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Impact of Fermented Urea-Untreated Orange Peel Meal on Haematological Parameters, Serum Biochemical Indices and Nutrient Metabolism in Broiler Chickens

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Abstract: In the face of escalating feed costs and protein scarcity in developing nations like Nigeria, orange peels a abundant agro-waste represent a promising alternative feed resource for broiler chickens when processed via solid-state fermentation to enhance nutritional value and reduce anti-nutritional factors. This study evaluated the impact of fermented urea-untreated orange peel meal (FUUOPM) included at 20% in broiler diets across varying fermentation durations (0, 3, 6 and 9 days) using Aspergillus niger on haematological parameters, serum biochemical indices and nutrient metabolism. 240-old Arbor Acre broilers were randomly allocated to four dietary treatments with four replicates of fifteen birds each, fed isonitrogenous and isocaloric starter (0–4 weeks) and finisher (5–8 weeks) diets formulated to NRC (2000) standards; blood samples were collected on day 56 for analysis and a 7-day digestibility trial was conducted using total faecal collection. Results showed that 3- and 6-day FUUOPM significantly ($P<0.05$) improved dry matter (87.24–89.12%), crude protein (84.44–85.73%) and crude fibre (85.17–87.22%) digestibility compared to control and 9-day treatments, enhanced mean corpuscular haemoglobin and volume and lowered serum cholesterol and triglycerides, particularly at 6 days; conversely, 9-day fermentation increased white blood cells (14,U14.95 × 10^9/L) and glucose (189.50 mg/dl) but reduced total protein and lipid profiles. Overall, 3- to 6-day fermentation optimized nutrient bioavailability and physiological responses without adverse health effects. It is recommended that FUUOPM be fermented for 3–6 days before 20% dietary inclusion in broiler rations to maximize digestibility, support growth performance and ensure haematological and biochemical stability.

Keywords: Health, Welfare, Diet, Livestock, Poultry.

Introduction

Nutrition is the most important consideration in the poultry industry and its survival is dependent on the availability of feedstuffs, which are mainly components of human food such as maize [1,2,3] reported that chicken is among the most frequently consumed animal proteins in developed nations, however, the scarcity of animal protein particularly in developing countries in Africa (Nigeria inclusive) has necessitated inquiries of several novel sources of alternative feed resources to enhance its availability at cheaper price [4,5]. This becomes more compelling now that the World Health Organization (WHO) (2017) had highlighted malnutrition as a major problem confronting the global population, particularly developing nations like Nigeria [6]. This problem is signaled by the chronic shortage in meeting the dietary protein demands and the consequent fierce competition between man and livestock [4].

[7] reported that peel which is also known as rind or skin as the outermost protective layer of the pericarp of fruits. In Botany, the peel, rind or skin is called the exocarp or epicarp, which includes the hard shell in fruits such as nuts [8]. In some fruits such as banana or grape, the peel is unpleasant or inedible; thus, it is removed and discarded [9,10] reported that most fruit peels are unwanted and serve as wastes after the inner fleshy portions have been consumed. Peels are detached from most fruits before consumption; and more importantly before using them in fruit juice industries because they serve no edible usage to humans [11].

Aspergillus niger as reported by [12] is a filamentous fungus commonly used in industrial fermentation processes for the production of enzymes, citric acids, organic acids and other metabolites. Aspergillus flavus on the other hand is not commonly used in fermentation as A. niger because its primary significance lies in its ability to produce secondary metabolites including toxins (aflatoxins) [13]. Aflatoxins are potent carcinogenic compounds that can contaminate crops like peanuts, corn, tree nuts and oranges [14]. Also, [15] stated that aflatoxins are not desirable in fermentation processes or food production due to their health risks compared to A. niger preferred for its efficiency and safety in fermentation process [16]. The use of Aspergillus niger in the fermentation of orange peel meal may lead to an increase in nutritional value due to the breakdown of complex compounds into simpler, more bioavailable forms [17]. Studies have shown that the fermentation of orange peel meal with Aspergillus niger can result in the production of beneficial metabolites such as citric acid, ascorbic acid and pectinase [18]. These metabolites have been shown to have various health benefits, including antioxidant and anti-inflammatory properties. Consequently, this study is aimed to determine the impact of fermented urea-untreated orange peel meal on haematological parameters, serum biochemical indices and nutrient digestibility in broiler chickens.

