair SS, Ally K, Dildeep V, Mathew JJ, Gleeja VL. Influence of different levels of dietary protein on serum biochemical parameters of dairy cows during the transition period. Journal of Veterinary and Animal Sciences. 2021;52(2):149-154.
9. Ndlovu T, Chimonyo M, Okoh AI, Muchenje V, Dzama K, Raats JG. Assessing the nutritional status of beef

cattle: current practices and future prospects. African Journal of Biotechnology. 2007;6(24):2727-2734.
10. Ojha P, Bhurtel Y, Karki R, Subedi U. Processing effects on anti-nutritional factors, phytochemicals, and functional properties of horse gram (Macrotyloma uniflorum) flour. Journal of Microbiology, Biotechnology and Food Sciences. 2020;9(6):1080-1086.
11. Park CS, Lindberg GL. The Mammary Gland and Lactation. In: Dukes Physiology of Domestic Animals. Edt. Reece, W. O., Edn. 12th., Panima Publishing Corporation, New Delhi; c2005. p. 720-741.
12. Praveen V, Swamy MN, Kalmath GP, Vinuthan MK, Narsimhamurthy Bellur SR, Sathisha KB. Biochemical components of milk during early lactation in Amrit Mahal, Holstein Friesian crossbred and jersey crossbred cows. Journal of Entomology and Zoological studies. 2020;8(3):122-125.
13. Reddy DV. Applied nutrition livestock, poultry, rabbits and laboratory animals. Edn. 3rd, Oxford and IBG publishing Co. Pvt. Ltd; c2015. p. 110.
14. Sherasia PL, Phondba BT, Hossain SA, Patel BP, Garg MR. Impact of feeding balanced rations on milk production, methane emission, metabolites and feed conversion efficiency in lactating cows. Indian J Anim. Res. 2016;50(4):505-511.
15. Tan J, Balasubramanian B, Sukha D, Ramkissoon S, Umaharan P. Sensing fermentation degree of cocoa (*Theobroma cacao* L.) beans by machine learning classification models based electronic nose system. Journal of Food Process Engineering. 2019;42(6):e13175.
16. Thapa P, Pandey T, Acharya R, Dhital B, Paklihawa R. Effect of by-pass protein supplements on milk production of dairy cattle. Journal of Agriculture and Natural Resources. 2019;2(1):171-179.