RESEARCH ARTICLE

Effect of Feeding Distiller's Dried Grains with Solubles (DDGS) and Legume Roughage on Serum Biochemical Profile in HF x Kankrej Crossbred Heifers

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Abstract

The study was undertaken on HF x K heifers (n=24) to evaluate the effect of feeding rice distiller's dried grains with soluble (RDDGS) and Soy DOC alone or in combination with cereal and/or legume straws as TMR from an average 8 months till 19 months of age on their health and serum biochemical profile. Heifers were uniformly distributed into three treatment groups. Further, each treatment group was subdivided into two roughage groups and were fed TMR having 50% concentrate and 50% roughage. The concentrates contain 0+40, 20+20, and 40+0% combination of RDDGS + Soy DOC, respectively in T1, T2, and T3 treatment. Each treatment was further subdivided to fed roughage as 50% wheat straw (R1) and another half as a mixture of wheat straw and groundnut gotar (R2). Heifers were also offered green hybrid Napier @ 2 kg/h/d up to 100 kg b.wt. and 4 kg/h/d for above 100 kg b.wt and Chelated Mineral Mixture @ 35 g/animal/d. Blood samples were collected in clot activator vials by jugular vein puncture from experimental heifers at the start and then at every month and serum was stored at -20°C till analysis. The collected serum samples were analyzed for biochemical constituents using suitable Coral and/or Tulip diagnostic kits on a chemistry analyzer. The data obtained were analyzed by 3 factors' factorial RBD. The results revealed that inclusion of RDDGS in concentrate and feeding a mixture of legume and cereal straws did not adversely affect the blood biochemical parameters, like glucose, total protein, albumin, globulin, cholesterol, BUN, and minerals calcium and phosphorus, all were within normal physiological limits. However, significantly reduced serum urea level and increased cholesterol levels on feeding RDDGS suggested it a good source of rumen degradable protein and fat enhancing steroidogenic function. Serum albumin, globulin, A:G ratio and minerals of heifers were within normal limit indicating no harmful effect of feeding RDDGS and mixture of groundnut straw and wheat straw on kidney and liver function. Thus, it can be concluded that feeding RDDGS in place of soy DOC in concentrate and feeding a mixture of groundnut gotar and wheat straw to growing crossbred heifers from 8 months till puberty/19 months maintains normal growth rate without adverse effect on the health and blood biochemical profile.

Key words: Crossbred heifers, Legume-nonlegume roughage, Serum biochemical profile, Rice distiller's dried grains with solubles (RDDGS), Soy DOC.

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Introduction

Adequate and balanced feeding to the young growing heifer is essential for proper body growth and development of the mammary gland, which can improve subsequent milk production (Hurley and Loor, 2011). Decreasing fodder land and increasing price of conventional feed grains are posing a threat to sustainable dairy farming, requiring investigation on alternate safe feed resources. India is one of the main rice-producing country and stands second after China in the world, it is also the largest producer of pulses and groundnut (GOI, 2019). During rice milling, around 9% of the total weight of paddy rice gets broken (Linscombe, 2006). Rice DDGS (RDDGS) is an important by-product of ethanol production plants, which uses damaged broken/low grade or unfit for human use rice for fermentation. RDDGS contains more than 40% protein and can replace highly prized soybean meal (Yogi *et al*., 2017). It is also rich in fat, vitamins, and minerals, and has been reported to be good source of rumen undegradable protein (RUP) and energy for ruminants (Ranathunga *et al*., 2018). The presence of dead yeast cells in DDGS improves amino acids composition of the protein, with better taste and nutritive value (Pecka-Kiełb *et al*., 2017). Due to limited availability of molasses, a large

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number of cereal-grain based ethanol production plants are established making huge quantity of DDGS as nutrient rich by-products (Sakthivel *et al.,* 2018).

Further, dairy animals are mainly offered cereal (paddy & wheat) straws as roughage owing to a greater availability, but lower in crude protein, essential amino acids, digestibility, and low voluntary intake, which is one of the reasons for energy-protein malnutrition in the dairy animals. Protein is extremely important in the diet of growing heifers to ensure optimum growth. RDDGS can be added to ration of growing heifers up to 40% of DMI to achieve improved growth rate and FCR (Kalscheur and Garcia, 2004) as it is an excellent source of protein and energy, and legume straw can supply more protein than cereal straw for growing heifers. The biochemical profiling of blood plays a crucial role in the judgment of physiological, nutritional, and pathological status, which can affect the growth, production, and reproduction of livestock (Aggarwal *et al*., 2016). Hence, this study was planned on crossbred heifers to study the effect of feeding RDDGS, Soya DOC, and roughage on health and serum biochemical profile.

