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Research Article

EFFECT OF FEEDING BREWERY WASTE ON SERUM MINERAL PROFILE AND BIOCHEMICAL PARAMETERS IN JERSEY CROSSBRED DAIRY CATTLE

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ABSTRACT

The study was conducted in twenty four Jersey crossbred dairy cows at Melvenkatapuram village, Ranipet district of Tamil Nadu in two farmer's fields for a period of one year with three treatments. The control group (T0) and brewery waste (T1) treatment was carried out in first farmer's field and balanced ration (T2) treatment was carried out in second farmer's field with eight animals in each group. The control, brewery and balanced ration animals were fed as per traditional and standard feeding practices. Blood samples were collected prior and after the end of experimentation to analyse the serum mineral profile and biochemical parameters. The study concluded that the brewery fed animals had marginal gain in serum mineral profile (Manganese, Copper, Cobalt, Iron and Zinc) and marginal loss in serum biochemical parameters (glucose, protein and globulin) after experimentation than the control animals.

Keywords: Balanced ration, Brewery waste, Serum glucose, Serum mineral profile, Serum protein

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Livestock sector plays a significant role in Indian economy and is an important sub-sector of Indian agriculture. Dairying in the recent decades has been considered a vital component in the diversification of Indian agriculture, where crop farming is beset with stagnating growth and low absorption of unskilled agricultural laborers. The cost of feeding is the single most important factor affecting the profitability of a dairy enterprise. Hence, appropriate use of relatively inexpensive agricultural and industrial by-products is of paramount importance for profitable livestock production.

Wet brewer's grain has been used in lactating cows ration as such or in compounded cattle feed formulations after drying (Dhiman *et al.*, 2003). The concentration of rumen degradable protein ranged from 28-43% (mean 35%), indicating that wet brewery grains are good sources of rumen un-degradable or "bypass-protein" and it also has 20-32 % dry matter (Thomas *et al.*, 2016). Currently, the interests on feeding of wet brewer's grains to dairy cattle have increased among dairy farmers, because of comparatively cheaper price. Hence, the experiment was carried out to study the effect of feeding brewery waste on serum mineral profile and biochemical parameters compared with traditional feeding practice and balanced ration feeding under field conditions in lactating Jersey crossbred dairy cattle.

MATERIALS AND METHODS

The present experimental work was carried out at Melvenkatapuram village, Ranipet district, Tamil Nadu in two farmer's field who owns dairy cattle which is the main source of income for their livelihood. Twenty four dairy cattle with uniform body weight and milk production from 2 farmer's fields were selected and randomly distributed into three experimental groups with 8 animals each. The first farmer possesses 16 dairy cattle and the second farmer possesses 8 dairy cattle where the experimental work was initiated. The selected dairy cattle were Jersey crossbred in 1st lactation of 3-4 years of age. The experimental work was carried out in animals which were calved around 45-60 days with an average milk yield of 5-5.5 kgs/day and mean body weight of 250.02 Kgs. The experimental work was initiated during the mid-month of September 2019. Deworming was carried out in all the dairy cattle as per the standard protocol using fenbendazole. A total of two weeks were given as adaptation period for all the dairy cattle prior to the experimentation which continued from October, 2019 to September, 2020.

The experimental work was initiated with three treatments *viz*. T0 (Control), brewery waste (T1) and balanced ration (T2). The control (T0) and brewery waste (T1) experimentation was carried out in 1st farmer's field with 16 dairy cattle and the balanced ration treatment (T2) was carried out in 2nd farmer's field who own rest of the 8 dairy cattle in the same village. The control (T0) dairy cattle were fed with rice bran/wheat bran and ground nut oil cake as per traditional practices followed by the farmer in field. The brewery waste (T1) and balanced ration (T2) were given to the dairy cattle based on the dry matter requirement and milk production during the feeding trial. The green fodder and dry fodder (paddy straw) @ 9 kgs & 5 kgs/animal/day, respectively were fed to all the dairy