Materials and Methods

The feeding trial was conducted at the Poultry Unit of the Department of Animal Science Teaching and Research Farm, Ahmadu Bello University, Zaria (ABU). The farm is located on latitude 11°.17''N, longitude 07°.64''E with an altitude of 671 m above sea level (Google map). The climate is characterized by well-defined dry and wet seasons with a mean annual rainfall ranging from 700-1400 mm. Onset of wet season begins in late April or early May, peaks between July and August and ends in mid-October. This is followed by the harmattan season which comprises of a cold and dry weather. The average minimum and maximum daily temperature vary from 15.6°C during the cold season and 38.5°C during the hot season. The area is characterized with a relative humidity of 36% in dry season and 78.5% for wet season as stated by Institute for Agricultural Research [19].

Fresh orange peels of mixed varieties were collected from orange vendors (mai-lemu) at Samaru, Sabon Gari and Randa Kano markets in Zaria, Kaduna State. The orange peels were washed thoroughly using tap water and immediately sundried at temperature of 28-30°C for 3 days to reduce their moisture content to less than 12%. This was done to remove all foreign materials such as dirt, debris and any residues from pesticides and chemicals from it. Once the peels are dried, they were ground into meal using Hammer mill (Model 912, Winona Attrition Mill Co, USA) with screen size of 2.36mm (14 mesh) so as to increase the surface area and easy handling during processing. The ground orange peel meal (OPM) was stored in sacks and kept under moisture free conditions pending solid state fermentation.

A quantity of 100 g of dried and finely powdered orange peel meal (OPM) was placed in sterilized aluminum foil and autoclaved. An inoculum prepared with Aspergillus niger was introduced at a volume of 10 ml to both urea-treated and untreated OPM. The mixtures were incubated under anaerobic conditions in containers sealed with cellophane, utilizing the methodology described by [20,21]. Fermentation was halted on the 3rd, 6th and 9th days, after which the substrates were sun-dried for two days at ambient temperatures ranging from 28 to 30°C to deactivate the microorganisms.

Two hundred and forty (240) day-old Arbor acre broiler chicks of mixed sexes were procured from Ottarb-Synergy Farms Limited in Ede, Osun State, Nigeria. The birds were randomly assigned to four (4) dietary treatments (i.e. 0-, 3-, 6- and 9-days fermentation period) after which they were distributed into four (4) replicates that contains fifteen (15) chicks per replicate. The diets were least-cost formulated to meet the nutrient requirement of broiler according to NRC [22] recommendation. Thereafter, fermented urea untreated orange peel meal (FUUOPM) was included as independent ingredient at 20% inclusion levels in the diets. The dietary formulae were balanced in such a way that the diets were isonitrogenous and isocaloric and the proximate composition determined using AOAC [23] methods. The same procedure was adopted for finisher diets. The formulation for both broiler starter and finisher diets is presented in Tables 1 and 2, respectively.

The birds were subjected to fasting period (no feeding between 7pm to 7am) before the collection of blood early in morning. Two (2) birds were selected per replicate to make a total of eight (8) birds per treatment group. Blood samples were collected at the end of the experimental period using a sterile hypodermic needle and syringe via the jugular vein puncture.

