

Article

Effects of Feeding Recycled Food Waste-Based Diets on Gut Health, Nutrient Digestibility, and Bone Quality in Laying Hens

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Abstract: This study explored feeding recycled food waste-based diets for gut health, nutrient digestibility, bone morphology, and bone mineral level in laying hens. Hy-Line Brown laying hens ($n = 150$) were randomly allocated to three experimental treatments consisting of a commercial control diet, a recycled food waste-based diet, and a 50:50 blend of the control and food waste-based diets, with 50 replicates of a single bird per cage per treatment from 24 to 63 weeks of age. Egg production was recorded daily and feed intake was measured weekly. The gut pH, jejunal and ileal morphology, nutrient digestibility, bone morphology, and mineral composition were measured at 63 weeks of age. Hens on the food waste-based treatment had similar egg production but lower feed conversion ratio (FCR, 1.948 vs. 2.172 kg feed/kg egg, $p < 0.001$) and higher ileal pH ($p < 0.001$) and bone ash content ($p < 0.001$) compared to birds on the control treatment. Moreover, hens fed the food waste-based diets had higher ileal digestible energy ($p < 0.001$); ileal energy digestibility ($p < 0.01$); tibia S, Fe, Mn, and Zn levels ($p < 0.05$); and Mg, K, S, Mn, and Mo digestibility ($p < 0.05$) compared to hens fed the control diets. Hens offered the 50:50 blend diets had higher tibia P, Mg, and Mo levels ($p < 0.05$) and higher Ca digestibility ($p < 0.05$) compared to those fed the control diets. Thus, feeding recycled food waste-based diets is effective to improve laying performance, nutrient digestibility, and bone mineralization in laying hens.

Keywords: chicken; digestibility; poultry nutrition; food waste; mineral



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1. Introduction

On a global scale, it has been estimated that 1.3 billion tons of food waste are disposed of in landfills, accounting for about a third of all food produced for human consumption annually [1,2]. This not only causes significant loss to the global economy but also serious environmental problems as food waste contributes to greenhouse gas emissions, soil pollution, and odor production [2–5]. Moreover, when food is wasted or lost, all costs involved in producing that food, including water, fertilizers, energy, and labor, are also wasted. It is estimated that the cropland area, fertilizer, and water exhausted to produce that amount of food that is wasted are approximately 198 million hectares, 28 million tons, and 173 billion cubic meters, respectively, each year on a global scale [6]. Meanwhile, food production to meet growing populations has faced challenges in many parts of the world in recent years due to adverse weather conditions such as drought, floods, and hurricanes [7,8].

Thus, it is no surprise that priority has been given to the prevention of food waste and food loss by the Food and Agriculture Organization of the United Nations [9].

Increasing consumer demand for animal products has put immense pressure on the industry as animal production relies on scarce natural resources, including land and water, which may become more limited due to the negative impacts of global climate change [10,11]. Increasing needs for animal products also induce a higher demand for feed ingredients—particularly cereal grains and protein meals—as these are the main energy and protein sources in pig and poultry diets [12–14]. The increasing price and/or limited availability of cereal grains and soybean meal in the regions where these feed ingredients are not produced locally have prompted the search for alternative feed ingredients for poultry production [9,15]. Recycling food waste into poultry feed is a feasible solution that would help to address issues associated with food security, waste management, environmental challenges, and resource scarcity while alleviating competition for food between animals and humans [9,16,17]. Additionally, incorporating food waste into poultry feed may help to reduce feed cost and increase production efficiency, as feed cost accounts for 55 to 75% of the total production costs in poultry farming and the cost to manufacture recycled food waste is generally lower than conventional feed ingredients [18,19].

Feeding animals with food scraps has been a common practice in many countries, particularly in the developing world [9]. This practice has several disadvantages, including nutrient variability, inconsistent waste supply, and risks associated with sanitary conditions and biological contaminants, such as live bacteria and viruses [20–23]. However, with the application of modern processing technologies, it has been demonstrated that poultry feed may be formulated from food waste materials that meet the birds' nutritional requirements and hygiene and chemical safety standards [9,22–25]. Various countries, including Japan, South Korea, Taiwan, and the US, have already implemented the utilization of recycled food waste as animal feed [26]. In South Korea and Japan, it is estimated that approximately 46% and 40%, respectively, of mixed food waste is recycled as poultry and livestock feed, leading to considerable reductions in animal feed costs (40 to 60%) in these countries [27]. In the US, it has been reported that a significant amount of food waste (84–86.8%) is diverted to land applications or animal feed from the processing sector [28]. Currently, there are various facilities converting food waste from processing, manufacturing, and/or harvesting sectors into animal feed in the US [9]. However, food waste must be processed by applying a minimum temperature of 100 °C, boiling for a minimum duration of 30 min, or using other processing methods approved by the state governments before it can be incorporated into animal feed [29].

The possibility of using recycled food waste as poultry feed has been demonstrated in previous studies [23,30,31]. For example, previous studies have shown that broilers fed diets added with 10 to 30% dried bakery waste had similar body weight and feed efficiency compared to those fed conventional diets based on corn and soybean meal [30,31]. Similarly, processed recycled food waste from various sources, such as fermented fish, leftover Korean food, fruits and vegetables, dried kitchen waste, fermented apple pomace, and restaurant food waste, has been illustrated to provide suitable ingredients for poultry diets [32–37]. However, previous research examined the effects of incorporating only food waste from one source into poultry diets at moderate inclusion rates. Recent research at our laboratory has illustrated the possibility of formulating a diet based almost 100% on food waste materials that meet the nutritional requirement of laying hens and safety and hygiene standards [23,38]. Furthermore, the results of our previous study showed that food waste-based diets maintained egg production and egg quality while improving the feed efficiency of laying hens compared to conventional control diets [23,38]. Although gut health and mineralization status play crucial roles in the nutrient digestibility, production performance, and general health conditions of laying hens, limited information could be found in the literature regarding the effects of feeding food waste-based diets on the gut health and mineralization status of laying hens. This study is an extension to our previous studies [23,38], and the main objectives are to explore the effects of feeding food

waste-based diets on gut pH and morphology, nutrient digestibility, bone morphology, bone mineral composition, and overall laying performance, which may elucidate the mechanisms generating the improved performance of laying hens offered food waste-based diets. To the best of our knowledge, this is the first study investigating the effects of feeding diets based almost 100% on food waste materials on the abovementioned health- and production performance-related parameters of laying hens.

