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Research Article

Serum biochemical and haematological status of growing calves fed graded level of animal feed grade wheat as partial replacement of maize

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Abstract

The present study was undertaken to evaluate the potential utilization of animal feed grade wheat in calf feeding. Animal feed grade wheat is unsuitable for human consumption but may serve as an economical feed resource for livestock. Experimental animal feed grade wheat contained 12.7% crude protein, and aflatoxin B1content was 25 µg/kg DM. Twenty-one growing Jersey crossbred male calves were divided into three groups and fed individually on the diet, comprising paddy straw and concentrate in a 40:60 ratio under stall feeding. Calves were fed three type of iso-nitrogenous concentrate mixture which contained 00, 12 and 20% animal feed grade wheat replacing equal amount of maize grain. Inclusion of animal feed grade wheat up to 20% in the concentrate mixture in calf's diet did not influence daily feed intake and haematological attributes such as haemoglobin concentration, total erythrocyte count, total leukocyte count, platelet count, and neutrophil percentage. Blood glucose concentration remained unaffected at 12% inclusion but was reduced ($P<0.05$) at 20% inclusion level of animal feed grade wheat. Serum IgG, alkaline phosphatase, and aspartate aminotransferase concentrations were not influenced by dietary treatments, while alanine aminotransferase concentration increased significantly at the 20% inclusion level of animal feed grade wheat.

It was concluded that animal feed grade wheat can be safely incorporated up to 12% of the concentrate mixture, replacing an equivalent quantity of maize grain, without adverse effects on feed intake, haematological and blood biochemical profile of growing calves to economize animal feed cost and cost of animal production.

Keywords: Animal feed grade wheat, Body immunity, Feed intake, Hematology, Liver enzymes

Highlights

- Inclusion of animal feed grade wheat at 20% of concentrate mixture by replacing equal quantity of maize grain had no adverse effect on daily feed intake of calves.
- RBC, WBC and platelet counts in blood were not affected by feeding animal feed grade wheat at 20% of concentrate mixture.
- Serum IgG concentration was not influenced by the inclusion of animal feed grade wheat at 20% of concentrate mixture in the cattle diet.

INTRODUCTION

Cost of animal feed is very high now-a-days and feed cost accounts for 65-70% of the total cost of animal production, while cereal grains itself constitute about 30-50% of most of the concentrate mixtures fed to different categories of livestock (Naik et al., 2025). Since the demand of food grains for human consumption is increasing due to the increasing human population and bioethanol production, it is neither logical nor desirable to feed the cereal grains to the animals. Therefore, it is necessary to replace certain proportion of conventional feed ingredients, particularly cereal grains (e.g., maize or barley as an energy source) of animal's diet by non-conventional feed resources that do not compete with human food to feed livestock to economize the cost of animal feed and animal production (Singh, 2018; Sime & Duguma, 2025).

About 23 million tonnes of cereal grains are lost in India annually due to lack of storage infrastructure and inefficiencies within food supply chain (Sharma et al., 2025). Among these cereal grains, wheat is the major cereal grain for human consumption. The Food Corporation of India (FCI) usually purchases a huge quantity of wheat grain from the local market every year and stores them in their warehouses. However, due to improper storage conditions, the quality of approximately 7–9% of the stored wheat deteriorates over time, rendering it unfit for human consumption and is designated as animal feed grade wheat

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(Tripathi et al., 2008). Market cost of animal feed grade wheat is very low in comparison to wheat that suitable for human consumption. However, nutrient density of animal feed grade wheat is as good as wheat for human consumption and can be used to replace the conventional source of dietary cereal grains like maize or barley grain up to certain percentage to economize the cost of animal feed as well as to economize the cost of animal production (Tripathi et al., 2008). Therefore, the present study was conducted to assess the effect of feeding animal feed grade wheat on blood biochemical parameters and body immune response in growing calves, with the objective of assessing its suitability as an economical feed alternative.

