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Status of Serum Mineral and Biochemical Parameters in Cross bred Cows fed Different Levels of Wet Distillers Grains with Solubles (WDGS)

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ABSTRACT

Serum mineral status and biochemical parameters were studied in crossbred dairy cows fed different levels of WDGS for 120 days. 24 Jersy × Sahiwal crossbred dairy cows were divided into 4 groups and randomly allotted to one of the dietary treatment containing APBN green fodder and paddy straw a s roughage and concentrate mixture (T1, control) or WDGS @ 15 (T2), 25 (T3) and 35 % (T4) of DM requirement. These animals were fed according ICAR (2013) feeding standards. Feeding WDGS did not affect the serum concentrations of Ca, Mg, Na, K, Cl, Fe, Mn and Co among treatments, but lowered serum P ($P<0.01$) from 7.4 to 5.73 mg/dl, serum copper ($P<0.05$) from 75.19 to 64.80 µg/dl and zinc ($P<0.05$) from 83.09 to 64.84 µg/dl was observed with the increase in level of WDGS inclusion from 0 (T1) to 35% (T4). Mean BUN (mg/dl) was significantly ($P<0.01$) lower in T2 compared to other treatments. The serum glucose and total protein did not differ significantly among groups, but significantly ($P<0.01$) increased serum albumin content was seen in T3 (3.72 g/dl). Serum SGOT, ALP and GGT activity was not different significantly among treatments, while serum SGPT activity was significantly ($P<0.01$) lower in T4 (35% WDGS) group compared to other treatments.

HIGHLIGHTS

- Inclusion of wet distillers grains with solubles (WDGS) in the diet of crossbred dairy cows significantly decreased the serum phosphorus, copper and zinc levels.
- WDGS significantly lowered the serum SGPT activity.

Keywords: Crossbred Dairy Cows, WDGS, Serum mineral status, Biochemical parameters

Livestock rearing is an integral component of rural living with cattle breeding and milk production being the important professions in rural India. India with only 2.29% of land area of the world, is maintaining nearly 17.4% of world human population and 10.7% of livestock (more than 510 million heads) creating a huge pressure on land, water and other resources (Roy *et al.,* 2019). There is a net deficit of 61.1% green fodder, 21.9% dry fodder (Datta, 2013) and the estimated annual availability of total concentrate feed is only 61 million tonnes against a demand of 96 million tonnes, indicating a deficit of 36% at national level (Anonymous, 2018). Feed cost accounts for about 65-70% of the total cost of livestock production, particularly in milch animals. So to increase the margin of profit from dairy farming, incorporation of agro-industrial waste in milch cow diets is one of the feeding strategy. One such example is wet distillers grains with solubles (WDGS) with good nutritive potential. Dietary factors change the supplementation levels of mineral requirements by altering absorption of minerals from the digestive tract (Faulkner *et al.,* 2017) and mineral deficiencies hampers performance and metabolism of the body cells,

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change the rumen fermentation, and can influence nutrient digestibility (Pino and Heinrichs, 2016). Consequently, use of blood metabolites in assessing nutritional status of cattle is becoming popular (Ndlovu *et al.,* 2007; Maurya and Singh, 2015). Hence, effect of feeding wet distillers grains with solubles (WDGS) on serum mineral status and serum biochemical parameters was studied.

MATERIALS AND METHODS

The present study was carried out at Department of LFC and Dept. of Animal Nutrition, College of Veterinary Science, Tirupati of Andhra Pradesh state, India for 120 days using twenty four lactating Jersy × Sahiwal crossbred cows (336.87 ± 9.96 kg) in late lactation (183.92 ± 2.38 days) that were divided into four groups of 6 cows each. The study investigated the effect of feeding wet distiller's grains with solubles (WDGS) on serum mineral status and bio chemical parameters. The cows were housed in a pucca shed with the provision for individual feeding and watering. The experimental animals had free access to clean, wholesome drinking water. Stall feeding was practiced throughout the experimental period. The four dietary treatments used in the feeding trial were formulated using Hybrid Napier (APBN-1) green fodder and paddy straw as roughages and concentrate mixture (T1, control) or WDGS @ 15 % (T2), 25% (T3) and 35 % (T4) of DMI. The chemical composition of feeds used for the experiment is presented in Table 1. The experimental diets were fed depending upon the body weight and level of production of animals as per ICAR (2013) recommendations. The WDGS was procured from a local supplier once in a week. Concentrate mixture was prepared by using 40 parts maize, 35.5 parts DORB, 21.5 parts soybean meal, 1 part urea and 2 parts mineral mixture. Paddy straw and chopped green fodder were given in divided lots every day to ensure minimum wastage. Feed samples (concentrate mixture, WDGS, paddy straw and APBN) were analyzed for proximate constituents according to AOAC (2005) methods. For mineral estimation, feed (0.5g) were digested in microwave sample digester (CEM Mars X-press) using 15 ml nitric acid. Digested samples were diluted with double glass distilled water and filtered through No. 1 Whatman filter paper. Calcium in feeds was estimated using atomic absorption spectrophotometer (Perkin Elmer, Avanta- PM-A-6287). Phosphorus was analyzed by AOAC (2005) procedure.