2. Materials and Methods

All experimental procedures were reviewed and approved by the University of New England Animal Ethics Committee (approval number: AEC20-042) and fulfilled the requirements of the Australian Code of Practice for Care and Use of Animals for Scientific Purposes [39].

2.1. Experimental Design and Diets

This study was conducted at the University of New England Laureldale Layer Cage facility in Armidale, New South Wales, Australia. The experimental design, dietary treatments, hen management, data collection, feed formulation, and analysis followed Dao et al. [23]. In brief, Hy-Line Brown laying hens ($n = 150$) at 23 weeks of age were randomly distributed to three dietary treatments including a standard/control diet, a recycled food waste-based diet, and a 50:50 blend of these two diets. Experimental diets were steadily increased over 10 days during weeks 23–24 as an adaptation period and were then offered to hens from week 24. Each treatment consisted of 50 replicate cages (30 cm width \times 50 cm depth \times 45 cm height) with a single hen per cage. The hens' starting weights were similar between the treatments. Specifically, the average weights of hens on the control, food waste, and 50:50 treatments were 1959 g, 1922 g, and 1926 g at 24 weeks of age, respectively. The study included 2 laying phases with 20 weeks each (24–43 and 44–63 weeks of age). Hens were raised in a curtain-sided house with two nipple drinkers and one feeder per hen over 40 weeks from 24 to 63 weeks of age. Hens had *ad libitum* access to the feed and clean water and were raised under 16 h of light and 8 h of dark throughout the experimental period. Temperature and relative humidity inside the hen house were recorded daily.

All diets were provided in mash form and satisfied the minimum nutrient recommendations for Hy-Line Brown laying hens per Hy-Line International [40]. Four feeding periods were applied: 24–37, 38–43, 44–58, and 59–63 weeks of age. Food waste streams were collected from various places, including bakeries, breweries, abattoirs, hospitals, nursing homes, fish processing facilities, pubs and restaurants, and vegetable and fruit markets. After collection, food waste streams were checked for foreign objects, separated into different categories, and processed by Food Recycle Ltd. (Candelo, New South Wales, Australia) using a patented production process to sterilize and dry the material into suitable granular powders for poultry feed (patent number P11980.WO) [41]. Specifically, the process comprises treating the categorized food waste separately to substantially destroy all pathogens by heating at 350 °C for less than 10 s, grinding the treated food waste, and finally mixing the treated ground food waste streams into the layer feed based on their nutrient contents [41]. Feed ingredients, including food waste streams, were analyzed for dry matter, gross energy (GE), crude fat, crude fiber, crude protein (CP), amino acids, and mineral levels following the AOAC [42] prior to diet formulation. Levels of metabolizable energy and total and digestible amino acids for the major feed ingredients used in the control diet were analyzed by near-infrared reflectance spectroscopy (Foss NIR 6500, Hillerød, Denmark). The levels of metabolizable energy and digestible amino acids in the food waste streams were set at 65% of the total measured energy and amino acids, following previous food waste diet research [33,35,43]. The analyzed nutritional composition of the feed ingredients and food waste streams was used to formulate the diets via feed formulation software (Concept 5 version 13.11.19, CFC Tech Services, Inc., Browerville, MN, USA). Minor ingredients, including crystalline L-lysine HCl, D,L-methionine, enzymes (xylanase, phytase), antioxidant, pigments, and layer vitamin–mineral premix, were supplemented

into the diets where necessary to satisfy the nutritional requirement of Hy-Line Brown laying hens. Titanium dioxide was supplemented in all diets at 0.5% during the last feeding period (59–63 weeks of age) as an inert marker for ileal protein and energy digestibility determination. The mixed diets were subjected to proximate and NSP analyses using standard methods [42] to confirm the formulation objectives. The diet composition and nutrient content are presented in the Appendix A in Tables A1–A8. Diet composition and nutrient content during the first phase of the study (the first 20 weeks from 24–43 weeks of age; see the Appendix A, Tables A1 and A3–A5) are available from Dao et al. [23].

2.2. Data Collection and Sampling

Laying performance parameters, including hen-day egg production, egg weight, egg mass, and mortality rate, were measured daily and feed consumption was measured weekly. The feed conversion ratio (FCR) was computed by dividing feed intake by egg mass. Hens were weighed individually at the start and end of the study.

Determination of mineral digestibility using internal markers, such as titanium dioxide, is generally difficult as it is affected by the secretion of endogenous minerals in the small intestine [44]. Thus, a total excreta collection method was used to measure mineral digestibility in this study instead of the internal marker method. At week 63, ten hens per treatment that had body weights close to the average body weight of the treatment were selected for determination of mineral digestibility through the total excreta collection method over 3 consecutive days, as described by Dao et al. [45].

Twenty hens per treatment were randomly selected and euthanized via electrical stunning (MEFE CAT 44N, Mitchell Engineering Food Equipment, Clontarf, QLD, Australia), followed by cervical dislocation, at week 63. The sampled birds were dissected and then the ileum and caeca were also removed for pH determination. The small intestine was removed and the ileum was demarcated using Meckel's diverticulum and the ileocaecal junction. The pH of digesta within the gizzard, crop, ileum, and caeca was immediately determined in situ using a digital pH meter (Mettler-Toledo Ltd., Leicester, UK) with a spear-tip piercing pH electrode (Sensorex, Garden Grove, CA, USA). The pH probe was carefully cleaned with ultra-pure water (ICW 3000 water purifier for ion chromatography; Millipore, Burlington, MA, USA) between the measurements. Ileal contents were obtained for ileal energy and protein digestibility analysis by gently squeezing the entire ileum into 50 mL containers. The samples were stored at -20°C prior to analysis. Approximately 2 cm of jejunal and proximal ileal tissues was collected for morphometric measurements. The samples were cleaned by flushing with phosphate-buffered saline (PBS), fixed with 10% buffered formalin, and kept in 50 mL containers until further processing.

