

Feed passage time, apparent metabolisable energy and apparent ileal amino acid digestibility of pretreated anchovy waste in quails

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ABSTRACT

This study investigated the effects of pre-treatment methods on the digestive utilisation of anchovy waste (*Engraulidae*) in Japanese quail (*Coturnix japonica*), evaluating feed passage kinetics, apparent Metabolisable energy (AME), and apparent ileal amino acid digestibility (AIAAD). Anchovy waste was subjected to desalting (DAW) by soaking in distilled water (1:3 w/v for 2 h) or *Lactiplantibacillus plantarum* fermentation (FAW) (5% inoculum, 10% molasses, 37°C for 48 h) and compared against untreated anchovy (UAW) and commercial fish meal (FM) as a reference. One-day-old quail chicks (n=140) were fed basal or experimental diets containing untreated, desalted, or fermented anchovy or commercial fish meal at 25% inclusion, with 0.5% TiO₂ as a marker. Rate of passage (T₅₀ and MRT), AME, and ileal crude protein and amino acid digestibility were determined using the difference and direct methods, with data analysed by one-way ANOVA (Tukey's HSD, p<0.05). Fermentation increased crude protein to 58.90% and reduced sodium by 60.8% compared to UAW (57,473 mg/kg DM). Desalting increased sodium concentration on a dry matter basis to 71,137 mg/kg DM whereby the apparent increase attributable to concentration of mineral residues following removal of water and soluble fractions as well as increased calcium to 61,627 mg/kg DM. UAW produced the shortest T₅₀ (2.313 h) but longest mean retention time (4.945 h), associated with its high-ash, high-mineral composition. Fermentation normalised passage kinetics. Desalted anchovy achieved the highest energy values at both the diet (2,870 kcal/kg) and ingredient (5,521 kcal/kg) levels, surpassing FM, attributable to compositional enrichment of energy-yielding fractions following aqueous washing. UAW showed the highest amino acid digestibility, matching or exceeding FM, while fermentation reduced digestibility. These findings indicate that anchovy waste represents a viable alternative protein ingredient for poultry, and that pre-treatment selection should be guided by whether energy optimisation or amino acid availability is the primary nutritional objective.

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1. INTRODUCTION

Anchovy waste (*Engraulidae*) represents a nutritionally promising alternative protein source for poultry, offering high crude protein content and a favourable amino acid profile comparable to conventional fish meal. However, its direct dietary application is constrained by several physicochemical limitations, including elevated salt concentrations arising from preservation processes, variable nutrient composition, and inconsistent protein digestibility (Awogbemi & Kallon, 2022; Peguero et al., 2022). Excess dietary salt may impair feed intake, osmotic regulation, and

nutrient absorption, while reduced protein accessibility limits digestive utilisation. These constraints have prompted interest in pre-treatment approaches, specifically aqueous desalting and microbial fermentation, as strategies to improve the nutritional quality and digestive compatibility of anchovy waste for use in poultry diets.

Desalting by aqueous washing reduces soluble salt content and may reconcentrate energy-yielding macronutrients in the residual dry matter, while fermentation with lactic acid bacteria such as *Lactiplantibacillus plantarum* can enhance protein hydrolysis, reduce anti-nutritional factors, and improve amino acid availability through microbial

enzymatic activity (Awogbemi & Kallon, 2022; Adebo et al., 2022). Although prior work has demonstrated that pre-treated anchovy waste improves growth performance and feed conversion in broilers (Nor et al., 2025), current knowledge remains largely confined to production-level responses. Whether these pre-treatments influence digestive utilisation, specifically feed passage kinetics, apparent metabolisable energy (AME), and apparent ileal amino acid digestibility (AIAAD), has not been established.

Japanese quail (*Coturnix japonica*) are widely used as a poultry model for digestive response studies due to their rapid growth, short production cycle, and sensitivity to dietary modifications; yet despite their suitability as an experimental model, no published study has evaluated the effects of pre-treated anchovy waste on gastrointestinal transit or nutrient digestibility in this species. This represents part of a broader set of unaddressed gaps in the literature, encompassing the effects of anchovy waste pre-treatment on feed passage kinetics (T_{50} and mean retention time, MRT), the absence of AME and AIAAD data specific to pre-treated anchovy waste in quail, and the lack of any direct comparison of desalting versus fermentation on digestive utilisation outcomes. The present study was therefore conducted to address these gaps by determining the effects of untreated, desalted, and fermented anchovy waste on feed passage time, AME, and AIAAD in Japanese quail, with the aim of providing evidence-based guidance for the practical dietary application of pre-treated anchovy waste in poultry production

2. MATERIALS AND METHODS

2.1. Ethical note

All experimental procedures involving animals were conducted in accordance with the guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of Universiti Malaysia Kelantan (UMK/FIAT/ACUE/PG/2025/02). The study complied with internationally accepted standards for the ethical treatment of animals in research. Throughout the experiment, birds were monitored daily to ensure their health and welfare, and all management practices were implemented to minimise stress and discomfort. Euthanasia for ileal digesta collection was performed by the intravenous administration of sodium pentobarbitone in accordance with approved humane procedures.

2.2. Anchovy waste treatment

Anchovy waste was purchased from a supplier in Bachok, Kelantan, Malaysia, and ground into flour before treatment. The anchovy was subjected to the following treatments: (1) desalting treatment (DAW) and (2)

Lactiplantibacillus plantarum bacteria fermentation (FAW). Desalting method (adapted from Marchetti et al., 2021; Boumendil et al., 2023) involved soaking ground anchovy in distilled water (1:3 w/v) for 2 h, followed by sieving and drying.

