



Feeding Rice Distillers Grain to Dairy Heifers

Pandey et al.

Effect of Feeding Rice Distillers Dried Grains and Mixture of Non-legume and Legume Straw on Hemato-Biochemical and Mineral Profile of Growing Dairy Heifers

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ABSTRACT

Under scarcity of livestock feeds and fodder, an investigation on alternate, conventional and non-conventional feed resources is essential. The present study was planned on crossbred heifers (HF×Kankrej) with average body weight of 116.1±4.74 kg for twelve fortnights to study the effect of feeding rice DDGS and a combination of straw (wheat + groundnut) on their hemato-biochemical and mineral profile. Blood samples for hemato-biochemical and mineral profile analysis were collected from heifers at the start of the experiment and thereafter at every fortnightly interval from the jugular vein in the sterilized blood collecting tubes (with and without anticoagulant). Auto-hematology-analyzer Mindray (BC-2800Vet) and Mindray BS-120 chemistry analyzer were used for hematology and serum biochemistry analysis, respectively. The RBC counts, Hb, PCV%, MCHC, Platelet count, MPV, WBCs and Granulocytes changed significantly ($P<0.05$) on feeding DDGS though their values were within normal physiological range. On feeding mixture of straw, platelets count and granulocytes changed significantly ($P<0.05$) but their values were also within normal physiological range. On inclusion of Rice DDGS, serum total protein, urea and phosphorus and on feeding mixture of straw serum glucose, albumin, urea and phosphorus values differ significantly ($P<0.05$). The serum total protein, glucose, albumin and urea values were within normal physiological interval on feeding DDGS or mixture of straw except phosphorus values (8.77-9.33 mg/dL) which was higher than normal range in all treatment groups irrespective of treatment. The results showed that rice DDGS in concentrate up to 40% and feeding a mixture of groundnut straw and cereal straw (1:1) did not adversely affect the hematology, blood biochemical parameters and mineral profile, all parameters were within normal physiological values except serum phosphorus which was higher than the normal physiological values.

KEYWORDS: Biochemical parameters, Groundnut straw, Hematology, Mineral profile, Rice DDGS

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INTRODUCTION

For sustainable livestock farming under scarcity of feed resources, an investigation on alternate, conventional and nonconventional feed resources is essential. Moreover, alternate feed resources must also be safe, when included in animal's diet and do not cause any harm to their normal body metabolism. Rice distillers dried grains (RDDGS) is a main by-product of rice grain based alcohol production plants which uses damaged poor grade rice grains for fermentation. Crude protein in RDDGS is in the

range of 40-45% protein and can substitute costly soybean meal (Yogi et al., 2017). Availability of DDGS from grains will increase in near future as the government is promoting the use of ethanol in blending with gasoline. The availability of molasses is limited hence, a large number of cereal-grain based ethanol production plants will be started (Sakthivel et al., 2018). Further, large numbers of dairy animals in India have mainly reared through feeding cereal straws based diet. The nutritive value and palatability of cereal straws are poor. India produces a notable

amount of pulses and groundnut around 30 MT (GOI, 2019). Legume straw contains higher crude protein than cereal straw and is more palatable.

As limited literature is available on long-term effect of feeding rice DDGS and mixture of non-legume and legume straw, it is essential to evaluate in detail their effect on hematology, blood biochemical and mineral profile. Hematology, biochemical and mineral profiling play a decisive role in the finding the general health status of livestock.

MATERIALS AND METHODS

Location of the experiment

The investigation was carried out at Livestock Research Station, College of Veterinary Science and Animal Husbandry, Anand Agricultural University(AAU), Anand, Gujarat.

Experimental animals and duration

The study was conducted on crossbred heifers

(HF×Kankrej) with 116.1 ± 4.74 kg and 281.9 ± 13.26 days, average body weight and average age, respectively (Table 1). Prior permission was taken from CPCSEA through the Institutional Animal Ethics Committee (IAEC) to perform the experiment (Project No: 313/LPM/2019). The experiment was started on June 24th and ended on December 9th of 2020 (a total of 12 fortnights).

Experimental treatments

Crossbred heifers were uniformly distributed on body weight basis in to six experimental groups with four heifers in each group. Three different concentrates-I, II and III containing 0, 20 and 40% rice DDGS (RDDGS) were offered to T1, T2 and T3 group heifers, respectively. Under individual concentrate type, four heifers were offered only wheat straw (R1 group) and other four heifers, a mixture of straw (wheat and groundnut in 1:1) as dry roughage (R2 group) (Table 2). The ingredients of different concentrate are presented in Table 3.

