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Hematological and Serum Biochemical Profiles of Hubbard Broiler Chickens Fed Fermented Brewers Dried Grain as Replacement for Maize

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Abstract

A 42-days trial evaluated the effects of replacing maize with Fermented Brewers Dried Grain (FBDG) on hematology, serum biochemistry, and electrolyte profiles of broiler chickens. 300 one-day-old Hubbard broiler chicks were assigned to five treatments (6 replicates of 10 birds each) under a Completely Randomized Design (CRD). Diets were formulated isonitrogenous and isocaloric according to NRC requirements. Treatment 1 (control) contained no FBDG, while T2, T3, T4, and T5 replaced maize with FBDG at 5%, 10%, 15%, and 20%, respectively. Solid-state fermentation significantly upgraded raw BDG: Crude Protein (18.0 to 27.1%), Ash (5.08 to 6.89%), and Metabolizable Energy (1980 to 2980 kcal/kg) increased, whereas Crude Fiber (16.0 to 10.01%) and Ether Extract (4.98 to 3.91%) decreased. Hematological indices (PCV, Hb, RBC, MCV, MCH, MCHC, WBC) were significantly higher ($P<0.05$) in FBDG groups (T2-T5) than T1. Similarly, serum total protein, albumin, globulin, calcium, phosphorus, potassium, and bicarbonate were elevated in T2-T5 relative to T1. Conversely, serum AST, ALT, ALP, urea, creatinine was not significantly affected. In conclusion, solid-state fermentation enhances the bio-nutritional value of BDG, and replacing maize up to 20% with FBDG improves oxygen-carrying capacity, protein metabolism, mineral balance, and hypocholesterolemic status without inducing hepato-renal stress.

Keywords: Brewers Dry Grain; Broilers; Blood; Fermentation; Yeast; Anti-Nutrients

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Introduction

Maize (Zea mays) is traditionally the primary energy constituent in poultry rations, often making up 50% to 70% of the total diet [1]. However, intense competition for maize between human nutrition, livestock feeds, and industrial applications such as biofuel production has inflated its market price [2, 3]. To ensure the financial viability and long-term sustainability of broiler enterprise, researchers and animal nutritionists are actively evaluating low-cost, non-conventional Agro-Industrial By-Products (AIBPs) capable of partially or fully replacing maize without compromising physiological health or growth performance [4].

The main constraint to using agro-industrial by-products like Brewers Dried Grain (BDG) as maize substitutes in monogastric diets is their high fibre composition and the presence of anti-nutrients [5]. BDG is the solid residue remaining after the extraction of wort from barley malt and secondary grains during brewing [6]. It is rich in crude protein (20%-28%) and minerals, but it also contains high levels of Crude Fiber (CF) and insoluble Non-Starch Polysaccharides (NSPs) such as cellulose, hemicellulose, and lignin [7]. Monogastric animals, including broiler chickens, lack endogenous enzymes (such as cellulases and xylanases) necessary to hydrolyze these complex plant cell wall structures. Consequently, high inclusion levels of raw BDG can impair the access of endogenous digestive enzymes (pepsin, trypsin, amylase, lipase) to ingested nutrients [8, 9], dilutes dietary metabolizable energy and accelerates gut transit time, limiting overall nutrient absorption [10].

Blood parameters serve as internal indicators of physiological health, metabolic status, and tissue safety [11]. Ingesting unprocessed or high-fiber agro-industrial residues can induce metabolic stress, subclinical anemia, organ toxicity, or impaired protein synthesis, which manifest as alterations in blood profiles [12]. Therefore, understanding the physiological boundary of maize replacement with

BDG requires thorough evaluation of systemic blood homeostasis.

Previous investigations have shown that substituting maize with cassava by-products reported that high cyanogenic glycoside content and elevated fiber led to reduced Packed Cell Volume (PCV), lower Hemoglobin (Hb) concentrations, and elevated serum transaminases (AST and ALT), signaling subclinical anemia and hepatocyte stress when fed raw or unfermented [13]. Research evaluating raw palm kernel cake as a replacement for maize observed a significant drop in Red Blood Cell (RBC) counts and serum total protein at inclusion levels exceeding 10%-15%. This was attributed to the high β-mannan fiber content, which restricted protein digestibility and mineral absorption required for erythropoiesis [13].

Investigations utilizing rice bran and wheat offal demonstrated that moderate inclusion levels maintained stable hematological profiles, whereas higher levels elevated serum urea and creatinine, indicating increased amino acid oxidation and elevated renal clearance burdens [14]. Conversely, trials utilizing biologically processed or solid-state fermented agro-wastes (such as fungal- or yeast-fermented cassava peels, cocoa pod husks, and rice bran) showed improved hematological values (elevated PCV, Hb, and RBC) and balanced serum proteins [15]. Fermentation reduced anti-nutritional factors, degraded complex NSPs, and released bioavailable minerals (Fe^{2+}, Cu^{2+}) necessary for hematopoiesis [15].