Fermentation was conducted following the method of Bueno-Solano et al. (2009) with minor modifications. Lactiplantibacillus plantarum obtained from Halways Sdn. Bhd. (Selangor, Malaysia) was cultured in de Man, Rogosa and Sharpe (MRS) broth and incubated at 37 °C for 24 h until reaching an optical density of $OD_{600} \approx 1.5$. A strain-level identifier was not provided by the commercial supplier; this limitation is acknowledged and future studies should employ characterised strains with deposited accession numbers. The fermentation mixture comprised 5% (v/v) inoculum, 10% (w/v) anchovy flour, and 10% (v/v) diluted molasses as a fermentable carbohydrate source. The anchovy waste was fully submerged in this liquid medium throughout the incubation period, yielding an approximate mixture moisture content of 78–82% (w/w).

Fermentation was carried out under strictly anaerobic conditions, achieved by sealing the fermentation vessel and purging headspace with nitrogen gas prior to incubation. The mixture was incubated at 37 °C for 48 h. At the end of the fermentation period, the pH of the fermented mixture was 4.2 ± 0.1 , confirming active lactic acid production; bacterial counts declined from 1.24×10^6 to 4.46×10^5 CFU/mL (64% reduction), consistent with substrate depletion and acidification inhibition of further growth. The fermented product was not sterilised prior to drying, in order to preserve heat-labile bioactive metabolites produced during fermentation. The product was dried in a forced-air oven at 50 °C for 72 h (three days) or until constant weight was achieved ($\leq 0.1\%$ mass change between consecutive 6-h intervals). The dried product was filtered and stored for further analysis and dietary inclusion.

2.3. Birds, housing, and management

One-day-old quail (*Coturnix japonica*) chicks ($n = 140$) were obtained from a local hatchery. On day 1, chicks were individually weighed and randomly assigned to 20 battery cages ($43 \times 57 \times 33$ cm; seven birds per cage) with wire flooring in an open-sided house. Ambient temperature ranged from 28°C to 38°C, and relative humidity from 62% to 80%. Feed and water were provided ad libitum under continuous lighting throughout the study (Hakim et al., 2020).

2.4 Experimental diets

The AME of anchovy was determined using the difference method as described by Abdollahi et al. (2015), whereas ileal crude protein (CP) and amino acid (AA)

digestibility were evaluated using the direct method according to Ahmed et al. (2014) and Jia et al. (2012). For the rate of passage (ROP) assay, the same test diet applied in the AME determination was utilised.

Under the difference method, a corn–soybean meal–based formulation served as the basal diet. Basal starter and finisher diets were formulated to meet or exceed the nutrient requirements established by the National Research Council (1994), as appropriate for each growth phase. From day 1 to 14, birds received a standard broiler starter diet containing 23.6% CP and 3,520 kcal/kg metabolisable energy (Table 1). Subsequently, five experimental diets were formulated by incorporating untreated anchovy, desalted anchovy, fermented anchovy, or commercial fish meal at 25% inclusion of the basal diet (Table 2).

Table 1. Ingredient and nutrient compositions of starter

Item	Starter
Ingredient (% fed basis)	
Corn	49.80
Soybean meal	42.64
Palm oil	3.81
DCP	1.54
Limestone	1.32
NaCl	0.34
DL-Methionine	0.19
L-Lysine.HCL	0.15
Mineral premix	0.10
L-Threonine	0.06
Vitamin premix	0.05
Nutrient composition (% unless stated otherwise)	
CP	23.6
CF	3.2
EE	4.3
ME (kcal kg ⁻¹)	3,520

DCP= Dicalcium Phosphate; NaCl= Sodium Chloride; CP = Crude Protein; CF= Crude Fiber; EE = Ether Extract; ME= Metabolisable Energy.

For the direct method, untreated anchovy, desalted anchovy, fermented anchovy, or commercial fish meal served as the sole source of CP and AA in the assay diets (Hakim et al., 2020) (Table 3) to ensure that all protein utilisation and digestibility responses are clearly attributable to that ingredient alone, avoiding confounding from other protein sources and enabling accurate comparison of processing effects. The high inclusion level of the test ingredient (~95%) in the direct-method assay diets is a standard feature of this methodology, ensuring that all protein and amino acids in the ileal digesta are solely attributable to the test ingredient. Small quantities of palm oil (energy), minerals, and vitamins were included to support basic physiological maintenance during the short assay period. This approach is consistent with validated direct-method protocols in the poultry nutrition literature (Zhang & Adeola, 2017; Hakim et al., 2020; Ahmed

et al., 2014; Jia et al., 2012). The assay diet was offered for a brief period (1.5 h after a 24-h fast), limiting the duration of dietary imbalance exposure.

Titanium dioxide (TiO₂) was included in all diets at 0.50% as an indigestible marker. Experimental diets were arranged in a completely randomised design with four replicates (cages) per treatment.

Table 2. Ingredient and nutrient compositions of finisher (difference method)

Item	C1	C2	T1	T2	T3
Ingredient (% fed basis)					
Corn	56.74	42.56	42.56	42.56	42.56
Soybean meal	35.70	26.78	26.78	26.78	26.78
Commercial Fish meal/Anchovy waste	-	25.00	25.00	25.00	25.00
Palm oil	3.81	2.86	2.86	2.86	2.86
DCP	1.54	1.16	1.16	1.16	1.16
Limestone	1.32	0.99	0.99	0.99	0.99
NaCl	0.34	0.26	0.26	0.26	0.26
DL-Methionine	0.19	0.14	0.14	0.14	0.14
L-Lysine.HCL	0.15	0.11	0.11	0.11	0.11
Mineral premix	0.10	0.08	0.08	0.08	0.08
L-Threonine	0.06	0.05	0.05	0.05	0.05
Vitamin premix	0.05	0.04	0.04	0.04	0.04
Nutrient composition (% unless stated otherwise)					
CP	21.6	33.42	20.70	20.73	23.58
CF	3.7	1.77	1.77	1.77	1.77
EE	0.5	6.20	9.04	2.60	10.80
ME (kcal kg ⁻¹)	3,540	2,747	2,359	2,864	2,517

C1 = Basal diet; C2 = Commercial fish meal diet; T1 = Untreated anchovy; T2 = Desalted anchovy; T3 = Fermented anchovy; DCP= Dicalcium Phosphate; NaCl= Sodium Chloride; CP = Crude Protein; CF= Crude Fiber; EE = Ether Extract; ME= Metabolisable Energy.