Table 1. Effect of energy and protein levels on dry matter intake (DMI) and body weight (BW) gain of buffalo calves

Groups	No. of heifers	Characters at beginning of a trial		
		Age (days)	Body Weight (kg)	
T1	R1	4	247.7 ± 46.2	115.8 ± 15.5
	R2	4	286.2 ± 16.1	116.3 ± 12.5
T2	R1	4	257.7 ± 32.3	116.0 ± 12.2
	R2	4	315.0 ± 22.8	116.5 ± 12.0
T3	R1	4	289.5 ± 47.1	116.0 ± 12.5
	R2	4	295.2 ± 29.4	116.0 ± 13.5
Total	24		281.9 ± 13.2	116.1 ± 4.7
Average \pm Standard Error				

T1: Group fed concentrate-I containing no RDDGS, T2: Group fed concentrate-II with 20% RDDGS, T3: Group fed concentrate-III containing 40% RDDGS, R1: Wheat straw fed group, R2: Mixture of straw fed group.

Table 2. Concentrate mixture and straw offered to crossbred heifers

Treatment Groups	No. of heifers	Type of Concentrate (50%)	Ingredients in TMR	
			Wheat Straw	Groundnut straw
T1	R1	I	50%	0%
	R2		25%	25%
T2	R1	II	50%	0%
	R2		25%	25%
T3	R1	III	50%	0%
	R2		25%	25%

*T1: Group fed concentrate-I containing no RDDGS, T2: Group fed concentrate-II with 20% RDDGS, T3: Group fed concentrate-III containing 40% RDDGS, R1: Wheat straw fed group, R2: Mixture of straw fed group

Table 3. Composition of different concentrate types

Concentrate Type	Ingredients (%)		
	Soya DOC	RDDGS	Amul Dan
I	40	0	60
II	20	20	60
III	0	40	60

*SsoyaDOC – soybean de-oiled cake, RDDGS- Rice DDGS and Amul Dan is concentrate mix sold by AMUL, Anand (Gujarat).

Experimental heifers were fed with concentrate and roughage in the form of total mixed ration (TMR). Details of proximate analysis of representative samples of feed ingredient, feeds and fodder offered are provided in Tables 4 and 5 was

done as per AOAC (2000). Rice DDGS used was procured from rice grain based distillery plant situated at Daman (India). The RDDGS manufacturing process is presented in Figure 1.

Table 4. Proximate composition (% DM basis) of various feed ingredients used in the experiment

Particulars	Rice DDGS	Soya DOC	Amul Dan	Groundnut Straw	Hybrid Napier	Wheat Straw
OM	95.1	87.6	86.2	90.7	80.1	87.5
CP	44.6	43.1	18.8	10.6	10.6	2.63
EE	7.27	0.15	2.79	2.24	2.32	3.33
CF	2.04	8.93	16.2	30.3	28.1	39.2
NFE	41.2	35.3	48.4	47.5	39.0	42.3
Ash	4.82	12.4	13.7	9.21	19.8	12.4

* CP-Crude protein, EE –Ether extract, CF- Crude fiber, NFE- Nitrogen Free Extract and OM -organic matter.

Table 5. Proximate composition (% DM basis) of concentrate mixture with RDDGS

Particulars	Concentrate -I (0% RDDGS)	Concentrate-II (20% RDDGS)	Concentrate -III (40% RDDGS)
CP	28.33	28.91	29.17
CF	12.66	11.36	10.48
EE	1.70	3.05	4.42
Ash	13.42	11.82	10.23
NFE	43.89	44.86	45.70
OM	86.58	88.18	89.77

* CP-Crude protein, CF- Crude fiber, EE –Ether extract, NFE- Nitrogen Free Extract and OM -organic matter.

**High EE in concentrate II and III is because of high EE content in rice DDGS

General and feeding management

Crossbred heifers were dewormed with Fenbendazole @ 7.5mg/kg BW before starting the research. They were housed in ventilated, hygienic shed under iso-managerial conditions. The crude protein requirements of the experimental heifers were met as per ICAR (2013) feeding standard. The ration was offered twice first after tying them at their respective place between 9:00 to 9:30 hours and then between 16:00 to 16:30 hours. In addition to TMR, heifers were offered fixed 2 kg/head/d and 4 kg/head/d green hybrid Napier up to 100kg and above 100kg BW, respectively and with a preset 35 gm/head/d mineral mixture throughout the experiment. The crude protein requirements of heifers were adjusted at every biweekly interval throughout the experiment as per change in body weight.