Blood samples were collected and processed from all the animals at 0, 30, 60, 90 and 120 day of experimentation from jugular vein into vacutainer (without anticoagulant) for smooth coagulation. After the clot retracted, the tubes were centrifuged at 3000 rpm for 15 minutes for serum separation. Serum was transferred to storage vials and stored at -20^{o}c for further studies. Serum mineral profile was studied in initial and final serum samples. Serum samples (1 ml) were digested in microwave sample digester (CEM Mars X press) using 15 ml nitric acid. Digested samples were diluted with double glass distilled water and filtered through Whatman filter paper no-1. Macro minerals, Ca and Mg as well as micro minerals Cu, Zn, Fe, Mn and Co were estimated using atomic absorption spectrophotometer (Perkin Elmer, Avanta- PM-A-6287). Serum phosphorus was estimated by ammonium molybdate method (Wang *et al.,* 1983) using kits (Erba test kits manufactured by Transasia bio-medicals ltd). Serum Na, K and Cl were estimated by using electrolyte analyser (Starlyte -3).

Table 1: Chemical composition (%) of feeds used in the experiment

	WDGS	**Concentrate mixture**	**APBN**	**Paddy straw**
Dry matter	28.22	91.55	24.77	90.70
Organic matter	94.75	89.65	89.29	85.59
Crude protein	36.72	21.83	7.31	4.44
Ether extract	8.74	1.64	2.35	1.34
Crude fibre	7.16	11.56	32.38	35.17
Total ash	5.25	10.39	10.70	14.41
Nitrogen free extract	42.12	54.60	47.25	44.63
Calcium	0.32	0.99	1.54	0.28
Phosphorus	0.67	0.43	0.59	0.11

* Mean of four values.

Blood urea nitrogen was estimated at monthly intervals using diagnostic kit (M/s. ERBA Diagnostics Mannheim GmbH) in the initial and final serum samples following enzymatic method as mentioned by Talke and Schubert (1965). Serum samples were analysed for bio chemical parameters at the end of the experiment like glucose by Trinder's method (Trinder, 1969), total proteins by Biuret method (Tietz, 1986), albumin by BCG dye method (Doumas *et al.,* 1972), serum glutamic oxaloacetic transaminase (SGOT) by International Federation of Clinical Chemistry method (IFCC method) (Tietz, 1986), serum glutamic pyruvic transaminase (SGPT) by IFCC method (Bradley *et al.,* 1972), Gamma-glutamyltransferase (GGT) by IFCC method (Kachmar and Moss, 1976) and Alkaline phosphatase (ALP) by Tris carbonate buffer method (Wilkinson *et al.,* 1969) using (Erba test kits manufactured by Transasia bio-medicals Ltd.) standard diagnostic procedures.

Statistical Analysis

The data of the trail was subjected to statistical analysis through software (version 23.0; SPSS, 2015) by applying one-way analysis of variance through generalized linear model and the treatment means were ranked using Duncan's multiple range test with a significance at P<0.05 (Duncan, 1955). All the statistical procedures were done as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Actual intake of different dietary components is given in Table 2. Dry matter intake was not affected by feeding different levels of WDGS. But with the increase in the level of WDGS in the diet crude protein intake was increased significantly (p<0.05). Piccolo *et al.* (2016) and Faccenda *et al.* (2017) also reported increased CP intake with the inclusion of wet brewers grain in the diets of cows. This increase in CP intake was due to the higher level of CP of WDGS.

Serum mineral status

The mineral profile in plasma is a good indicator for assessing the mineral status in animals, in spite of homeostatic mechanism in the system (Mc Dowell, 1992). Critical values of 8.0 mg/dl for calcium, 4.5 mg/dl for phosphorus, 1.0 mg/dl for magnesium, 80 µg/dl for zinc, 65 µg/dl for copper, 100 µg/dl for iron and 20 µg/ml for manganese in serum were given by McDowell, 1985. Critical level of cobalt in serum is 2 µg/dl (Radostits *et al.*, 1999).