MATERIALS AND METHODS

Experimental animals and feeding management: Animal experiment was conducted in the animal farm (cattle yard) at Eastern Regional Station (ERS), ICAR-National Dairy Research Institute (ICAR-NDRI), Kalyani, West Bengal, India. Twenty-one growing Jersey crossbred (Jersey X Tharparkar) male calves about seven months age and having initial average body weight of 86.6±4.02 kg, were adopted from the herd of ERS, ICAR-NDRI, Kalyani, West Bengal, India for conducting the experiment. These calves were randomly divided into three groups (G1, G2 and G3) of seven animals each, so that average body weight of each group becomes similar. All the experimental calves were vaccinated against prevailing diseases like FMD, HS and anthrax, and were also dewormed with ivermectin (Ivectin®) and albendazole (Wormar®) before starting of the experiment. Calves of each experimental group maintained under individual feeding on roughage (paddy straw) and concentrate based ration under stall feeding for 150 days to meet out maintenance and growth (600 g average daily gain) requirement (National Research Council [NRC], 2001). Roughage and concentrate mixture were offered separately, and their ratio was maintained at 40:60 throughout the experimental period. Three type of iso-nitrogenous concentrate mixtures (C1, C2 and C3) which contained 00, 12 and 20% animal feed grade wheat by replacing same amount of maize grain, were prepared. Concentrate mixture C1, C2 and C3 were fed to the experimental calves from G1, G2 and G3 groups, respectively. Experimental calves were offered weighed quantity of feed once a day at 9:00 AM every day and the residue which left was recorded the next day at 8:30 AM. The offered feed and residue were sampled at weekly intervals throughout the experimental period for subsequent analysis of dry-matter (DM) to assess average DM intake during the experimental period. Collected feed samples for three-week collection were pooled together for further chemical analysis. Clean and fresh drinking water was offered ad libitum twice to each animal daily at 10.00 and 16.00 h.

Blood samples collection: Blood samples were obtained from each experimental calf at 1, 30, 60, 90, 120 and 150 days of experimental feeding. Before offering feed, blood samples were collected from the jugular vein into 10 ml tube containing Na heparin as anticoagulant. Blood samples were centrifuged (5000 x g for 15 minutes at 4°C) within 10 minutes, and separated plasma was collected. Blood samples were also collected without any anticoagulant to separate serum. Separated serum and plasma were frozen immediately at -20°C until analyzed.

Chemical analysis of feeds and fodder: The chemical composition of offer feeds was analyzed for estimating its dry matter (DM) content by drying at 100°C for 24 h, organic matter (OM) by ashing at 550°C for 4 h and crude protein (CP) by the Kjeldahl technique (Association of Official Analytical Chemists [AOAC], 2023). Fibre fractions of the offered feed, e.g., NDF, ADF and ADL content were estimated following the method of Van Soest et al. (1991). Cellulose content of the offered feed (paddy straw and concentrate mixture) was calculated by subtracting ADL from ADF. DM content of the residue feed was also estimated. Starch content of the animal feed grade wheat was estimated by the method of Raghuramalu et al. (1983), while aflatoxin B1 content of the animal feed grade wheat and concentrate mixtures was estimated by HPLC method with the principles described by Pans et al. (1966).

Analysis of haematological and blood biochemical parameters: Haematological attributes, e.g., haemoglobin, red blood cells, white blood cells, platelets, PCV, MCV and MCH were estimated in whole blood immediately after collection of blood following the methods of Jain (1986). Blood glucose (Cooper & McDaniel, 1970), urea nitrogen (Rahamatullah & Boyde, 1980), total protein and albumin (Annino, 1976) were estimated in separated blood plasma. Serum ALP, serum ALT and serum AST were estimated using a commercially available test kit from M/S Coral Clinical Systems, Tulip Diagnostics (P) Ltd., India. Immunoglobulin (IgG) concentration in serum was evaluated using the ELISA Kit (M/S ELK Biotechnology, India), following the manufacturer's instructions.

Statistical analysis: Statistical analyses of the data were carried out according to ANOVA – a one-way analysis of variance (Snedecor & Cochran, 1994). The difference between the treatments mean was tested using the Duncan's multiple range test (Duncan, 1955).

RESULTS

Chemical composition of experimental ration and feed intake: The animal feed grade wheat used in the present study contained 94.8% dry matter (DM), 96.8% organic matter (OM) and 12.7% crude protein (CP). The starch content was 728 g/kg DM. The level of aflatoxin B1in animal feed grade wheat in the present experiment was 25 µg/kg while in all the three concentrate mixture it was undetectable. The formulated concentrate mixtures, designated as C1, C2, and C3, contained 16.6%, 16.7%, and 16.4% CP and 14.6, 14.8 and 15.1% cellulose on DM basis, respectively (Table 1). The paddy straw offered to the animals contained 3.3% CP, 81.1% Total-CHO, 36.9% cellulose and 6.0% lignin on a DM basis.

