

Article

Nutritional Profile and Digestibility of Feather Meal and Mixed Meal in Broiler Chickens

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Abstract

This study evaluated the nutritional composition, nutrient digestibility, and effects on small intestinal morphology of feather meal (100% feather meal) and a mixed meal consisting of 90% feather meal and 10% offal meal. A total of 300 twenty-two-day-old male Ross 308 broilers were randomly allocated into two dietary treatment groups (93% feather meal- or mixed meal-based diets). Each treatment had six replicates of 25 birds in each replication, and the experiment lasted 7 days using a direct feeding approach for nutrient digestibility evaluation. Data were analyzed using the General Linear Model (GLM) of the Statistical Analysis System (SAS), and treatment means were compared using a two-sample *t*-test (5%). Results showed that feather meal had higher crude protein and gross energy, while mixed meal contained greater ash, crude fiber, and unsaturated fatty acids. Feather meal was rich in methionine and lysine, whereas mixed meal had higher levels of valine, leucine, and serine. Standardized ileal digestibility was higher for fiber and most amino acids in the mixed meal, while protein digestibility and metabolizable energy were similar ($p > 0.05$) between treatments. Additionally, birds fed the mixed meal exhibited improved intestinal morphology, with greater jejunum and ileum villus height. In summary, the mixed meal showed better digestibility and gut morphology, indicating greater potential as a sustainable protein source.

Keywords: feather meal; mixed meal; broiler chickens; nutrient digestibility; amino acids; intestinal morphology



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1. Introduction

In poultry production systems, feed costs account for the dominant financial input, nearly 70% of total production expenses [1]. This challenge has sparked growing interest in low-cost, locally available protein alternatives to partially replace conventional ingredients, such as soybean meal [2]. Animal by-products have attracted particular attention due to their potential to reduce feed costs and improve resource utilization efficiency [3].

Among these, feather meal and mixed meal are two widely discussed by-products [4]. Feather meal (100% feather-derived product) has an extremely high crude protein content,

but its utilization is limited by the presence of indigestible keratin and an unbalanced amino acid profile [5,6]. In this study, mixed meal refers to a combination of 90% feather meal and 10% offal meal. Mixed meal, produced from rendered animal by-products after fat removal, provides a more diverse nutrient composition but suffers from inconsistent quality and unstable nutritional value [3,7].

Previous studies have shown that increasing the dietary inclusion of feather meal markedly reduces the apparent ileal digestibility (AID) of nutrients, particularly protein [4]. AID is an indicator used to assess the digestibility of nutrients, representing the proportion of nutrients that disappear from the digestive tract before the end of the ileum. It is typically calculated from nutrient and marker concentrations in both feed and ileal digesta, reflecting the animal's overall nutrient absorption. However, AID includes endogenous nutrient losses—such as digestive enzymes, desquamated epithelial cells, and mucoproteins—which can lead to an underestimation of the true nutritive value of nutrients [8,9].

To achieve a more accurate assessment, the concept of standardized ileal digestibility (SID) was introduced. By accounting for basal endogenous losses (BELs), SID provides a more accurate estimate of feed ingredient digestibility than AID. Thus, it provides a more precise estimation of the actual nutrient utilization by animals. This correction better reflects the true efficiency of nutrient absorption, making it a more consistent and reliable measure for comparing the nutritional value of different feed ingredients [10,11].

However, comprehensive SID data for feather meal and mixed meal remain scarce, underscoring the importance of systematically evaluating their nutrient composition and digestibility [7,12]. Furthermore, assessing small intestinal morphology is essential when evaluating alternative protein sources, as it directly reflects gut health and nutrient absorption capacity in broilers.

Therefore, the current study aimed to systematically evaluate and compare the nutritional composition, AID and SID values, and small intestinal morphology of broilers fed feather meal- or mixed meal-based diets. The findings will provide updated insights into the nutritive value of these animal by-products and support the development of cost-effective and sustainable feeding strategies in poultry production [1,2].

2. Materials and Methods

2.1. Experimental Setup and Feeding Regimens

A total of 300 one-day-old male broiler chicks (Ross 308) were procured from a local hatchery (FFM Farms Sdn. Bhd., 47000 Sungai Buloh, Selangor, Malaysia) and housed in a closed commercial poultry facility with controlled temperature and humidity, following the Ross 308 management guidelines. Environmental conditions were initially maintained at 31 ± 1 °C on day 1 and gradually reduced to approximately 23 ± 1 °C by the end of the trial, with adjustments made to account for relative humidity, which averaged $70 \pm 5\%$ throughout the experimental period. Continuous lighting was provided throughout the study. The birds were accommodated in a penning cage system. Vaccinations were administered against Newcastle disease and Infectious Bronchitis at 7 and 22 days of age, respectively, and against Infectious Bursal Disease at 13 days of age. From day 1 to 21, all birds were fed a standard commercial diet to meet their nutritional requirements. The experimental diets containing feather meal or mixed meal were introduced only from day 22 to 28 as a short-term feeding period for digestibility and intestinal morphology evaluation. On day 22, birds were randomly allocated to two experimental dietary groups based on feather meal (100% feather-derived) and mixed meal (90% feather meal and 10% offal meal) inclusion (93%), with six replicates per treatment and 25 birds per replicate. Throughout the 7-day feeding trial, birds had unrestricted access to the experimental diets and drinking water. Titanium dioxide (TiO₂) was incorporated at 0.3% in the diets as

an indigestible marker to evaluate nutrient digestibility. The experimental diets (Table 1) were not formulated to be isonitrogenous or isoenergetic, as the primary objective was to evaluate the intrinsic nutritional value and digestibility of the test ingredients. While this direct feeding approach provides valuable insight into nutrient digestibility, it may also influence physiological responses, which we acknowledge as a limitation of the study.

Table 1. Nutrient composition of feed in week 4 (day 22–28).