Blood collection for hemato-biochemical and mineral analysis

Blood samples for hemato-biochemical and mineral profiles were collected from heifers at the beginning and thereafter every fortnightly interval from the jugular vein in the two sterilized tubes (with and without anticoagulant). Fresh blood in tubes with anticoagulant was used for hematological examination (RBC count ($10^6/\mu\text{l}$), Hb (g/dL), PCV (%), MCV (fL), MCH (pg), MCHC (g/dL), Platelets ($10^3/\mu\text{L}$), Mean Platelets Volume (fL), WBC count ($10^3/\mu\text{L}$), Lymphocytes ($10^3/\mu\text{L}$), Monocytes ($10^3/\mu\text{L}$) and Granulocytes ($10^3/\mu\text{L}$)) immediately after the collection. Other tubes without anticoagulant were allowed to clot at room temperature and serum

was separated by centrifugation. Serum samples were then stored at $-20^\circ\text{R}^\circ\text{C}$ till analysis of various biochemical parameters viz. serum glucose (mg/dL), protein (g/dL), albumin (g/dL), urea (mg/dL), (mg/dL) and mineral profile viz. serum calcium (mg/dL) and phosphorus (mg/dL) using suitable diagnostic kits. Auto-hematology-analyzer Mindray (BC-2800Vet) and Mindray BS-120 chemistry analyzer were used for hematology and serum biochemistry analysis, respectively. The hematology and chemistry analyzer was calibrated before starting analysis of biochemical parameters as well as on changing reagent. The observed hematological, biochemical and mineral profile were then compared with normal physiological values for cattle depicted in Veterinary hematology by Schalm (1961), Clinical Biochemistry of Domestic Animals by Kaneko et al. (2008) and Merck's Veterinary Manual by Kahn and Line (2010) as well as reported values for Indian, exotic and crossbred cattle from different authors.

Statistical Analysis

The trial data were analyzed at Dept. of Agricultural Statistics, BACA, AAU, Anand (Gujarat) using in-house tested software. Design of the experiment was factorial RBD and analyzed as per the statistical method described by Snedecor and Cochran (2002). Comparison was done between different concentrate and between roughage sources. When comparison was done between three different concentrate, roughage source was avoided. When different roughage source was compared, concentrate source was ignored. The difference was considered significant at $P < 0.05$.

Table 6. Normal hematological reference range for cattle depicted in Schalm's Veterinary Hematology and Merck's Veterinary Manual

S.No.	Particulars	Veterinary Hematology by Schalm (1961)	Merck's Veterinary Manual by Kahn and Line (2010)
1	RBC ($10^6/\mu\text{L}$)	5-10	5-10
2	Hemoglobin (g/dL)	8-14	8-15
3	PCV (%)	24-48	24-46
4	MCV (fL)	40-60	40-60
5	MCH (pg)	11-17	11-17
6	MCHC (g/dL)	26-34	30-36
7	WBC ($10^3/\mu\text{L}$)	4-12	4-12
8	Lymphocytes	45-75%	2.5-7.5($10^3/\mu\text{L}$)
9	Monocytes	2-7%	0.025-0.85 ($10^3/\mu\text{L}$)
10	Segmented Neutrophils	15-45%	0.6-4 ($10^3/\mu\text{L}$)
11	Band Neutrophils	0-2%	0-0.12 ($10^3/\mu\text{L}$)
12	Eosinophils	2-20%	0-2.4 ($10^3/\mu\text{L}$)
13	Basophils	0-2%	0-0.2 ($10^3/\mu\text{L}$)
14	Platelets ($10^3/\mu\text{L}$)	-	100-800
15	MPV (fL)	-	3.5-6.5

Table 7. Normal blood biochemical and mineral reference interval in cattle

S. No.	Particulars	Clinical Biochemistry of Domestic Animals (Kaneko et al., 2008)	Merck's Veterinary Manual (Kahn and Line, 2010)
1	Glucose (mg/dL)	45-75 (57.4±6.8)	41.4-73.9
2	Total protein (g/dL)	6.0-7.9 (7.2±0.52)	6.2-8.2
3	Albumin (g/dL)	2.4-3.0 (2.7±0.19)	2.5-3.8
4	Creatinine (mg/dL)	1.2-1.9	0.5-2.2
5	Total cholesterol (mg/dL)	52-76 (64±12)	71-156
6	Urea (mg/dL)	20-30	7.8-25
7	Calcium (mg/dL)	4-7	8.4-11.00
8	Phosphorus (mg/dL)	1.2-3.5	4.3-7.8

RESULTS AND DISCUSSION

The average hematological parameters with standard error are shown in Table 8. The RBC count ($10^6/\mu\text{L}$) in heifers differs significantly ($P<0.05$) due to inclusion of RDDGS in diet, but the change was non-linear and of no practical importance as RBC count was within the normal physiological range $5-10 \times 10^6/\mu\text{L}$ for cattle as depicted in Schalm's Veterinary Hematology and Merck's Veterinary Manual as well as normal range reported by Kraft and Durr (2005). The RBC ($10^6/\mu\text{L}$) also did not get affected by feeding mixture of straw. George et al. (2010) and Wood and Quirzo-Rocha (2010) in healthy cattle reported a lower reference range for