DM intake through roughage (paddy straw) and concentrate mixture by the experimental calves as well as daily DM intake per unit body weight was not affected significantly due to replacement of maize grain with animal feed grade wheat in the experimental ration (Table 2). On average, experimental calves consumed 2.0 kg dry matter through paddy straw and 3.1 kg dry matter through concentrate mixture per day.

Haematological attributes: The haematological profiles of Jersey crossbred calves are presented in

Table 1. Composition of ration fed to the experimental calves

Attributes	Concentrate mixtures			Paddy straw
	C1	C2	C3	
Ingredient composition (on % air dry basis)				
Maize	40	28	20	-
Animal feed grade wheat	00	12	20	-
Wheat bran	37	39	43	-
Mustard oil cake	20	18	14	-
Mineral mixture	2	2	2	-
Salt	1	1	1	-
Vitamin (Vitablend®AD3)	@ 20 g/100 kg concentrate	@ 20 g/100 kg concentrate	@ 20 g/100 kg concentrate	
Chemical composition (on % DM)				
Organic matter (OM)	93.2	93.3	93.6	85.9
Crude protein (CP)	16.6	16.7	16.4	3.3
Ether extract (EE)	4.3	4.2	3.9	1.5
Total carbohydrate (Total-CHO)	72.3	72.4	73.2	81.1
Neutral detergent fibre (NDF)	36.2	37.5	39.2	73.5
Acid detergent fibre (ADF)	18.8	19.2	19.6	42.9
Cellulose	15,1	14.8	14.6	36.9
Acid detergent lignin (ADL)	3.7	4.0	4.2	6.0
Aflatoxin (ppm)	ND	ND	ND	-

ND: Non detectable.

Table 2. Effect of feeding animal feed grade wheat on dry-matter intake of calves

Attributes	Concentrate mixtures			SEM	P - value
	C1	C2	C3		
Dry matter intake (kg/d)					
Roughage (Paddy straw)	2.2	2.0	1.8	0.18	0.36
Concentrate mixture	3.2	3.2	2.9	0.23	0.27
Total	5.4	5.2	4.7	0.33	0.49
Roughage: concentrate ratio	40.7:59.3	38.5:61.5	38.3:61.7	0.02	0.36
DMI (kg/100 kg body weight/d)	3.1	3.1	3.0	0.05	0.48
DMI (g/kg $W^{0.75}$/d)	112.9	111.8	105.9	3.14	0.51

DMI: Dry matter intake, SEM: Standard error of means.

Table 3. Effect of feeding animal feed grade wheat on haematology of calves

Attributes	Experimental groups			SEM	P-value
	G1	G2	G3		
Haemoglobin (%)	12.1	11.9	11.8	0.19	0.15
Red blood cells (x10^6/mmL)	8.1	7.8	7.6	0.41	0.18
White blood cells (x10^3/mmL)	8.6	8.4	7.9	0.87	0.26
Platelets (lakhs /mmL)	4.2	3.9	3.6	0.69	0.17
Lymphocyte	61.4	62.2	60.8	0.96	0.35
Neutrophils	30.4	30.9	31.4	0.62	0.29
Monocytes	3.1	2.2	2.6	0.26	0.14
Eosinophils	5.1	4.7	5.2	0.37	0.28
Haematocrit / PCV (%)	33.7	33.2	31.5	1.04	0.37
Mean corpuscular volume / MCV (fL)	42.4	41.6	40.9	0.75	0.52
Mean cell haemoglobin / MCH (pg)	15.4	15.1	14.9	0.19	0.25

SEM: Standard error of means

Table 4. Effect of feeding animal feed grade wheat on blood biochemical parameters in calves

Attributes	Experimental groups			SEM	P-value
	G1	G2	G3		
Glucose (mg/dL)	64.1^b	65.1^b	59.8^a	0.77	<0.05
BUN (mg/dL)	34.2	34.9	36.1	0.73	0.08
Total Protein (g/dL)	6.9	7.1	6.8	0.11	0.21
Plasma albumin (g/dL)	3.2	3.2	3.1	0.04	0.19
Plasma globulin (g/dL)	3.7	3.9	3.7	0.11	0.28
Albumin: Globulin	0.86	0.82	0.83	0.03	0.36
Serum ALP (IU/L)	159.6	161.8	163.2	2.41	0.39
Serum ALT (IU/L)	16.9^a	17.4^a	19.4^b	0.74	<0.05
Serum AST (IU/L)	41.7	42.9	45.4	2.62	0.11
Serum IgG (g/L)	26.2	25.3	24.5	1.38	0.09

SEM: Standard error of mean, Mean with different superscript in a row differ significantly (P<0.05).