2.3. Nutrient Digestibility

Excreta samples collected at week 63 were freeze-dried (Christ Alpha 1-4 LDplus, Osterode am Harz, Germany) and milled using a 0.5 mm screen. Feed samples were also ground using a 0.5 mm screen. Then, mineral levels in feed and excreta were analyzed using an inductively coupled plasma-optical emission spectrometry instrument (Agilent, Mulgrave, VIC, Australia) according to procedures previously described by Dao et al. [46]. The apparent mineral digestibility at week 63 was calculated following Dao et al. [47]. All data were calculated on a dry matter basis.

The ileal digesta samples were freeze-dried (Christ Alpha 1-4 LD plus, Osterode am Harz, Germany). The dried ileal digesta and feed samples were ground to particle sizes of ≤ 0.5 mm. Then, titanium dioxide concentration was measured in both ileal digesta and feed samples using the colorimetric method described by Short et al. [48]. Nitrogen concentration in the digesta and feed samples was measured using a nitrogen analyzer (LECO Corporation, St. Joseph, MI, USA) with EDTA as the calibration standard. The CP levels of the diet and ileal digesta samples were calculated by multiplying the diet nitrogen content by 6.25. The GE levels in the digesta and feed samples were measured using a Parr adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA) calibrated

using benzoic acid as the standard. The equations below, described by Jasek et al. [49], were used to calculate the apparent ileal digestible energy (IDE) level and coefficients of protein (IDPC) and energy digestibility (IDEC).

$$\text{IDE} = \text{GE}_{\text{diet}} - \left(\text{GE}_{\text{digesta}} \times \left(\frac{\text{Ti}_{\text{diet}}}{\text{Ti}_{\text{digesta}}} \right) \right)$$

$$\text{IDPC} = 1 - \left(\frac{\text{Ti}_{\text{diet}} \times \text{CP}_{\text{digesta}}}{\text{Ti}_{\text{digesta}} \times \text{CP}_{\text{diet}}} \right)$$

$$\text{IDEC} = 1 - \left(\frac{\text{Ti}_{\text{diet}} \times \text{GE}_{\text{digesta}}}{\text{Ti}_{\text{digesta}} \times \text{GE}_{\text{diet}}} \right)$$

where GE_{diet} and $\text{GE}_{\text{digesta}}$ represent the GE values of the diets and ileal digesta, respectively. Ti_{diet} and $\text{Ti}_{\text{digesta}}$ represent titanium dioxide concentrations in the diet and ileal digesta, respectively. CP indicates either feed or ileal digesta CP level.

2.4. Gut Morphology

The jejunal and ileal tissue samples were sectioned and processed using a standard hematoxylin and eosin assay following Golder et al. [50]. Then, the jejunal and ileal histological slides were scanned using a NanoZoomer 2.0-RS (Model C10730-12, Hamamatsu Photonics K.K., Hamamatsu, Japan). Finally, slide readings were carried out using the NDP.scan 2.5.8 software provided by the same company.

2.5. Bone Morphology and Mineral Composition

The tibia and femur were collected during the sampling at week 63, cleaned using a knife and scissors, and dried in a fume hood for 48 h. Weights of wet and air-dried bones were measured and then samples were stored at 4 °C until further measurement. Then, air-dried bones were measured for length, diameter, breaking strength, and ash content. Tibia and femur length and diameter were measured using a digital caliper. The tibia and femur breaking strength were determined using a Lloyd Testing Instrument (model 1000R, Lloyd Instruments Ltd., Fareham, Hampshire, UK). Bone samples were oven-dried at 105 °C for 24 h and ashed at 600 °C for 13 h. Additionally, the bone Seedor index (mg/mm) was calculated using an equation proposed by Seedor et al. [51]:

$$\text{Bone Seedor index (mg/mm)} = \text{Weight of oven-dry bone (mg)} / \text{Bone length (mm)}$$

The mineral composition in the tibia samples at week 63 was analyzed using an inductively coupled plasma-optical emission spectrometry instrument (Agilent, Mulgrave, VIC, Australia) following procedures previously described by Dao et al. [46].

2.6. Statistical Analysis

All data were analyzed using R Commander (version 3.3.1, R Foundation for Statistical Computing, Vienna, Austria). Firstly, a quantile comparison plot was used to test the approximately normal distribution of the data. Then, data were subjected to Levene's test to determine whether or not the treatment groups had approximately equal variances. Depending on the results of these tests, either one-way ANOVA or non-parametric ANOVA (Kruskal–Wallis test) were employed to test statistical differences among the treatment groups. If significant differences were obtained from ANOVA tests, Tukey's post hoc test was employed to identify pairwise differences between the treatment means (p -value ≤ 0.05). The data on mortality rates were analyzed for statistical differences using the Chi-squared test as the data were non-continuous.

3. Results

3.1. Housing Environment and Mortality Rate

The temperature and humidity conditions inside the hen house over the experimental period are described in Figure 1. The mean temperature was 13.5 °C, ranging from 7.4 to 19.7 °C, and the mean relative humidity was 61.9%, ranging from 49.3% to 76.1%, over the entire study. The maximum temperature ranged from 11.0 to 29.0 °C (average 18.6 °C) and the minimum temperature ranged from 3.0 to 14.0 °C (average 8.7 °C) during the experimental period. Mortality rates were not different between the experimental treatments over the entire study (Table 1). Only one mortality was observed in the 50:50 blend treatment at 35 weeks of age.