RBC $5.1-7.6 \times 10^6/\mu\text{L}$ and $4.9-7.5 \times 10^6/\mu\text{L}$ in cattle, respectively. The certain amount of variation in present finding and reported value could be because of physiological variability exists in hematologic profiles of cattle (Krimer, 2011). The Hb (g/dL) of experimental heifers changed significantly ($P<0.05$) due to feeding of RDDGS in heifers diet but it also changed in a non-linear way. The Hb (g/dL) was statistically similar between wheat straw and straw mixture fed heifers. The average Hb content in crossbred heifer's blood was within the normal range 8-14 and 8-15 g/dL as depicted in Schalm's Veterinary Hematology and Merck's Veterinary Manual, respectively for healthy cattle. Kraft and Durr (2005), George et al. (2010) and Wood and

Quirzo-Rocha (2010) reported a higher reference range i.e. 9-14, 8.5-12.2 and 8.4-12 g/dL, respectively for healthy bovine. The PCV (%) of heifers also varied significantly on feeding RDDGS but the change was nonlinear in the same way as RBC count. Average PCV (%) of different group heifers remained within the normal reference range as depicted in Schalm's Veterinary Hematology & Merck's Veterinary Manual i.e. 24-48 and 24-46, respectively for healthy cattle. The observed PCV (%) in experimental heifers was also within the normal reference range reported by Kraft and Durr (2005) and George et al. (2010) i.e. 28-38 and 22-33, respectively for healthy bovine. MCH (fL) did not change on feeding RDDGS however it changed significantly ($P < 0.05$) on feeding mixture of straw but the change has no implication as difference was of only 0.45 fL. The observed MCV (fL) was within reference interval for normal MCV (fL) in bovine reported by George et al. (2010) and Wood and Quirzo-Rocha (2010) i.e. 38-50 and 36-50, respectively. However, the MCV (fL) of experimental heifers was below the normal reference range (40-60 fL) depicted in Schalm Veterinary Hematology & Merck's Veterinary Manual for healthy cattle. The variation in the MCV (fL) of experimental heifers and other reported values could be because of physiological variability that exists in hematologic profiles of cattle (Krimer, 2011). The MCH (pg) did not change on feeding RDDGS as well as on feeding straw mixture. Average MCH (pg) of crossbred heifers was close to the lower limit of normal reference interval depicted in Schalm's Veterinary Hematology & Merck's Veterinary Manual and that was reported by Kraft and Durr (2005) for healthy bovines i.e. 11-17. George et al. (2010) and Wood and Quirzo-Rocha (2010) however reported an even higher reference range for MCH (pg) i.e. 14-18 and 14-19, respectively for healthy bovine. The MCHC (g/dL) changed linearly on increasing inclusion of RDDGS as well as on feeding straw mixture. The change in MCHC is of less importance as the observed MCHC (g/dL) of experimental heifers fall within the normal reference range depicted in Schalm Veterinary Hematology

i.e. 26-34. While, a higher normal reference interval is mentioned in Merck Veterinary Manual (30-36). Kraft and Durr (2005), George et al. (2010) and Wood and Quirzo-Rocha (2010) also reported a higher reference range for MCHC (g/dL) in normal healthy bovine i.e. 31-34, 36-39 and 38-43, respectively. Platelets ($10^3/\mu\text{L}$) count of experimental heifers also changed significantly ($P < 0.05$) on feeding RDDGS and feeding mixture of straw but were within normal reference interval as depicted in Merck's Veterinary Manual i.e. 100-800. The observed platelet count of heifers was also within the normal reference range reported by Kraft and Durr (2005), George et al. (2010) and Wood and Quirzo-Rocha (2010) i.e. 300-800, 193-637 and 160-650 for healthy cattle, respectively. The MPV (fL) changed statistically ($P < 0.05$) on feeding RDDGS but did not change on feeding straw mixture. The observed MPV in heifers was within the normal reference range depicted in Merck's Veterinary Manual for cattle (3.5-6.5 fL) and that was reported by George et al. (2010), Wood and Quirzo-Rocha (2010) for healthy bovine i.e. 4.5-7.5 and 4.6-7.4 fL, respectively.

WBC ($10^3/\mu\text{L}$) changed significantly ($P < 0.05$) when RDDGS was included in the diet but was statistically similar on feeding straw mixture. The observed average WBC of experimental heifers was within the normal reference range depicted in Schalm Veterinary Hematology and Merck's Veterinary Manual i.e. $4-12 \times 10^3/\mu\text{L}$ for cattle. The observed WBCs in experimental heifers were also within the normal reference range reported by George et al. (2010) and Wood and Quirzo-Rocha (2010) i.e. 4.9-12 and 5.1-13.3, respectively. Aggarwal et al. (2016) also reported similar WBC count during hot humid conditions in growing Karan-fries cattle ($11.13 \times 10^3/\mu\text{L}$) managed under standard farm feeding regime. The lymphocytes ($10^3/\mu\text{L}$) in heifers remained statistically same on feeding RDDGS and mixture of straw. The lymphocyte (%) of total WBC) of heifers was within the normal reference range depicted in Schalm's Veterinary Hematology i.e. 45-75%. Average lymphocytes ($10^3/\mu\text{L}$) of crossbred heifers were also within the normal