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cattle during the experimental period. The brewery waste was given @ 1 kg/kg of milk production in T1 group dairy cattle and the concentrate feed was provided @ 400 gms/kg of milk production in T2 group dairy cattle. For every 1 kg of milk production, approximately 1000 kcal of gross energy is required and the brewery waste (T1) contained 1931 kcal/kg on dry matter basis. Hence, for every 1 kg of milk production, 1 kg of brewery waste was fixed in the current experiment. In case of balanced ration (T2), as per the thumb rule, for every 2.5 kg of Milk production, 1 kg of concentrate feed has to be given. Hence for every 1 kg of milk production, 400 grams of concentrate feed was fixed in the present study and this could almost equate with ICAR 2013 standard. During the dry period, the dairy cattle were fed with 4 kgs of brewery waste per day per animal in T1 group and 1.5 kgs of balanced ration feed per day per animal in T2 group as maintenance requirement for dairy cattle.

Initially the experimental diet on control (T0-control), brewery waste (T1) and balanced ration (T2) were analysed for proximate principles at animal feed analytical and quality assurance laboratory, Namakkal (AOAC, 1990) and are presented in Table 1.

Collection of Blood samples and analysis

10 ml of blood samples were collected from jugular vein using non-heparinised vacutainers before and at the end of experimentation by aseptic venipuncture technique. The collected blood samples were centrifuged at 3000 rpm for 10 minutes and the clear supernatant serum was transferred to serum storage vials and stored at - 30° C for further serum mineral profile (trace minerals) and biochemical parameters estimation. Mineral profile (trace minerals) in serum such as manganese, cobalt, copper and selenium were estimated with Inductively Coupled Plasma Mass Spectrometry (ICPMS) at Pharmaco vigilance laboratory for Animal feed and food safety (PLAFFS), Tamil Nadu Veterinary and Animal Sciences University (TANUVAS).

Estimation of serum biochemical parameters viz. serum glucose, serum protein and serum albumin was carried out in serum auto analyzer (Biosystem 320) using commercial reagent kit. Serum glucose was estimated by glucose oxidase method. The serum protein and albumin level in the samples were estimated based on direct biuret method.

The data collected on mineral profile (trace minerals) and biochemical parameters, by the Jersey crossbred cattle under different treatment regimens were subjected to one-way Analysis of Variance (ANOVA) by the statistical software, IBM SPSS version 20.0 for Windows to find the significant difference between treatments. Also interpretation of data was done as per the procedure described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Serum Mineral Profile

The gain in serum mineral (manganese and selenium) profile was significantly higher for balanced ration (T2) followed by brewery (T1) and control (T0) in the descending order of magnitude (Table 2). The loss of serum minerals (Table 2) in control (T0) animals could be due to feeding of imbalanced ration containing rice or wheat bran, rice gruel and ground nut oil cake. The mineral content present in the imbalanced ration would be the plausible reason to show its presence in the blood serum at the end of experiment (Yao *et al.*, 2019). However, the negative values obtained in them could be due to feeding of imbalanced proportion of the feed ingredients in the concentrate feed. In brewery (T1) fed animals, there was a marginal gain in serum mineral profile after the end of experiment period (Table 2). This was in accordance with the findings of Senthil Murugan *et al.* (2015) who observed elevated levels of serum minerals while studying the serum mineral profile of feeding brewery spent grains to dairy cattle.

In balanced ration (T2), significant gain in manganese and selenium content was observed (Table 2) in the serum profile of the Jersey crossbred cattle. The increase of serum minerals in T2 fed animals could be due to feeding of balanced ration which included recommended level of mineral mixture. The additive effects of these ingredients could be the probable reason for the increased level of serum manganese and copper in the blood serum at the end of experiment. This was in agreement with the findings of Biswanath *et al.* (2017) who reported the effect of supplementing area specific mineral mixture on productive performance of crossbred cows in Uttarakhand and tested for its efficacy on nutrient utilization, mineral bioavailability and milk production performance in lactating cows. They concluded that the serum mineral content significantly ($P<0.05$) increased for treatment animals and for the control it decreased. Agrawalla *et al.* (2018) studied the combined effect of area specific mineral mixture (ASMM) and bypass protein (BPP) supplementation with traditional feeding practices (TP) on the performance of crossbred cattle. They recorded significantly ($P<0.05$) higher serum manganese content in T1 group (TP + ASMM-50gm/day) and T2 group (TP + ASMM-50gm/day + BPP @ 100 g/day) than control group (TP) on 60th day after experiment implying the significance of mineral