Although Brewers Dried Grain is widely available and cost-effective, its direct application in broiler diets is limited by fiber constraints and variable energy density. Processing strategies such as solid-state microbial fermentation can upgrade its nutritional value and reduce their level of anti-nutrients. Therefore, evaluating the effects of replacing maize with Brewers Dried Grain on the hematological and serum biochemical profiles of broiler chickens is necessary to confirm that this feed ingredient supports optimal growth without inducing physiological stress, organ damage, or metabolic impairment.

Materials and Methods

Experimental site and environmental conditions

The feeding trial was conducted from March to May 2025 at the Poultry Unit, Section of Animal Husbandry and Animal Nutrition, Gandhi College of Agriculture, Rajasthan, India. The farm is geographically positioned at latitude 26°55'N and longitude 75°49'E. The area experiences a semi-arid climate; during the experimental period (March to May), the average daily ambient temperature ranged between 28.5°C and 41.2°C, with a mean relative humidity ranging from 32% to 58%. The region receives an average annual rainfall of approximately 500 mm (Encyclopedia Britannica, 2026). Brooding and rearing temperatures were maintained using automated thermostatic controllers within the environmentally controlled house.

Institutional ethical approval

All animal handling protocols, surgical procedures, and blood sampling techniques were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Gandhi College of Agriculture (Approval Protocol No.: GCA/IAEC/2025/POUL-04). Birds were humanely cared for in strict accordance with national guidelines for laboratory animal welfare.

Pre-experimental operations and sanitation

Two weeks prior to bird arrival, the environmentally controlled battery cage and poultry house was thoroughly swept, washed with high-pressure hot water, and disinfected using a broad-spectrum disinfectant solution containing 2% glutaraldehyde and benzalkonium chloride (Kohrsolin TH, Bode Chemie GmbH, Hamburg, Germany). Feed troughs, nipple drinkers, tray liners, and brooding equipment were scrubbed, disinfected, and sun-dried. The pen was kept sealed for 10 days before chick placement.

Collection, processing, and *saccharomyces cerevisiae* fermentation of BDG

To process Brewers Dried Grain (BDG) *via* fungal solid-state fermentation, fresh wet spent grain is first collected from a brewery, pressed or spun to drain excess liquor, and oven-dried or sun-dried to a stable moisture level (~10-12%) for storage before being rehydrated with sterile distilled water to achieve an optimal moisture content of 65%. The rehydrated substrate is pasteurized or steam-sterilized at 121°C for 15-20 minutes to eliminate indigenous microbes, cooled to room temperature (~28-30°C), and inoculated with an active culture of *Saccharomyces cerevisiae* (live baker's or brewer's yeast) at a rate of 1-5% (w/w, providing approximately 1×10^7 CFU/g of dry substrate). The inoculated mass is mixed thoroughly, spread into shallow anaerobic or micro-aerophilic fermentation vessels fitted with one-way airlocks or gas-permeable covers, and incubated at a controlled temperature of 28-32°C for 48 to 72 hours. During this incubation, *S. cerevisiae* actively metabolizes simple sugars, secretes exogenous enzymes, synthesizes B-complex vitamins, and converts non-protein nitrogen into single-cell microbial protein while producing organic acids that lower substrate *pH*. Finally, the fermented biomass is oven-dried at low heat (50-55°C) to arrest yeast metabolic activity while preserving heat-sensitive enzymes, vitamins, and probiotic cell wall components (such as β-glucans and mannan-oligosaccharides), before being ground into a fine, nutrient-enriched meal for feed incorporation.

Experimental animals, design, and housing

300 one-day-old unsexed Hubbard broiler chicks were purchased from a reputable hatchery in Rajasthan, India within the month of September, 2025. On arrival, average initial body weight of chicks was recorded using a digital electronic balance (Model A&D FX-300i, A&D Company Ltd., Tokyo, Japan; accuracy: 0.001 g). Birds were randomly allocated to 6 dietary treatments in a Completely Randomized Design (CRD). Each treatment consisted of 6 replicates with 10 birds per replicate. Birds were housed in three-tier galvanized battery cages (100 cm×60 cm×45 cm per pen) equipped with nipple drinkers and trough feeders, providing a floor space allowance of approximately 600 cm^2/bird. Starter (Days 1-21) and Finisher (Days 22-42) diets were formulated to be isonitrogenous and isocaloric, fulfilling NRC [16] nutritional requirements for broiler chickens. Feed and clean drinking water were offered at all times. Routine vaccinations were administered according to the prevalent disease profile in the region: At day 5: Newcastle Disease (ND) vaccine (Lasota strain, Hester Biosciences Ltd., Gujarat, India) via eye-drop. Day 12: Infectious Bursal Disease (IBD) vaccine (Intermediate strain, Venkateshwara Hatcheries Pvt. Ltd., Pune, India) via drinking water. Day 19: IBD Booster vaccine via drinking water. Day 28: Newcastle Disease (Lasota strain) booster via drinking water. The replacement level of maize with FBDG (percentage) includes (Table 1-3):

T1 (Control) - 0% FBDG (100% Maize control)

T2 - 5% FBDG replacing Maize

Table 1: Composition of the experimental diets (Starter) from 0-28 days.