Ingredients (%)	Treatment Diets	
	Feather Meal	Mixed Meal
Feather meal	93.00	0.00
Mixed meal	0.00	93.00
Palm oil	3.30	3.30
CaCO ₃	1.70	1.70
Salt	0.40	0.40
Vitamin premix ¹	0.50	0.50
Mineral premix ²	0.50	0.50
Choline-Cl	0.30	0.30
TiO ₂ ³	0.30	0.30
Total	100.0	100.0

^{1,2} Vitamin and Mineral premixes: [13]. ³ TiO₂: Titanium dioxide.

2.2. Sampling

At the end of the experiment, a total of 60 birds were randomly selected from each dietary treatment, with 10 birds per replicate, for sample collection. The selected birds were euthanized following halal procedures in accordance with the Department of Standards Malaysia guidelines. Ileal digesta were collected from Meckel's diverticulum to approximately 1 cm proximal to the ileocecal junction, gently expressed into plastic containers, and subsequently freeze-dried for analysis. Feed samples were obtained immediately after complete mixing of the diet ingredients, ground to a fine powder, and stored at −20 °C until further chemical analysis.

2.3. Chemical Analysis and Calculations

2.3.1. Proximate Analysis

The proximate composition of the samples was determined following the official methodologies prescribed by the Association of Official Analytical Chemists (AOAC) [14]. The contents of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) in the feed samples were quantified using the analytical procedures outlined by Van Soest [15].

2.3.2. Amino Acid Analysis

Amino acid concentrations were determined by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) using previously established protocols [16]. After sample hydrolysis and derivatization with AccQ-Fluor reagent (Waters Corporation, Milford, MA, USA), separation was performed on an AccQ-Tag column, 3.9 × 150 mm, 4 µm (Waters Corporation, Milford, MA, USA), and quantification was performed using an external standard method with an internal standard (L-2-aminobutyric acid). The content of each amino acid is expressed as a percentage by mass (% w/w).

2.3.3. Fatty Acid Analysis

Fatty acid analysis employs gas chromatography (GC) as previously explained [17]. Briefly, the total lipids are extracted from the sample, and then triglycerides and other

components are converted into highly volatile fatty acid methyl ester (FAME) derivatives via an alkali-catalyzed transesterification reaction. The prepared sample was injected into a gas chromatograph equipped with a polar capillary column and a flame ionization detector (FID) for compound separation and quantification. Finally, compounds were identified by matching their retention times to those of known standards, and the relative proportions of each fatty acid were determined using an area normalization approach.

2.3.4. Assessment of Gross Energy Content

Gross energy (GE) content was measured using a fully automated C300 basic combustion oxygen bomb calorimeter manufactured by IKA (Staufen, Germany), as explained earlier [18]. Briefly, approximately 1 g of the sample was compressed into a pellet and introduced into the calorimeter chamber. Following complete combustion, during which the sample was oxidized to carbon dioxide and water as end products, the instrument automatically measured and recorded the gross energy value.

2.3.5. Titanium Dioxide Analysis

The TiO_2 concentration was quantified following the analytical approach described by Short et al. [19]. Briefly, approximately 0.1 g of feed or ileal digesta was weighed into pre-dried, pre-weighed crucibles and incinerated at 600 °C for 6 h to obtain ash. After cooling, 10 mL sulfuric acid (7.4 M) was added, and the samples were heated gently on a hot plate for about 60 min to ensure complete dissolution of the residue. Once cooled, the solutions were transferred into beakers containing 25 mL of distilled water and filtered through Whatman No. 541 filter paper (Whatman International Ltd., Maidstone, UK) into 100 mL volumetric flasks. Subsequently, 20 mL of 30% hydrogen peroxide was added to each flask, and the volume was adjusted to 100 mL using distilled water. A stock TiO_2 standard solution (0.3 mg/mL) prepared in concentrated sulfuric acid was serially diluted to construct a calibration curve, while a TiO_2 -free blank solution served as the reference. The absorbance of the prepared samples was then measured at 410 nm using a Multiskan GO spectrophotometer (Thermo Scientific, Waltham, MA, USA) [16].

2.3.6. Calculations of AID, Basal Endogenous Losses (BELs), and SID

Calculations were conducted based on data stated on a dry matter basis [10,20]. The AID of nutrients was calculated according to the following equation:

$$AID (\%) = \frac{(\text{nutrient}/\text{TiO}_2)_{\text{diet}} - (\text{nutrient}/\text{TiO}_2)_{\text{ileal digesta}}}{(\text{nutrient}/\text{TiO}_2)_{\text{diet}}} \times 100$$

The BELs flow was expressed as mg of nutrient flow per kg of dry matter intake and was determined as

$$\text{BELs flow (mg/kg)} = \text{nutrients in ileal digesta (mg/kg)} \times \frac{\text{TiO}_2 \text{ in diet (mg/kg)}}{\text{TiO}_2 \text{ in digesta (mg/kg)}}$$

The AID values of nutrients were then standardized by correcting for the BEL flow to obtain the SID using the following formula:

$$SID (\%) = AID (\%) + \frac{\text{BEL flow (mg/kg)}}{\text{Ingredients (mg/kg)}}$$

2.3.7. Apparent Metabolizable Energy Measurement

Apparent metabolizable energy (AME) was determined following the method of Scott and Boldaji [21], using the equation provided below:

$$AME (j/g) = GE_{diet} (j/g) - [GE_{digesta}(j/g) \times \left(\frac{TiO_2 \text{ in diet}}{TiO_2 \text{ in digesta}} \right)]$$

2.3.8. Small Intestine Histomorphology

Intestinal samples were from the duodenum (mid-duodenal loop), jejunum (midway between the duodenal loop endpoint and Meckel's diverticulum), and ileum (midway between Meckel's diverticulum and the ileocaecal junction). These segments were promptly flushed with 10% neutral buffered formalin. From each, 3 mm pieces were then trimmed, placed in plastic cassettes, and fixed overnight in the same formalin solution. Tissue processing included dehydration using a tissue processor (Leica ASP 3000, Tokyo, Japan) and embedding in paraffin wax (Leica EG 1160, Tokyo, Japan) at an embedding station. Sections were initially trimmed to 30 µm, then cut to 4 µm, mounted on slides, and dried on a 60 °C hot plate. After hematoxylin and eosin staining, the slides were coverslipped and examined under light microscopy. Morphometric parameters—specifically crypt depth (defined as the depth of invagination between adjacent villi) and villus height (measured from the villus tip to the villus-crypt junction)—were quantified using an image analysis system [22,23].