Materials and Methods

This research work was carried out from June 2020 to May 2021 at Livestock Research Station, Anand Agricultural University, Anand (Gujarat, India) on 24 HF × Kankrej crossbred heifers of average 8 months of initial age with average body weight of 116.13±4.74 kg until 19 months of age or puberty and sexual maturity. Prior permission to perform this experiment was taken from CPCSEA through institutional animal ethics committee (IAEC). The selected heifers were divided randomly on the basis of body weight and age into six isometric groups under factorial RBD. Each group of four heifers was fed with 50% concentrate and 50% roughage TMR for a period of 10-12 months. Three different concentrates containing 0, 20 and 40% rice DDGS (RDDGS), and 40, 20, 0% Soy DOC, were offered to T1, T2, and T3 group heifers, respectively. Within each concentrate type, half of the heifers were offered wheat straw (R1 group) and another half a mixture of wheat straw and groundnut straw (R2 group) as dry roughage.

All the animals were de-wormed and kept tied under iso-managerial conditions in well ventilated hygienic shed and fed individually. The animals were left loose for 2 h both in the morning and evening, and closely observed for heat/estrus signs. Known quantity of ration as TMR was offered twice a day at 9.00-9.30 h and 16.00-16.30 h after tying them at respective places. Heifers were also offered green hybrid Napier @ 2 and 4 kg/d up to 100 kg and above 100 kg b.wt., respectively, and chelated mineral mixture @ 35 g/d. The protein requirement was met as per the ICAR (2013) feeding standard. Next day the heifers were offered measured extra wheat straw for rumen fill if no leftover was observed. Wholesome drinking water was provided to the animals 3-4 times a day.

Representative samples of individual feed ingredients, concentrate mixtures and TMR offered were analyzed for proximate principles as per AOAC (2000), which revealed very high CP (43-44%) and low CF (2-9%) in the rice DDGs and Soy DOC over other feeds. Moreover, the concentrate containing 40% RDDGS had 3-fold higher ether extract and 15% lower CF than the feed containing 40% Soy DOC. The proximate composition of six TMR used has been reported earlier (Dhami *et al*., 2023).

The general health and behaviour of all animals were observed regularly. Blood samples were collected by Jugular vein-puncture from all heifers at monthly intervals in clot activator vials. The serum separated out by centrifugation of clotted samples was stored at -20°C until analyzed for various biochemical profiles, *viz.,* glucose, cholesterol, total protein, albumin, globulin, urea, and minerals calcium-phosphorus on chemistry analyzer (Mindray BS-120) using standard procedures and assay kits procured from Coral Clinical System and/or Tulip Diagnostic (p) Ltd, India. The data were analyzed using factorial RBD, Duncan's NMRT and CD tests on SPSS software (Snedecor and Cochran, 2002).

Results and Discussion

The findings on serum biochemical profiles are presented in Tables 1, 2 and Figure 1. Period, Treatment, Roughage and Period x Treatment, Period x Roughage effects were highly significant at P<0.01 on most of these parameters.

The monthly mean blood glucose (BG) concentrations in heifers under different treatment regimens varied significantly (p<0.01) between 8 and 19 months of age, being lower initially and showed a sudden drop around 12 months of age probably due to severe winter season fell during that time requiring more feed intake, but it was offered in fixed quantity (Table 1). Further, the effect of concentrate treatment, irrespective of roughage source, was also significant (p<0.05) on this trait, BG being lowest in T1 and highest in T2, however roughage source and all interactions did not influence the BG concentration (Table 2, Fig. 1). The observed serum glucose was within the reference range as depicted in Merck's Veterinary Manual (41.4-73.9 mg/dL) by Kahn and Line (2010) and that reported by Garnsworthy *et al*. (2021) for healthy cattle. Significantly (p<0.05) higher serum glucose in T2 group heifers might be because of balanced consumption of soy DOC and rice DDGS providing needed fat and yeast. Our findings also concurred well with those reported by Mahima *et al*. (2013) and Mayengbam (2014) in Hariana and Frieswal cattle.