range as depicted in Merck's Veterinary Manual and reported by Wood and Quirzo-Rocha (2010) for healthy cattle i.e. 2.5-7.5 and 1.8-8.1, respectively. Mayengbam (2014) in healthy Frieswal cattle reported similar lymphocytes count ($6.31 \times 10^3/\mu\text{L}$ during summer; $6.90 \times 10^3/\mu\text{L}$ during winter) as observed in present study. Kraft and Durr (2005) and George et al. (2010) reported lower normal reference interval for cattle i.e. 2.5-5.5 and 1.5-5.6, respectively. The monocytes ($10^3/\mu\text{L}$) did not change on the inclusion of RDDGS in the diet of heifers however it differs between heifers that were offered wheat straw and mixture of straw. This variation in monocytes ($10^3/\mu\text{L}$) could be because of a certain amount of physiological variability in hematologic profiles that exist in cattle. Average % monocyte of experimental heifers was close to the upper limit of the reference range depicted in Schalm's Veterinary Hematology i.e. 2-7% and

average monocytes count ($10^3/\mu\text{l}$) was within the reference range as mentioned in Merck's Veterinary Manual for healthy cattle i.e. 0.025-0.85. The observed monocytes count ($10^3/\mu\text{l}$) in crossbred heifers was also near to the upper limit of normal reference range reported by George et al. (2010) for healthy cattle i.e. 0-0.8 $\times 10^3/\mu\text{L}$. Mayengbam (2014) in Frieswal heifers during summer reported a similar monocytes count ($0.76 \times 10^3/\mu\text{l}$) as found in this study. The granulocytes ($10^3/\mu\text{l}$) changed significantly ($P < 0.05$) on feeding RDDGS but the change was non-linear. The granulocytes ($10^3/\mu\text{l}$) did not get affected statistically by feeding straw mixture. The granulocytes count ($10^3/\mu\text{L}$) in crossbred heifers was within the reference range reported by Kraft and Durr (2005), George et al. (2020), and Wood and Quirzo-Rocha (2010) for healthy bovine i.e. 1.3-5.3, 1.8-7.5, 0.6-6.72 and 1.8-7.6, respectively.

Table 8. Hematological profile of crossbred heifers

Particulars	Group						R1	R2
	T1		T2		T3			
	R1	R2	R1	R2	R1	R2		
RBC ($10^6/\mu\text{L}$)	7.86 ± 0.07	7.95 ± 0.15	8.03 ± 0.08	8.32 ± 0.13	8.18 ± 0.07	7.43 ± 0.07	8.02 ± 0.04	7.90 ± 0.07
CD	7.91 $\pm 0.08^A$		8.17 $\pm 0.08^B$		7.80 $\pm 0.06^A$		NS	
Hb (g/dL)	8.30 ± 0.10	8.24 ± 0.12	8.62 ± 0.09	8.49 ± 0.12	8.19 ± 0.09	7.99 ± 0.09	8.37 ± 0.05	8.24 ± 0.07
CD	8.27 $\pm 0.08^B$		8.56 $\pm 0.07^C$		8.09 $\pm 0.06^A$		NS	
PCV (%)	29.9 ± 0.28	30.0 ± 0.40	31.5 ± 0.34	31.1 ± 0.46	29.7 ± 0.27	29.5 ± 0.22	30.4 ± 0.18	30.2 ± 0.22
CD	29.98 $\pm 0.24^A$		31.37 $\pm 0.28^B$		29.64 $\pm 0.17^A$		NS	
MCV (fL)	38.3 ± 0.51	38.1 ± 0.52	39.4 ± 0.40	37.6 ± 0.36	36.5 ± 0.38	39.9 ± 0.34	38.1 ^a ± 0.27	38.5 ^b ± 0.25
CD	38.25 ± 0.36		38.53 ± 0.28		38.21 ± 0.30		0.445	
MCH (pg)	10.5 ± 0.14	10.4 ± 0.13	10.7 ± 0.11	10.1 ± 0.09	9.97 ± 0.11	10.7 ± 0.09	10.4 ± 0.07	10.4 ± 0.06
CD	10.47 ± 0.10		10.46 ± 0.08		10.35 ± 0.08		NS	
MCHC (g/dL)	27.7 ± 0.34	27.3 ± 0.33	27.3 ± 0.37	27.3 ± 0.34	27.5 ± 0.37	27.0 ± 0.33	27.5 ^b ± 0.21	27.2 ^a ± 0.19
CD	27.56 $\pm 0.24^B$		27.35 $\pm 0.25^A$		27.33 $\pm 0.25^A$		0.162	
Platelets ($10^3/\mu\text{l}$)	589.1 ± 31.78	625.6 ± 21.68	558.5 ± 22.03	557.6 ± 26.54	541.2 ± 29.20	654.1 ± 24.65	562.9 ^a ± 1 6.12	612.4 ^b ± 1 4.35
CD	607.3 $\pm 19.22^B$		558.0 $\pm 17.16^A$		597.7 $\pm 19.81^{AB}$		33.405	
MPV (fL)	4.77 ± 0.08	4.82 ± 0.07	4.65 ± 0.06	4.60 ± 0.06	4.56 ± 0.08	4.65 ± 0.08	4.66 ± 0.04	4.69 ± 0.04
CD	4.80 $\pm 0.05^B$		4.63 $\pm 0.04^A$		4.60 $\pm 0.06^A$		NS	
WBC ($10^3/\mu\text{L}$)	10.5 ± 0.36	12.1 ± 0.43	11.0 ± 0.31	10.0 ± 0.31	12.0 ± 0.33	10.8 ± 0.2 3	11.24 ± 0.20	11.02 ± 0.20
CD	11.3 $\pm 0.03^B$		10.5 $\pm 0.02^A$		11.4 $\pm 0.02^B$		NS	
Lymphocytes ($10^3/\mu\text{l}$)	6.37 ± 0.27	7.45 ± 0.30	6.93 ± 0.24	6.04 ± 0.19	7.36 ± 0.26	6.45 ± 0.20	6.89 ± 0.15	6.65 ± 0.14
CD	6.91 ± 0.48		6.48 ± 0.49		6.91 ± 0.34		NS	
Monocytes ($10^3/\mu\text{l}$)	0.80 ± 0.06	0.75 ± 0.03	0.82 ± 0.04	0.69 ± 0.04	0.81 ± 0.04	0.72 ± 0.03	0.81 ^b ± 0.03	0.72 ^a ± 0.02
CD	0.78 ± 0.04		0.76 ± 0.03		0.76 ± 0.03		0.063	
Granulocytes ($10^3/\mu\text{l}$)	3.39 ± 0.14	3.96 ± 0.15	3.32 ± 0.15	3.35 ± 0.23	3.92 ± 0.17	3.66 ± 0.10	3.54 ± 0.09	3.66 ± 0.10
CD	3.67 $\pm 0.11^B$		3.34 $\pm 0.14^A$		3.79 $\pm 0.10^B$		NS	