Ingredients	T1	T2	T3	T4	T5
Maize	51.33	48.76	46.2	43.63	41.06
Fermented Brewers Dry Grain (FBDG)	0	2.57	5.13	7.7	10.27
Wheat bran	4.11	4.11	4.11	4.11	4.11
Fish meal	4.00	4.00	4.00	4.00	4.00
Soybean meal	35.01	35.01	35.01	35.01	35.01
Limestone	1.50	1.50	1.50	1.50	1.50
Mono Calcium Phosphate	3.00	3.00	3.00	3.00	3.00
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Growers Mineral-Vitamin Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30	0.30
Total	100.0	100.0	100.0	100.0	100.0
Determined analysis					
Crude protein	23.44	23.21	23.13	23.08	23.02
Crude fibre	3.96	3.87	3.85	3.83	3.82
Ether extract	4.13	4.08	4.05	4.03	4.01
Ash	5.06	6.02	6.02	6.02	6.02
Energy (Kcal/kg)	2998.7	2996.3	2995.1	2993.6	2991.4

Each 2.5 kg contain: 6000000 IU Vit. A; 900000 IU Vit. D3; 40000 mg Vit. E; 2000 mg Vit. K3; 2000 mg Vit. B1; 4000 mg Vit. B2; 2000 mg Vit. B6; 10 mg Vit. B12; 50 mg Biotin; 10000 mg Pantothenic acid; 50000 Niacin; 3000 mg Folic acid; 250000 mg Choline; 8500 mg Mn; 50000 mg Zn; 50000 mg Fe; 200 mg I; 100 mg Se, 5000 mg Cu

Table 2: Composition of the experimental diets (Finisher) from 28-42 days.

Ingredients	T1	T2	T3	T4	T5
Maize	53.8	51.11	48.52	45.73	43.04
Fermented Brewers Dry Grain (FBDG)	0.00	2.69	5.38	8.07	10.76
Wheat bran	4.00	4.00	4.00	4.00	4.00
Soya meal	35.1	35.1	35.1	35.1	35.1
Fish meal	2.00	2.00	2.00	2.00	2.00
Limestone	4.00	4.00	4.00	4.00	4.00
Mono Calcium Phosphate	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Mineral-Vitamin Premix	0.35	0.35	0.35	0.35	0.35
Salt	100.0	100.0	100.0	100.0	100.0
Total					
Determined analysis					
Crude protein	21.11	21.02	20.92	20.88	20.83
Crude fibre	4.34	3.94	3.89	3.87	3.86
Ether extract	3.93	3.78	3.75	3.73	3.71
Ash	5.87	6.83	6.83	6.83	6.83
Energy (Kcal/kg)	3110.2	3108.9	3106.7	3102.6	3100.2

Each 2.5 kg contain 6000000 IU Vit. A; 900000 IU Vit. D3; 40000 mg Vit. E; 2000 mg Vit. K3; 2000 mg Vit. B1; 4000 mg Vit. B2; 2000 mg Vit. B6; 10 mg Vit. B12; 50 mg Biotin; 10000 mg Pantothenic acid; 50000 Niacin; 3000 mg Folic acid; 250000 mg Choline; 8500 mg Mn; 50000 mg Zn; 50000 mg Fe; 200 mg I; 100 mg Se, 5000 mg Cu

T3 - 10% FBDG replacing Maize

T4 - 15% FBDG replacing Maize

T5 - 20% FBDG replacing Maize

Data collection

Hematological determination: Blood (2.5 mL) was drawn from the bronchial vein into K2-EDTA vacuum tubes (BD Vacutainer, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Samples were analyzed within 2 hours using an Abacus 380 Automated Veterinary Hematology Analyzer (Diatron MI PLC, Budapest, Hungary): Technical Specifications: 3-part WBC differential; 22 parameters; impedance method for cell counting (RBC, WBC, Platelets); light absorption photometry at 540 nm for Hemoglobin

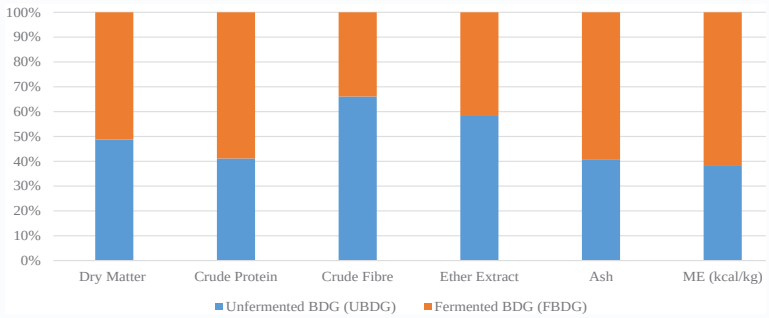


Table 3: Proximate composition of Unfermented (UBDG) and Fermented Brewers Dry Grain (FBDG).