Serum total cholesterol (TC) concentrations varied significantly (p<0.05) between periods/age, treatment, roughage, and treatment x roughage and treatment x period interactions. The TC levels were higher at 13th month of age followed by 10th month with lowest on 16th month of age (Table 1). Irrespective of roughage source, the TC concentration was significantly higher in T3 than T2 and T1,

Table 1: Overall monthly mean (±SE) plasma biochemical profile of experimental HF x K heifers from 8 months to 19 months of age

Parameters	Age in months												
	8.0	9.0	10.0	11.0	12.0	13.0	14.0	15.0	16.0	17.0	18.0	19.0	Overall
Blood glucose (mg/dL)	46.33 ±1.69	48.59 ±1.58	55.28 ±1.70	48.08 ±1.43	38.80 ±1.69	49.92 ±1.84	61.79 ±1.16	59.41 ±1.73	58.53 ±1.67	64.69 ±1.35	60.81 ±1.55	61.56 ±1.51	54.63 ±0.79
Total cholesterol (mg/dL)	108.77 ±4.31	109.92 ±4.36	117.06 ±5.21	93.24 ±3.82	102.67 ±3.88	124.09 ±4.82	87.84 ±3.44	90.63 ±3.53	82.00 ±3.23	95.55 ±5.66	90.78 ±2.79	96.65 ±6.37	100.60 ±0.83
Total protein (g/dL)	6.07 ±0.09	5.95 ±0.06	5.87 ±0.06	5.97 ±0.07	6.20 ±0.08	6.17 ±0.11	7.45 ±0.17	8.47 ±0.26	8.29 ±0.26	8.27 ±0.26	8.39 ±0.20	8.50 ±0.20	7.13 ±0.05
Albumin (g/dL)	3.31 ±0.03	3.32 ±0.02	2.81 ±0.04	2.65 ±0.03	2.91 ±0.05	3.10 ±0.04	4.11 ±0.06	4.45 ±0.05	4.56 ±0.04	4.35 ±0.09	4.53 ±0.06	4.61 ±0.06	3.73 ±0.03
Globulin (g/dL)	2.76 ±0.07	2.63 ±0.05	3.06 ±0.06	3.32 ±0.07	3.30 ±0.09	3.07 ±0.12	3.33 ±0.18	4.02 ±0.28	3.73 ±0.25	3.93 ±0.27	3.86 ±0.21	3.89 ±0.20	3.41 ±0.03
A:G ratio (AGR)	1.22 ±0.03	1.27 ±0.03	0.93 ±0.03	0.80 ±0.02	0.90 ±0.04	1.09 ±0.11	1.35 ±0.10	1.29 ±0.13	1.35 ±0.09	1.28 ±0.13	1.25 ±0.07	1.25 ±0.07	1.17 ±0.01
BUN (mg/dL)	34.48 ±1.73	41.03 ±1.79	44.69 ±2.33	44.17 ±2.15	47.75 ±2.43	43.92 ±1.75	41.93 ±1.19	48.59 ±1.59	49.29 ±1.48	52.73 ±1.44	44.52 ±1.93	43.86 ±1.57	44.67 ±0.86
Calcium (mg/dL)	10.62 ±0.19	11.79 ±0.24	9.35 ±0.21	10.34 ±0.24	10.98 ±0.35	10.08 ±0.27	12.00 ±0.28	11.98 ±0.37	11.71 ±0.27	12.03 ±0.33	12.19 ±0.37	12.44 ±0.28	11.29 ±0.07
Phosphorus (mg/dL)	9.16 ±0.13	8.71 ±0.08	8.66 ±0.10	8.99 ±0.13	8.98 ±0.23	9.22 ±0.13	8.35 ±0.20	7.38 ±0.23	7.24 ±0.16	8.04 ±0.22	7.57 ±0.20	8.68 ±0.24	8.42 ±0.04

Period effect was highly significant at $p<0.01$ on most of these parameters.

Table 2: Average serum biochemical profile of crossbred heifers fed soy DOC, rice DDGS and legume, non-legume straws from 8 months to 19 months of age