2.4. Statistical Analysis

All data were analyzed using the General Linear Model (GLM) procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Treatment means were compared using a two-sample *t*-test, with statistical significance declared at $p < 0.05$. The variables analyzed included AID, BELs, SID, GE, AME, DM, CP, CF, Ash, NDF, ADF, ADL, AA profile, FA profile, and villi structure across dietary treatments.

3. Results

3.1. Chemical

3.1.1. Proximate Composition

The proximate and fiber contents of feather meal and mixed meal are shown in Table 2. Feather meal had significantly higher crude protein content compared to mixed meal ($p = 0.0412$), while its Ash content was significantly lower ($p = 0.0197$). No significant differences were detected between the two meals with respect to dry matter, organic matter, ether extract, crude fiber, neutral detergent fiber, acid detergent lignin, or gross energy content.

Table 2. Proximate and fiber contents of feather meal and mixed meal.

Nutrient (%)	Feather Meal	Mixed Meal	<i>p</i> -Value
Dry matter %	96.22 ± 0.07	97.28 ± 0.11	0.4548
Organic matter %	91.15 ± 0.06	84.39 ± 0.24	0.0550
Ash %	5.08 ± 0.04 ^b	12.89 ± 0.19 ^a	0.0197
Ether extract %	9.33 ± 0.20	11.17 ± 0.10	0.2431
Crude protein %	94.36 ± 0.12 ^a	69.66 ± 0.51 ^b	0.0412
Crude fiber %	0.48 ± 0.05	0.47 ± 0.01	0.0513
Neutral detergent fiber %	29.43 ± 0.10	39.89 ± 0.05	0.3529
Acid detergent lignin	0.06 ± 0.00	0.06 ± 0.001	0.0950
Gross energy (J/g)	22,317.0 ± 26.21	23,435.5 ± 21.59	0.5549

^{a,b} Means in the same row bearing different superscripts indicate a statistically significant difference ($p < 0.05$). All data are presented as mean ± standard error.

3.1.2. Amino Acid

The amino acid contents of feather meal and mixed meal are shown in Table 3. Significant differences were observed in all amino acids between the two meals. Mixed meal exhibited significantly higher levels of most amino acids, except for lysine and methionine, which showed higher content in feather meal. Notably, the serine content in mixed meal was significantly greater than that in feather meal ($p < 0.0001$).

Table 3. Amino acid contents of feather meal and mixed meal.

Amino Acids (%)	Feather Meal	Mixed Meal	<i>p</i> -Value
Essential amino acids			
Threonine	1.51 ± 0.01 ^b	2.78 ± 0.05 ^a	0.0024
Valine	1.41 ± 0.02 ^b	3.40 ± 0.03 ^a	0.0004
Leucine	2.96 ± 0.05 ^b	5.38 ± 0.05 ^a	0.0053
Phenylalanine	1.90 ± 0.03 ^b	3.65 ± 0.03 ^a	0.0008
Lysine	0.92 ± 0.03 ^a	0.65 ± 0.01 ^b	0.0148
Isoleucine	1.23 ± 0.02 ^b	2.95 ± 0.03 ^a	0.0005
Methionine	2.05 ± 0.01 ^a	1.58 ± 0.01 ^b	0.0005
Arginine	2.86 ± 0.04 ^b	4.53 ± 0.08 ^a	0.0021
Histidine	1.01 ± 0.01 ^b	1.29 ± 0.03 ^a	0.0157
Non-essential amino acids			
Proline	2.11 ± 0.03 ^b	4.71 ± 0.07 ^a	0.0009
Aspartic acid	1.60 ± 0.01 ^b	1.89 ± 0.04 ^a	0.0379
Alanine	1.55 ± 0.02 ^b	1.65 ± 0.02 ^a	0.0093
Tyrosine	1.49 ± 0.02 ^b	2.32 ± 0.03 ^a	0.0002
Serine	1.52 ± 0.03 ^b	6.48 ± 0.05 ^a	<0.0001
Glycine	4.60 ± 0.06 ^b	5.57 ± 0.05 ^a	0.0048
Glutamic acid	2.96 ± 0.03 ^b	4.12 ± 0.06 ^a	0.0077
Cystine	1.42 ± 0.02 ^b	2.23 ± 0.06 ^a	0.0086

^{a,b} Means in the same row bearing different superscripts indicate a statistically significant difference ($p < 0.05$). All data are presented as mean ± standard error.

3.1.3. Fatty Acid Contents

The fatty acid composition of feather meal and mixed meal is shown in Table 4. Significant differences were observed in multiple fatty acids between the two meals. Mixed meal exhibited significantly higher levels of most unsaturated fatty acids (e.g., oleic acid, $p = 0.0007$; linoleic acid, $p = 0.0010$) and specific saturated fatty acids, including butyric acid ($p < 0.0001$) and myristic acid ($p = 0.0015$). Conversely, feather meal showed higher contents of capric acid ($p = 0.0037$), nervonic acid ($p < 0.0001$), and lignoceric acid ($p = 0.0324$).

Table 4. Fatty acid contents of feather meal and mixed meal.