Serum macro and micro mineral concentration of different treatments with varying levels of WDGS feeding during the trial is presented in Table 3 and 4, respectively. Serum concentrations of different macro and micro minerals at the beginning of the study did not differ between groups. At the end of the study, serum Ca, Mg, Na, K, Cl, Fe, Mn, and Co concentrations did not differ between treatments, and the values were higher than the critical values reported (McDowell, 1985 and Radostits *et al.*, 1999). In accordance with the results of the present study Belibasakis and Tsirgogianni (1996) reported no change in serum calcium, magnesium, sodium and potassium contents of the wet brewers grain fed group in comparison with the control cows.

Serum P concentration differed significantly (P<0.01) towards the end of the experiment with the highest concentration (7.40) in the control (T1) group compared to WDGS included groups, but the values in all the treatments were above the critical level (4.5 mg/dl) of serum P. Serum copper concentration was (µg/dl) significantly (P<0.05) decreased with the inclusion of WDGS in T3 and T4. Decreased (P<0.01) liver Cu levels in steers fed 60% of DM with WDGS compared to control and 30% WDGS fed group and increased liver S concentration in 30% WDGS and 60% WDGS groups compared to control group (Ponce *et al.,* 2014) was reported. Interaction of excess dietary

Table 2: Feed and nutrient intake of lactating crossbred cows fed different levels of WDGS

Parameter	T1	T2	T3	T4
Feed intake (kg DM/d) APBN	1.95 ± 0.15	2.14 ± 0.18	2.15 ± 0.09	2.15 ± 0.19
Paddy straw	3.08 ± 0.03	3.06 ± 0.07	3.00 ± 0.09	2.93 ± 0.09
Concentrate mixture**	3.94^{a} ± 0.29	1.83^{b} ± 0.14	0.79^{c} ± 0.04	0.00
WDGS**	0.00	1.36^{c} ± 0.29	2.20^{b} ± 0.21	3.37^{a} ± 0.69
Total DMI(kg/d)	8.98 ± 0.47	8.39 ± 0.45	8.27 ± 0.22	8.45 ± 0.50
DMI as % of B.wt.	2.68 ± 0.16	2.56 ± 0.20	2.40 ± 0.09	2.53 ± 0.20
CPI (kg/d)*	1.14^{b} ± 0.08	1.19^{b} ± 0.09	1.27^{ab} ± 0.05	1.53^{a} ± 0.12

[ab] Values in row bearing different superscripts differ significantly *(P<0.05), ** (P<0.01).

Table 3: Serum macro mineral content of lactating crossbred cows fed different levels of WDGS

Parameter	T1	T2	T3	T4
Ca (mg/dl)				
Initial	8.51 ± 0.22	8.80 ± 0.30	8.66 ± 0.39	8.48 ± 0.23
Final	8.28 ± 0.18	8.75 ± 0.09	8.44 ± 0.08	8.63 ± 0.10
P (mg/dl)				
Initial	5.48 ± 0.13	5.62 ± 0.26	5.83 ± 0.16	6.08 ± 0.07
Final**	7.40^{a}± 0.10	6.11^{b}± 0.47	6.14^{b}± 0.23	5.73^{b}± 0.31
Mg (mg/dl)				
Initial	3.83 ± 0.35	3.67 ± 0.19	3.95 ± 0.23	3.60 ± 0.15
Final	3.73 ± 0.20	3.86 ± 0.21	3.81 ±0.10	3.67 ± 0.06
Na (meq/l)				
Initial	146.98 ± 4.09	149.29 ± 6.22	147.24 ± 3.57	146.35 ± 4.81
Final	143.56 ± 3.70	153.74 ±5.33	150.13 ± 4.39	143.81 ± 3.50
K (meq/l)				
Initial	4.73 ± 0.14	4.65 ± 0.18	4.61 ± 0.13	4.56 ± 0.10
Final	4.62 ± 0.11	4.79 ± 0.22	4.67 ± 0.11	4.44 ± 0.11
Cl (meq/l)				
Initial	114.57 ± 5.82	117.29 ± 3.64	113.47 ± 3.89	115.06 ± 5.28
Final	111.60 ± 2.98	128.46 ± 4.23	116.01 ± 4.39	111.84 ± 3.50

^{ab} values in a row not sharing common superscripts differ significantly **(P<0.01).