Particulars	Group						Overall	
	T1		T2		T3		R1	R2
	R1	R2	R1	R2	R1	R2		
Blood glucose (mg/dL)	52.81 ±1.61	53.33 ±1.59	56.47 ±1.74	55.33 ±1.49	53.37 ±1.67	56.41 ±1.34	54.22 ±1.08	55.02 ±0.98
	53.07^{B} ±1.19		**55.90^{A} ±1.17**		**54.89^{AB} ±1.09**		**54.63 ±0.79**	
Total cholesterol (mg/dL)	90.87 ±3.49	103.04 ±2.47	84.04 ±2.67	103.15 ±3.95	114.90 ±3.56	107.61 ±3.28	96.61^{Y} ±1.08	104.59^{X} ±1.12
	96.95^{B} ±1.58		**93.59^{B} ±1.71**		**111.26^{A} ±1.88**		**100.60 ±0.83**	
Total protein (g/dL)	7.18 ±0.31	7.04 ±0.20	7.29 ±0.23	7.10 ±0.19	7.19 ±0.21	7.00 ±0.17	7.22 ±0.09	7.05 ±0.08
	7.11 ±0.18		**7.20 ±0.13**		**7.09 ±0.12**		**7.13 ±0.05**	
Albumin (g/dL)	3.75 ±0.11	3.78 ±012	3.63 ±0.11	3.74 ±0.11	3.66 ±0.10	3.79 ±0.12	3.68^{Y} ±0.06	3.77^{X} ±0.05
	3.77^{A} ±0.08		**3.69^{B} ±0.07**		**3.72^{AB} ±0.09**		**3.73 ±0.03**	
Globulin (g/dL)	3.43 ±0.15	3.25 ±0.12	3.67 ±0.17	3.36 ±0.14	3.53 ±0.14	3.21 ±0.11	3.54^{X} ±0.07	3.27^{Y} ±0.06
	3.34 ±0.09		**3.51 ±0.10**		**3.37 ±0.08**		**3.41 ±0.03**	
A:G ratio (AGR)	1.19 ±0.08	1.21 ±0.05	1.09 ±0.07	1.18 ±0.05	1.08 ±0.04	1.17 ±0.03	1.12^{Y} ±0.03	1.21^{X} ±0.02
	1.20 ±0.04		**1.14 ±0.03**		**1.16 ±0.02**		**1.17 ±0.01**	
BUN (mg/dL)	46.38 ±1.76	52.73 ±1.89	43.21 ±1.75	46.16 ±1.56	41.67 ±1.58	39.92 ±1.41	43.42^{Y} ±0.98	45.94^{X} ±1.08
	49.54^{A} ±1.08		**44.68^{B} ±1.12**		**40.81^{C} ±1.18**		**44.67 ±0.86**	
Calcium (mg/dL)	11.58 ±0.29	11.57 ±0.23	11.24 ±0.24	11.09 ±0.22	11.11 ±0.23	11.16 ±0.24	11.33 ±0.09	11.27 ±0.11
	11.58^{A} ±0.17		**11.16^{B} ±0.13**		**11.13^{B} ±0.16**		**11.29 ±0.07**	
Phosphorus (mg/dL)	8.41 ±0.16	8.43 ±0.15	8.48 ±0.13	8.39 ±0.18	8.38 ±0.17	8.41 ±0.16	8.42 ±0.07	8.41 ±0.06
	8.42 ±0.09		**8.44 ±0.10**		**8.39 ±0.10**		**8.42±0.04**	

Means with different superscripts (A, B) within the row differ significantly for concentrate and (X,Y) differ significantly for roughage source ($p<0.05$).