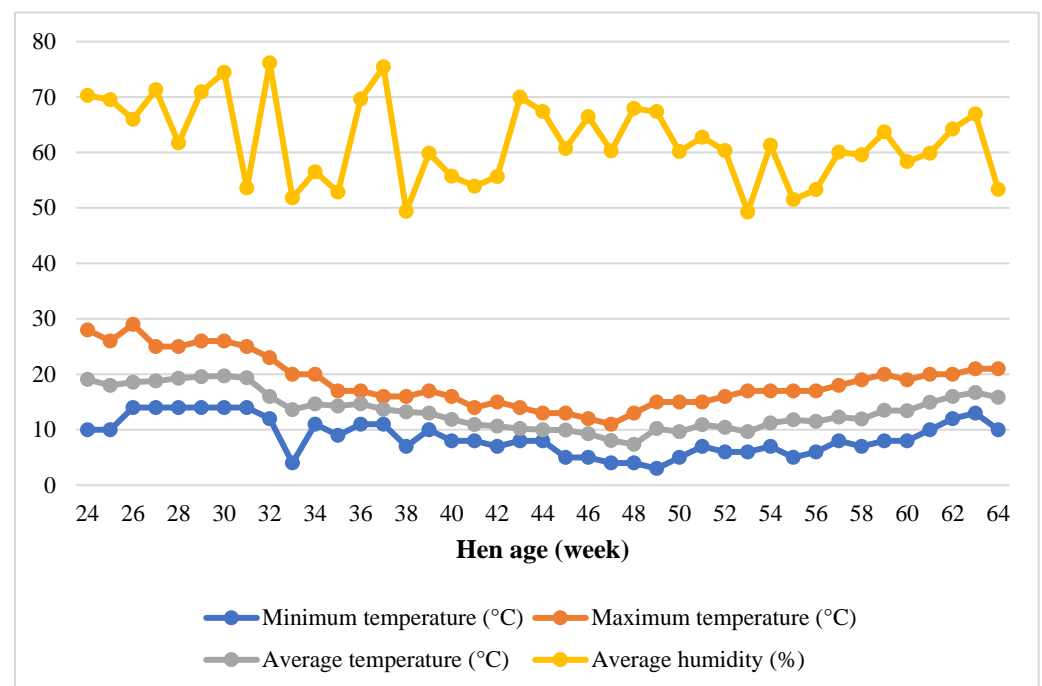


Figure 1. Temperature and relative humidity of the hen house over the entire study from weeks 24 to 63.

Table 1. Laying performance and hen weight for experimental treatments from 24 to 63 weeks of age.

Variable	Control	Food Waste	50:50 Blend	SEM	<i>p</i> -Value
Hen weight at week 63 (g)	2317	2318	2357	15.82	0.509
Weight change for weeks 24–63 (g)	358 ^a	396 ^{ab}	433 ^b	10.05	0.010
Egg weight (g)	61.7	61.2	61.6	0.29	0.747
Hen-day egg production (%)	95.9	96.2	96.6	0.34	0.534
Egg mass (g/hen/day)	59.1	58.8	59.6	0.35	0.709
Feed intake (g/hen/day)	128 ^c	114 ^a	121 ^b	0.84	<0.001
FCR (kg feed/kg egg)	2.172 ^c	1.948 ^a	2.044 ^b	0.015	<0.001
Mortality rate (%)	0.00	0.00	2.00	0.67	0.365

^{a–c} Means within rows not sharing a common suffix were significantly different at the 5% level of probability.

3.2. Laying Performance and Hen Weight

Laying performance parameters and hen weight over the entire study from 24 to 63 weeks of age are presented in Table 1. The results showed that feeding food waste-based diets reduced feed intake by 10.9% ($p < 0.001$) without affecting egg production and egg weight, thereby improving FCR by 10.3% (1.948 vs. 2.172; $p < 0.001$) compared to the control diets over the entire study. The 50:50 blend treatment showed intermediate effects. The hen weight did not differ between the dietary treatments at week 63. However, hens offered the

50:50 blend diets had higher weight gain compared to those offered the control diets over the entire study from 24 to 63 weeks of age ($p = 0.01$).

3.3. Nutrient Digestibility

Apparent ileal energy and protein digestibility at week 63 are reported in Table 2. Higher total ileal digestible energy ($p < 0.001$) and ileal energy digestibility coefficients ($p < 0.01$) were observed in hens on the food waste treatment compared to hens on the control treatment at week 63. Protein digestibility did not differ between the dietary treatments at week 63.

Table 2. Apparent ileal energy and protein digestibility for experimental treatments at week 63.

Variable	Control	Food Waste	50:50 Blend	SEM	<i>p</i> -Value
Digestible energy (kcal/kg)	2218 ^a	3001 ^c	2658 ^b	76.54	<0.001
Energy digestibility coefficient	0.57 ^a	0.66 ^b	0.62 ^{ab}	0.01	0.009
Protein digestibility coefficient	0.48	0.51	0.50	0.01	0.580

^{a-c} Means within rows not sharing a common suffix were significantly different at the 5% level of probability.

The mineral intake, excretion, retention, and digestibility for the dietary treatments as ascertained by the total tract collection method at week 63 are reported in Tables 3–6, respectively.

Table 3. Mineral intake for the experimental treatments at week 63.

Mineral	Control	Food Waste	50:50 Blend	SEM	<i>p</i> -Value	
Macro minerals (mg/g)	Ca	6160 ^c	4211 ^a	5263 ^b	168	<0.001
	P	587 ^a	1074 ^c	871 ^b	38.7	<0.001
	Na	134 ^a	396 ^c	284 ^b	20.2	<0.001
	Mg	216 ^c	118 ^a	168 ^b	7.92	<0.001
	K	735 ^c	445 ^a	596 ^b	23.9	<0.001
	S	285 ^b	254 ^a	277 ^{ab}	4.59	0.009
Micro minerals (µg/g)	Fe	29.0 ^c	22.7 ^a	26.4 ^b	0.62	<0.001
	Mn	13.5 ^c	10.1 ^a	11.9 ^b	0.31	<0.001
	Mo	0.71 ^c	0.53 ^a	0.63 ^b	0.02	<0.001
	Al	26.9 ^c	20.2 ^a	24.0 ^b	0.62	<0.001
	Cr	0.24 ^a	0.28 ^b	0.27 ^b	0.005	<0.001
	Cu	1.25 ^c	0.96 ^a	1.13 ^b	0.03	<0.001

^{a-c} Means within rows not sharing a common suffix were significantly different at the 5% level of probability.

Table 4. Mineral excretion for the experimental treatments at week 63.