(Hb); throughput of 80 tests/hour. Packed Cell Volume (PCV) and erythrocyte indices (MCV, MCH, and MCHC) were computed automatically.

Serum biochemistry, mineral electrolytes, and enzymes: Blood (2.5 mL) was collected into plain serum separation tubes, clotted at 25°C for 30 minutes, and centrifuged at 3,000×g for 10 minutes at 4°C (Eppendorf Centrifuge 5810 R, Eppendorf AG, Hamburg, Germany). Serum was analyzed on an Erba XL 200 Fully Automated Clinical Chemistry Analyzer (Erba Lachema Brno, Czech Republic) using pre-packaged colorimetric diagnostic kits (Erba Diagnostics Mannheim GmbH, Mannheim, Germany): Technical Specifications: Photometric throughput of 200 tests/hour; diffraction grating spectrophotometer (340-800 nm wavelength range); Peltier-controlled cuvette temperature (37°C±0.1°C).

Specific Assay Methods: Total Protein (Biuret method), Albumin (Bromocresol Green method), AST, ALP and ALT (UV kinetic IFCC method without pyridoxal phosphate), Urea (Urease-GLDH kinetic method), Creatinine (Modified Jaffe kinetic method), Cholesterol (CHOD-PAP enzymatic colorimetric method), Calcium (Arsenazo III method), and Phosphorus (Molybdate UV method).

Electrolytes (K^+ and HCO^{3-}): Measured using an InLyte Automated Ion-Selective Electrode (ISE) Electrolyte Analyzer (Fisher Scientific Inc., Waltham, MA, USA) with solid-state crown-ether membrane electrodes.

Proximate analysis of experimental diet and test material was carried out according to the procedure of AOAC [17].

Statistical analysis

One-Way Analysis of Variance (ANOVA) analyzed data for a Completely Randomized Design using SPSS software (Version 26.0, IBM Corp., Armonk, NY, USA). Means were separated using Duncan's Multiple Range Test with statistical significance established at $P<0.05$.

Results and Discussion

Solid-State Fermentation (SSF) of Brewer's Dried Grains (BDG) using (*Saccharomyces cerevisiae*) serves as a highly effective processing method to improve low-quality agro-industrial byproducts into high-value feedstuffs [18]. The increase in crude protein from 16.86% to 24.11% is primarily driven by the rapid proliferation of yeast biomass and the concentrating effect resulting from fungal and yeast enzymes metabolizing soluble carbohydrates. Simultaneously, the reduction in crude fiber from 19.42% down to 9.97% highlights the enzymatic breakdown of lignocellulosic structures, turning indigestible fractions into nutrients that can be easily utilized by the birds [19].

Table 4: Haematological indices of broiler chickens fed fermented Brewers dry grain.

Parameters	T1	T2	T3	T4	T5	SEM
RBC (× 10^{12}/L)	2.37^b	3.12^a	3.21^a	3.35^a	3.46^a	0.02
Hb (g/dL)	8.97^b	10.98^a	11.12^a	11.35^a	11.47^a	0.03
Haematocrit (%)	27.18^b	32.22^a	32.80^a	32.91^a	32.98^a	1.66
WBC (×10^9/L)	15.06^b	19.97^a	20.03^a	20.09^a	20.15^a	0.98
MCV (fl)	100.2^b	121.6^a	123.8^a	124.5^a	124.8^a	7.91
MCH (pg)	39.88^b	46.73^a	47.11^a	47.18^a	47.39^a	1.87
MCHC (g/dL)	30.09^b	37.18^a	37.37^a	37.81^a	37.89^a	1.25
Lymphocytes (%)	59.81^b	69.87^a	70.07^a	70.12^a	70.15^a	2.31
Monocytes (%)	0.93^b	1.97^a	1.99^a	2.02^a	2.04^a	0.02
Neutrophils (%)	20.87	20.33	20.14	20.09	20.03	0.85

Note on Superscripts: ^{a}, ^{b} Means along the same row with different superscripts are significantly different ($p<0.05$). Rows without superscripts show no significant difference ($p>0.05$); SEM: Standard error of mean.

T1 (Control)	0% FBDG (100% Maize control)
T2	5% FBDG replacing Maize
T3	10% FBDG replacing Maize
T4	15% FBDG replacing Maize
T5	20% FBDG replacing Maize

Furthermore, the increase in dry matter (86.22% to 90.56%) and ash content (4.86% to 7.12%) reflects moisture removal during processing and the relative concentration of essential minerals as organic matter is oxidized [20, 21]. Similarly, the fibrolytic activity directly accounts for the significant boost in metabolizable energy from 1873.1 kcal/kg in the unfermented brewer's dry grain to 2997.0 kcal/kg post-fermentation as complex polysaccharides were hydrolyzed into fermentable sugars and easily digestible organic acids [22, 23].