*Superscripts (A, B) and (a, b) in a row indicate significant difference (P<0.05) for concentrate and roughage source, respectively. *CD- Critical Difference

The observed average serum biochemical parameters with standard error are shown in Table 9. Serum glucose of experimental heifers remained statistically similar on feeding RDDGS in place of soya DOC in concentrate. However, offering a mixture of straw resulted in a significant ($P<0.05$) increase serum glucose. Certain amount of variation in serum glucose could occur because of different diet (Ekman, 1976). The observed serum glucose (mg/dL) was within the reference range 45-75 mg/dL and 41.4-73.9 mg/dL as depicted in Clinical Biochemistry of Domestic Animals and Merck's Veterinary Manual, respectively for healthy cattle. Garnsworthy et al. (2021) in their study on dairy cows found similar values. Serum total protein increased significantly ($P<0.05$) when soyaDOC was replaced with DDGS, although the increase was non-linear. Serum total protein did not change significantly in experimental heifers fed straw mixture. The total protein (g/dL) values of experimental heifers were close to the lower limit of normal values depicted in Clinical Biochemistry of Domestic Animals and Merck's Veterinary Manual i.e. 6.0-7.9 and 6.2-8.2, respectively. Contrarily, Garnsworthy et al. (2021) in dairy cows reported lower and Dey et al. (2020) in crossbred calves reported higher serum total protein than the present findings. The serum albumin was statistically similar between heifers fed different concentrates. Serum albumin was found to be significantly ($P<0.05$) higher in heifers fed straw mixture. The observed serum albumin (g/dL) in crossbred heifers was within the normal range as mentioned in Clinical Biochemistry of Domestic Animals and Merck's Veterinary Manual for cow i.e. 2.4-3.0 and 2.5-3.8, respectively. Dey et al. (2020) in crossbred calves and Garnsworthy et al. (2021) in dairy cows reported lower serum albumin. Serum urea level of heifers reduced significantly ($P<0.05$) and linearly on replacing soyaDOC with RDDGS. Feeding mixture of groundnut 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 suggested in

Clinical Biochemistry of Domestic Animals and Merck's Veterinary Manual for cows i.e. 20-30 and 7.8-25 mg/dL, respectively. Similar to the present findings, Yan et al. (2010) in Chinese Holstein cows found a 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) in crossbred calves found a numerical decrease in blood urea on feeding RDDGS against soybean meal. Reduction in blood urea nitrogen was significant ($P<0.05$) in dairy cows when wheat DDGS was included at the rate of more than 160 g/kg diet DM (Garnsworthy et al., 2021). Feeding mixture straw to heifers resulted in significantly ($P<0.05$) higher serum urea, the parallel finding was found by Shelke et al. (2015), who observed significantly higher blood urea in soybean straw fed group over Jowar straw fed group.