Mineral	Control	Food Waste	50:50 Blend	SEM	<i>p</i> -Value	
Macro minerals (mg/g)	Ca	2455 ^b	1448 ^a	1632 ^a	108	<0.001
	P	396 ^a	761 ^c	602 ^b	32.5	<0.001
	Na	59.4 ^a	206 ^c	146 ^b	12.1	<0.001
	Mg	157 ^c	66.7 ^a	107 ^b	7.28	<0.001
	K	558 ^c	250 ^a	394 ^b	24.6	<0.001
	S	137 ^b	95.6 ^a	105 ^a	4.58	<0.001
Micro minerals (µg/g)	Fe	20.6 ^b	15.5 ^a	17.1 ^a	0.63	0.002
	Mn	11.5 ^c	7.34 ^a	9.11 ^b	0.41	<0.001
	Mo	0.30 ^b	0.18 ^a	0.22 ^a	0.01	<0.001
	Al	16.2 ^b	12.6 ^a	13.5 ^{ab}	0.51	0.042
	Cr	0.10 ^{ab}	0.13 ^b	0.07 ^a	0.01	0.014
	Cu	1.02 ^b	0.72 ^a	0.85 ^{ab}	0.04	0.001

^{a-c} Means within rows not sharing a common suffix were significantly different at the 5% level of probability.

Table 5. Mineral retention for the experimental treatments at week 63.

Mineral		Control	Food Waste	50:50 Blend	SEM	p-Value
Macro minerals (mg/g)	Ca	3705 ^b	2763 ^a	3631 ^b	108	<0.001
	P	191 ^a	312 ^b	269 ^{ab}	17.1	0.012
	Na	74.8 ^a	190 ^c	139 ^b	9.69	<0.001
	Mg	58.9	51.0	60.2	2.34	0.182
	K	177	195	202	7.34	0.520
	S	148	158	171	4.14	0.061
	Fe	8.35	7.19	9.24	0.42	0.120
Micro minerals (µg/g)	Mn	2.02	2.76	2.77	0.18	0.182
	Mo	0.41 ^b	0.35 ^a	0.41 ^b	0.01	0.023
	Al	10.7 ^b	7.51 ^a	10.4 ^b	0.45	0.003
	Cr	0.14 ^a	0.14 ^a	0.20 ^b	0.01	0.005
	Cu	0.23	0.24	0.29	0.02	0.484

^{a-c} Means within rows not sharing a common suffix were significantly different at the 5% level of probability.

Table 6. Total tract mineral digestibility for the experimental treatments at week 63.

Mineral		Control	Food Waste	50:50 Blend	SEM	p-Value
Macro minerals (mg/g)	Ca	60.3 ^a	65.6 ^{ab}	69.1 ^b	1.26	0.011
	P	32.9	29.1	30.9	1.68	0.695
	Na	55.7	48.0	48.9	2.61	0.468
	Mg	27.1 ^a	43.3 ^c	35.9 ^b	1.65	<0.001
	K	23.8 ^a	43.8 ^c	33.9 ^b	1.82	<0.001
	S	51.7 ^a	62.3 ^b	62.0 ^b	1.37	<0.001
	Fe	28.9	31.6	35.1	1.51	0.259
Micro minerals (µg/g)	Mn	15.4 ^a	27.4 ^b	23.3 ^{ab}	1.72	0.015
	Mo	57.4 ^a	66.3 ^b	64.6 ^b	1.21	0.003
	Al	40.1	37.3	43.5	1.49	0.243
	Cr	58.9 ^{ab}	52.3 ^a	73.6 ^b	3.52	0.007
	Cu	18.9	24.9	25.2	1.91	0.369

^{a-c} Means within rows not sharing a common suffix were significantly different at the 5% level of probability.

The results showed that hens fed the food waste-based diets had lower intake and excretion for all measured minerals ($p < 0.05$) compared to those offered the control diets, except for P, Na, and Cr, where mineral intake and excretion were higher for the food waste group ($p < 0.05$; Tables 3 and 4). Hens fed the 50:50 blend showed intermediate responses. Hens fed the food waste-based diets had lower retention of Ca ($p < 0.001$), Mo ($p < 0.05$), and Al ($p < 0.01$) but higher retention of Na ($p < 0.001$) compared to hens fed the control and 50:50 blend diets. Additionally, hens on the food waste treatment had higher P retention than the control treatment ($p < 0.05$) and lower Cr retention than the 50:50 blend treatment ($p < 0.01$, Table 5). Consequently, hens fed the food waste-based diets showed higher Mg ($p < 0.001$), K ($p < 0.001$), S ($p < 0.001$), Mn ($p < 0.05$), and Mo ($p < 0.01$) digestibility compared to the control counterparts at week 63 (Table 6). In contrast, hens offered the 50:50 blend diets had higher Ca ($p < 0.05$), Mg ($p < 0.001$), K ($p < 0.001$), S ($p < 0.001$), and Mo ($p < 0.01$) digestibility than those offered the control diets and higher Cr digestibility ($p < 0.01$) compared to those fed the food waste-based diets at week 63 (Table 6).

3.4. Gut pH and Morphology

The results for pH along the intestinal tract at week 63 are shown in Table 7. Hens offered food waste based-diets had a significantly higher pH in the ileum than hens offered the control or 50:50 blend diets ($p < 0.001$), but there were no significant differences in pH for the crop, gizzard, or caeca.

Table 7. pH along intestinal tract for experimental treatments at week 63.

Variable	Control	Food Waste	50:50 Blend	SEM	p-Value
Crop	4.33	4.24	4.33	0.03	0.343
Gizzard	4.22	4.36	4.37	0.07	0.615
Ileum	5.41 ^a	6.18 ^b	5.58 ^a	0.07	<0.001
Ceca	5.90	5.82	5.77	0.05	0.548

^{a,b} Means within rows not sharing a common suffix were significantly different at the 5% level of probability.

The jejunal and ileal morphologies of the dietary treatments at week 63 are reported in Tables 8 and 9, respectively. The results showed that hens offered the 50:50 blend diets tended to have higher crypt depth in both the jejunum ($p = 0.083$, Table 8) and ileum ($p = 0.054$, Table 9) compared to those fed the control diets at week 63. Other gut morphological parameters did not differ between the dietary treatments at week 63.

Table 8. Jejunal morphological measurements for experimental treatments at week 63.