Table 3. Ingredient and nutrient compositions of finisher (direct method)

Item	C2	T1	T2	T3
Ingredient (% fed basis)				
Corn	-	-	-	-
Soybean meal	-	-	-	-
Commercial Fish meal/Anchovy waste	95.15	95.15	95.15	95.15
Palm oil	2.00	2.00	2.00	2.00
DCP	1.35	1.35	1.35	1.35
Limestone	1.00	1.00	1.00	1.00
NaCl	0.35	0.35	0.35	0.35
DL-Methionine	-	-	-	-
L-Lysine.HCL	-	-	-	-
Mineral premix	0.05	0.05	0.05	0.05
L-Threonine	-	-	-	-
Vitamin premix	0.1	0.1	0.1	0.1
Nutrient composition (% unless stated otherwise)				
CP	69.863	52.785	54.195	51.506
CF	0.00	0.00	0.00	0.00
EE	9.900	2.185	0.725	2.035
ME (kcal kg ⁻¹)	4830	3360	5370	3960

C2 = Commercial fish meal diet; T1 = Untreated anchovy; T2 = Desalted anchovy; T3 = Fermented anchovy; DCP= Dicalcium Phosphate; NaCl= Sodium Chloride; CP = Crude Protein; CF= Crude Fiber; EE = Ether Extract; ME= Metabolisable Energy.

2.5 Experimental procedure

From days 15–18, birds were adapted to experimental diets. On day 19, the ROP assay was performed per Rochell et al. (2012). Each anchovy-based treatment included four replicates (cages) of seven birds. After a 2-hour feed withdrawal, birds received TiO_2 -marked diets for 2 hours, followed by the same diets without TiO_2 ad libitum. Marker intake was quantified, and excreta were collected at 1-hour intervals over 12 hours, then weighed and frozen.

On day 20, birds from each ROP treatment were divided into two subgroups (seven birds per cage). One subgroup underwent an AME assay from days 20–23, receiving the same TiO_2 -marked diets. Daily feed intake was recorded, and excreta were pooled by cage, homogenized, subsampled, freeze-dried, ground, and stored at -20°C for DM and gross energy (GE) analysis.

The remaining subgroup (excluding the basal diet group) received direct-method assay diets using the test ingredient as the sole protein and energy source to isolate its nutrient contributions (Zhang & Adeola, 2017). After a 4-day adaptation (days 20–23), birds fasted for 24 hours to clear the gastrointestinal tract (Wang et al., 2022). On day 25, birds received assay diets ad libitum for 1.5 hours. Four hours later, they were euthanised via sodium pentobarbitone injection into the wing vein. Ileal digesta were flushed with distilled water, pooled by cage, freeze-dried, and stored at -20°C for analysis of DM, CP, amino acids (AA), and TiO_2 to determine apparent ileal CP and AA digestibility.

All anchovy-based diet samples were analysed for DM, CP, crude fiber (CF), ether extract (EE), ash, GE, amino acid composition, and titanium concentration.

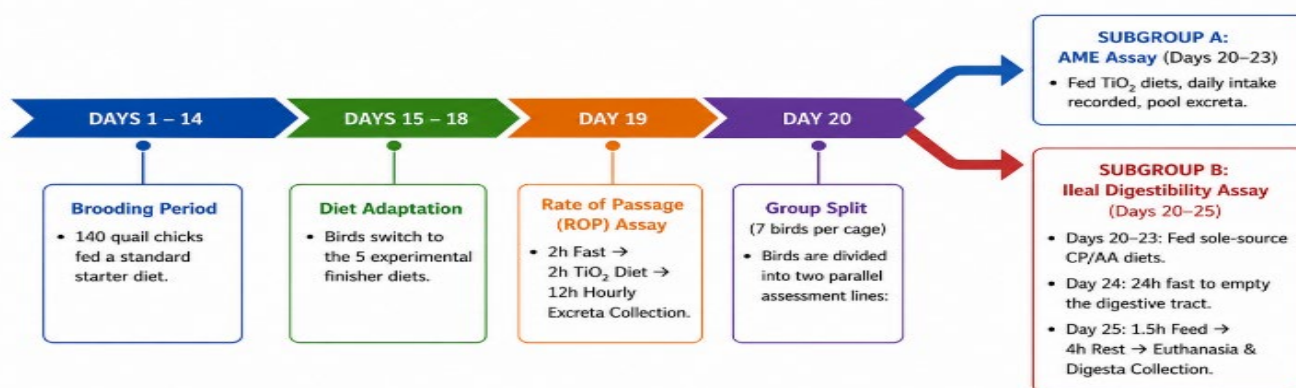


Figure 1: Schematic representation of the experimental timeline and subgroup assessments

2.6. Laboratory analyses

Dry matter (DM) was determined by oven-drying samples at 90°C until a constant weight was achieved. CP was quantified using the Kjeldahl nitrogen method, and CP content was calculated by multiplying total nitrogen by a conversion factor of 6.25. Ash content was measured by incineration in a muffle furnace at 550°C for 24 h. Ether extract (EE) was determined using a Soxtec extraction system in accordance with AOAC (2000) procedures. Gross energy (GE) was measured using a bomb calorimeter. Mineral concentrations were analysed by atomic absorption spectrophotometry.

2.7. Calculations

Total titanium excretion was standardised to unity, and the quantity of titanium recovered at each hourly interval was expressed as the cumulative proportion of total titanium excreted. These cumulative values were plotted against time to generate an excretion curve. The time required for 50% of the ingested titanium to be excreted (T_{50}) was subsequently

estimated from the fitted cumulative curve using the model described by Almirall and Esteve-Garcia (1994):

$$y = \frac{t^m}{t^m + k}$$

At $y = 0.5$,

$$0.5 = \frac{T_{50}^m}{T_{50}^m + k} \Rightarrow T_{50} = (k)^{1/m}$$

where t is time (hour), y is the cumulative proportion of excreted titanium, m is the slope of the line, and k is the correlation coefficient.