As revealed in Table 4, haematological indices of broiler chickens fed fermented Brewers dry grain. Red blood cell, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell, lymphocytes and monocytes were influenced ($p<0.05$) by the treatment except neutrophil count ($p>0.05$). However, all values recorded were within the standard reference range for healthy birds reported by Campbell [24]. Red blood cell, haematocrit and haemoglobin values were within the normal range [(2.00 – 4.50 (× 1012/L)], 22.00 – 36.00% and 7.80-15.00 g/dL reported by [25, 26]. Red blood cells are responsible for carrying oxygen from lungs to tissues and return carbon dioxide to lungs [24]. This outcome

Table 5: Serum biochemical indices of broiler chickens fed fermented Brewers dry grain.

Parameters	T1	T2	T3	T4	T5	SEM
Total protein (g/dL)	5.05[b]	6.12[a]	6.19[a]	6.25[a]	6.29[a]	0.03
Albumin (g/dL)	2.61[b]	3.07[a]	3.11[a]	3.15[a]	3.18[a]	0.02
Globulin (g/dL)	2.44[b]	3.05[a]	3.08[a]	3.10[a]	3.11[a]	0.02
Creatinine (mg/dL)	0.71	0.71	0.73	0.75	0.76	0.01
Urea (mg/dL)	4.72	4.65	4.88	4.92	4.95	0.02
HDL (mg/dL)	32.11[b]	40.82[a]	44.15[a]	44.20[a]	44.31[a]	2.11
Triglycerides (mg/dL)	48.92[b]	61.12[a]	63.08[a]	63.21[a]	63.25[a]	4.05

Note on Superscripts: [a, b] Means along the same row with different superscripts are significantly different (p<0.05). Rows without superscripts show no significant difference (p>0.05); SEM: Standard Error of Mean, HDL: High-Density Lipoprotein

T1 (Control)	0% FBDG (100% Maize control)
T2	5% FBDG replacing Maize
T3	10% FBDG replacing Maize
T4	15% FBDG replacing Maize
T5	20% FBDG replacing Maize

indicates efficient oxygen circulation to drive absorbed nutrients in the tissue of birds. Abnormal mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration suggests iron, folate, vitamin B12 deficiency and other blood disorders [27]. Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration recorded in this study aligns with the range 98.22-200 fl, 35.00-85.00 pg and 25.00-70.00 g/dL cited by [28, 29]. Fermentation lowers anti-nutrients and fibre fractions that typically chelate ions, thereby enhancing the bioavailability of iron, copper, and amino acids essential for hemoglobin synthesis [30, 31]. Low white blood cells and its differentials suggest weak immunity. Elevated white blood cell counts in fermented brewers dry grain groups suggest an active, strengthened cellular immune system without systemic inflammation, supported by prebiotics present in the fermented substrate [32]. White blood cell, lymphocytes, monocytes and neutrophil count were within normal range: 14.50 – 26.00 (×109/L), 40.00-75.00%, 0.01-4.00% and 10.20-25.00% recorded by [33, 34].

Serum biochemical indices of broiler chickens fed fermented Brewers dry grain is presented in Table 5. Total protein (5.05-6.29 g/dL), albumin (2.61-3.18 g/dL), globulin (2.44-3.11 g/dL), high-density lipoprotein (32.11-44.31 mg/dL) and triglyceride concentrations (48.92-63.25 mg/dL) were affected (p<0.05) by the treatment except creatinine (0.71-0.76 mg/dL) and urea (4.72-4.95 mg/dL) (p>0.05). Serum total protein, albumin and globulin concentrations were within standard range: 3.80-7.00 g/dL, 2.00-4.00 g/dL and 1.80-4.50 g/dL reported by [30, 35]. Elevated total protein concentrations indicate enhanced intestinal absorption of amino acids from fermented brewer's dry grain [24, 36]. The increase in globulin reflects enhanced humoral immune response and immunoglobulin synthesis [37, 38]. Lower serum urea and creatinine indicate efficient protein utilization and minimal amino acid degradation, resulting in a reduced renal filtration workload [26]. Urea and creatinine values were within 3.70-5.00 mg/dL and 0.03-1.00 mg/dL recorded by [19]. High-density lipoproteins carry cholesterol from the arteries back to the liver while triglycerides are needed for energy in the body of birds [16, 39]. Though High-density lipoprotein and triglycerides concentrations were higher in groups fed fermented Brewers dry grain (T2-T5) compared to T1, however, values were within the standard reference range reported by [18]. This suggests that the outcome promotes better heart health [12, 40].

Table 6: Serum electrolytes and enzymes of broiler chickens fed fermented Brewers dry grain.

