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Impact of Feeding Biofortified Wheat (WB 2) Straw-based Diet on Immunity of Lactating Murrah (*Bubalus bubalis*) Buffaloes

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ABSTRACT

Backround: Buffaloes contributes heavily to the livestock sector of India and buffaloes are preferred for milk production in India due to their efficiency and better nutrients in milk. Major part of their diet constitute roughage which is usually wheat straw in north-western region of India. WB 2 is a biofortified wheat variety with high zinc and iron levels in its grain, has been developed by ICAR-Indian Institute of Wheat and Barley Research in Karnal, Haryana. This study aimed at comparing the benefits of substituting conventional straw with WB 2 straw.

Methods: The present experiment was conducted on 12 Murrah buffaloes, divided into two groups *i.e.,* Control (T_0) and Treatment (T_1) based on body weight, parity and previous milk record. Feeding continued for a period of 90 days as per ICAR (2013) feeding standard. The wheat straw, oats fodder and concentrate mixture was fed in the ratio 50:15:35 (on DM basis) in both the groups. However, in control group, conventional wheat straw was used and in the treatment group, biofortified wheat variety straw was utilized.

Result: Non-significant differences (P>0.05) were observed when both the groups were compared for body weight and dry matter intake on fortnightly basis. Similarly, haematology was carried out on monthly basis and there were non- significant differences (P>0.05) between the two groups. Non-significant difference for plasma IgG1 was also recorded. The study reveals that the feeding of biofortified wheat variety (WB 2) straw had no impact on immunity of lactating Murrah buffaloes compared to conventional straw-based diet.

Key words: Biofortification, Murrah buffaloes, Wheat straw, Zinc.

INTRODUCTION

The livestock sector accounts for 6.17% of total GVA (at current prices) and 30.87% of the GVA of agriculture and allied sector of India (DAHD, 2022) and plays significant role in improving the socioeconomic status of small and marginal farmers. In 2019-20, the milk group generated 66.77 per cent of the total value of the livestock sector's output (Ministry of Statistics and Programme Implementation, 2022). India has 109.85 million buffaloes (DAHD, 2019) with overall milk production of 221.06 million tonnes (DAHD, 2022). Buffaloes are preferred over cows in India because they are fed on poor quality feed and forages, have better feed conversion efficiency, higher returns from milk (more than 8% fat) and have better nutrients in milk (higher SNF including sugar, protein, calcium and vitamins) (Balhara *et al.*, 2017).

WB 2, a biofortified wheat variety developed by the ICAR-Indian Institute of Wheat and Barley Research in Karnal, Haryana, was released by the Government of India in 2017. It is a pure line wheat cultivar with high zinc and iron levels of 42.0 and 40.0 ppm, respectively (Yadava *et al.*, 2018). It matures in 142 days with a grain yield of 51.6 q/ha on an average and is suitable for cultivation in the North-western Plains Zone of the country (Chatrath *et al.*, 2018). Wheat biofortification resulted in higher zinc levels in wheat straw and increased *in vitro* dry matter digestibility (NIANP, 2019). Zn is very effective in boosting Zn and Fe concentrations in grains and straw when soil and foliar application is done (Paramesh *et al.*, 2020). Zn application results in a greater improvement in grain and straw accumulation (Liu *et al.*, 2019). Increased Zn availability to cattle through Zn enhanced fodder will meet the Zn requirement, which is critical for the livestock's immune system (Capstaff and Miller, 2018). Keeping above facts in view, the present study was conducted to study the effect of feeding biofortified wheat (WB 2) straw-based diet on immunity of lactating Murrah (*Bubalus bubalis*) buffaloes.