Fatty Acid (%)	Feather Meal	Mixed Meal	<i>p</i> -Value
Butyric acid	0.06 ± 0.01 ^b	0.62 ± 0.09 ^a	<0.0001
Caproic acid	0.32 ± 1.16 ^a	0.09 ± 0.002 ^b	<0.0001
Caprylic acid	0.51 ± 0.08 ^a	0.14 ± 0.002 ^b	<0.0001
Capric acid	3.09 ± 0.46 ^a	0.37 ± 0.03 ^b	<0.0001
Undecylic acid	0.22 ± 0.04 ^a	0.02 ± 0.002 ^b	<0.0001
Lauric acid	1.03 ± 0.16 ^a	0.34 ± 0.03 ^b	<0.0001
Tridecylic acid	0.09 ± 0.01 ^a	0.02 ± 0.001 ^b	<0.0001
Myristoleic acid	1.18 ± 0.12	0.99 ± 0.04	0.0069
Myristic acid	0.26 ± 0.01 ^b	0.55 ± 0.02 ^a	<0.0001
Pentadecylic acid	0.35 ± 0.02 ^a	0.21 ± 0.01 ^b	<0.0001
Pentadecenoic acid	0.01 ± 0.001	0.004 ± 0.0004	0.1399
Palmitic acid	29.26 ± 0.61 ^b	34.72 ± 0.49 ^a	<0.0001

Table 4. Cont.

Fatty Acid (%)	Feather Meal	Mixed Meal	p-Value
Palmitoleic acid	2.18 ± 0.07 ^b	5.99 ± 0.07 ^a	<0.0001
Margaric acid	2.52 ± 0.05 ^a	0.30 ± 0.01 ^b	<0.0001
cis-10-Heptadecenoic acid	0.07 ± 0.001 ^b	0.17 ± 0.01 ^a	<0.0001
Stearic acid	12.14 ± 0.12 ^a	10.31 ± 0.02 ^b	<0.0001
Elaidic acid	20.86 ± 0.44 ^a	2.49 ± 0.09 ^b	<0.0001
Oleic acid	10.77 ± 0.23 ^b	20.12 ± 0.28 ^a	<0.0001
Linolelaidic acid	0.02 ± 0.001 ^b	0.11 ± 0.01 ^a	<0.0001
Linoleic acid	9.11 ± 0.12 ^b	17.51 ± 0.08 ^a	<0.0001
γ-Linolenic acid	0.12 ± 0.01 ^b	0.314 ± 0.02 ^a	<0.0001
α-Linolenic acid	0.57 ± 0.03 ^b	1.81 ± 0.08 ^a	<0.0001
Arachidic acid	2.82 ± 0.23 ^a	0.49 ± 0.03 ^b	<0.0001
cis-11-Eicosenoic acid	0.22 ± 0.01 ^b	0.41 ± 0.03 ^a	<0.0001
cis-11,14-Eicosadienoic acid	0.06 ± 0.005 ^b	0.14 ± 0.01 ^a	<0.0001
Dihomo-γ-linolenic acid	0.08 ± 0.001 ^b	0.22 ± 0.02 ^a	<0.0001
Arachidonic acid	0.48 ± 0.02 ^b	0.92 ± 0.05 ^a	<0.0001
cis-11,14,17-Eicosatrienoic acid	0.26 ± 0.21	0.20 ± 0.35	0.7529
Behenic acid	0.62 ± 0.03 ^a	0.21 ± 0.01 ^b	<0.0001
Erucic acid	0.02 ± 0.001	0.02 ± 0.001	0.0012
Eicosapentaenoic acid	0.01 ± 0.001 ^b	0.03 ± 0.003 ^a	<0.0001
cis-13,16-Docosadienoic acid	0.01 ± 0.001	0.01 ± 0.003	0.1544
Tricosylic acid	0.07 ± 0.004 ^a	0.01 ± 0.003 ^b	<0.0001
Lignoceric acid	0.50 ± 0.03 ^a	0.07 ± 0.004 ^b	<0.0001
Nervonic acid	0.02 ± 0.001 ^b	0.05 ± 0.003 ^a	<0.0001
Docosahexaenoic acid	0.02 ± 0.001 ^b	0.05 ± 0.003 ^a	<0.0001

^{a,b} Means in the same row bearing different superscripts indicate a statistically significant difference ($p < 0.05$). All data are presented as mean ± standard error.

3.2. Apparent and Standardized Ileal Nutrient Digestibility

3.2.1. Digestibility of Proximate Composition

According to the AID and SID data shown in Table 5, mixed meal showed significantly higher digestibility of ash (AID, $p = 0.0013$; SID, $p = 0.0013$), crude fiber (AID, $p = 0.0427$; SID, $p = 0.0427$), neutral detergent fiber (AID, $p = 0.0271$; SID, $p = 0.0270$), and acid detergent fiber (AID, $p = 0.0090$; SID, $p = 0.0090$) compared to feather meal. In contrast, feather meal had significantly higher digestibility of acid detergent lignin (AID, $p = 0.0274$; SID, $p = 0.0274$). The digestibility of dry matter, organic matter, ether extract, crude protein, or metabolizable energy showed no significant variation among treatments.

Table 5. Apparent and standard digestibility of nutrients in broilers fed feather meal and mixed meal.