The goodness of fit of the data to the Hill-type equation was tested. The curves were then transformed into a linear equation to determine the values of m and k as follows:

$$\ln\left(\frac{y}{1-y}\right) = m \ln(t) - \ln(k)$$

which can be expressed as:

$$Y = aX + b$$

where $Y = \ln \left(\frac{y}{1-y} \right)$, $a = m$ (the gradient of the line), $X = \ln(t)$, and $b = -\ln(k)$.

The mean retention time (MRT) was calculated according to the method of Coombe and Kay (1965):

$$MRT = \frac{\sum(x_i \times t_i)}{\sum x_i}$$

where x_i is the amount of excreted titanium at the

i^{th} collection and t_i is the corresponding time (hour).

The AME, apparent ileal crude protein digestibility (AICPD), and AIAAD of anchovy meal and its treated forms were calculated in accordance with the methodologies outlined by Abdollahi et al. (2015) and Awad et al. (2016) as below;

$$AME = \frac{(Gross\ Energy_{diet} \times Feed\ Intake) - (Gross\ Energy_{excreta} \times Excreta\ Output)}{Gross\ Energy_{diet} \times Feed\ Intake} \times AME_{anchovy}$$

$$= \frac{AME_{anchovy\ diet} - (0.75 \times AME_{basal\ diet})}{0.25} \quad AICPD\ or\ AIAAD = 100 - \left(\frac{CP\ or\ AA_{digesta}}{CP\ or\ AA_{anchovy\ diet}} \times \frac{Ti_{anchovy\ diet}}{Ti_{digesta}} \times 100 \right)$$

2.8 Nutritional characterization of treated anchovy waste

The compositional analyses were conducted to characterize the nutritional quality of the alternative feed

ingredients prior to dietary inclusion. Proximate composition and gross energy are shown in Table 4, mineral composition in Table 5, and amino acid profiles in Table 6.

Table 4. Proximate composition of commercial fish meal and pre-treated anchovy ingredients (mean $\pm$ SEM).

Treatment	GE (kcal/kg)	DM (%)	CP (%)	EE (%)	Ash (%)
Commercial Fish Meal	4259.3 $\pm$ 7.1 ^a	89.65 $\pm$ 0.18 ^a	69.76 $\pm$ 0.23 ^d	8.73 $\pm$ 0.27 ^c	15.57 $\pm$ 0.57 ^a
Untreated Anchovy Waste	4432.5 $\pm$ 2.7 ^c	92.76 $\pm$ 0.32 ^b	51.64 $\pm$ 0.56 ^a	4.70 $\pm$ 0.25 ^b	44.98 $\pm$ 0.30 ^d
Desalted Anchovy Waste	4394.8 $\pm$ 1.8 ^{bc}	88.78 $\pm$ 0.36 ^a	54.63 $\pm$ 0.61 ^b	3.32 $\pm$ 0.15 ^a	35.41 $\pm$ 0.77 ^b
Fermented Anchovy Waste	4373.1 $\pm$ 9.0 ^b	94.77 $\pm$ 0.03 ^c	58.90 $\pm$ 0.40 ^c	3.64 $\pm$ 0.16 ^a	38.20 $\pm$ 0.16 ^c
P-Value	<0.001	<0.001	<0.001	<0.001	<0.001

GE = Gross Energy; DM = Dry Matter; CP = Crude Protein; EE = Ether Extract. Within each column, means with different superscript letters differ significantly ($p < 0.05$; Tukey's HSD).

Table 5. Mineral composition of pre-treated anchovy ingredients (mean $\pm$ SEM; mg/kg DM).

Treatment	Ca (mg/kg)	K (mg/kg)	Na (mg/kg)	Mg (mg/kg)	Zn (mg/kg)
Commercial Fish meal	27,180 $\pm$ 249	5,040 $\pm$ 86	4,580 $\pm$ 42	3,160 $\pm$ 38	179 $\pm$ 2
Untreated anchovy waste	47,103 $\pm$ 768 ^b	9,356 $\pm$ 30 ^c	57,473 $\pm$ 2,592 ^b	3,043 $\pm$ 23 ^a	155 $\pm$ 4 ^a
Desalted anchovy waste	61,627 $\pm$ 1,262 ^c	4,946 $\pm$ 1 ^a	71,137 $\pm$ 1,601 ^c	3,550 $\pm$ 13 ^b	383 $\pm$ 10 ^b
Fermented anchovy waste	43,030 $\pm$ 270 ^a	6,687 $\pm$ 15 ^b	22,530 $\pm$ 240 ^a	3,429 $\pm$ 20 ^b	140 $\pm$ 2 ^a
P-Value	<0.001	<0.001	<0.001	<0.001	<0.001

Ca = Calcium; K = Potassium; Na = Sodium; Mg = Magnesium; Zn = Zinc. All mineral values are expressed on a dry matter (DM) basis (mg/kg DM). Note: The higher Na concentration in desalted anchovy (T2) compared with untreated anchovy (UAW) is a dry matter concentration artefact: aqueous washing removes water and soluble fractions, increasing residual mineral concentrations per kg DM even when absolute sodium is partially reduced. Within each column, means with different superscript letters differ significantly ($p < 0.05$; Tukey's HSD).

2.9 Statistical analysis

All data were analysed by one-way analysis of variance (ANOVA) using SPSS software (Version 26.0; IBM Corp., Armonk, NY, USA). The experimental unit was the cage ($n = 4$ cages per treatment), with data pooled or averaged per cage prior to statistical analysis in accordance with the completely randomised design. Individual bird observations were pooled by cage before analysis to account for the non-independence of birds within the same housing unit; therefore, four cage means per treatment were used as the unit of

replication in all statistical tests. Treatment means were compared using Tukey's honest significant difference (HSD) multiple range test. Differences were considered statistically significant at $p < 0.05$. Prior to ANOVA, the assumptions of normality and homogeneity of variance were assessed using the Shapiro–Wilk test and Levene's test, respectively. All variables satisfied these assumptions ($p > 0.05$), confirming the appropriateness of parametric analysis. Digestibility values are reported as mean $\pm$ SEM based on four biological replicates (cages) per treatment.