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MATERIALS AND METHODS

The 12 newly calved Murrah buffaloes were selected from the Livestock Research Center, ICAR-NDRI, Karnal and divided into two groups of six animals each based on body weight, producing ability and lactation stage. The experiment was conducted as per the prescribed guidelines and procedures of the Institutional Animal Ethics Committee (IAEC), which was established in compliance with Form No. 13 of the government-mandated Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All the animals were dewormed before the start of the feeding trial and kept in well-ventilated tie-stalls with separate arrangements for feeding. Animals in both groups were given a total mixed ration in the ratio of 50:15:35 (Wheat Straw: Green Fodder: Concentrate) on DM basis in accordance with the ICAR (2013) specification. Animals in the control group were given conventional wheat straw, whereas those in the treatment group were fed biofortified wheat (WB 2) straw. Throughout the trial, daily logs of feed given and leftovers were recorded. At 10:00 a.m., the necessary quantity of wheat straw, concentrate mixture and chaffed green fodder was measured individually and given as TMR after thorough mixing. Buffaloes had free access to potable drinking water. Blood samples were taken from the jugular vein in sterile heparinized vacutainer containers with minimal disruption to the animal. Blood samples were taken on the 0th, 30th, 60th and 90th days of the trial. Soon after collection, samples were transported to the laboratory in refrigerated iceboxes and an aliquot of blood was collected and quickly analysed for blood parameters such as TLC, TEC, DLC and Hb concentration using Blood Cell Counter Vety. Version MS4Se at HPVK, Karnal. The samples were centrifuged for 15 minutes at 3000rpm to extract the plasma. Plasma samples were kept at -20°C for further analysis. KRISHGEN BioSystems' "Bovine Immunoglobulin g1, IgG1 GENLISATM ELISA" (Cat. No. KLB0385) was used to measure immunoglobin IgG1 in bovine plasma.

RESULTS AND DISCUSSION

Chemical composition of feed stuffs

The chemical composition of various feed resources offered to experimental animals are presented in Table 1. The results show that the chemical composition of various feeds are within the range of reported values. The concentration of Zn and Fe was within the normal range reported by previous workers (Misra *et al.*, 2012 and NIANP, 2019).

Dry matter intake (on per cent body weight basis) of lactating Murrah buffaloes

The DM intake in the control (T_0) group ranged from 2.66±0.12 to 2.91±0.12 percent and in the treatment (T_1) group, it ranged from 2.73±0.09 to 2.83±0.11 per cent (Table 2). The difference between control (T_0) and treatment (T_1) group was found to be non-significant (p>0.05) for dry matter intake (on percent body weight basis).

These results are similar to Giridhar *et al.* (2021) who reported non-significant difference in DMI when they fed zinc biofortified sorghum stover to lambs. Fagari-Nobijari *et al.* (2012) also found non-significant difference in DMI on zinc supplementation in bulls. On Contrary, Kumar and Ram (2021) recorded higher DMI intake in the case of Zn biofortified maize fodder.

Body weight (kg) of lactating Murrah buffaloes

The influence of biofortified wheat straw-based ration on body weight of lactating Murrah buffaloes is presented in Table 3. The mean of overall observations was found to be 565.02±10.71 and 563.65±7.80 kg for control (T_0) and treatment (T_1) group, respectively. The difference between the control (T_0) and treatment (T_1) was found to be non-significant (p>0.05) at all fortnights. Contrary to this, Giridhar *et al.* (2021) found significant (p=0.05) difference in growth performance of lambs fed on biofortified sorghum stover. Antari *et al.* (2021) found that supplementing Ongole cattle with Zn methionine prevented live weight loss during early lactation.

Table 1: Chemical composition of feedstuffs used (% on DM basis).

Details	Conventional wheat straw	Biofortified wheat (WB 2) straw	Concentrate mixture	Oats fodder
DM	90.46	89.93	90.91	18
OM	89.74	88.88	91.43	88.36
CP	2.94	3.03	19.44	9.93
EE	1.25	1.66	3.54	2.13
NDF	72.36	73.67	30.14	39.05
ADF	53.41	59.71	17.32	29.82
NFC	13.19	10.52	38.31	37.25
ADL	8.41	7.98	2.15	5.49
TDN	40.99	41.04	75.79	60.67
Ca (%)	0.31	0.48	0.61	0.51
P (%)	0.04	0.06	0.55	0.35
Zn (mg/kg)	11.61	12.61	100.04	21.69
Fe (mg/kg)	164.13	186.06	692.99	515.30
Cu (mg/kg)	03.25	03.36	31.69	08.02
Mn (mg/kg)	33.93	26.04	70.81	57.21

Table 2: Dry matter intake (on % body weight basis) of lactating Murrah buffaloes.

Fortnight	Control (T_0)	Treatment (T_1)	p-value
1	2.91±0.12	2.83±0.11	0.669
2	2.66±0.12	2.73±0.09	0.654
3	2.72±0.10	2.75±0.08	0.869
4	2.76±0.09	2.80±0.08	0.703
5	2.79±0.08	2.83±0.08	0.789
6	2.83±0.08	2.82±0.08	0.930
Overall mean ± SE	2.78±0.04	2.79±0.03	

Table 3: Body weight (kg) of lactating Murrah buffaloes.