Variable	Control	Food Waste	50:50 Blend	SEM	p-Value
Villus height (μm)	935	1075	998	31.15	0.181
Crypt depth (μm)	130	149	154	4.78	0.083
Villus height/crypt depth	7.64	7.53	7.02	0.25	0.581
Basal width (μm)	143	145	165	5.47	0.192
Middle width (μm)	136	138	155	4.82	0.228
Apical width (μm)	125	125	142	4.53	0.205
Apparent villus area ($\mu\text{m}^2 \times 10^3$)	125	146	154	6.69	0.143

Table 9. Ileal morphological measurements for experimental treatments at week 63.

Variable	Control	Food Waste	50:50 Blend	SEM	p-Value
Villus height (μm)	527	545	633	25.90	0.238
Crypt depth (μm)	108	111	162	10.35	0.054
Villus height/crypt depth	5.00	5.17	4.23	0.29	0.431
Basal width (μm)	114	99.2	107	4.08	0.310
Middle width (μm)	106	93.6	102	3.94	0.456
Apical width (μm)	91.3	85.6	101	5.02	0.521
Apparent villus area ($\mu\text{m}^2 \times 10^3$)	55.3	51.4	66.8	4.25	0.362

3.5. Bone Morphology and Mineral Composition

Bone morphology, breaking strength, and other characteristics at week 63 are described in Table 10.

Table 10. Bone morphology and strength for experimental treatments at week 63.

Variable	Control	Food Waste	50:50 Blend	SEM	p-Value
Tibia					
Fresh weight (g)	12.9	12.5	12.9	0.12	0.341
Air-dry weight (g)	9.60	8.98	9.35	0.11	0.054
Oven-dry weight (g)	8.95 ^b	8.34 ^a	8.71 ^{ab}	0.10	0.041
Relative oven-dry weight ¹ (g/kg)	3.86	3.64	3.68	0.04	0.053
Ash ² (%)	37.6 ^a	42.0 ^b	39.8 ^{ab}	0.44	<0.001
Length ³ (mm)	125 ^b	122 ^a	124 ^{ab}	0.51	0.034
Diameter (mm)	8.38	8.08	8.15	0.07	0.163
Seedor index ⁴ (mg/mm)	71.3	68.3	70.4	0.62	0.132
Breaking strength (N)	253	248	256	9.82	0.941
Femur					
Fresh weight (g)	11.2	11.0	11.2	0.10	0.697
Air-dry weight (g)	7.62	7.34	7.54	0.09	0.489
Oven-dry weight (g)	6.74	6.37	6.52	0.09	0.218
Relative oven-dry weight (g/kg)	2.90	2.78	2.76	0.03	0.176

Table 10. *Cont.*

Variable	Control	Food Waste	50:50 Blend	SEM	<i>p</i> -Value
Ash (%)	44.4 ^a	50.3 ^b	48.8 ^b	0.57	<0.001
Length (mm)	90.0 ^b	88.0 ^a	89.5 ^{ab}	0.32	0.024
Diameter (mm)	8.64	8.60	8.52	0.04	0.448
Seedor index (mg/mm)	74.7	72.3	72.8	0.85	0.483
Breaking strength (N)	285	302	324	8.33	0.170

^{a,b} Means within rows not sharing a common suffix were significantly different at the 5% level of probability.

¹ Relative tibia and femur weights were calculated as per unit of body weight. ² Bone ash was expressed as a percentage of oven-dry bone (%). ³ Bone length, diameter, and breaking strength were measured on air-dry bones.

⁴ Bone Seedor index was calculated as the weight of oven-dry bone divided by the bone length (mg/mm).

Hens offered the food waste-based diets had a lower absolute oven-dry weight for the tibia compared to those offered the control diets ($p < 0.05$). However, when the oven-dry weight of the tibia was expressed as per unit of hens' body weight, the tibia oven-dry weights were not different between the dietary treatments. Higher ash content in both the tibia and femur was observed in hens offered the food waste-based diets compared to the control group ($p < 0.001$). Hens offered the 50:50 blend diets had higher ash content in the femur compared to those offered the control diets ($p < 0.001$). Additionally, hens offered the food waste-based diets had shorter tibias and femurs compared to hens offered the control diets at week 63 ($p < 0.05$).

The tibia mineral composition for the dietary treatments at week 63 is given in Table 11. Hens fed the food waste-based diets had increased S ($p < 0.05$), Fe ($p < 0.01$), Mn ($p < 0.001$), and Zn ($p < 0.001$) levels in the tibia compared to hens fed the control diets at week 63. Hens offered the 50:50 blend diets had higher tibia P, Mg, and Mo compared to those fed the control diets at week 63 ($p < 0.05$). Additionally, higher tibia Ca and P levels were observed in hens fed the 50:50 blend diets compared to those fed the food waste-based diets at week 63 ($p < 0.05$).

Table 11. Tibia mineral composition for experimental treatments at week 63.

Mineral	Control	Food Waste	50:50 Blend	SEM	<i>p</i> -Value	
Macro minerals (mg/g)	Ca	361 ^{ab}	358 ^a	375 ^b	2.93	0.030
	P	163 ^a	163 ^a	170 ^b	1.32	0.042
	Na	9.53	9.86	10.04	0.09	0.060
	Mg	4.81 ^a	5.09 ^{ab}	5.14 ^b	0.06	0.028
	K	3.29	3.46	3.53	0.07	0.413
	S	1.36 ^a	1.55 ^b	1.49 ^{ab}	0.03	0.021
Micro minerals (µg/g)	Fe	143 ^a	194 ^b	173 ^{ab}	7.20	0.008
	Mn	15.0 ^a	24.5 ^b	20.5 ^b	1.07	<0.001
	Mo	58.9 ^a	59.5 ^{ab}	62.9 ^b	0.66	0.021
	Zn	398 ^a	530 ^b	486 ^b	13.07	<0.001

^{a,b} Means within rows not sharing a common suffix were significantly different at the 5% level of probability.