Table 6. Essential and non-essential amino acid content of commercial fish meal and pre-treated anchovy ingredients (mg/100g DM; mean $\pm$ SEM).

Amino Acid	Fish Meal	Untreated	Desalted	Fermented	P-Value
Essential Amino Acids (EAA)					
Histidine (His)	669.28 $\pm$ 71.88	399.48 $\pm$ 23.78	423.99 $\pm$ 49.59	340.39 $\pm$ 81.09	0.063
Isoleucine (Ile)	2917.95 $\pm$ 145.85 ^a	1346.49 $\pm$ 93.09 ^b	1640.72 $\pm$ 198.12 ^b	1552.48 $\pm$ 134.48 ^b	0.005
Leucine (Leu)	3757.86 $\pm$ 22.46 ^a	1578.51 $\pm$ 57.81 ^b	1965.42 $\pm$ 199.32 ^b	1899.63 $\pm$ 157.33 ^b	<0.001
Lysine (Lys)	3900.74 $\pm$ 60.84 ^a	1361.69 $\pm$ 64.00 ^b	1701.67 $\pm$ 139.17 ^b	1431.08 $\pm$ 198.18 ^b	<0.001
Methionine (Met)	1276.39 $\pm$ 47.29 ^a	800.21 $\pm$ 26.11 ^b	776.57 $\pm$ 63.97 ^b	685.36 $\pm$ 24.86 ^b	0.002
Phenylalanine (Phe)	1948.00 $\pm$ 56.00 ^a	1115.38 $\pm$ 30.43 ^b	1251.00 $\pm$ 117.00 ^b	1114.93 $\pm$ 58.03 ^b	0.003
Threonine (Thr)	2961.14 $\pm$ 31.46 ^a	1400.66 $\pm$ 41.46 ^b	1749.44 $\pm$ 30.24 ^b	1662.97 $\pm$ 24.23 ^b	<0.001
Valine (Val)	3993.94 $\pm$ 149.64 ^a	1929.49 $\pm$ 118.19 ^b	2300.03 $\pm$ 249.23 ^b	2181.59 $\pm$ 177.99 ^b	0.004
Arginine (Arg)	1416.97 $\pm$ 23.77 ^a	667.31 $\pm$ 24.91 ^b	786.76 $\pm$ 67.36 ^b	732.80 $\pm$ 37.10 ^b	<0.001
Non-Essential Amino Acids (NEAA)					
Alanine (Ala)	4552.97 $\pm$ 21.87 ^a	2080.63 $\pm$ 48.63 ^b	2544.19 $\pm$ 193.39 ^b	2448.70 $\pm$ 53.10 ^b	<0.001
Aspartic Acid (Asp)	4741.83 $\pm$ 37.43 ^a	2401.56 $\pm$ 54.86 ^b	2778.57 $\pm$ 170.27 ^b	2602.77 $\pm$ 75.77 ^b	<0.001
Cysteine (Cys)	276.65 $\pm$ 23.35 ^a	177.22 $\pm$ 17.32 ^b	195.39 $\pm$ 10.29 ^{ab}	175.88 $\pm$ 9.28 ^b	0.031
Glutamic Acid (Glu)	6870.23 $\pm$ 86.83 ^a	3022.10 $\pm$ 59.70 ^b	3570.97 $\pm$ 249.57 ^b	3422.43 $\pm$ 94.83 ^b	<0.001
Glycine (Gly)	5870.59 $\pm$ 74.79 ^a	3622.89 $\pm$ 130.79 ^b	4394.56 $\pm$ 321.86 ^b	3830.41 $\pm$ 118.20 ^b	0.003
Serine (Ser)	2618.76 $\pm$ 96.65 ^a	1244.16 $\pm$ 12.16 ^b	1472.04 $\pm$ 75.54 ^b	1376.32 $\pm$ 6.32 ^b	<0.001
Tyrosine (Tyr)	1791.79 $\pm$ 61.79 ^a	1030.51 $\pm$ 71.61 ^b	1236.22 $\pm$ 108.62 ^b	1055.09 $\pm$ 22.59 ^b	0.005

Within each row, means with different superscript letters differ significantly ($p < 0.05$; Tukey's HSD). Rows without superscripts indicate no statistically significant difference among groups.

3. RESULT AND DISCUSSION

3.1 Feed Passage Time

The mean retention time (MRT) and time to 50% marker excretion (T50) are presented in Table 7. Significant treatment effects were observed for both MRT ($p < 0.001$) and T50 ($p < 0.001$). The commercial fish meal diet (C2) produced the shortest MRT (1.820 $\pm$ 0.048 hours) and T50 (2.643 $\pm$ 0.039 hours) of all treatments. The basal diet (C1) yielded intermediate values of MRT (3.060 $\pm$ 0.077 hours) and T50 (3.125 $\pm$ 0.038 hours). Untreated anchovy (T1) and desalted anchovy (T2) produced the longest MRT values (4.945 $\pm$ 0.101 and 5.118 $\pm$ 0.073 hours, respectively), which were not significantly different from each other but significantly exceeded all other treatments ($p < 0.05$). Of particular note, T1 simultaneously recorded the shortest T50 of all groups (2.313 $\pm$ 0.054 hours), indicating a marked dissociation between early and overall digesta transit. Fermented anchovy (T3) produced an MRT (3.015 $\pm$ 0.053 hours) that was statistically comparable to the basal diet, while its T50 (3.373 $\pm$ 0.046 hours) was significantly higher than that of C1 ($p < 0.05$).