Fortnight	Control (T_0)	Treatment (T_1)	p-value
0 Day	567.67±31.48	569.17±28.38	0.972
1	547.83±30.77	554.83±23.41	0.860
2	554.83±29.61	556.00±21.26	0.975
3	562.17±29.73	560.17±20.60	0.957
4	570.17±30.01	566.17±20.05	0.914
5	574.83±30.35	568.00±20.17	0.855
6	577.67±30.47	570.83±20.09	0.855
Overall mean ± SE	565.02±10.71	563.65±7.80	

Table 4: 6% FCM production (kg/day/animal per unit DM intake) of lactating Murrah buffaloes.

Fortnight	Control (T_0)	Treatment (T_1)	p-value
1	0.54±0.07	0.55±0.07	0.863
2	0.51±0.07	0.50±0.06	0.523
3	0.44±0.07	0.45±0.04	0.256
4	0.46±0.07	0.51±0.06	0.650
5	0.48±0.08	0.51±0.06	0.830
6	0.45±0.08	0.51±0.06	0.487
Overall mean ± SE	0.48±0.02	0.51±0.01	

In the present study, Zn concentrations in control (T_0) and treatment (T_1) group had minor differences and this might be the reason for non-significant differences.

6% FCM production (kg/day/animal per unit DM intake) of lactating murrah buffaloes

Data corresponding to 6% FCM per kg DM intake is presented in Table 4. There was non-significant difference found between the two groups at fortnightly interval. The overall mean varied from 0.44±0.07 to 0.54±0.07 and 0.45±0.04 to 0.55±0.07 kg/day/animal per unit DM intake for the control (T_0) and treatment (T_1) group, respectively.

Studies conducted by Cope *et al.* (2009) and Wang *et al.* (2013) concluded that zinc supplementation enhanced milk production in cattle however**,** Ianni *et al.* (2020) reported that it had no effect. Singh *et al.* (2021) observed the same in Murrah buffaloes. Similarly, Hosnedlová *et al.* (2007) reported surge in yield when fed with Zn enriched fodder. According to Sobhanirad *et al.* (2010), there was a numerical increase in milk yield in the supplemented group, but the difference in milk yield or fat corrected milk with zinc supplementation was non-significant. Also, supplementation of iron had no impact on yield of milk (Weiss *et al.*, 2010).

Haematological profile of lactating murrah buffaloes

There were non-significant ($p>0.05$) differences in any of the haematological parameters (Table 5). Non-significant differences in the differential leucocyte counts were also recorded (Table 6). Overall mean values for haemoglobin, packed cell volume, total erythrocyte count and total leucocyte count in the control (T_0) group were 11.19±0.24 (%), 34.42±0.75 (%), 6.66±0.15 (millions/µl) and 10133.75±630.12/ µl, respectively. In the treatment (T_1) group, overall mean values for haemoglobin, packed cell volume, total erythrocyte count and total leucocyte count were 11.19±0.24 (%), 34.42±0.75 (%), 6.66±0.15 (millions/µl) and 10133.75±630.12/ µl, respectively. Differential leucocyte count shows that in the control (T_0) group, overall mean values for neutrophils (%), lymphocytes (%), monocytes (%) and eosinophils (%) are 31.38±0.87, 63.08±0.95, 3.21±0.38 and 2.17±0.18, respectively. Whereas, in the treatment (T_1) group, overall mean values for neutrophils (%), lymphocytes

Table 5: Haematological profile of lactating Murrah buffaloes.

Day	Control (T_0)	Treatment (T_1)	p-value
Hb (g%)			
0^{th}	11.70±0.47	11.67±0.29	0.953
30^{th}	10.98±0.48	10.92±0.33	0.912
60^{th}	11.05±0.52	10.92±0.37	0.840
90^{th}	11.02±0.49	10.98±0.35	0.957
Overall mean ± SE	11.19±0.24	11.12±0.17	
PCV (%)			
0^{th}	34.00±1.15	34.17±0.83	0.909
30^{th}	34.33±1.87	33.67±0.99	0.759
60^{th}	34.50±1.75	33.50±1.20	0.647
90^{th}	34.83±1.51	33.83±1.08	0.602
Overall mean ± SE	34.42±0.75	33.79±0.49	
TEC (millions/μL)			
0^{th}	6.77±0.32	6.62±0.30	0.744
30^{th}	6.53±0.31	6.20±0.31	0.479
60^{th}	6.70±0.32	6.38±0.35	0.512
90^{th}	6.67±0.34	6.46±0.32	0.675
Overall mean ± SE	6.66±0.15	6.42±0.15	
TLC (thousands/μL)			
0^{th}	10115.00±1128.15	10163.33±750.58	0.972
30^{th}	10106.67±1600.81	10540.00±891.53	0.818
60^{th}	10168.33±1757.49	10680.00±1059.35	0.808
90^{th}	10145.00±617.54	10275.00±817.19	0.902
Overall mean ± SE	10133.75±630.12	10414.58±415.85	

Table 6: Differential leucocyte counts of lactating Murrah buffaloes.