Content (%)	AID ¹			SID ²		
	Feather Meal	Mixed Meal	p-Value	Feather Meal	Mixed Meal	p-Value
Dry matter	33.10 ± 0.44	35.53 ± 0.97	0.2220	33.77 ± 0.43	36.17 ± 0.96	0.2220
Organic matter	34.65 ± 0.53	38.64 ± 1.28	0.1813	35.31 ± 0.52	39.25 ± 1.27	0.1813
Ash	2.86 ± 0.203 ^b	17.29 ± 2.77 ^a	0.0013	3.83 ± 0.44 ^b	18.82 ± 2.75 ^a	0.0013
Ether extract	94.63 ± 0.12	90.49 ± 0.32	0.1240	94.69 ± 0.11	90.58 ± 0.32	0.1241
Crude protein	36.82 ± 0.82	37.06 ± 1.57	0.3199	37.45 ± 0.82	37.69 ± 1.55	0.3199
Crude fiber	30.72 ± 6.55 ^b	61.19 ± 1.57 ^a	0.0427	31.41 ± 6.48 ^b	61.58 ± 1.56 ^a	0.0427
Neutral detergent fiber	60.57 ± 0.14 ^b	87.89 ± 0.30 ^a	0.0271	61.57 ± 0.56 ^b	87.78 ± 0.38 ^a	0.0270
Acid detergent fiber	6.97 ± 5.26 ^b	57.72 ± 0.74 ^a	0.0090	7.90 ± 5.21 ^b	58.14 ± 0.73 ^a	0.0090
Acid detergent lignin	66.54 ± 1.81 ^a	58.85 ± 8.80 ^b	0.0274	66.88 ± 1.79 ^a	59.26 ± 8.71 ^b	0.0274
Metabolizable energy (J/g)	10,009.8 ± 98.50	9983.30 ± 263.60	0.1402			

^{a,b} Means in the same row bearing different superscripts indicate a statistically significant difference ($p < 0.05$) for each variable. All data are presented as mean ± standard error. ¹ AID = Apparent ileal digestibility. ² SID = Standardized ileal digestibility.

3.2.2. Amino Acids Digestibility

The digestibility of the amino acid contents of feather meal and mixed meal is shown in Table 6. Significant differences were detected between the two feed types in the digestibility of several amino acids. Mixed meal exhibited significantly higher AID and SID for valine ($p = 0.0212$), phenylalanine ($p = 0.0433$), isoleucine ($p = 0.0172$), histidine ($p = 0.0007$), and serine ($p = 0.0163$) compared to feather meal. In contrast, feather meal showed significantly higher digestibility for aspartic acid ($p = 0.0231$). No alterations ($p > 0.05$) were found in the digestibility of threonine, leucine, lysine, methionine, arginine, proline, alanine, tyrosine, glycine, glutamic acid, or cystine between the two meals.

Table 6. Digestibility of amino acids in broilers fed feather meal and mixed meal-based diets.

Content (%)	AID ¹			SID ²		
	Feather Meal	Mixed Meal	<i>p</i> -Value	Feather Meal	Mixed Meal	<i>p</i> -Value
Essential amino acids						
Threonine	37.93 ± 1.45	43.46 ± 0.75	0.1970	38.55 ± 1.44	44.03 ± 0.74	0.1967
Valine	31.01 ± 1.37 ^b	52.27 ± 0.94 ^a	0.0212	31.69 ± 1.35 ^b	52.74 ± 0.93 ^a	0.0212
Leucine	34.83 ± 3.29	50.49 ± 0.95	0.1354	35.48 ± 3.26	50.99 ± 0.94	0.1354
Phenylalanine	44.37 ± 1.07 ^b	56.35 ± 0.94 ^a	0.0433	44.93 ± 1.06 ^b	56.79 ± 0.93 ^a	0.0429
Lysine	26.83 ± 1.03	20.38 ± 1.37	0.0967	27.56 ± 1.01	21.18 ± 1.36	0.0963
Isoleucine	30.95 ± 1.59 ^b	59.85 ± 0.77 ^a	0.0172	31.64 ± 1.58 ^b	60.24 ± 0.94 ^a	0.0172
Methionine	39.10 ± 3.56	33.94 ± 0.49	0.5055	39.81 ± 3.53	34.60 ± 0.49	0.5054
Arginine	41.62 ± 1.08	47.85 ± 0.78	0.1929	42.21 ± 1.07	48.37 ± 0.77	0.1921
Histidine	7.17 ± 1.40 ^b	28.09 ± 1.94 ^a	0.0007	8.09 ± 1.38 ^b	28.80 ± 1.92 ^a	0.0008
Non-essential amino acids						
Proline	38.37 ± 2.09	40.39 ± 1.26	0.6873	38.98 ± 2.07	41.52 ± 1.24	0.6877
Aspartic acid	28.51 ± 2.21 ^b	6.35 ± 1.47 ^a	0.0231	29.23 ± 2.18 ^b	7.28 ± 1.46 ^a	0.0245
Alanine	43.47 ± 1.36	49.54 ± 1.86	0.4532	44.03 ± 1.34	50.05 ± 1.85	0.4523
Tyrosine	42.74 ± 1.67	39.46 ± 1.10	0.4753	43.31 ± 1.65	40.07 ± 1.09	0.4763
Serine	30.31 ± 0.66 ^b	46.63 ± 0.75 ^a	0.0163	31.01 ± 0.65 ^b	47.17 ± 0.74 ^a	0.0167
Glycine	44.61 ± 0.83	46.23 ± 1.58	0.3303	45.17 ± 0.83	46.77 ± 1.56	0.3299
Glutamic acid	39.84 ± 1.37	35.87 ± 1.93	0.4340	40.44 ± 1.36	36.51 ± 1.91	0.4339
Cystine	42.69 ± 1.18	41.67 ± 1.50	0.9672	43.26 ± 1.17	42.25 ± 1.49	0.9671

^{a,b} Means in the same row bearing different superscripts indicate a statistically significant difference ($p < 0.05$) for each variable. All data are presented as mean ± standard error. ¹ AID = Apparent ileal digestibility. ² SID = Standardized ileal digestibility.

3.2.3. Digestibility of Fatty Acids

The fatty acid digestibility of feather meal and mixed meal is shown in Table 7. Significant differences in the digestibility of most fatty acids were detected between the two feed types. Mixed meal exhibited significantly higher AID and SID for the majority of fatty acids, particularly short- and medium-chain fatty acids, including butyric acid ($p = 0.0006$), caproic acid ($p = 0.0005$), caprylic acid ($p = 0.0001$), and capric acid ($p = 0.0016$). Several unsaturated fatty acids, including linoleic acid ($p = 0.0418$), linoleic acid ($p = 0.0377$), and α -linolenic acid ($p = 0.0428$), also demonstrated significantly higher digestibility in mixed meal. In contrast, feather meal showed significantly higher digestibility for lignoceric acid ($p < 0.0001$). No significant differences were observed in the digestibility of tridecyl acid, pentadecyl acid, palmitic acid, heptadecanoic acid, palmitoleic acid, stearic acid, oleic acid, elaidic acid, arachidic acid, and several other fatty acids between the two meals.