Table 7. Mean retention time (MRT) and time to 50% marker excretion (T50) of pre-treated anchovy diets fed to Japanese quail (mean $\pm$ SEM).

Treatment	MRT (hours)	T50 (hours)
C1	3.060 $\pm$ 0.077 ^b	3.125 $\pm$ 0.038 ^c
C2	1.820 $\pm$ 0.048 ^a	2.643 $\pm$ 0.039 ^b
T1	4.945 $\pm$ 0.101 ^c	2.313 $\pm$ 0.054 ^a
T2	5.118 $\pm$ 0.073 ^c	3.033 $\pm$ 0.037 ^c
T3	3.015 $\pm$ 0.053 ^b	3.373 $\pm$ 0.046 ^d
P-Value	<0.001	<0.001

C1 = Basal diet; C2 = Commercial fish meal diet; T1 = Untreated anchovy; T2 = Desalted anchovy; T3 = Fermented anchovy. Within each column, means with different superscript letters differ significantly ($p < 0.05$; Tukey's HSD).

The shorter MRT observed in the commercial fish meal diet is consistent with previous reports documenting the high apparent ileal amino acid digestibility and rapid gastrointestinal transit associated with fish meal-based diets in poultry (Ravindran et al., 2005; Lemme et al., 2004). In contrast, both untreated and desalted anchovy diets prolonged MRT relative to the controls, suggesting that processing characteristics of anchovy waste influenced overall digesta retention. Although T1 produced the shortest T50, its MRT remained among the highest, indicating that early marker excretion did not correspond to faster complete passage of digesta. This dissociation is consistent with the possibility that early marker excretion reflected rapid transit of a liquid or fine-particle fraction, while bulkier or more slowly processed material prolonged overall retention (Svihus, 2014; Svihus et al., 2013). However, fractional flow rates and digesta particle size were not measured in the present study; this interpretation therefore remains speculative and cannot be confirmed from the available data.

The prolonged MRT in T2 corresponded with an altered mineral profile following desalting, particularly elevated calcium and ash concentrations (Table 5). It is plausible that these compositional changes influenced digesta characteristics and gastrointestinal transit behaviour (Svihus, 2014). However, direct measurement of digesta viscosity or gut motility was not conducted in the present study, and this association is therefore inferred from compositional data rather than directly established. The MRT and T50 of fermented anchovy approached those of the basal diet (C1), suggesting that fermentation modified the digestive behaviour of the ingredient relative to untreated anchovy (T1).

Fermentation has previously been reported to modify nutrient composition and digestibility characteristics of feed ingredients through microbial activity, including effects on apparent ileal amino acid digestibility in broilers (Adebo et al., 2022; Ahmed et al., 2014). However, gut morphology, digesta viscosity, and microbial activity were not assessed in the present study, and any mechanistic link between fermentation-induced physicochemical changes and passage kinetics therefore remains inferred rather than confirmed.

Importantly, the passage kinetics data carry direct implications for the AME and AICPD outcomes reported in Sections 3.2 and 3.3. The conventional expectation is that prolonged MRT increases the duration of enzyme-substrate contact and thereby improves nutrient digestion and absorption. However, as discussed below, this relationship did not hold consistently across treatments in the present study, suggesting that the cause of prolonged retention, whether productive enzymatic digestion or passive physical obstruction by high-mineral digesta, is a critical determinant of whether extended transit translates into improved nutrient utilisation).

3.2 Apparent metabolisable energy and apparent ileal crude protein digestibility

The AME values at both diet and ingredient levels, together with apparent ileal crude protein digestibility (AICPD), are presented in Table 8. Significant treatment effects were observed for all parameters ($p < 0.001$). Desalted anchovy (T2) produced the highest AME at both diet (2870.33 ± 7.75 kcal/kg) and ingredient levels (5521.33 ± 31.01 kcal/kg), significantly exceeding all other treatments. Untreated anchovy (T1) recorded the lowest AME values, whereas fermented anchovy (T3) showed intermediate values. In contrast, the highest AICPD was observed in commercial fish meal ($98.26 \pm 0.35\%$), followed by untreated anchovy, fermented anchovy, and desalted anchovy. Thus, the treatment with the highest AME (T2) produced the lowest AICPD among all groups.

Table 8. Apparent metabolisable energy (AME) and apparent ileal crude protein digestibility (AICPD) of pre-treated anchovy in Japanese quail (mean $\pm$ SEM).

Treat- ment	AME of Diet (kcal/kg)	AME of Ingredient (kcal/kg)	AICPD (%)
C2	2747.00 ± 4.51^c	5028.00 ± 18.04^c	98.26 ± 0.35^a
T1	2359.00 ± 10.21^a	3476.00 ± 40.86^a	97.53 ± 0.42^b
T2	2870.33 ± 7.75^d	5521.33 ± 31.01^d	96.07 ± 0.48^d
T3	2516.67 ± 13.86^b	4106.67 ± 55.44^b	97.05 ± 0.39^c
P- Value	<0.001	<0.001	<0.001

C2 = Commercial fish meal; T1 = Untreated anchovy; T2 = Desalted anchovy; T3 = Fermented anchovy. AICPD = Apparent Ileal Crude Protein Digestibility. Within each column, means with different superscript letters differ significantly ($p < 0.05$; Tukey's HSD).

The high AME values observed in desalted anchovy indicate that the desalting process substantially altered the energy utilisation characteristics of the ingredient. Aqueous washing removed soluble fractions, reconcentrating energy-yielding macronutrients (lipids, proteins) relative to total dry matter. It must be noted that the sodium concentration of T2 on a dry matter basis ($71,137$ mg/kg DM; Table 5) was numerically higher than that of untreated anchovy ($57,473$ mg/kg DM), not lower. This apparent increase is a well-recognised concentration artefact: as water and soluble low-energy fractions are removed during washing and drying, residual mineral concentrations per unit DM increase even if total sodium was partially leached in absolute terms. Accordingly, the improved AME of T2 cannot be attributed to a reduced osmotic or sodium load and should instead be interpreted in terms of relative enrichment of energy-yielding macronutrients in the residual dry matter. Because direct quantification of absolute sodium removal, lipid fractionation, and osmotic responses was not performed in the present study, the precise mechanisms underlying the increased AME of T2 cannot be conclusively established.