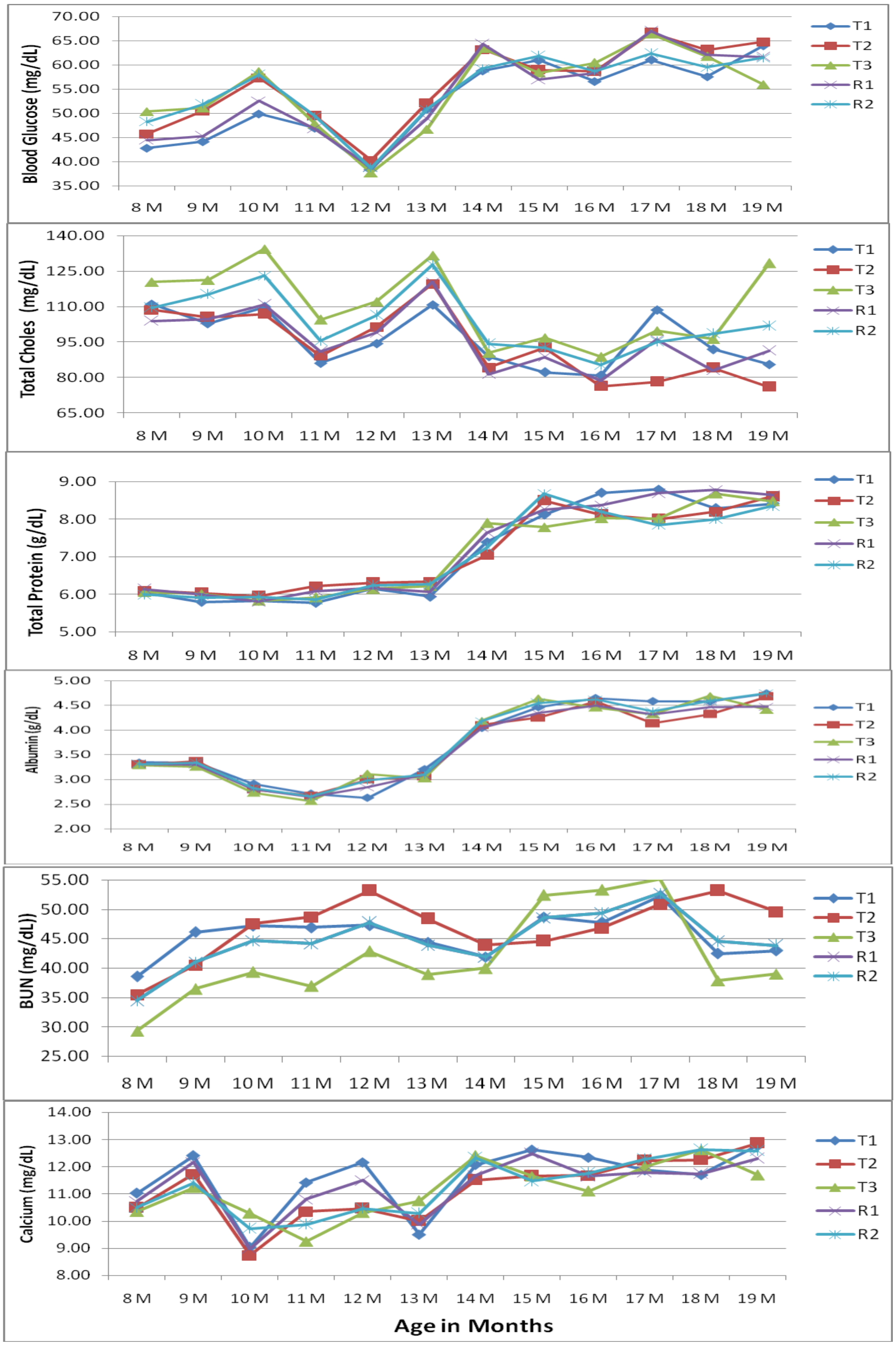

Fig. 1: Overall effect of Concentrate (T) and Roughage (R) on various blood biochemical parameters over the period of experiment in CB heifers

and also it was higher in R2 group heifers that were offered a mixture of wheat straw and groundnut straw than R1 group offered only wheat straw (Table 2, Fig. 1). The lowest serum TC concentration noted around 16 months of age in heifers may be associated with increased steroidogenesis and its utilization in the gonads. The observed average serum cholesterol (mg/dL) of experimental heifers was within the normal range (71-156 mg/dL) as depicted in Merck's Veterinary Manual for cows by Kahn and Line (2010). Serum cholesterol increased significantly ($p<0.05$) when soy DOC was replaced with RDDGS in concentrate. This might be possible because of the higher fat content in RDDGS; however, serum cholesterol remained statistically the same for soy DOC and half of the soy DOC replaced with RDDGS group of concentrate. Similar to the present study, Ranathunga *et al*. (2018) found greater plasma total cholesterol concentration in cows fed DDGS in the diet compared with cows fed no DDGS ($p=0.01$; 151 *vs.* 132 mg/ dL). However, contrary to the significant increase in serum cholesterol in R2 group heifers, Shelke *et al*. (2015) found a non-significant change in serum cholesterol (69.18 *vs*. 68.64 mg/dL) on feeding leguminous straw in cows.

Serum total protein levels showed a gradual and significant ($p<0.05$) increase from 8 months to 15 months of age and then remained more or less static, while the effects of treatment and roughage source were non-significant, though the values were higher in T2 and R1 groups (Table 1). Serum total protein increased when soya DOC was replaced with DDGS, although the increase was non-linear. Serum total protein did not change significantly in R2 group heifers that were offered wheat straw and groundnut straw mixture. The serum total protein values of heifers were close to the lower limit of reference range (6.2-8.2 g/dL) depicted in Merck's Veterinary Manual (Kahn and Line, 2010). Increase in serum total protein in heifers on feeding RDDGS might be observed because of higher protein digestibility (Dey *et al*., 2020).