Two (2) mls of blood from each bird was collected in ethylenediaminetetraacetic acid (EDTA) bottles for haematological evaluation. Blood was collected in a bottle without anticoagulants and placed in slanted form for serum biochemical analyses. All blood samples collected was placed in an ice-packed cooler to maintain the cold chain of the blood cell constituents and transported by road to the Veterinary Clinical Pathology Laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The haematological parameters determined was Packed cell volume (PCV), Erythrocytes count (RBC), Haemoglobin (Hb), White blood cell (WBC), Mean cell volume (MCV), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC). The serum biochemical indices determined will be Total Protein, Glucose, Globulin, Albumin, Creatinine and lipid profiles: Total Cholesterol (TC), Triglycerides (TG), High- Density Lipoproteins (HDL) and Low-Density Lipoproteins (LDL). The serum enzymes: Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were also obtained using commercial kits (Randox test kits).

Table 1: Ingredients composition of broiler starter diets containing fermented urea untreated orange peel meal (0-4 weeks).

Ingredient composition (%)				
Ingredients	**Control**	**3 Days**	**6 Days**	**9 Days**
Maize	58.60	40.90	39.60	38.80
Soybean meal	30.00	30.00	30.00	30.00
Groundnut cake	7.20	9.54	6.20	7.00
FUUOPM	**0.00**	**20.00**	**20.00**	**20.00**
Lysine	0.10	0.10	0.10	0.10
Methionine	0.20	0.20	0.20	0.20
Bone Meal	3.00	3.00	3.00	3.00
Limestone	0.40	0.40	0.40	0.40
Vit/Min Premix[1]	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis				
Parameters	**Control**	**3 Days**	**6 Days**	**9 Days**
ME (Kcal/kg)	3012.21	3002.39	3009.28	3008.45
Crude Protein (%)	23.0	23.0	23.0	23.0
Ether Extract (%)	3.23	3.23	3.23	3.23
Crude Fibre (%)	3.52	3.31	3.46	3.97
Calcium (%)	1.31	1.31	1.31	1.31
Phosphorus (%)	0.87	0.87	0.87	0.87
Lysine (%)	1.26	1.26	1.26	1.26
Methionine + Cysteine (%)	0.55	0.55	0.55	0.55
Cost (₦/kg)	712.85	685.38	668.52	650.25
Proximate Analysis				
Parameters (%)	**Control**	**3 Days**	**6 Days**	**9 Days**
Dry Matter	82.38	83.75	84.61	83.95
Crude Protein	24.85	24.51	24.56	23.15
Ash	6.91	6.94	4.39	5.85
Crude Fibre	5.4	5.26	5.33	7.73
Ether Extract	2.0	2.05	2.14	2.08
Nitrogen Free Extract	68.62	69.8	67.29	66.13

FUUOPM: Fermented urea untreated Orange Peel Meal, ME: Metabolizable Energy, Met: Methionine, Cys: Cysteine, [1]Vitamin/Mineral Premix (Bio-mix®) Each 2.5kg supplied: Vit A 10,000,000 iu; Vit D^3 2,000,000 iu; Vit E 23,000mg; Vit K_3 2,000mg; Vit B_1 1,800mg; Vit B_2 5,500mg; Niacin 27,500mg; Pantothenic acid 7,500mg; Vit B_6 3,000mg; Vit B_{12} 15mg; Folic acid 750mg; Biotin 60mg; Choline Chloride 300, 000mg; Cobalt 200mg, Copper 3,000mg; Iodine 1,000mg; Iron 20, 000mg; Manganese 40,000mg; Selenium 200mg; Zinc 30,000mg, Antioxidant 1,250mg.

Table 2: Ingredient composition of broiler finisher diets containing fermented urea untreated orange peel meal (5–8 weeks).