Parameters	T1	T2	T3	T4	T5	SEM
Calcium (mg/dL)	8.21[b]	12.34[a]	12.56[a]	12.84[a]	12.92[a]	0.96
Phosphorus (mg/dL)	4.32[b]	6.09[a]	6.17[a]	6.21[a]	6.23[a]	0.02
Potassium (mmol/L)	2.33[b]	4.46[a]	4.52[a]	4.58[a]	4.61[a]	0.02
Magnesium (mg/dL)	2.12[b]	3.54[a]	3.72[a]	3.78[a]	3.91[a]	0.02
Sodium (mmol/L)	120.4	125.6	128.1	128.7	128.9	8.54
Chloride (mmol/L)	100.8	102.3	105.8	106.2	106.7	11.08
Bicarbonate (mmol/L)	12.33[b]	18.71[a]	19.02[a]	19.55[a]	19.56[a]	0.86
Alanine Amino transferase (IU/L)	48.71	50.03	51.12	51.27	51.41	0.73
Aspartate Amino transferase (IU/L)	86.11	87.09	87.22	87.45	87.51	0.61
Alanine phosphatase (IU/L)	61.13	65.08	67.18	68.09	68.11	0.54

Note on Superscripts: [a, b] Means along the same row with different superscripts are significantly different (p<0.05). Rows without superscripts show no significant difference (p>0.05); SEM: Standard error of mean.

T1 (Control)	0% FBDG (100% Maize control)
T2	5% FBDG replacing Maize
T3	10% FBDG replacing Maize
T4	15% FBDG replacing Maize
T5	20% FBDG replacing Maize

Serum electrolytes and enzymes of broiler chickens fed fermented Brewers dry grain is presented in Table 6. Calcium, phosphorus, potassium, magnesium, bicarbonate concentrations were higher in T2-T5 than T1 (p<0.05). However, sodium, chloride, Alanine amino Transferase (ALT), Aspartate amino Transferase (AST) and Alanine Phosphatase (ALP) concentrations were not affected (p>0.05). Fermentation lowers phytic acid concentration, freeing bound minerals (calcium, phosphorus, potassium and magnesium ion) for systemic intestinal absorption [26]. Higher serum bicarbonate indicates effective acid-base buffering and metabolic stability in Fermented Brewer's Dry Grain (FBDG) fed birds [29, 44]. Calcium, phosphorus and potassium concentration recorded in this experiment are within the normal range 7.00-15.00 mg/dL, 4.00-16.00 mg/dL and 2.00-8.50 mmol/L cited by Viñuela *et al.,* [40]. Magnesium, sodium, chloride and bicarbonate values were within 2.10-6.00 mg/dL, 100.2-200.4 mmol/L, 87.11-150.6 mmol/L and 10.00-30.00 mmol/L reported by [39-51]. Alanine amino Transferase (ALT), Alanine Phosphatase (ALP) and Aspartate amino Transferase (AST) leak into circulation during hepatocyte membrane damage. The non-significant difference in these parameters confirm that FBDG contains no hepatotoxins, preserving liver tissue integrity.

Conclusion

This study confirms that Fermentation can reduce the anti-nutrients and boost the nutritional profile brewer's dry grain. Replacing maize with fermented brewer's dry grain up to 20% enhanced physiological and immunological state of birds. It also enhanced nutrient absorption and metabolic balance. Haematological and serum biochemical parameters in all the treatments were within the standard reference range for healthy birds.