Serum albumin levels dropped significantly ($P<0.05$) around 11 months of age (*i.e.,* at 4^{th} month of feeding trial) compared to initial values and then increased by 15-16 month and then remained static. Similarly, irrespective of roughage source, albumin levels were significantly higher in T1, at par with T3, and lower in T2, and so also it was higher in group fed a mixture of wheat straw and groundnut gotar (R2), with inverse findings on the globulin levels (Table 2, Fig. 1). The observed serum albumin in crossbred heifers was within the normal range as mentioned in Merck's Veterinary Manual by Kahn and Line (2010) for cows, *i.e*., 2.5-3.8 g/dL. Dey *et al*. (2020) in crossbred calves and Garnsworthy *et al*. (2021) in dairy cows reported lower serum albumin.

The seum A-G ratio was around 1.25:1 initially, which dropped significantly within 2 months of feeding trial in all groups, remained low till 6^{th} month of trial (13^{th} month of age) and then again spiked-up to normal initial values till the end of experiment. This was probably due to reduced albumin and increased globulin concentrations during 3^{rd} to 6^{th} month of trial, perhaps for adaptive mechanism to RDDGS solvent, and A-G ratio was significantly higher in R2 than R1 roughage, though the concentrate treatment with Soy DOC or DDGS did not influence it (Table 2). Mahima *et al.* (2013) and Mayengbam (2014) also recorded similar values in Hariana and Frieswal cattle.

The BUN concentration varied significantly ($p<0.01$) between periods, treatments, roughages, as well as their all interactions. The value increased significantly from 8^{th} to 10^{th} to 12^{th} month of age, dropped around 13^{th}-14^{th} month of age, again reached to peak at 17^{th} month of age, and then dropped in next 2 months of the study (Table 1). Irrespective of roughage source, it was significantly lower in T2 and lowest in T3, and irrespective of concentrate treatment, it was significantly higher in R2 than R1 (Table 2, Fig. 1). Serum urea level of heifers reduced significantly ($p<0.05$) and linearly on replacing soya DOC with RDDGS. Feeding a mixture of groundnut straw and cereal straw also resulted in a significantly ($p<0.05$) higher serum urea in R2 group heifers. The observed serum urea of experimental heifers was higher than the reference interval (7.8-25.0 mg/dL) suggested by Kahn and Line (2010). Similar to present findings, Yan *et al.* (2010) in Chinese Holstein cows found linear decrease in blood urea and it decreased significantly ($p<0.05$) when DDGS was included at the rate of 34.1% of diet DM. Furthermore, Dey *et al*. (2020) found a numerical decrease in blood urea in crossbred calves on feeding RDDGS against soybean meal. Reduction in BUN was significant in dairy cows when wheat DDGS was included in ration @ >160 g/kg diet DM (Garnsworthy *et al*., 2021). A significant increase in serum urea on feeding a mixture of wheat straw and groundnut straw to heifers observed in R2 group, paralleled the finding of Shelke *et al*. (2015), who observed significantly higher blood urea in soybean straw fed group over Jowar straw fed group. Serum urea level reduced significantly ($p<0.05$) and linearly on replacing soy DOC with RDDGS, might be due to higher rumen un-degradable protein in RDDGS.

Serum calcium concentration increased significantly ($p<0.05$) from 14^{th} month of age and remained more or less at the same level compared to earlier age, with increasing trend of plasma inorganic phosphorus levels (Table 1). Further, the calcium was significantly higher in concentrate treatment T1 than in T2 and T3, irrespective of roughage source, while roughage source did not influence calcium levels significantly, and so also the inorganic phosphorus concentrations remained unaffected with concentrate or roughage source, irrespective of counter component (Table 2, Fig. 1). The present observations on mean values of various biochermical profiles concurred well with those reported by Mahima *et al.* (2013) and Mayengbam (2014) in Hariana and Frieswal cattle.

Not only the serum biochemical profile, but significantly enhanced growth rate, ovarian dynamics and age at puberty were also observed with significant influence on some of the hormonal and haematological parameters with these treatments in the said HFxK heifers (Dhami *et al.,* 2021, 2023), proving that RDDGS is a very good nutritional alternate resource with legume-nonlegume mixed straw in raising growing crossbred heifers.

Conclusions

From the findings of the present study, it can be concluded that rice DDGS in place of soy DOC in concentrate and a mixture of legume and cereal straw (50:50) can be fed to growing crossbred heifers without any deleterious effect on kidney and liver functions, thereby the health and serum biochemical profile.

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