Table 1

Proximate Principles Analysis (in %)

S.No.	Particulars	Control feed (T0)	Brewery waste (T1)	Balanced ration (T2)
1.	Moisture	9.15	73.17	12.22
2.	Crude protein	7.36	13.90	19.18
3.	Crude Fibre	5.95	6.40	9.02
4.	Ether Extract	4.95	5.13	6.09
5.	Total Ash	5.02	5.76	7.19
6.	Gross Energy (K.Cal/kg)	1323	1931	3708

mixture supplementation. Sharma *et al.* (2006) observed an increase in the level of cobalt content in blood serum of animals while studying the status of mineral profile in cattle reared under organized farms and unorganized farms in Aligarh and Mathura district of western Uttar Pradesh. Also the addition of commercially available mineral mixture in the balanced ration fed to cattle in organized farms could be the probable reason for increase in cobalt content in serum of the animals.

Serum biochemical parameters

a. Serum glucose

A marginal decrease in the blood glucose level at the end of the experiment from the initial values for treatment groups was evident for control (T0) and brewery (T1) fed animals. The decrease of blood glucose was greater for control (T0-1.58 mg %) than the brewery fed animals (T1-0.02 mg %) in the experimentation. It was an interesting fact to be noted that there was a marginal increase in the blood glucose for balanced ration (T2) fed animals at the end of experiment (Table 3).

The negative balance of glucose in control (T0) animals was due to feeding of imbalanced ration without meeting the nutritional requirement of the animals. Also the concentrates fed to the control animals contained only 1323 K.cal/kg energy, 7.36% crude protein and 5.95% crude fibre to meet out the lower requirement of the animals. Further the marginal loss of blood glucose in control animals could be due to the fact that they would have been in negative energy balance (NEB) during the first 3 months of lactation period (up to the peak milk production). Moreover, if animals were unable to get enough energy from rations, then they start to mobilise body fat reserves to meet its energy demand and enter in a state of NEB. Such cows under NEB will have lower plasma glucose. This was in accordance with the findings of Gross *et al.* (2015). In case of brewery fed animals (T1) the concentrate feed contained 1931 K.cal/kg energy, 13.90% crude protein and 6.40% crude fibre to meet out the dietary requirement of the animals. As narrated for the control treated, the brewery treated animals would have been in negative energy balance up to peak lactation period and during this period, the brewery waste wouldn't have met the dietary requirement. In view of the above, there was a marginal loss in the serum glucose as exhibited in the blood.

The increased serum glucose level in T2 animals was due to supplementation of the balanced diet which contained 3708 K.cal/kg energy, 19.18% crude protein and 9.02% crude fibre to meet out the dietary requirement. Also the balanced ration would have enhanced the overall status of the animal in terms of immune function. Further, feeding of balanced ration provided all the nutrients and minerals required for the functionality of numerous structural proteins, enzymes and cellular proteins. Garg *et al.* (2016) reported that feeding of balanced ration increased the blood glucose level from 45.58 mg% to 47.07 mg% in the dairy animals implying the importance of balanced ration on augmenting serum glucose under field conditions. The increase in serum glucose may also be due to effect of minerals either as cofactors and/or activators of many enzymatic systems associated with the metabolism of nutrients. It was reported that the Zn alters the molar proportion of VFA in the rumen with an increase of propionate production (Arelovich *et al.*, 2000) resulting in an increased glucose level in the serum (Aliarabi and Chhabra, 2006). Moreover, the balanced ration fed animals contained mineral mixture as part of the important component which helps in improving the blood glucose level. Debasish *et al.* (2016) studied the effect of supplementation of area specific mineral mixture on performance of crossbred cows with reproductive disorders in Kakatpur Block and reported that the blood glucose level increased significantly ($P<0.05$) from 44.55 to 54.85 mg/dl in mineral mixture supplemented cows. Agrawalla *et al.* (2018) studied the combined effect of area specific mineral mixture and bypass protein supplementation on the performance of crossbred cattle. They observed on 60^{th} day the treatment groups, T1-Traditional practices (TP) & area specific mineral mixture (ASMM-50gm/day) and T2-TP + ASMM + bypass protein @ 100 g/day) recorded significantly ($P<0.05$) higher blood glucose (54.55 & 55.73 mg %) than control group (TP-50.56 mg %) implying the significance of mineral mixture supplementation in increased blood glucose level.