Ingredient composition (%)				
Ingredients	**Control**	**3 Days**	**6 Days**	**9 Days**
Maize	56.0	38.4	37.8	35.1
Wheat bran	5.5	5.5	5.5	5.5
Soybean meal	23.0	23.0	23.0	23.0
Groundnut cake	11.3	8.9	9.5	12.2
FUUOPM	0.0	20.0	20.0	20.0
Lysine	0.1	0.1	0.1	0.1
Methionine	0.2	0.2	0.2	0.2
Bone meal	3.0	3.0	3.0	3.0
Limestone	0.4	0.4	0.4	0.4
Vitamin/Mineral Premix[1]	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	**100.0**	**100.0**	**100.0**	**100.0**
Calculated Analysis				
Parameters	**Control**	**3 Days**	**6 Days**	**9 Days**
ME (Kcal/kg)	3100.36	3100.47	3102.49	3102.46
Crude Protein (%)	20.0	20.0	20.0	20.0
Ether Extract (%)	3.53	3.53	3.53	3.53
Crude Fibre (%)	3.91	3.91	3.91	3.91
Calcium (%)	1.29	1.29	1.29	1.29
Phosphorus (%)	0.85	0.85	0.85	0.85
Lysine (%)	1.02	1.02	1.02	1.02
Methionine + Cysteine (%)	0.5	0.5	0.5	0.5
Cost (₦/kg)	644.11	619.45	606.91	596.21
Proximate Analysis				
Parameters (%)	**Control**	**3 Days**	**6 Days**	**9 Days**
Dry Matter	84.64	84.68	82.98	84.4
Crude Protein	29.83	27.47	33.95	29.13
Ash	5.88	7.51	7.69	10.01
Crude Fibre	5.4	5.25	5.0	5.42
Ether Extract	2.09	1.98	2.14	1.86
Nitrogen Free Extract	67.96	69.82	69.94	66.66

[1]Vitamin/Mineral Premix (Bio-mix®): Each 2.5 kg supplied Vit A 8,500,000 IU; Vit D_3 1,500,000 IU; Vit E 10,000 mg; Vit K_3 1,500 mg; Vit B_1 1,600 mg; Vit B_2 4,000 mg; Niacin 20,000 mg; Pantothenic acid 5,000 mg; Vit B_6 1,500 mg; Vit B_{12} 10 mg; Folic acid 500 mg; Biotin 50 mg; Choline chloride 175,000 mg; Cobalt 200 mg; Copper 3,000 mg; Iodine 1,000 mg; Iron 20,000 mg; Manganese 40,000 mg; Selenium 200 mg; Zinc 30,000 mg; Antioxidant 1,250 mg.

On day 49 of feeding trial, a nutrient digestibility trial was carried out. After three (3) days acclimatization period, two (2) birds were randomly selected from each replicate pen to make a total of eight (8) birds in each treatment. The birds were housed in individual clean and disinfected cages with wire mesh bottoms. Polythene was placed under each cage for faecal collection. The birds were given a known quantity of experimental diet that represents the average weight of daily intake of birds in each treatment group and offered to the birds for three (5) days. Water was also provided during the experiment. Feed consumed was measured by weighing the left-over feed daily and subtracting from amount of feed provided. Total faeces collection was done for 7 days during which excreta were collected and then freeze-dried in a freeze-dryer cabinet at the Product Development Research Programme (PDRP) Laboratory, Institute for Agricultural Research, Zaria (IAR).

Contaminants will be carefully removed and the faeces will be sundried for proximate composition analysis (Dry matter, Crude protein, Crude fibre, Ether extract, Ash retention and Nitrogen free extract) in the Nutrition Laboratory, Institute of Agricultural Research, Zaria using the methods by AOAC [23] while the metabolizable energy will be calculated using the equation;

$$ME\ (kcal/kg) = 37 \times \%CP + 81.1 \times \%\ EE + 35.5 \times \%\ NFE \quad \text{(Pauzenga, 1985)}$$

The percentage coefficient of digestibility will be calculated using the below equation:

$$\text{Coefficient of Digestibility (\%)} = \frac{(Nutrient\ Intake\text{-}Nutrient\ Output)}{(Nutrient\ Intake)} \times 100$$

Where;
Nutrient Intake (g) = Feed Intake × Nutrient in diet,
Nutrient Output (g) = Faecal Output × Nutrient in faeces.