References

1. Alagbe JO, Oluwafemi RA. Growth performance of weaner rabbits fed Noni (Morinda citrifolia) and Moringa olifera leaf mixture as partial replacement of soya bean meal. Int J Adv Biol Biomed Res. 2019; 7(2): 185-195.
2. Alagbe JO, Omokore EA. Effect of replacing soya bean meal with Indigofera zollingeriana leaf meal on the performance and carcass characteristics of growing rabbits. Int J Multidisciplin Res Dev. 2019; 6(5): 74-77.
3. Abbasi H, Seidavi AR, Liu W, Asadpour L. Investigation on the effect of different levels of dried sweet orange (Citrus sinensis) pulp on performance, carcass characteristics and physiological and biochemical parameters in broiler chicken. Saudi J Biol Sci. 2015; 22: 139-146.
4. Olusiyi JA. Performance and carcass charac teristics of broiler finisher fed graded levels of Detarium microcarpium seed meal fortified with brewers' dried grain. Taraba J Agric Res. 22014; 2: 90-101.
5. Uchegbu MC, Omedec AA, Chiedozie IC, Nwaodu CH, Ezeokeke CT. Performance of finisher broilers fed varying level of raw (sun-dried) cocoyam (Xanthosoma sagittifolium) corm meals. Rep Opin. 2010; 2: 22-25.
6. Okereke CO. Utilization of cassava, sweet potato, and cocoyam meals as dietary sources for poultry. World J Eng Pure Appl Sci. 2012; 2: 67.
7. Alagbe JO. Nutritional evaluation of sweet orange (*Citrus sinensis*) fruit peel as replacement for maize in the diet of weaner grass cutters. Schol J Agric Sci. 2017; 6(8): 277-282.
8. Alagbe JO, Anuore DN. Growth performance and egg quality of laying hens fed diet supplemented with date palm seed powder. J Trends Life Sci. 2024; 2(2): 31-38.
9. Alagbe OJ, Anuore DN. Effect of Doum palm mesocarp meal (Hyphaene thebaica) as partial replacement for maize on growth performance and heamatological indices of weaned pigs. J Biotechnol Bioinform Res. 2023; 5(3): 1-6.
10. Shittu MD, Alagbe JO, Alaba O, Okanlawon EO, Adelakun FA, Emmanuel EO, et al. Effect of ginger, garlic and Negro pepper on the gut microbes, gut histomorphometry and pathological assessment of selected organs of broiler chickens. Asso Deans Agric Nig Uni. 2024; 5: 105-121.
11. Singh S, Alagbe OJ, Liu X, Sharma R, Kumar A. Comparative analysis of ethanolic Juniperus thurifera leaf, stem bark and root extract using gas chromatography and mass spectroemetry. Int J Agric Ani Prod. 2022; 2(6): 18-27.
12. Adewale AO, Alagbe JO, Adeoye K. Dietary Supplementation of Rauvolfia vomitoria Root extract as a Phytogenic Feed Additive in Growing Rabbit Diets: Haematology and Serum Biochemical Indices. Concept Dairy Vet Sci. 2021; 4(2): 20-28.
13. Singh AS, Alagbe JO, Sharma S, Oluwafemi RA, Agubosi OCP. Effect of dietary supplementation of melon (Citrallus linatus) seed oil on the growth performance and oxidative status of growing rabbits. J Multidimensional Res Rev. 2021; 2(1): 78-95.
14. Ojediran TK, Emiola IA, Durojaye V, Alagbe JO. Proximate, vitamin and GC-MS profiling of *Kigella africana* powder. Cerrado. 2024; 1(1): 13-20.
15. Ojediran TK, Emiola IA, Durojaye V, Alagbe JO. Analysis of *Kigellia africana* fruit's powder antioxidant and phytochemical properties. Braz J Sci. 2024; 3(7): 38-49.
16. NRC. Nutrient Requirements of Poultry (19th rev. ed.). J Appl Poultry Res. 1994; 3: 101.
17. AOAC. Official methods of analysis, Association of Official Analytical Chemists, Washington, D.C., USA. 15th Edition. 1990.
18. Daniel NA, Friday U, Alagbe O J. Investigating the effects of pawpaw (*Carica papaya*) essential oil dietary supplementation on the growth performance and carcass characteristics of broilers. Res Agric Vet Sci. 2023; 7(3): 164-174.
19. Muritala DS, Alagbe JO, Ojebiyi OO, Ojediran TK, Rafiu TA. Growth performance and haematological and serum biochemical parameters of broiler chickens given varied concentrations of *Polyalthia longifolia* leaf extract in place of conventional antibiotics. Ani Sci Gen. 2022; 18(2): 57-71.
20. Shittu MD, Alagbe JO, Adejumo DO, Ademola SO, Abiola AO, Samson BO, et al. Productive performance, caecal microbial population and immune-modulatory activity of broiler chicken fed different levels of *Sida acuta* leaf extract in replacement of antibiotics. Bioinform Prot Open Access J. 2021; 5(1): 000143.
21. Zubairu H, Mubarak AA, Alagbe JO. Effect of partial replacement of Soymeal with Sphenocentrum jollyanum seed meal on the growth performance and haematological constituents of broiler chickens. Int J Integr Res. 2025; 3(10): 741-752.
22. Olafadehan OA, Oluwafemi RA, Alagbe JO. Performance, haemato-biochemical parameters of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative. Adv Res Rev. 2020.
23. Alagbe JO. Haemato-biochemical indices of growing rabbits fed diets containing Hunteria umbellata seed meal as replacement for Soybean meal. Hematol Disord. 2026; 5(1): 018.
24. Campbell TW, Ellis CK. Avian and exotic animal hematology and cytology. 4th edition. Wiley-Blackwell. 2015.
25. Musa B, Alagbe JO,Adegbite MB, Omokore EA. Growth performance, caeca microbial population and immune response of broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture. Unite J Res Technol. 2020; 2(2): 13-21.
26. Polo FJ, Peinado VI, Viscor G, Palomeque J. Hematologic and plasma chemistry values in captive psittacine birds. Avian Dis. 1998; 42(3): 523-535.
27. Ammersbach M, Beaufrère H, Gionet Rollick A, Tully T. Laboratory blood analysis in Strigiformes-Part I: Hematologic reference intervals and agreement between manual blood cell counting techniques. Vet Clin Pathol. 2015; 44(1): 94-108.
28. Gaspar H, Bargallo F, Grifols J, Correia E, Pinto M. Age and sex-related differences in the haematological parameters of captive African grey parrots (*Psittacus erithacus*). Vet Med-Czech. 2021; 66(8): 330-341.
29. Nwaigwe CU, Ihedioha JI, Shoyinka SV, Nwaigwe CO. Evaluation of the hematological and clinical biochemical markers of stress in broiler chickens. Vet World. 2020; 13(10): 2294-2300.
30. Townsend AK, Wheeler SS, Freund D, Sehgal RNM, Boyce WM. Links between blood parasites, blood chemistry, and the survival of nestling American crows. Ecol Evol. 2018; 8: 8779-8790.
31. Agubosi OCP, Soliu MB, Alagbe JO. Effect of dietary inclusion levels of Moringa oleifera oil on the growth performance and nutrient retention of broiler starter chicks. Central Asian J Theoret Appl Sci. 2022; 3(3): 30-39.
32. Van Balveren JA, Huijskens MJAJ, Gemen EFA, Péquériaux NC, Kusters R. Effects of time and temperature on 48 routine chemistry, haematology and coagulation analytes in whole blood samples. Ann Clin Biochem. 2017; 54: 448-462.
33. Chin YL, Chai KF, Chen WN. Upcycling of brewers' spent grains via solid-state fermentation for the production of protein hydrolysates with antioxidant and techno-functional properties. Food Chem X. 2021; 13: 100184.
34. Tan YX, Mok WK, Lee J, Kim J, Chen WN. Solid state fermentation of brewers' spent grains for improved nutritional profile using Bacillus subtilis WX-17. Fermentation. 2019; 5(3): 52.
35. Olatunji AK, Alagbe JO, Hammed MA. Effects of varying levels of Moringa