4. Discussion

This study illustrated that recycled food waste is a promising ingredient for poultry feed that can meet all the requirements of hens and, if suitable waste streams are available, provide whole diets for laying hens that meet their needs throughout production. Food waste-based diets had higher levels of energy, crude protein, and fat compared to the control diets. Furthermore, hens offered the food waste-based diets had higher energy digestibility compared to those offered the control diets. These are likely the reasons for the higher feed efficiency in hens offered food waste-based diets compared to their control counterparts in this study. The results of this study are supported by previous findings [52,53]. In addition, it may be worth noting that current diet-formulation practice feeds birds as if they are strictly granivores, neglecting the fact that they are actually omnivores [54]. Thus, modern poultry nutrition typically relies on grain-based diets. Food waste-based diets may allow

optimization of the genetic potential of birds as omnivores, as shown by the results of this study. With the opportunities that food waste provides, there are several aspects that are yet to be further refined. For example, several food waste streams, such as pub and restaurant meal and hospital and nursing home meal, may be highly variable in relation to practical commercial use. However, this issue can be attenuated by focusing on more reliable waste streams that are available in large quantities and by investing in an NIR calibration for valuable waste stream sources. Furthermore, it is recommended that the digestibility and availability of nutrients within key waste streams should be evaluated to ensure accurate diet formulation. Nevertheless, this study demonstrates that food waste is a suitable feedstuff and, with some further research input, such as investment into NIR calibrations allowing the rapid determination of nutrient content to combat any variability issues, would likely become a preferable ingredient compared to traditional foodstuffs in the future with its reduced cost and high nutrient value.

As the food waste-based diets were rather energy-dense, it is understandable that the total ileal digestible energy level was increased in hens fed the food waste-based diets compared to hens offered the control diets in this study. Additionally, the higher apparent ileal energy digestibility in hens offered the food waste-based diets compared to the control groups may be attributed to the lower fiber and non-starch-polysaccharide (NSP) levels in food waste diets compared to the control diets at week 63 in the current study. This is interesting and consistent with the results of our previous research where feeding food waste-based diets with higher fiber and NSP levels compared to the conventional control diets decreased dry matter digestibility and tended to decrease energy digestibility of laying hens at 43 weeks of age [23]. Similarly, other studies have shown higher dry matter and energy digestibility in birds fed diets with lower fiber or NSP levels [55–57]. This is understandable as increases in dietary fiber and/or NSP levels—particularly soluble NSP—may increase digesta viscosity, resulting in reduced nutrient utilization in the gut [58–60]. Additionally, it has been suggested that energy originating from fat may be more digestible than that from carbohydrates, as it may reduce competition between glucose and amino acids for absorption sites in the small intestine, resulting in increased nutrient utilization [61]. Thus, food waste diets may deliver an advantage by providing proportionally more energy as fat rather than carbohydrates. Due to the variability in fiber composition, use of commercial food waste diets will require monitoring of fiber levels within ingredients and supplementation with NSPase enzymes accordingly.

The idea of long-life laying hens that can produce 500 eggs or more in a single production cycle of 100 weeks has become a reality thanks to advances in hen genetics [62]. However, despite the benefits of improving the utilization of scarce resources and reducing waste and the carbon footprint, the extension of the laying cycle may also induce challenges for hen health, particularly with respect to bone metabolism, as large amounts of Ca are mobilized from the skeleton for eggshell formation, which may increase the incidence of bone fractures and osteoporosis during the late laying phase [63,64]. Nutritional strategies that could improve the accumulation of medullary bone in the early laying cycle may help to maintain eggshell quality and skeletal integrity in aging laying hens [65]. In the current study, hens offered food waste-based diets and 50:50 blend diets had increased mineral digestibility for various minerals, which explains the improved bone ash and mineral levels in the bones of hens that received these diets compared to the control group. Thus, it is speculated that feeding food waste-based diets would be even more advantageous for egg production and hens' health and welfare during the late laying phase when the hens' physiological conditions and bone mineralization status worsen. Besides the minerals provided in the vitamin–mineral premix, the mineral sources in the food waste-based diets mainly originated from the organic sources presented in fish offal and spent brewers' grain blend, pub and restaurant meal, recycled meat and bone meal, hospital and nursing home meal, vegetable and fruit meal, and oyster shell meal. In contrast, the minerals presented in the control diets were mainly from inorganic sources, such as limestone and di-calcium phosphate. It is widely accepted that organic minerals are more bioavailable and digestible

compared to inorganic mineral sources [44,66,67]. This may be due to the effects of organic minerals in diminishing the complexation of trace minerals with phytate, fiber, and macro minerals, such as Ca and/or P, leading to fewer antagonistic reactions with these dietary components during feed digestion [68]. This may explain the higher mineral digestibility in hens fed the food waste-based diets and 50:50 blend diets compared to those fed the control diets in the current study. Nevertheless, despite the increased mineralization, the bone breaking strength did not differ between the dietary treatments in the current study. This may be explained by the fact that bone strength in birds is not only influenced by bone mineral density but also by organic factors, such as collagen crosslinkage in bone [69].

Accumulation of minerals—particularly P, Cu, Mn, Zn, and Fe—in the poultry litter has raised public concern due to its negative impacts on the environment and crop yield [70,71]. For example, excess P in poultry manure may cause eutrophication, leading to the overgrowth of aquatic plants, such as algae [72,73]. Excess manure P in soil may also contaminate ground water [74]. Also, using livestock and poultry manure containing high trace mineral content as fertilizer may increase toxicity risks to plants and soil microorganisms reducing crop yield [70,75]. This has raised concerns for the current practice of oversupplying inorganic minerals in poultry diets and necessitated mineral sources that can be incorporated at lower levels but still satisfy birds' nutritional requirements [76]. This problem may be solved by replacing inorganic with organic minerals in poultry diets, which are more bioavailable and digestible than inorganic mineral sources [66]. In the current study, feeding food waste-based diets that contained more organic minerals than the control diets reduced the excretion of nine minerals but also increased the excretion of three minerals (P, Na, and Cr) into the environment. The reduction in the amounts of various minerals in the excreta of hens offered food waste-based diets may be attributed to the lower intake but higher or similar mineral digestibility of these minerals in food waste diets compared to the control group. In contrast, the higher P, Na, and Cr levels in the excreta of hens fed the food waste-based diets were likely associated with the higher intake but similar mineral digestibility of these minerals in the food waste group compared to the control group in the current study. Thus, feeding food waste-based diets may have both positive and negative effects on mineral excretion. Reducing inclusion rates or replacement of food waste streams with high Na (salt), P, and Cr content (e.g., hospital and nursing home meal, pub and restaurant meal, and oyster shell meal) with waste streams without high contents of these minerals may help to solve this issue and provide a more balanced mineral profile, leading to further improvements in the nutrient digestibility, laying performance, and environmental outcomes. Additionally, analyzing phytate-P levels in the food waste streams and mixed complete diets and optimizing phytase enzyme inclusions for food waste-based diets are necessary to improve P digestibility and reduce P excretion. Other nutritional strategies, such as adjusting dietary Ca to P ratios and/or supplementation of vitamin D, may also help to increase P digestibility in the food waste diets [77].