All data that were collected were subjected to General Linear Model (GLM) procedure of SAS (version 9.2) [24]. Significant treatment means were compared using New Duncan's Multiple Range Test [25] of the same package. Significance was accepted at 5% level of probability. The statistical model adopted is shown below:

$$Yij = \mu + Ti + +eij$$

Where;
Y_{ij} = performance of j^{th} animal fed i^{th} levels of FOPM;
μ= Population means of all observation;
T_i = i^{th} effect of the FOPM levels on the birds;
e_{ij} = experimental error (assumed to be identically, independently and normally distributed with zero mean and constant variance (iinDoσ^2).

Materials and Methods

Haematological Indices

Table 3 shows the haematological indices of broiler chicken fed diets contacting 20% inclusion level of FOPM at varying fermentation periods. 9-day FOPM diet significantly (P<0.05) increased white blood cell count to 14.95 x109/l, followed by 3-day diet (13.95 x109/l), control diet (12.78 x109/l) and 6-day FOPM diet (11.05 x109/l) and monocytes to 2.00% from 0.75% (control diet), 0.25% (3-day diet) and 0.25% (6-day diet). 6- and 9-day FOPM diet significantly (P<0.05) reduced packed cell volume to 28.50% and 29.00% from 30.00% (control diet) and 29.25% (3-day diet) and lymphocytes to 69.25% and 70.25% from 74.25% (control diet) and 73.75% (3-day diet). Three- and 6-day FOPM diet significantly (P<0.05) increased mean corpuscular haemoglobin to 30.34 pg/cell and 30.93 pg/cell from 28.58 pg/cell (control diet) and 28.02 pg/cell (9-day diet) and mean corpuscular volume to 91.46 fl (3-day diet) and 93.07 fl (6-day diet) from 85.96 fl (control diet) and 84.20 fl (9-day diet). Other indices were similar (P>0.05). Elevated white blood cell counts (11.05–14.95 x109/l, above 1.90–9.50 x109/l; 26) in 9-day The elevated white blood cell counts in the 9-day FOPM diet, above the normal range of 1.90–9.50 x109/l, are consistent with mild immune activation from microbial metabolites, such as flavonoids, enhancing immune responses, as supported by [27]. The reduced packed cell volume and lymphocytes in 6- and 9-day FOPM diets correspond to modulated hematopoiesis, while improved red cell quality in 3- and 6-day FOPM diets is in agreement with better nutrient absorption, as noted by [28].

Table 3: Haematological indices of broiler chicken fed diets containing 20% inclusion level of FOPM at varying fermentation periods.

Parameters	Control	3 Days	6 Days	9 Days	SEM (±)	P-value	Reference Value
Packed Cell Volume (%)	30.00[a]	29.25[ab]	28.50[b]	29.00[b]	0.20	0.04	24.00-40.00[x]
Haemoglobin (g/dl)	9.98	9.70	9.48	9.65	0.13	0.68	7.00-15.00[x]
Red Blood Cell ($x10^{12}$/l)	3.53	3.23	3.10	3.45	0.13	0.71	1.59-4.10[x]
White Blood Cell ($x10^9$/l)	12.78[c]	13.95[b]	11.05[d]	14.95[a]	0.45	0.01	1.90-9.50[y]
Lymphocytes (%)	74.25[a]	73.75[a]	69.25[c]	70.25b	0.66	0.01	40.00-100.00[z]
Basophils (%)	0.00	0.00	0.00	0.00	-	-	0.10-7.00[z]
Monocytes (%)	0.75[b]	0.25[b]	0.25[b]	2.00[a]	0.25	0.01	0.10-2.00[x]
Eosinophils (%)	0.25	0.50	0.00	0.75	0.15	0.35	1.50-6.00[y]
MCH (pg/cell)	28.58[b]	30.34[a]	30.93[a]	28.02[b]	0.38	0.01	33.00-49.00[z]
Mean Corpuscular Volume (fl)	85.96[c]	91.46[b]	93.07[a]	84.20[d]	1.12	0.01	90.00-140.00[y]
MCHC (g/dl)	33.25	33.16	33.24	33.28	0.12	0.10	26.00-85.00[z]

abc means on the same row with different superscript are significantly different (P<0.05) SEM: Standard Error of Mean; FOPM: Fermented Orange Peel Meal, MCH: Mean Corpuscular Haemoglobin, MCHC: Mean Corpuscular Haemoglobin Concentration; Reference values: x Mitruka and Rawnsley [26]: y Simrak et al. [29]: z Jain [30].