Observed average serum calcium and phosphorus of heifers with standard error are shown in Table 9. Serum calcium did not get affected on feeding RDDGS and a mixture of straw. The calcium level of experimental heifers was within the normal reference interval 8.4-11.00 mg/dL depicted in Merck's Veterinary Manual. However, lower normal reference range 4-7 mg/dL is depicted in Clinical Biochemistry of Domestic Animals for healthy cattle. Serum calcium levels did not change on feeding RDDGS; a similar finding was reported by Stewart et al. (2017) in heifers. Serum phosphorus of experimental heifers that were fed RDDGS in concentrate and straw mixture was significantly ($P<0.05$) higher. The observed serum phosphorus higher than the reference range reported by Clinical Biochemistry of Domestic Animals and Merck's Veterinary Manual i.e. 1.2-3.5 and 4.3-7.8 mg/dL, respectively. Similar higher serum phosphorus in growing cattle was reported by Palmer et al. (1930), Bide and Tumbleson (1976) as well as Shah (1990) in healthy crossbred heifers (8.424 ± 0.20). Serum phosphorus was significantly ($P<0.05$) higher in the RDDGS fed group heifers. This could be because of the high phosphorus content in DDGS (Council, U. G., 2012).

Table 9. Biochemical and mineral profile of crossbred heifers

Particulars	Group						R1	R2
	T1		T2		T3			
	R1	R2	R1	R2	R1	R2		
Glucose (mg/dL)	47.3 ±1.20	46.6 ±1.35	47.6 ±1.24	50.7 ±1.37	43.7 ±1.27	51.1 ±1.58	46.2 ^a ±0.72	49.5 ^b ±0.84
CD	47.00±0.09		49.23±0.09		47.43±0.10		1.946	
Total protein (g/dL)	5.92 ±0.06	5.88 ±0.05	6.16 ±0.05	6.18 ±0.06	6.04 ±0.09	6.14 ±0.06	6.04 ±0.04	6.07 ±0.03
CD	5.90±0.04 ^A		6.17±0.04 ^B		6.09±0.05 ^B		NS	
Albumin (g/dL)	3.02 ±0.05	3.04 ±0.04	3.00 ±0.04	3.07 ±0.04	2.98 ±0.04	3.03 ±0.04	3.00 ^a ±0.02	3.04 ^b ±0.02
CD	3.03±0.03		3.03±0.03		3.00±0.03		NS	
Urea (mg/dL)	48.6 ±1.77	61.4 ±1.50	38.9 ±1.07	49.1 ±1.40	36.8 ±0.81	37.8 ±0.76	41.4 ^a ±0.84	49.5 ^b ±1.0 6
CD	55.06±1.31 ^C		44.06 ±1.01 ^B		37.33 ±0.56 ^A		1.882	
Calcium (mg/dL)	10.5 ±0.18	10.6 ±0.17	10.6 ±0.13	10.2 ±0.15	10.3 ±0.17	10.3 ±0.14	10.5 ±0.09	10.4 ±0.09
CD	10.64±0.13		10.42±0.10		10.37±0.11		NS	
Phosphorus (mg/dL)	8.77 ±0.12	8.93 ±0.10	9.01 ±0.10	9.22 ±0.09	8.94 ±0.13	9.33 ±0.11	8.91 ^a ±0.07	9.17 ^b ±0.06
CD	8.85±0.09 ^A		9.12±0.07 ^B		9.15±0.08 ^B		0.148	

*Superscripts (A, B) and (a, b) in a row indicate significant difference (P<0.05) for concentrate and roughage source, respectively. *CD- Critical Difference

CONCLUSIONS

The present study showed that feeding RDDGS at the rate of 40% in concentrate in place soya DOC and feeding a mixture of groundnut straw and wheat straw in 1:1 to crossbred heifers did not adversely affect the blood hematological, biochemical and mineral profile as these were within the normal physiological range. Hence, RDDGS can be included in concentrate at the rate of 40% and can replace high priced soya DOC in the diet of growing heifers and a mixture of wheat straw and groundnut straw in 1:1 is safe included in the diet of heifers.