Table 3. Inclusion of animal feed grade wheat at levels up to 20% of the concentrate mixture, as a replacement for an equal proportion of maize grain, did not influence haemoglobin concentration, red blood cell (RBC) count, white blood cell (WBC) count, or platelet count. Similarly, the proportions of different leucocytes, haematocrit percentage, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) values remained comparable across all experimental groups. On average, the blood of experimental calves contained 11.9 g/dL haemoglobin, 7.8 × 10^6/µL RBCs, and 8.3 × 10^3/µL WBCs. Platelet counts were recorded as 4.2, 3.9, and 3.6 lakh/µL in the G1, G2, and G3 groups, respectively.

Blood metabolites, liver enzymes and body immunity: The concentrations of blood biochemical metabolites, including blood urea nitrogen, total protein, albumin, globulin, albumin: globulin ratio, serum alkaline phosphatase (ALP), and serum aspartate aminotransferase (AST) or SGOT, did not differ significantly among the experimental groups (Table 4). However, blood glucose concentration was significantly higher (P<0.05) in group G3, followed by G2 and G1. Serum alanine aminotransferase (ALT) / SGPT concentration was also greater (P<0.05) in calves from group G3 compared with those in G1 and G2. Similarly, serum IgG concentrations did not differ significantly among the groups, with mean values of 26.2, 25.3 and 24.5 g/L in G1, G2, and G3, respectively. All biochemical parameters measured in this study remained within the normal physiological range.

DISCUSSION

Composition of experimental feed and feed intake: Almost every year Food Corporation of India (FCI) discarded certain amount of wheat which are become unfit for human consumption and sell them as animal feed grade wheat in very cheaper price in comparison

to wheat use as human food. Animal feed grade wheat generally contain aflatoxin in variable amount due to fungal/mould infestation (Tripathi et al., 2008). Aflatoxin content in the concentrate mixtures in the present experiment was so low that it was not detected even using high performance liquid chromatography (HPLC) method. Animal feed grade wheat in the present experiment, contained less amount of aflatoxin B_1 compare to earlier reported by Tripathi et al. (2008). The maximum permissible level of Aflatoxin B_1 in cattle feed according to Bureau of Indian Standards (BIS), is 20 parts per billion (ppb) or 20 µg/kg. Heath condition of the experimental calves during 150 days of experiment was excellent and showed no sign of ill health indicated feeding of concentrate mixture, having 20% animal feed grade wheat had no apparent effect on the health of growing calves. The chemical composition of paddy straw used in the present experiment was comparable to the values reported earlier by Santra et al. (2021) and Gani et al. (2025).

Similar dry matter intake among calves from the three experimental groups indicated that inclusion of animal feed–grade wheat up to 20% of the concentrate mixture in the ration of growing calves had no effect on daily dry matter intake. In agreement with this finding, Tripathi et al. (2008) reported that in lambs, daily dry matter intake was not affected by the inclusion of animal feed–grade wheat up to 47% of the concentrate mixture. On average, the experimental crossbred calves in the present study consumed 3.1 kg dry matter per 100 kg body weight per day. Previously, at the same experimental site, Santra et al. (2012) observed that Jersey male crossbred calves consumed 3.4 kg dry matter per 100 kg body weight per day under stall-feeding conditions.

Haematological parameters: Despite feeding animal feed grade wheat in different proportion in concentrate mixture, the blood parameters remained stable, suggesting that animal feed grade wheat up to 20% of concentrate mixture had minimal or no effect on the haematological profile of growing Jersey crossbred calves under the conditions studied. The lack of significant differences in Hb levels indicated that feeding of animal feed grade damage wheat has a limited effect on blood parameters. The mean Hb concentration in the blood of experimental calves was within the normal range of cattle as reported by Jain (1986), and it was ranged from 11.5±0.56 to 15.06±1.36 g/dL. Earlier, Raghunandanan et al. (1977) and Mahima et al. (2013) in Hariana Cattle, Pyne and Maitra (1981) in Sahiwal cattle, Deshpande et al. (1986) in Red Kandhari cattle and Srinivasan and Sathiamoorthy (2021) in Pulikulam cattle also reported similar range on Hb concentration in blood. The Hb level in the present study also similar to the earlier report of Rewar et al. (2025) in Rathi calves.