Serum Biochemical Indices

Table 4 presents the serum biochemical indices of broiler chicken fed diets containing 20% inclusion level of FOPM at varying fermentation periods. Control diet significantly (P<0.05) increased total protein to 6.50 g/dl from 4.25 g/dl (3-day diet), 4.50 g/dl (6-day diet) and 3.80 g/dl (9-day FOPM diet), total cholesterol to 363.25 mg/dl from 237.25 mg/dl (3-day diet), 218.50 mg/dl (6-day diet) and 276.50 mg/dl (9-day diet), triglycerides to 86.50 mg/dl from 81.75 mg/dl (3-day diet), 76.75 mg/dl (6-day diet) and 66.75 mg/dl (9-day diet), low-density lipoprotein to 99.25 mg/dl from 94.25 mg/dl (3-day diet), 16.25 mg/dl (6-day diet) and 50.50 mg/dl (9-day diet) and aspartate aminotransferase to 92.50 IU/l from 81.50 IU/l (3-day diet), 80.25 IU/l (6-day diet) and 86.50 IU/l (9-day diet). 9-day FOPM diet significantly (P<0.05) increased glucose to 189.50 mg/dl from 151.50 mg/dl (3-day diet), 159.75 mg/dl (6-day diet) and 172.00 mg/dl (control diet), high-density lipoprotein to 31.05 mg/dl from 23.63 mg/dl (3-day diet), 29.20 mg/dl (6-day diet) and 22.65 mg/dl (control diet), alanine aminotransferase to 24.50 IU/l from 19.00 IU/l (3-day diet), 21.75 IU/l (6-day diet) and 23.25 IU/l (control diet) and alkaline phosphatase to 64.00 U/l from 54.75 U/l (3-day diet), 61.50 U/l (6-day diet) and 52.25 U/l (control diet). Other indices were similar (P>0.05). The lower total protein in FOPM diets suggests altered protein absorption, consistent with findings by [31]. The elevated glucose in the 9-day FOPM diet corresponds to microbial starch breakdown, as supported by [31]. Reduced lipid levels in the 6-day FOPM diet are in agreement with improved metabolism from fermented bioactive compounds, as reported by [32]. The elevated liver enzymes in the 9-day FOPM diet, though within safe limits, suggest mild stress, consistent with [33].

Table 4: Serum biochemical indices of broiler chicken fed diets containing 20% inclusion level of FOPM at varying fermentation periods.

Parameters	Control	3 Days	6 Days	9 Days	SEM (±)	P-value	Reference Value
Total Protein (g/dl)	6.50^{a}	4.25^{b}	4.50^{b}	3.80^{b}	0.34	0.01	6.00-8.30^{x}
Albumin (g/dl)	1.30	1.23	1.13	1.18	0.12	0.98	1.20-3.20^{y}
Globulin (g/dl)	1.16	1.63	1.25	1.56	0.14	0.61	1.10-2.20^{x}
Glucose	172.00^{b}	151.50^{d}	159.75^{c}	189.50^{a}	4.31	0.01	-
Creatinine (Mmol/l)	0.26	0.26	0.27	0.19	0.12	0.10	-
Urea (Mmol/l)	4.78^{ab}	5.00^{ab}	4.48^{b}	5.60^{a}	0.17	0.12	-
Cholesterol (mg/dl)	363.25^{a}	237.25^{c}	218.50^{d}	276.50^{b}	16.79	0.01	129-237^{z}
Triglyceride (mg/dl)	86.50^{a}	81.75^{b}	76.75^{c}	66.75^{d}	2.21	0.01	<135.00^{z}
HDL (mg/dl)	22.65^{d}	23.63^{c}	29.20^{b}	31.05^{a}	1.08	0.01	>90.00^{z}
LDL (mg/dl)	99.25^{a}	94.25^{b}	16.25^{d}	50.50^{c}	10.24	0.01	<130.00^{z}
AST (IU/l)	92.50^{a}	81.50^{c}	80.25^{d}	86.50^{b}	1.46	0.01	100-400^{y}
ALT (IU/l)	23.25^{b}	19.00^{d}	21.75^{c}	24.50^{a}	0.63	0.01	-
ALP (U/l)	52.25^{d}	54.75^{c}	61.50^{b}	64.00^{a}	1.45	0.01	10-106^{z}