Table 7. Digestibility of fatty acids in broilers fed feather meal and mixed meal-based diets.

Fatty Acids (%)	AID ¹			SID ²		
	Feather Meal	Mixed Meal	<i>p</i> -Value	Feather Meal	Mixed Meal	<i>p</i> -Value
Butyric acid	43.10 ± 11.95 ^b	96.32 ± 1.22 ^a	0.0006	43.67 ± 11.83 ^b	96.36 ± 1.21 ^a	0.0006
Caproic acid	57.75 ± 4.84 ^b	92.76 ± 0.46 ^a	0.0005	58.17 ± 4.79 ^b	92.83 ± 0.46 ^a	0.0005
Caprylic acid	29.92 ± 3.67 ^b	95.97 ± 0.24 ^a	0.0001	30.62 ± 3.63 ^b	96.01 ± 0.23 ^a	0.0001
Capric acid	17.70 ± 7.26 ^b	85.11 ± 0.93 ^a	0.0016	18.52 ± 7.19 ^b	85.26 ± 0.92 ^a	0.0016
Undecylic acid	23.50 ± 10.64 ^b	81.80 ± 1.12 ^a	0.0007	24.26 ± 10.54 ^b	81.99 ± 1.11 ^a	0.0007
Lauric acid	37.13 ± 3.50 ^b	80.30 ± 0.96 ^a	0.0279	37.76 ± 3.47 ^b	80.5 ± 0.95 ^a	0.0279
Tridecylic acid	15.66 ± 2.71	78.83 ± 1.26	0.1662	16.51 ± 2.68	79.04 ± 1.25	0.1662
Myristoleic acid	40.81 ± 4.55 ^b	78.18 ± 1.00 ^a	0.0124	41.41 ± 4.51 ^b	78.39 ± 0.99 ^a	0.0124
Myristic acid	79.65 ± 2.57 ^b	90.93 ± 1.29 ^a	0.0081	79.85 ± 2.55	91.02 ± 1.27	0.2082
Pentadecylic acid	51.73 ± 4.84	79.18 ± 1.74	0.0725	52.21 ± 4.8	79.39 ± 1.72	0.0725
Pentadecenoic acid	68.14 ± 8.19	81.93 ± 1.56	0.0872	68.46 ± 4.06	82.11 ± 1.54	0.0872
Palmitic acid	55.47 ± 4.87	72.35 ± 1.73	0.0698	55.91 ± 4.82	72.62 ± 1.71	0.0698
Palmitoleic acid	88.22 ± 1.47	89.55 ± 0.61	0.1158	88.34 ± 1.46	89.65 ± 0.60	0.1158
Heptadecanoic acid	48.17 ± 5.44	71.55 ± 2.67	0.1966	48.69 ± 5.39	71.84 ± 2.64	0.1966
Heptadecenoic acid	86.27 ± 2.21 ^b	89.57 ± 0.67 ^a	0.0395	86.4 ± 2.18 ^b	89.67 ± 0.66 ^a	0.0395
Stearic acid	35.45 ± 6.70	62.99 ± 2.77	0.0998	36.19 ± 6.93	63.36 ± 2.74	0.0998
Elaidic acid	86.13 ± 1.75	87.70 ± 3.19	0.2701	86.27 ± 1.73	87.83 ± 3.16	0.2701
Oleic acid	87.11 ± 1.99	83.18 ± 0.84	0.1278	87.23 ± 1.96	83.35 ± 0.84	0.1278
Linolelaidic acid	82.22 ± 2.99 ^b	86.13 ± 0.92 ^a	0.0418	82.4 ± 2.96 ^b	86.27 ± 0.91 ^a	0.0418
Linoleic acid	80.77 ± 3.17 ^b	82.41 ± 0.95 ^a	0.0377	80.96 ± 3.14 ^b	82.59 ± 0.94 ^a	0.0377
γ-Linolenic acid	84.86 ± 3.04 ^b	92.73 ± 0.63 ^a	0.0097	85.02 ± 3.01 ^b	92.8 ± 0.62 ^a	0.0097
α-Linolenic acid	89.56 ± 1.71 ^b	91.90 ± 0.53 ^a	0.0428	89.67 ± 1.69 ^b	91.98 ± 0.52 ^a	0.0428
Arachidic acid	17.17 ± 5.94	60.93 ± 3.43	0.3135	18 ± 5.88	61.32 ± 3.4	0.3135
cis-11-Eicosenoic acid	79.44 ± 3.06	80.55 ± 1.15	0.0854	79.65 ± 3.03	80.75 ± 1.14	0.0854
Eicosadienoic acid	70.92 ± 5.93 ^b	82.51 ± 1.01 ^a	0.0046	71.21 ± 5.87 ^b	82.68 ± 1.00 ^a	0.0046
Dihomo-γ-linolenic	51.83 ± 11.06 ^b	83.21 ± 1.26 ^a	0.0010	52.31 ± 10.95 ^b	83.38 ± 1.25 ^a	0.0010
Arachidonic acid	27.03 ± 10.55 ^b	53.07 ± 3.23 ^a	0.0415	27.76 ± 10.44 ^b	53.54 ± 3.19 ^a	0.0415
Eicosatrienoic acid	28.76 ± 10.85 ^b	53.07 ± 3.23 ^a	0.0374	29.47 ± 10.74 ^b	53.54 ± 3.19 ^a	0.0374
Behenic acid	17.68 ± 8.93	60.70 ± 3.23	0.0742	18.5 ± 8.84	61.09 ± 3.2	0.0742
Erucic acid	68.88 ± 4.50 ^b	72.11 ± 1.44 ^a	0.0489	69.19 ± 4.45 ^b	72.39 ± 1.43 ^a	0.0489
Eicosapentaenoic acid	35.98 ± 3.72	59.96 ± 2.32	0.3806	36.62 ± 3.69	60.36 ± 2.29	0.3806
Docosadienoic acid	50.58 ± 9.10	76.30 ± 10.12	0.8427	51.07 ± 9.01	76.53 ± 10.02	0.8427
Tricosylic acid	17.14 ± 9.31	42.66 ± 10.95	0.7612	37.63 ± 13.25	70.59 ± 3.09	0.1293
Lignoceric acid	54.99 ± 5.50 ^a	47.17 ± 11.34 ^b	<0.0001	17.7 ± 8.81 ^b	84.62 ± 8.46 ^a	<0.0001
Nervonic acid	19.62 ± 8.72	35.46 ± 3.98	0.1582	28.92 ± 11.83	45.56 ± 5.04	0.1271
Docosahexaenoic acid	29.43 ± 11.21	31.73 ± 4.45	0.1014	24.65 ± 5.06	32.23 ± 4.36	0.7809