b. Serum protein, albumin and globulin:

The results revealed that there was a decrease in the serum protein and serum globulin level at the end of the experiments from their initial values for treatments,

Table 2

Serum mineral profile and Gain/loss (in ppm) in Jersey crossbred cattle under different treatment regimens (Mean ±S.E.)

S.No.	Parameters	Before experimentation			F value	At the end of experimentation			F value	Mean gain/ loss in serum mineral profile			F value
		Control (T0)	Brewery (T1)	Balanced Ration (T2)		Control (T0)	Brewery (T1)	Balanced Ration (T2)	(T0)	Control (T1)	Brewery Ration (T2)	Balanced	
1.	Manganese	0.1036 ± 0.008	0.091 ± 0.007	0.091 ± 0.007	0.82^{NS}	0.1023 ± 0.003^{d}	0.094 ± 0.009^{d}	0.141 ± 0.004^{c}	15.73^{**}	- 0.0003 ± 0.011^{b}	0.003 ± 0.014^{b}	0.05 ± 0.009^{a}	5.67^{**}
2	Cobalt	0.00047^{b}	0.0006^{ab}	0.0007^{a}	5.00^{*}	0.00042^{d}	0.0018^{cd}	0.0022^{c}	3.43^{NS}	- 0.0005^{b}	0.0012^{ab}	0.0015^{a}	2.97^{NS}
3	Copper	0.415 ± 0.06^{ab}	0.494 ± 0.024^{a}	0.375 ± 0.003^{b}	2.71^{NS}	0.562 ± 0.069	0.661 ± 0.029	0.608 ± 0.073	0.66^{NS}	0.147± 0.038	0.167± 0.015	0.233 ± 0.066	1.02^{NS}
4	Selenium	0.213 ± 0.016^{a}	0.186 ± 0.021^{ab}	0.148 ± 0.006^{b}	4.33^{*}	0.169 ± 0.011	0.182 ± 0.015	0.190 ± 0.008	0.73^{NS}	- 0.044 ± 0.025^{b}	- 0.004 ± 0.022^{ab}	0.042 ± 0.009^{a}	4.51^{*}
5.	Chromium	0.004 ± 0.0001^{a}	0.003 ± 0.0004^{ab}	0.002 ± 0.0005^{b}	3.40^{NS}	0.002 ± 0.0004	0.002 ± 0.0004	0.003 ± 0.0006	0.38^{NS}	– 0.002 ± 0.0007^{b}	– 0.001 ± 0.0017^{ab}	0.001 ± 0.00085^{a}	3.22^{NS}
6.	Iron	1.057 ± 0.084	1.172 ± 0.063	0.953 ± 0.111	1.54^{NS}	1.248 ± 0.127	1.392 ± 0.082	1.368 ± 0.102	0.53^{NS}	0.192 ± 0.167	0.220 ± 0.117	0.415 ± 0.145	0.71^{NS}
7.	Zinc	0.966 ± 0.12	0.809 ± 0.094	0.688 ± 0.053	2.26^{NS}	0.918 ± 0.046	0.941 ± 0.069	0.985 ± 0.030	0.45^{NS}	– 0.048 ± 0.156	0.132 ± 0.104	0.297 ± 0.071	2.21^{NS}

Means bearing same superscripts within rows do not differ significantly

** - Highly Significant (P<0.01), * - Significant (P<0.05), NS - Non Significant (P>0.05)

Table 3

Serum biochemical parameters and gain/ loss in Jersey crossbred cattle under different treatment regimens (Mean ±S.E.)