abc means on the same row with different superscript are significantly different (P<0.05) SEM: Standard Error of Mean; FOPM: Fermented Orange Peel Meal;, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, AST: Aspartate-amino Transferase, ALT: Alanine-amino Transferase, ALP: Alkaline Phosphate Reference values: x Ross et al. [34]: y LAVC [33]: z Bounous and Stedman [35].

Apparent Nutrient Metabolism

Table 5 reveals the nutrient metabolism of broiler chicken fed diets containing 20% inclusion level of FOPM at varying fermentation periods. 3 and 6-day FOPM diet significantly (P<0.05) increased dry matter digestibility to 89.12% and 87.24% from 78.24% (control diet) and 78.79% (9-day diet), crude fibre digestibility to 85.17% (3-day diet) and 87.22% (6-day diet) from 79.88% (control diet) and 77.97% (9-day diet) and crude protein digestibility to 84.44% (3-day diet) and 85.73% (6-day diet) from 82.88% (9-day diet). 3-day FOPM diet significantly (P<0.05) increased ether extract digestibility to 87.05% from 78.66% (9-day diet), with 85.28% (control diet) and 81.79% (6-day diet) intermediate. Control diet significantly (P<0.05) increased nitrogen free extract digestibility to 89.29% from 69.40% (9-day diet). The enhanced digestibility of dry matter, crude fiber and crude protein in 3- and 6-day FOPM diets is consistent with microbial breakdown improving nutrient bioavailability, as supported by [31,32].

The lower digestibility in the 9-day FOPM diet corresponds to nutrient degradation from over-fermentation, contrasting with [36], who found no digestibility differences and [37], who noted reduced digestibility in rabbits due to high fiber, unlike broilers.

Table 5: Nutrient metabolism of broiler chicken fed diets containing 20% inclusion level of FOPM at varying fermentation periods

Parameters	Control	3 Days	6 Days	9 Days	SEM (±)	P-value
Dry matter	78.24^{b}	89.12^{a}	87.24^{a}	78.79^{b}	3.12	0.01
Crude protein	84.16^{ab}	84.44^{ab}	85.73^{a}	82.88^{b}	4.19	0.02
Crude fibre	79.88^{bc}	85.17^{b}	87.22^{a}	77.97^{c}	2.65	0.01
Ether extract	85.28^{a}	87.05^{a}	81.79^{b}	78.66^{c}	5.65	0.04
Nitrogen free extract	89.29^{a}	80.45^{b}	84.51^{ab}	69.40^{c}	4.36	0.01

^{abc} means on the same row with different superscript are significantly different (P<0.05) SEM: Standard Error of Mean; FOPM: Fermented Orange Peel Meal.

Conclusion

The inclusion of 20% fermented urea-untreated orange peel meal in broiler diets, particularly at 3- and 6-day fermentation periods, enhanced nutrient digestibility, improved red blood cell quality and modulated lipid metabolism without compromising overall health, while the 9-day fermentation elicited mild immune activation and glucose elevation but reduced protein and lipid profiles, indicating an optimal fermentation window for balancing nutritional benefits and physiological responses in broiler chickens. It is recommended that a 3- to 6-day fermentation period be adopted when incorporating fermented urea-untreated orange peel meal at 20% in broiler diets to maximize nutrient utilization and maintain haematological and biochemical stability.

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