^{a,b} Means in the same row bearing different superscripts indicate a statistically significant difference ($p < 0.05$) for each variable. All data are presented as mean ± standard error. ¹ AID = Apparent ileal digestibility. ² SID = Standardized ileal digestibility.

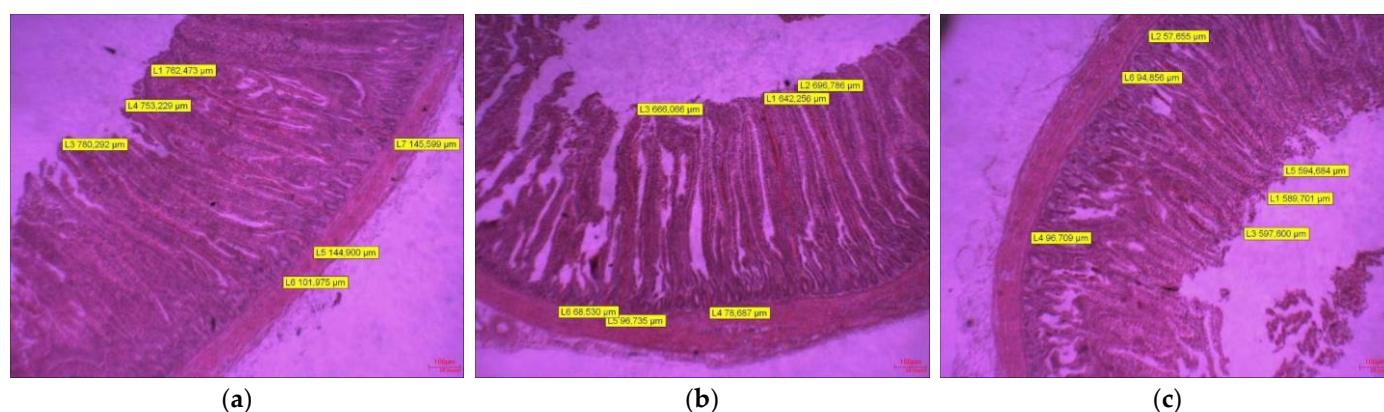
3.3. Small Intestinal Histomorphology

Table 8 presents the histomorphological characteristics of the small intestine of broiler chickens fed diets based on feather meal or mixed meal (Figure 1). Birds fed the mixed meal diet exhibited significantly greater villus height in the jejunum and ileum, along with increased crypt depth in the ileum, compared with those fed feather meal ($p < 0.05$). In contrast, the villus height-to-crypt depth ratio in the jejunum was significantly higher in the feather meal group. No differences ($p > 0.05$) in duodenal morphology were detected between the two dietary treatments.

Table 8. Histomorphology of the small intestine in broiler chicken fed feather meal and mixed meal.

Parameters	Feather Meal	Mixed Meal	<i>p</i> -Value
Villi height, μm			
Duodenum	1132.43 $\pm$ 29.81	1073.27 $\pm$ 22.99	0.6274
Jejunum	796.41 $\pm$ 2.93 ^b	850.89 $\pm$ 16.51 ^a	0.0055
Ileum	445.19 $\pm$ 6.78 ^b	665.63 $\pm$ 23.32 ^a	0.0346
Crypt depth, μm			
Duodenum	102.51 $\pm$ 3.71	96.16 $\pm$ 2.10	0.2949
Jejunum	80.67 $\pm$ 0.37	97.22 $\pm$ 0.63	0.3273
Ileum	33.64 $\pm$ 0.15 ^b	109.21 $\pm$ 0.64 ^a	0.0165
Villi height: Crypt depth			
Duodenum	11.08 $\pm$ 0.36	11.18 $\pm$ 0.31	0.7922
Jejunum	9.87 $\pm$ 0.05 ^b	8.76 $\pm$ 0.22 ^a	0.0119
Ileum	13.23 $\pm$ 0.17	6.10 $\pm$ 0.21	0.6626

^{a,b} Means in the same row bearing different superscripts indicate a statistically significant difference ($p < 0.05$). All data are presented as mean $\pm$ standard error.

**Figure 1.** (a) Duodenum, (b) Jejunum, (c) Ileum villi heights and crypt depth of small intestines.

4. Discussion

4.1. Proximate Composition

The compositional variation between the two animal by-products likely arises from differences in raw material sources and processing conditions; mixed meal is typically produced from a blend of diverse animal tissues, whereas feather meal consists almost exclusively of poultry feathers that require specific hydrolysis to disrupt keratin, resulting in compositional differences [24,25].