S.No.	Parameters	Before experimentation			F value	At the end of experimentation			F value	Mean gain/ loss in serum mineral profile			F value
		Control (T0)	Brewery (T1)	Balanced Ration (T2)		Control (T0)	Brewery (T1)	Balanced Ration (T2)	(T0)	Control (T1)	Brewery Ration (T2)	Balanced	
1.	Serum glucose (mg%)	41.00 ± 0.45	42.00 ± 1.18	40.83 ± 1.01	0.05^{NS}	39.42 ± 0.42	41.98 ± 1.88	42.67 ± 2.79	0.77^{NS}	- 1.58 ± 0.14	- 0.02± 0.07	1.83 ± 0.17	0.49^{NS}
2.	Serum protein (g/dl)	7.57 ± 0.15	7.43 ± 0.48	6.93 ± 0.40	0.81^{NS}	7.52 ± 0.22	7.37 ± 0.21	7.73 ± 0.42	0.38^{NS}	- 0.05 ± 0.03	- 0.06 ± 0.03	0.80 ± 0.11	1.07^{NS}
3.	Serum albumin (g/dl)	2.72 ± 0.17	2.62 ± 0.28	2.73 ± 0.21	0.08^{NS}	3.60 ± 0.11	3.17 ± 0.16	3.34 ± 0.19	1.95^{NS}	0.88 ± 0.17	0.55± 0.12	0.60 ± 0.22	0.29^{NS}
4.	Serum globulin (g/dl)	4.85 ± 0.25	4.82 ± 0.54	4.20 ± 0.39	0.80^{NS}	3.92 ± 0.22	4.20 ± 0.25	4.40 ± 0.38	0.68^{NS}	- 0.93 ± 0.28	- 0.62± 0.13	0.19 ± 0.04	1.02^{NS}
5.	Albumin globulin ratio	0.57 ± 0.06	0.59 ± 0.10	0.68 ± 0.08	0.45^{NS}	0.93 ± 0.05	0.78 ± 0.08	0.79 ± 0.09	1.15^{NS}	0.36 ± 0.09	0.19 ± 0.04	0.11 ± 0.03	0.70^{NS}

NS - Non Significant (P>0.05)

control (T1) and brewery (T1) fed dairy animals (Table 3). On the contrary, serum protein and serum globulin for balanced ration fed (T2: 0.80 & 0.19 g/dl) animals exhibited a remarkable increase from their initial values, but non-significant (P>0.05) among the treatment groups. Also a marginal increase in the serum albumin content at the end of the experiments from their initial values for all the treatments was noticed. The gain in serum albumin, loss in serum protein and serum globulin of control animals was mainly due to supplementation of imbalanced ration which was not fulfilling the nutrient requirement of the dairy cattle. The ration also contained very low level of energy (1323 K.cal/kg), crude protein (7.36%) and crude fibre (5.95%), which would not have met the dietary requirement of the animals. This could be the plausible reason of the changes of serum protein, albumin and globulin levels in control animals. In case of brewery waste fed animals (T1) the feed contained 1931 K.cal/kg energy, 13.90% crude protein and 6.40% crude fibre which wouldn't have met the dietary requirement of the animals as like control and hence the loss in serum protein and serum globulin was noticed in T1.

In case of balanced ration fed animals (T2), the concentrate feed contained 3708 K.cal/kg energy, 19.18% crude protein and 9.02% crude fibre to meet out the dietary requirement of the animals. The increased serum protein level in T2 animals could be due to feeding of the animals with balanced ration which would have enhanced the overall status of the animal in terms of immune function. Also feeding balanced ration to animal provides all the nutrients and minerals required for the functionality of numerous structural proteins, enzymes and cellular proteins. Further the animals fed with balanced diet containing sufficient energy and protein along with

recommended level of mineral mixture, the animals exhibited a positive state of equilibrium. When animals were fed with balanced ration, the serum protein and globulin significantly (P<0.01) increased, respectively from 6.02 g/dl to 6.94 g/dl and from 2.85 to 3.82 g/dl (Garg *et al.*, 2016). Agrawalla *et al.* (2018) reported that the serum protein significantly (P<0.05) increased to 6.32 g/dl from initial values of 5.52 g/dl, serum albumin to 3.75 g/dl from 3.19 g/dl for area specific mineral mixture supplementation treated animals and for control animals a marginal non-significant increase of serum protein and albumin was evident implying the importance of mineral mixture supplementation to the dairy animals.