Table 4: Serum micro mineral content of lactating crossbred cows fed different levels of WDGS

Parameter	T1	T2	T3	T4
Cu (µg/dl)				
Initial	77.47 ± 3.78	72.16 ± 5.04	76.50 ± 1.49	79.80 ± 1.61
Final*	75.19^{a} ± 2.45	71.23^{ab} ± 2.62	65.00^{b} ± 2.10	64.80^{b} ± 2.98
Zn (µg/dl)				
Initial	76.84 ± 6.34	70.99 ± 5.12	75.55 ± 3.73	70.50 ± 4.37
Final*	83.09^{a} ± 4.56	75.86^{ab} ± 5.08	70.03^{ab} ± 2.11	64.84^{b} ± 5.60
Fe (µg/dl)				
Initial	116.22 ± 8.19	114.20 ± 5.43	112.04 ± 6.38	117.34 ± 3.05
Final	115.12 ± 6.19	116.60 ± 4.96	115.96 ± 6.67	119.66 ± 8.84
Mn (µg/dl)				
Initial	21.11 ± 5.21	20.21 ± 4.48	24.62 ± 2.11	24.60 ± 1.29
Final	25.00 ± 2.15	23.91 ± 1.86	20.30 ± 1.55	23.77 ± 2.98
Co (µg/dl)				
Initial	3.45 ± 0.35	4.13 ± 0.33	4.25 ± 0.35	4.54 ± 0.43
Final	3.41 ± 0.37	3.88 ± 0.36	4.42 ± 0.49	4.35 ± 0.24

^{abc} Values in a row not sharing common superscripts differ significantly *(P<0.05).

S with Mo and formation of thio-molybdates in rumen fermentation might be the reason for decreased absorption of Cu (Suttle, 1991). Serum Zn concentration (µg/dl) was significantly (P<0.05) lower (70.50) in T4 (35% WDGS), compared control (83.09). In contrast to the serum Cu and Zn concentration in the present study, Moriel *et al.* (2015) found no significant difference in serum Cu and Zn in spite of the increased (P<0.05) intake of S, Cu, and Zn in beef heifers supplemented with WBG replacing corn at 0, 50 and 100 per cent.

The result on serum P and Zn concentrations of the present study can be supported by the findings of Espinoza *et al*. (1991), who studied the supplementation of mineral supplement with 4 or 6%, 8% and 12% phosphorus on free choice for three years in grazing cattle and reported higher (P<0.01) mean serum P in 8% P group (5.37 mg/dl) than in 4% P (4.12 mg/dl) and 12% P (3.9 mg/dl) groups during first year, higher (P<0.01) serum P concentrations in low P supplement group (6% P) during second and third compared to other two (8% P and 12% P) groups and lower serum Zn concentrations (P<0.05) for 12% P group for the first year. Noureddini and Dang (2009) stated that phytic acid content of corn milling co products of ethanol production ranges from 50-80% of the total P available, inhibits the absorption of zinc (Lonnerdal, 2000).

Blood Biochemistry

Blood urea nitrogen (BUN) of experimental animals is presented in Table 5. During study period mean BUN (mg/dl) was significantly (P<0.01) lower in T2. Mean BUN (mg/dl) values were significantly (P<0.01) lower on 0 and 30 day compared to 60, 90, 120 days of experiment. Inclusion of WDGS in the diet increased mean BUN levels increased towards the end of the experiment. West *et al.* (1994) and Chibisa *et al.* (2012) also found increased plasma urea nitrogen with the inclusion of WBG or wheat DDGS. Urea N in serum reflects dietary CP content because excess ruminal NH_3 from protein degradation enters the blood stream for conversion to urea. CP intake increased with the level of WDGS in the present study and might have contributed to the elevated BUN levels in WDGS fed animals.

No significant differences in blood glucose and serum total protein (Table 5) were observed among different treatments. There was a significant (P<0.01) increase of serum albumin in 25% WDGS fed group (T3), compared to other treatments. Non- significant differences in blood glucose levels were found by Belibasakis and Tsirgogianni (1996), Kazemi *et al.* (2009) and Mjoun *et al.* (2010) by feeding of WDGS or DDGS at varying levels to cattle. In contrast Oba *et al.* (2010) reported lower (P<0.05) plasma

Table 5: Blood urea nitrogen (BUN) levels (mg/dl) of lactating crossbred cows during the study