The lowest AME observed in untreated anchovy (T1) is notable given that T1 simultaneously recorded the longest MRT alongside the shortest T50 among all treatments. This pattern illustrates why MRT alone is an insufficient predictor of nutrient utilisation efficiency. The uniquely short T50 of T1 (2.313 h) suggests that a rapidly transiting liquid or fine-particle fraction carried soluble nutrients, including free amino acids and short-chain energy substrates, past the absorptive surface of the small intestine before enzymatic hydrolysis could be completed, thereby limiting energy recovery despite the high overall MRT. This interpretation is consistent with Rochell et al. (2012), who demonstrated in broiler chicks that diet composition influences both rate of passage and nutrient digestibility simultaneously, and with Svihus (2014), who noted that the nutritional consequence of passage depends not only on transit duration but on which digesta fraction is moving and when. In T1, the prolonged bulk retention did not compensate for the rapid early loss of the most readily absorbed fraction, resulting in the lowest AME of all treatments.

The inverse relationship between AME and AICPD in T2, where the highest AME coincided with the lowest crude protein digestibility, reflects the fact that aqueous washing affected energy-yielding and protein fractions differently. Washing selectively removed soluble nitrogenous compounds, including free amino acids and short peptides, which are the most readily absorbed forms of dietary nitrogen and contribute substantially to measured ileal digestibility.

Their removal reduced AIAAD without a corresponding reduction in AME, because the energy contribution of the removed nitrogenous fractions was proportionally small relative to the lipid and structural macronutrient fractions retained and concentrated in the residual dry matter. Because lipid yields approximately 2.25 times more metabolisable energy per gram than protein, even a modest enrichment of the lipid fraction relative to total dry matter substantially elevates measured AME independently of protein digestibility (Ravindran et al., 2005). Similar reductions in protein digestibility following aqueous processing of fish-derived ingredients have been attributed to losses of soluble nitrogenous fractions during washing (Ravindran et al., 2005; Parsons et al., 1997). These findings demonstrate that AME and AIAAD measure nutritionally distinct properties of an ingredient and can respond in opposite directions to the same processing treatment, underscoring the importance of evaluating both parameters simultaneously when characterising alternative protein sources.

Fermented anchovy (T3) produced intermediate AME values and a passage profile resembling the basal diet, suggesting that fermentation improved the overall digestive compatibility of the ingredient. However, fermentation did not restore AME to the level of desalted anchovy or commercial

fish meal, indicating that the fermentation process, despite normalising transit kinetics, did not fully compensate for the energy losses associated with the fermentation substrate and processing conditions.

3.3 Apparent ileal amino acid digestibility

The apparent ileal amino acid digestibility (AIAAD) coefficients are presented in Table 9. Significant treatment effects were observed for all amino acids analysed ($p < 0.001$). Untreated anchovy (T1) consistently produced the highest AIAAD values, with several amino acids approaching 99–100% digestibility, including methionine, and isoleucine. Commercial fish meal (C2) also exhibited uniformly high digestibility values across all amino acids. For most amino acids, T1 values were comparable to or higher than those of C2, although lysine digestibility remained slightly higher in C2.

Desalted anchovy (T2) produced intermediate digestibility values that were significantly lower than both T1 and C2 for all amino acids. Fermented anchovy (T3) consistently produced the lowest AIAAD coefficients among all treatments. Marked reductions were observed for several amino acids, including cysteine, lysine, serine, threonine, and histidine.

Table 9. Apparent ileal amino acid digestibility (AIAAD, %) of pre-treated anchovy in Japanese quail (mean $\pm$ SEM).

Amino Acid	C2	T1	T2	T3	P-Value
Alanine (Ala)	97.40 $\pm$ 0.41 ^a	99.29 $\pm$ 0.28 ^a	96.25 $\pm$ 0.53 ^b	93.33 $\pm$ 0.61 ^c	<0.001
Phenylalanine (Phe)	97.61 $\pm$ 0.38 ^a	99.23 $\pm$ 0.31 ^a	96.60 $\pm$ 0.49 ^b	92.04 $\pm$ 0.67 ^c	<0.001
Methionine (Met)	98.66 $\pm$ 0.33 ^a	99.48 $\pm$ 0.26 ^a	96.55 $\pm$ 0.44 ^b	91.51 $\pm$ 0.72 ^c	<0.001
Isoleucine (Ile)	98.42 $\pm$ 0.36 ^a	99.43 $\pm$ 0.27 ^a	97.59 $\pm$ 0.43 ^b	93.47 $\pm$ 0.58 ^c	<0.001
Histidine (His)	98.74 $\pm$ 0.40 ^a	98.92 $\pm$ 0.37 ^a	96.98 $\pm$ 0.51 ^b	88.94 $\pm$ 0.83 ^c	<0.001
Valine (Val)	98.13 $\pm$ 0.39 ^a	99.29 $\pm$ 0.28 ^a	97.26 $\pm$ 0.46 ^b	92.39 $\pm$ 0.64 ^c	<0.001
Tyrosine (Tyr)	98.53 $\pm$ 0.37 ^a	99.32 $\pm$ 0.29 ^a	96.43 $\pm$ 0.50 ^b	91.64 $\pm$ 0.69 ^c	<0.001
Threonine (Thr)	98.23 $\pm$ 0.38 ^a	98.89 $\pm$ 0.35 ^a	96.58 $\pm$ 0.47 ^b	88.68 $\pm$ 0.79 ^c	<0.001
Serine (Ser)	97.44 $\pm$ 0.43 ^a	98.69 $\pm$ 0.36 ^a	94.98 $\pm$ 0.56 ^b	88.12 $\pm$ 0.81 ^c	<0.001
Lysine (Lys)	98.76 $\pm$ 0.34 ^a	98.62 $\pm$ 0.38 ^b	96.70 $\pm$ 0.48 ^c	88.06 $\pm$ 0.82 ^d	<0.001
Leucine (Leu)	98.43 $\pm$ 0.35 ^a	99.27 $\pm$ 0.30 ^a	97.05 $\pm$ 0.45 ^b	93.38 $\pm$ 0.60 ^c	<0.001
Glutamic Acid (Glu)	97.34 $\pm$ 0.44 ^a	99.24 $\pm$ 0.30 ^a	96.28 $\pm$ 0.52 ^b	93.06 $\pm$ 0.62 ^c	<0.001
Cysteine (Cys)	97.48 $\pm$ 0.46 ^a	98.69 $\pm$ 0.37 ^a	95.50 $\pm$ 0.55 ^b	85.29 $\pm$ 0.94 ^c	<0.001
Aspartic Acid (Asp)	97.13 $\pm$ 0.45 ^a	99.23 $\pm$ 0.29 ^a	96.37 $\pm$ 0.51 ^b	92.77 $\pm$ 0.63 ^c	<0.001
Arginine (Arg)	98.66 $\pm$ 0.36 ^a	98.92 $\pm$ 0.34 ^a	95.30 $\pm$ 0.57 ^b	90.68 $\pm$ 0.74 ^c	<0.001
Glycine (Gly)	97.59 $\pm$ 0.43 ^a	99.17 $\pm$ 0.31 ^a	95.32 $\pm$ 0.55 ^b	93.01 $\pm$ 0.61 ^c	<0.001