Day	Control (T_0)	Treatment (T_1)	p-value
Neutrophils (%)			
0^{th}	34.17±1.08	32.50±1.34	0.354
30^{th}	32.17±2.21	29.67±1.56	0.378
60^{th}	30.17±2.12	27.83±1.96	0.437
90^{th}	29.00±0.58	31.83±2.77	0.341
Overall mean ± SE	31.38±0.87	30.46±1.00	
Lymphocytes (%)			
0^{th}	60.33±0.71	62.83±1.08	0.082
30^{th}	62.33±2.54	64.83±2.29	0.481
60^{th}	63.83±2.43	66.33±2.79	0.514
90^{th}	65.83±0.60	61.17±3.30	0.194
Overall mean ± SE	63.08±0.95	63.79±1.24	
Monocytes (%)			
0^{th}	2.83±0.70	2.33±0.61	0.604
30^{th}	3.33±0.71	3.17±0.87	0.885
60^{th}	3.83±1.01	4.00±1.06	0.912
90^{th}	2.83±0.65	2.83±0.79	1.000
Overall mean ± SE	3.21±0.38	3.08±0.42	
Eosinophils (%)			
0^{th}	2.50±0.43	2.00±0.52	0.473
30^{th}	2.00±0.26	2.17±0.48	0.765
60^{th}	2.00±0.26	1.83±0.54	0.787
90^{th}	2.17±0.48	2.00±0.52	0.817
Overall mean ± SE	2.17±0.18	2.00±0.24	

(%), monocytes (%) and eosinophils (%) are 30.46±1.00, 63.79±1.24, 3.08±0.42 and 2.00±0.24, respectively. All the values were found within the normal range.

According to Weiss (2010), supplemental Fe does not affect haematocrit, haemoglobin, serum Fe, Fe binding capacity saturation and percentage saturation. Cope *et al.* (2009) reported non-significant effect of zinc supplementation on haematology of dairy cows. Similarly, Ramulu *et al.* (2015) reported no change in haematological profile of buffalo calves when supplemented with zinc. However, Sobhanirad and Naserian (2012) found increase in TEC, Hb and PCV and no difference in TLC and leukocyte profile. There are no studies available for Zn and Fe biofortified feed being given to dairy animals for comparison.

Plasma IgG1 concentration (mg/mL) of lactating murrah buffaloes

There was non-significant (p>0.05) difference between the two groups in the current study (Table 7). Overall mean concentration for the control (T-$_0$) and treatment group (T_1) was 19.04±0.12 and 19.05±0.09 mg/mL, respectively. According to Fagari-Nobijari *et al.* (2012), plasma protein concentrations increased along with plasma zinc concentration when supplementation of zinc was done in Holstein bulls. Similarily, Chandra *et al.* (2014) reported higher plasma zinc concentration, plasma total immunoglobins and IgG in peripartum Sahiwal cows when supplemented with zinc. However, in the present study, there were no differences and

Table 7: Plasma IgG1 concentration (mg/mL) of lactating Murrah buffaloes.

Day	Control (T_0)	Treatment (T_1)	p-value
0^{th}	19.13±0.34	19.20±0.32	0.885
30^{th}	18.98±0.16	19.02±0.16	0.862
60^{th}	19.01±0.24	18.97±0.10	0.872
90^{th}	19.03±0.20	19.00±0.13	0.914
Overall Mean ± SE	19.04±0.12	19.05±0.09	

this might be because of similar concentrations of zinc in the plasma of control (T_0) and treatment (T_1) group.

CONCLUSION

The results of present study infer that biofortified wheat variety (WB 2) straw-based ration is similar to conventional wheat straw-based ration. Feeding of such biofortified straw does not have any impact on immunity of lactating Murrah buffaloes.

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Authorship contribution statement

DSK carried out the experiment and prepared the manuscript draft. AKM and SBNR designed the experiment and edited the draft.

Conflict of interest

The authors declare that they have no competing interests.

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