olifera leaf meal on performance and blood profile of weaner rabbits. Int J Sci Res. 2016; 5(6): 803-806.

36. Szabo A, Mezes M, Horn P, Suto Z, Bazar G, Romvari R. Developmental dynamics of some blood biochemical parameters in the growing turkey (*Meleagris gallopavo*). Acta Vet Hung. 2005; 53(4): 397-409.

37. Terefe G, Walelgne M, Fekadu D, Kitaw G, Dejene M, Kehaliu A, et al. Effect of sun dry brewer spent yeast on chemical composition, in vitro digestibility, and ruminal degradation kinetics of wheat straw. CABI Agric Biosci. 2023; 4: 22.

38. Mitchell EB, Johns J. Avian haematology and related disorders. Vet Clin Exot Anim Pract. 2008; 11: 501-522.

39. Yitayew T, Moges D, Satheesh N. Effect of brewery spent grain level and fermentation time on the quality of bread. Int J Food Sci. 2022.

40. Viñuela J, Ferrer M, Recio F. Age-related variations in plasma levels of alkaline phosphatase, calcium and inorganic phosphorus in chicks of two species of raptors. Comp Biochem Physiol. 1991; 99: 49-54.

41. Café MB, Rinaldi FP, Morais HR, Nascimento MR, Mundim AV, Marchini CFP. Biochemical blood parameters of broilers at different ages under thermoneutral environment. World Poultry Science Journal. 2012.

42. Oladele SB. The significance of haematology in the diagnosis and therapy of avian disease: a review. Niger Vet J. 2009; 30(2): 24-39.

43. Kilgas P, Mand R, Magi M, Tilgar V. Haematological parameters in brood rearing Great Tits in relation to habitat, multiple breeding and sex. Comp Biochem Physiology A Mol Integr Physiol. 2006; 144: 224-231.

44. Latimer KS, Mahaffey EA, Prasse KW, Duncan JR. Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology. Iowa State University Press. 2003.

45. Lloyd S, Gibson JS. Haematology and biochemistry in healthy young pheasants and redlegged partridges and effects of spironucleosis on these parameters. Avian Pathol. 2006; 35(4): 335-340.

46. American Metabolic Testing Laboratories, Inc. Chemistry Profile. 2001.

47. Alagbe JO, Anaso EU, Shittu MD, Ojediran TK, Emiola IA. Nutritional evaluation of fermented Hunteria umbellata seed meal as partial substitute for Soybean meal in growing rabbits. Clin Investig Research. 2026; 3(1): 004.

48. Alagbe JO, Anorue DN. Effect of Replacing Soybean meal with fermented Hydrocotyle asiatica seed meal on the growth performance, semen quality and oxidative stress markers of Rabbit bucks. Biomed Biotechnol Sci. 2026; 2(1): 011.

49. Alagbe JO. Effect of Partial Replacement of Soyameal with Albizia lebbeck seed meal on the growth performance, egg quality and haemato-biochemical constituents of Nera black hens. Discov Agric. 2025; 11(24): e18da3161.

50. Alagbe JO. Effects of feeding varying levels of Tigernut (*Cyperus esculentus*) seed meal on the performance and blood profile of weaner grass cutters. Scholar J Agric Sci. 2021; 7(1): 15-19.

51. Alagbe JO. Studies on growth performance, nutrient utilization and haematological characteristics of broiler chickens fed different levels of Azolla-Moringa olifera mixture. Greener J Agric Sci. 2017; 7(6): 145-156.