C2 = Commercial fish meal; T1 = Untreated anchovy; T2 = Desalted anchovy; T3 = Fermented anchovy. Within each row, means with different superscript letters differ significantly ($p < 0.05$; Tukey's HSD).

The high AIAAD observed in untreated anchovy, despite its prolonged MRT and atypical T50 dissociation, reinforces the interpretation from Section 3.2 that the extended MRT in T1 reflected passive physical retention rather than productive enzymatic digestion. The high inherent digestibility of anchovy protein was preserved in T1, suggesting that the protein matrix itself was readily hydrolysable, but the abnormal transit profile, particularly the

rapid early fraction, may have limited energy recovery by reducing the effective absorption window for soluble nutrients, as reflected in the low AME of T1. Protein digestibility and energy utilisation therefore diverged in T1 for mechanistically distinct reasons: protein digestibility was governed by the intrinsic accessibility of the protein substrate, while AME was constrained by the biphasic transit pattern that reduced exposure time for soluble energy substrates.

The reduced AIAAD in desalted anchovy is consistent with the interpretation in Section 3.2 that aqueous washing removed soluble free amino acids and short peptides, which are among the most bioavailable nitrogenous fractions. The simultaneous decrease in AIAAD and increase in AME in T2 is therefore not paradoxical but reflects the divergent fate of soluble nitrogenous and energy-yielding fractions during washing. Previous studies in poultry have reported that processing of fish-derived ingredients may remove soluble nitrogenous compounds, thereby reducing apparent ileal amino acid digestibility in broilers (Ravindran et al., 2005; Parsons et al., 1997).

The lowest AIAAD observed in fermented anchovy (T3), despite its normalised passage kinetics, indicates that improved transit compatibility did not translate into improved amino acid availability. Fermentation and subsequent drying appear to have reduced amino acid digestibility through modification of the protein matrix, particularly for heat-sensitive amino acids including lysine, cysteine, serine, and threonine. Processing conditions involving fermentation and drying have previously been associated with such reductions, particularly through Maillard reactions between reducing sugars and free amino groups at elevated temperatures (Ravindran, 2013; Abraha et al., 2018). This finding highlights that passage kinetics and amino acid digestibility are not always aligned, T3 achieved the most physiologically compatible transit profile among the anchovy treatments yet produced the poorest amino acid availability, indicating that the rate of digesta movement and the biochemical accessibility of amino acids are governed by independent mechanisms.

Taken together, the results across Sections 3.1–3.3 reveal that the three nutritional parameters, passage kinetics, AME, and AIAAD, did not respond uniformly to pre-treatment and cannot be predicted from one another. Each parameter captures a distinct aspect of nutritional quality: MRT and T50 reflect the physical interaction between the diet and the gastrointestinal tract; AME reflects the gross recovery of metabolisable energy regardless of macronutrient source; and AIAAD reflects the site-specific availability of individual amino acids at the ileal level. The data demonstrate that pre-treatment selection involves inherent trade-offs among these dimensions, and that comprehensive nutritional characterisation of alternative protein ingredients requires simultaneous assessment of all three.

4. CONCLUSION

Anchovy waste is a viable alternative protein source for Japanese quail, but its nutritional value depends heavily on pre-treatment. Desalting maximised AME at diet (2,870

kcal/kg) and ingredient (5,521 kcal/kg) levels; this improvement reflects compositional enrichment of energy-yielding macronutrients in the residual dry matter following aqueous washing and is not attributable to sodium reduction, as sodium concentration on a dry matter basis was higher in desalted anchovy (71,137 mg/kg DM) than in untreated anchovy (57,473 mg/kg DM). Untreated anchovy matched commercial fish meal in AIAAD, demonstrating inherently high protein digestibility despite the prolonged MRT and altered transit kinetics associated with its high-ash, high-mineral composition. Fermentation reduced sodium by 60.8% and normalised passage kinetics, improving physiological gut compatibility, but it slightly reduced amino acid digestibility.

These findings highlight a clear nutritional trade-off between energy utilisation and amino acid availability across pre-treatment methods. From a practical perspective, desalting may be preferred when maximising dietary energy is the primary objective, whereas fermentation offers a more balanced approach by improving physiological tolerance and mineral profile. Future studies should focus on optimising processing conditions, particularly fermentation and drying parameters, to minimise amino acid losses and enhance overall protein quality.

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DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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