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REFERENCES

- Aggarwal, A., Singh, V., Khan, F. B., Renuka and Kumar, A. 2016. Haematological and hormonal profile of various breeds of cattle and buffalo under varied seasons and environmental conditions. ICAR-National Dairy Research Institute, Karnal. NDRI Publication No. 146: 47p.
- Association of Analytical Chemists. 2000. Official Methods of Analysis (17th Edn.) Washington, D.C., U.S.A.
- Bide, R. W. and Tumbleson, M. E. 1976. Age related variations in plasma electrolytes of Hereford cattle under range conditions. Comparative

- Biochemistry and Physiology:Part A: Physiology. 54(3): 365-371.
- Dey, D., Sharma, B., Mohammad, A., Mandal, D. K., Bhakat, C., Dutta, T. K. and Chatterjee, A. 2020. Effect of feeding rice distillers dried grain with solubles as major protein source on nutrient digestibility and growth performance of Jersey crossbred calves. *Indian Journal of Animal Research*. 54(4): 446-451.
- Ekman, L. 1976. Variation of some blood biochemical characteristics in cattle, horses and dogs, and causes of such variations. In *Annales de Recherches Vétérinaires*. 7(2), pp. 125-128.
- Garnsworthy, P. C., Marsden, M., Goodman, J. R. and Saunders, N. 2021. Inclusion of wheat dried distillers' grains with solubles from bioethanol plants in diets for dairy cows. *Animals*. 11(1): 70.
- George, J. W., Snipes, J. and Lane, V. M. 2010. Comparison of bovine hematology reference intervals from 1957 to 2006. *Veterinary Clinical Pathology*. 39(2): 138-148.
- Government of India. 2019. *Pocket Book of Agricultural Statistics- 2019*. Ministry of Agriculture & Farmers Welfare, Department of Agriculture, Cooperation & Farmers Welfare, Directorate of Economics & Statistics, GOI, New Delhi.
- ICAR. 2013. *Nutrients Requirements of Cattle and Buffalo*. Official Publication. Nutrients Requirement of Animals. ICAR, New Delhi, India.
- Kahn, C. M. and Line, S. 2010. *The Merck Veterinary Manual*. Whitehouse Station, NJ: Merck. & Co. p 2584-2586.
- Kaneko, J. J., Harvey, J. W. and Bruss, M. L. (Eds.). 2008. *Clinical Biochemistry of Domestic Animals*. Academic press.
- Kraft, W. and Dürr, U. M. 2005. *Clinical Laboratory Diagnostics in Veterinary Medicine (6th Edn.)*. Schattauer, Stuttgart, Germany.
- Krimer, P.M. 2011. Generating and interpreting test results: test validity, quality control, reference values, and basic epidemiology. Latimer KS. Duncan & Prasses: *Veterinary Laboratory Medicine Clinical Pathology*. 5th Edn. Blackwell Publishing. pp.365-382.
- Mayengbam, P. 2014. *Haematological and blood biochemical profile of Frieswal cattle*. Scholar's Press, Germany.
- Palmer, L. S., Cunningham, W. S. and Eckles, C. H. 1930. Normal variations in the inorganic phosphorus of the blood of dairy cattle. *Journal of Dairy Science*. 13(3): 174-195.
- Sakthivel, P., Subramanian, K. A. and Mathai, R. 2018. Indian scenario of ethanol fuel and its utilization in automotive transportation sector. *Resources, Conservation and Recycling*. 132:102-120.
- Schalm, O. W. 1961. *Schalm's Veterinary Hematology*. Lea & Febiger, Philadelphia.
- Shah, S. V. 1990. *Efficiency of Growth and Related Blood Profile in Inter-se Mated Jersey × Kankrej F2 and F3 crossbred calves*. M.V.Sc. Thesis, Gujarat Agricultural University, Anand Campus, Anand.
- Shelke, R. R., Chavan, S. D., Waghmare, S. P. and Kahate, P. A. 2015. Effect of feeding of urea ammoniated soybean straw on blood biochemical parameters of lactating cows. *Asian Journal of Animal Science*. 10(2): 140-145.
- Snedecor, G. W. and Cochran, W. G. 2002. *Statistical methods (7th Edn.)*. The Iowa State University Press, Ames, Iowa, USA.
- Stewart, R. L., Segers, J. R., Hammond K, Cheely, T., Ward, B. and Allen, B. 2017. Using distillers grains in beef cattle diets. *UGA Cooperative Extension Bulletin*. 1482.
- Wood, D. and Quiriz-Rocha, G. F. 2010. Normal hematology in cattle. In: D.J. Weiss & K.J. Wardrop (Eds.). *Schalm vet. Hematology*. ed. 829-835.
- Yan, Z. H., Wang, J. Q., Wang, M., Bu, D. P., Wei, H. Y., Zhou, L. Y., Zohu, Z. F. and Sun, P. 2010. Effect of feeding dried distillers grains

- with solubles on the blood metabolites of chinese holstein cows. *Acta Agriculturae Boreali-Sinica*. 25: 179-183.
- Yogi, R. K., Thakur, S. S., Mohini, M., Singh, S. K. and Malik, T. 2017. *In vitro* evaluation of concentrate mixtures containing incremental level of rice dried distillers grain with solubles replacing oil cakes in concentrate mixture. *Indian Journal of Animal Nutrition*. 34 (2): 163-168.