CONCLUSION

It was inferred from the study that feeding brewery waste had marginal gain in serum mineral profile (Manganese, Copper, Cobalt, Iron and Zinc) and marginal loss of serum biochemical parameters (serum glucose, serum protein and serum globulin) after experimentation compared with the control treated animals. Hence, feeding of brewery waste to lactating dairy animals does not affect the serum mineral profile and biochemical parameters to a great extent which could influence the reproductive performance and fertility status of dairy cattle.

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REFERENCES

AOAC. (1990). Official Methods of Analysis, Association of Analytical Chemists. (15th Edn.), Washington, D.C.

Agrawalla, J., Sethy, K., Swain, R.K., Mishra, S.K., Behera1, K., Debata, N.R. and Mishra, B.K. (2018). Combined effect of area specific mineral mixture and bypass protein supplementation on the performance of crossbred cattle. *Int. J. Livest. Res.* **8**: 92-98.

Aliarabi, H. and Chhabra, A. (2006). Effect of inorganic and chelated zinc supplementation on the performance of cross bred calves. *Indian J. Anim. Nutr.* **23**: 141-145.

Arelovich, H.M., Owens, F.N., Horn, G.W. and Vizcarra, J.A. (2000). Effect of supplemental zinc and manganese on ruminal fermentation, forage intake and digestion by cattle fed prairi hay and urea. *J. Anim. Sci.* **78**: 2972- 2979.

Biswanath, S., Kumar, R., Garg, A.K. Ranjan, K., Mohanta, Agarwal, A. and Sharma, A.K. (2017). Effect of supplementing area specific mineral mixture on productive performance of crossbred cows. *Indian J. Anim. Nutr.* **34(4)**: 414-419.

Debasish, S., Mishra, S.K., Swain, R.K., Kamdev, S. and Sahoo, G.R. (2016.) Effect of supplementation of area specific mineral mixture on performance of crossbred cows with reproductive disorders in Kakatpur block. *Indian J. Anim. Nutr.* **33(3)**: 279-284.

Dhiman, T.R., Bingham, H.R. and Radloff, H.D. (2003). Production response of lactating cows fed dried versus wet brewers' grain in diets with similar dry matter content. *J. Dairy Sci.* **86**: 2914–2921.

Garg, M.R., Bhanderi, B.M., Goswami, A. and Shankhpal, S. (2016). Effect of balanced feeding on SNF content of milk: A field study. *Int. J. Dev. Res.* **6(11)**: 10054-10059.

Gomez, K.A and Gomez, A.A. (1984). Statistical Procedure for Agricultural Research Hand Book. John Wiley & Sons, New York.

Gross, J.J., Kessler, E.C., Albrecht, C. and Bruckmaier. R.M. (2015). Response of the cholesterol metabolism to a negative energy balance in dairy cows depends on the lactational stage. *PLoS One.* **21956**: 1-17.

Senthil Murugan, S., Sakkariya Ibrahim, Seethalakshimi, M., Ramanathan, A., Raja, T.V. and Joseph, M. (2015). Influence of feeding wet brewer's grains on dry matter intake and milk quality and quantity in lactating cows. *Indian J. Nat. Sci.* **5(29)**: 4322-4328.

Sharma, M.C., Kumar, P., Joshi, C. and Kumar, H. (2006). Status of serum minerals and biochemical parameters in cattle of organized and unorganized farms of western Uttar Pradesh. *Asian J. Anim. Vet. Adv.* **1(1)**: 33-41.

Thomas, M., Hersom, M., Thrift, T. and Yelich, J. (2016). Wet Brewers' Grains for Beef Cattle, Animal Sciences Department, UF/ IFAS Extension, University of Florida.

Yao, B.M., Chen, P. and Sun, G. (2019). Distribution of elements and their correlation in bran, polished rice, and whole grain. *Food Sci. Nutr.* **8**: 982–992.