Blood lymphocyte counts indicate the body immune status. Similar lymphocyte counts among the all-experimental calves in present study indicated that feeding of animal feed grade wheat did not alter the body immune status of the experimental calves. Monocyte and neutrophil content in blood indicates the inflammatory response in the body. Similar monocyte and neutrophil content in blood among the calves from three experimental groups in present study, suggests that feeding of animal feed grade damage wheat had no impact on inflammatory response of calves. Tripathi et al. (2008) also reported in growing Malpura lamb that inclusion of animal feed grade wheat up to 23% of concentrate mixture by replacing equal proportion of maize grain had no effect on blood Hb, RBC, WBC, PCV, MCV and MCH content as well as differential blood WBC count.

Plasma and serum biochemical parameters: Blood composition data were used as an indication of the health status of the experimental animals. Feeding animal feed grade wheat up to 20% of concentrate mixture in the present experiment did not affect health status of experimental calves. Battacone et al. (2003) reported that ruminants are more tolerant to the dietary mycotoxin feeding. During the experiment, all the blood values recorded were within the normal range of variation for animals of similar age group. The concentration of different blood metabolites in the present study is in agreement with the values reported earlier for cross-bred calves (Sahoo, 1994; Santra et al., 1999). Earlier, it was also reported that concentration of plasma urea, total protein and albumin was not influenced by the replacement of dietary barley grain up to 100% level by animal feed grade wheat in the ration of growing lambs (Santra et al., 2001-2002). Lower blood glucose level in the calves of G3 group might be due to higher content of animal feed grade wheat by replacing maize grain in their ration which leads to lower starch intake in comparison to other two experimental groups as animal feed grade wheat content lesser amount of starch than maize grain. Mould/insect infestation reduced the starch content by 5% or more in animal feed grade wheat, compared to human food grade wheat (Tripathi et al., 2008). Therefore, starch content of the ration becomes decrease if the ration content more

amount of animal feed grade wheat by replacing maize grain resulting in lower blood glucose level.

Activities of liver enzymes in blood generally increase following intake of dietary mycotoxin (Pier, 1992). Generally, activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the blood is studied to evaluate the function of liver. Symptoms of early liver damage generally unapparent and that's why elevated activities of different liver enzymes like ALT, ALP and AST is the only means to detect early liver damage (Casteel et al., 1995). Higher ($P<0.05$) activities of alanine aminotransferase (ALT) enzymes or SGPT in the blood of experimental calves fed with concentrate mixture having 20% animal fed grade wheat indicated adverse effect on liver function. Tripathi et al. (2008) observed that ALT level in blood increased with increasing level of animal feed grade wheat (up to 25%) in the diet of lambs while, blood concentration of ALP and ASP remain unaffected. The concentration of IgG in blood of experimental calves was similar to the IgG concentration reported by Costa et al. (2021) and Martin et al. (2021) in dairy calves. Similar immunoglobulin (IgG) level in the blood of experimental calves from three experimental groups in the present experiment indicated that dietary feeding of animal feed grade wheat up to 20% of concentrate mixture by replacing equal amount of maize grain had no effect on body immune status of the animal. Earlier, Tripathi et al. (2008) also observed that replacement of conventional cereal grain, e.g., maize grain by animal feed grade wheat up to 23% had no effect on body immunity in growing lambs.

The findings of the present study suggest that the inclusion of animal feed-grade wheat containing aflatoxin B1 at a concentration of 25 µg/kg dry matter (DM) is safe for use in the diets of growing calves when incorporated at levels up to 12% of the concentrate mixture. This level of inclusion did not exert any adverse effects on blood biochemical profiles or immune responses. However, it is essential to ensure that the total dietary aflatoxin content remains within the maximum permissible limits established by the regulatory authorities of the respective countries.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: AS, DCK, SKD: Involved in investigation, conceptualization and methodology; TT: Analysis of biological samples; DKM: Performed statistical analysis.

Data availability statement: The data mentioned in the article will be available with the corresponding author.

Ethical Approval: Experiment was conducted in accordance with the established guidelines set forth by the institution's (ICAR-NDRI, Karnal, Haryana) animal ethics committee.

Use of artificial intelligence tools: Artificial intelligence tools was not used for writing the research paper.

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