Treatment	Day					
	0	**30**	**60**	**90**	**120**	**Mean****
T1*	$16.71^{B} \pm 1.12$	$20.38^{AB} \pm 1.67$	$22.90^{A} \pm 1.68$	$23.80^{A} \pm 2.22$	$22.51^{A} \pm 1.36$	$21.26^{a} \pm 0.83$
T2	15.49 ± 1.05	17.31 ± 1.64	18.99 ± 1.21	19.04 ± 1.88	19.88 ± 1.44	$18.14^{b} \pm 0.68$
T3**	$15.77^{D} \pm 0.88$	$19.13^{C} \pm 0.65$	$21.70^{BC} \pm 0.61$	$22.35^{B} \pm 1.43$	$25.49^{A} \pm 0.60$	$20.89^{a} \pm 0.71$
T4**	$17.56^{B} \pm 0.89$	$21.41^{AB} \pm 1.45$	$23.26^{A} \pm 1.49$	$24.90^{A} \pm 1.59$	$25.92^{A} \pm 1.91$	$22.61^{a} \pm 0.83$
Mean**	$16.38^{B} \pm 0.49$	$19.56^{B} \pm 0.73$	$21.71^{A} \pm 0.71$	$22.52^{A} \pm 0.96$	$23.45^{A} \pm 0.83$	

^{abcd} values in a column, ^{ABCD} values in a row not sharing common superscripts differ significantly *(P<0.05) ** (P<0.01).

Table 6: Blood metabolites of lactating Crossbred cows fed different levels of WDGS

Parameter	T1	T2	T3	T4
Glucose (mg/dl)	59.27 ± 2.22	61.61 ± 1.62	57.32 ± 2.34	60.03 ± 2.90
Protein (g/dl)	7.82 ± 0.29	7.83 ± 0.49	8.05 ± 0.13	7.98 ± 0.09
Albumin (g/dl)**	$3.15^{c} \pm 0.10$	$3.43^{b} \pm 0.10$	$3.72^{a} \pm 0.06$	$3.48^{ab} \pm 0.10$
SGPT (IU/L)**	$18.70^{a} \pm 1.09$	$21.63^{a} \pm 0.54$	$21.39^{a} \pm 2.01$	$14.83^{b} \pm 0.64$
SGOT (IU/L)	33.31 ± 2.00	32.41 ± 3.91	38.90 ± 4.38	42.84 ± 1.59
ALP (IU/L)	28.34 ± 1.61	25.75 ± 2.53	23.51 ± 1.14	25.95 ± 2.17
GGT (IU/L)	11.06 ± 0.83	10.65 ± 0.43	11.19 ± 0.72	12.50 ± 0.38

^{abc} values in a row not sharing common superscripts differ significantly ** (P<0.01).

glucose in dairy cows fed diets containing corn DDGS to supply 30% of the dietary CP compared to triticale DDGS or SBM supplemented groups. West *et al.* (1994), Belibasakis and Tsirgogianni (1996) and Obeidat (2018) found similar serum protein content with the inclusion of brewers grain in the rations of cattle and sheep. Kazemi *et al*. (2009) reported non-significant differences in the serum albumin content.

The range of 14.83 to 21.63, 32.41 to 36.84, 23.51 to 28.34 and 10.65 to 12.50 were seen for SGPT (IU/L), SGOT (IU/L), ALP (IU/L) and GGT (IU/L) among different treatments (Table 6). SGPT activity was significantly (P<0.01) lowered in 35% WDGS fed groups and the other enzymes (SGOT, ALP and GGT) were not differed significantly among groups. Kazemi *et al.* (2009) reported no significant differences in serum SGOT and SGPT levels in dairy cattle fed ensiled barley distillers grains with varying levels of beet pulp and stated that as SGOT and SGPT are indicators of liver health the non-significant differences in the activity of these enzymes showed that ensiled WBG had no negative effect because of mycotoxin or any other toxins. Obeidat (2018) reported no significant difference in serum SGOT, SGPT and ALP levels with the inclusion of varying levels of DDGS in the rations of in Awassi lambs. Ashour *et al.* (2019) found gradual elevation ($P = 0.019$) in the activity of glutathione peroxidase enzymes and a depression ($P<0.01$) in the serum AST activity with the increasing level of the dietary dried brewers grain and non-significant differences in the activity of ALT with the level of DBG inclusion in broilers. The results of the present study and that of earlier workers revealed no change in SGOT and SGPT or decrease in the activity of these enzymes due to feeding of WDGS indicating that liver health is not affected.

Based on the results of the study it can be concluded that feeding WDGS up to 35% of dry matter intake had no deleterious effects on liver health and decreased serum copper and zinc levels. Addition of copper and zinc sources to trace mineral mixture may be suggested to improve respective mineral elements in serum.

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