Feather meal and mixed meal exhibited distinct differences in proximate composition. Feather meal contained substantially higher crude protein (94.36%) and gross energy, whereas mixed meal showed higher ash, crude fiber, and unsaturated fatty acid contents. These results are consistent with those of Wang and Parsons [2] and Papadopoulos [26], who reported that the elevated protein concentration of feather meal results from its keratin structure, which is highly resistant to hydrolysis. The higher ash and unsaturated fatty acid contents in mixed meal reflect the retention of mineral and lipid fractions during rendering [27].

4.2. Composition and Digestibility of Amino Acids

Recent advances in precision nutrition emphasize that ingredient composition and processing strongly influence amino acid digestibility and overall protein utilization in broiler diets, reinforcing observed differences between feather meal and mixed meal [28].

The amino acid composition showed that feather meal was rich in methionine and lysine, while mixed meal contained higher levels of valine, leucine, isoleucine, and serine. Despite the higher crude protein content of feather meal, its amino acid balance was markedly poorer. The SID results further revealed that mixed meal exhibited greater digestibility of several essential amino acids, including valine, phenylalanine, isoleucine, and histidine. These findings are consistent with Lemme et al. [7] and Adedokun et al. [12], who reported that keratin-based ingredients exhibit low amino acid digestibility due to extensive disulfide cross-linkages, making the keratin matrix resistant to enzymatic degradation. Conversely, mixed meal contains a higher proportion of connective tissue proteins, which are generally more easily digestible. Processing methods such as enzymatic hydrolysis or alkaline treatment may improve the availability of amino acids in feather meal and enhance its efficiency in broiler diets [4].

The observed differences in amino acid digestibility can be largely attributed to the inherent physical and chemical structures of the proteins in the respective ingredients. Feather meal is predominantly composed of keratin, which contains dense disulfide cross-linkages that strongly resist degradation by gastrointestinal digestive enzymes. In contrast, the mixed meal provides a more balanced amino acid profile with protein structures (such as connective or muscle tissues) that are more readily accessible to enzymatic hydrolysis, thereby leading to significantly higher AID and SID values.

4.3. Composition and Digestibility of Fatty Acid

Emerging research highlights that the structural and physicochemical properties of fatty acids play a crucial role in digestibility and lipid utilization in poultry, aligning with the observed differences between feather meal and mixed meal [29,30].

The fatty acid profiles of feather meal and mixed meal differed markedly. Mixed meal exhibited both higher proportions and greater digestibility of unsaturated fatty acids, particularly linoleic, α -linolenic, and γ -linolenic acids, which are essential for metabolic regulation and membrane integrity in poultry. These findings correspond with those of Izuddin et al. [17], who reported that dietary inclusion of unsaturated fats improved lipid absorption and metabolism in laying hens. The superior digestibility of unsaturated fatty acids in mixed meal may be attributed to their lower melting points and greater solubility, which facilitate micelle formation and intestinal absorption. In contrast, the predominance of long-chain saturated fatty acids in feather meal could explain its relatively lower digestibility.

Furthermore, the digestibility of fatty acids is closely related to their structural and physicochemical properties, particularly their degree of saturation. Ingredients with a higher proportion of unsaturated fatty acids exhibit better lipid emulsification and micelle formation in the avian gastrointestinal tract. Consequently, the distinct fatty acid profile in the mixed meal facilitates more efficient lipid absorption, resulting in higher overall digestibility compared to the lipid fractions primarily found in feather meal.

4.4. Fiber and Energy Digestibility

The structural and physicochemical characteristics of feed ingredients can modulate both enzymatic digestibility and gut microbial interactions, shaping nutrient utilization efficiency in broilers [30,31].

Mixed meal showed significantly higher apparent and standardized ileal digestibility values for ash and fibers than feather meal, indicating improved utilization of mineral and fibrous components. This improvement could be linked to the heterogeneous structure and residual lipid content of mixed meal, which may enhance enzymatic exposure and microbial fermentation. Similar patterns were described by Barua et al. [10], who emphasized the

influence of ingredient structure on nutrient accessibility. The higher digestibility of ADL in feather meal could be due to partial keratin breakdown during thermal processing. Nevertheless, no changes were detected between feather meal and mixed meal in crude protein or metabolizable energy digestibility, suggesting that both can serve as viable protein and energy sources when appropriately processed.

4.5. Intestinal Morphology

Morphological assessment of the small intestine revealed that broilers fed mixed meal-based diets exhibited significantly greater villus height in the jejunum and ileum, as well as deeper crypts in the ileum, suggesting enhanced absorptive surface area and epithelial regeneration. These observations are consistent with those of Gilani et al. [32], who reported that improved nutrient digestibility supports intestinal development and function. Conversely, the higher villus height-to-crypt depth ratio observed in the jejunum of feather meal-fed birds is likely a mathematical consequence of their shallower crypt depths, as the lower nutrient digestibility of feather meal may not provide sufficient substrates to stimulate rapid intestinal crypt cell proliferation compared to mixed meal. Overall, the morphological advantages observed in the mixed meal-fed group align with its superior nutrient digestibility and utilization efficiency.

5. Conclusions

This study demonstrates that although feather meal has high crude protein, its amino acid composition is unbalanced and its nutrient digestibility is relatively low. In contrast, mixed meal not only has a more balanced nutritional composition but also shows significantly higher AID and SID values for most nutrients (particularly fiber components and essential amino acids). Additionally, mixed meal promotes intestinal development, as evidenced by significantly increased villus height in the jejunum and ileum. Therefore, under the conditions of this short-term study, mixed meal demonstrated better overall nutrient digestibility and beneficial effects on intestinal morphology compared to feather meal, suggesting its potential as a viable alternative protein source in broiler diets. Building on existing research, further efforts should continue to optimize strategies to enhance the utilization efficiency of feather meal, particularly through its combination with complementary protein sources, through the application of processing techniques such as enzymatic treatment.

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