Gut pH may be influenced by various nutritional factors, including feed form and particle size, dietary fiber, protein, Ca, and Na levels [78–80]. Generally speaking, lower gut pH is more favorable as it may facilitate nutrient digestibility and diminish the growth of acid-sensitive pathogenic bacteria [81]. In this study, the pH values were relatively unchanged throughout the gastrointestinal tract with the exception of the ileum, which showed higher pH in hens fed the food waste-based diets compared to those fed the control or 50:50 blend diets at week 63. It could be seen that the food waste-based diet used in the last period of this study (weeks 59–63) was generally more complex and contained higher levels of CP, Na, and P but a lower level of fiber compared to the control diet during the same period. In a recent review, Desbruslais et al. [80] indicated that increased intestinal pH values in birds are often associated with a reduction in dietary fiber. Additionally, higher dietary CP levels may also raise intestinal pH by increasing the amounts of undigested protein entering the hindgut, which may in turn also increase microbial fermentation of nitrogenous waste, resulting in greater ammonia production [82,83]. Previous research has shown that ammonia production increased intestinal pH [84,85]. These findings may partly

explain the higher ileal pH in hens fed food waste-based diets compared to the control group in this study.

Feeding food waste-based diets did not affect gut morphological parameters in the current study. However, hens offered the 50:50 blend diets tended to have increased crypt depth in both the jejunum and ileum compared to hens fed the control diets at week 63. Greater crypt depth may not be preferred as it reflects a higher tissue turnover, leading to more energy requirements for body maintenance [86,87]. The mechanisms of this effect on birds fed 50:50 blend diets in this study were unclear. It could be seen that the 50:50 blend diet was the most complex diet in this study. The combination of both conventional feed ingredients and food waste streams; organic and inorganic matter; processed (e.g., hospital and nursing home meal, pub and restaurant meal) and raw, unprocessed ingredients (e.g., sorghum, wheat); and animal and plant oil in these diets may have both positive and negative impacts on bird nutrient digestibility and physiological condition. Further studies on this matter are necessary to understand possible interactions between dietary components when food waste streams are incorporated into conventional diets.

5. Conclusions

Feeding recycled food waste-based diets is effective to improve laying performance, nutrient digestibility and bone mineralization in laying hens. The findings of the current study demonstrate that hens offered the food waste-based diets had higher digestibility for energy and various minerals, resulting in increased bone ash and mineral levels compared to the control group. This would be advantageous for egg production and hen health during the late laying period when the hen's physiological conditions and bone mineralization status worsen. Furthermore, the current findings showed that feeding food waste-based diets improved feed efficiency by 10.3% without compromising egg production compared to the control diets, while the 50:50 blend treatment showed intermediate effects over 40 weeks of the study. Thus, using the food waste-based diets would be more advantageous to reduce the feed cost, improve production efficiency, and reduce environmental impacts from the poultry production compared to the 50:50 blend diets. However, the inclusion of recycled food waste materials at 50% or less is still beneficial for poultry producers who may want to replace a part of their commercial diet with the food waste diet to reduce the feed cost. Removal or reducing dietary inclusion rates of food waste streams with high Na, P, and Cr content would improve the mineral profile of the food waste-based diets, leading to further improvements in laying hens' performance and nutrient digestibility. Furthermore, determination of digestible values and development of near-infrared reflectance spectroscopy (NIR) calibration for rapid nutrient analysis of food waste materials, as well as determination of the economic benefits of food waste-based diets, may help to expand the industry adoption on this feed.

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Institutional Review Board Statement: The Animal Ethics Committee of the University of New England approved all the experimental design and procedures (approval number: AEC 20-042), and the experimental procedures met the requirements of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

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Data Availability Statement: The research data that support this study will be shared upon reasonable request to the corresponding author.

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Conflicts of Interest: Author Nishchal K. Sharma is employed by the Ridley AgriProducts Pty Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A

Table A1. Diet composition for experimental treatments during the first phase of the study from 24 to 43 weeks of age (as-is basis, %, otherwise as indicated).

Dietary Treatment	Weeks 24 to 37			Weeks 38 to 43		
	Control ¹	Food Waste ²	50:50 Blend	Control	Food Waste	50:50 Blend
Ingredients						
Wheat	40.75	0.00	20.38	46.30	0.00	23.15
Sorghum	20.00	0.00	10.00	20.00	0.00	10.00
Soybean meal	13.51	0.00	6.76	9.63	0.00	4.82
Canola meal	10.00	0.00	5.00	10.00	0.00	5.00
Commercial meat and bone meal	2.00	0.00	1.00	3.11	0.00	1.56
Canola oil	2.96	0.00	1.48	0.55	0.00	0.28
Limestone	9.60	0.00	4.80	9.80	0.00	4.90
Di-calcium phosphate	0.48	0.00	0.24	0.00	0.00	0.00
Salt	0.24	0.00	0.12	0.20	0.00	0.10
Vegetable and fruit meal	0.00	5.00	2.50	0.00	5.00	2.50
Spent brewers' grain	0.00	34.90	17.45	0.00	28.19	14.10
Fish offal and spent brewers' grain blend ³	0.00	15.00	7.50	0.00	15.00	7.50
Hospital and nursing home meal	0.00	15.00	7.50	0.00	15.00	7.50
Pub and restaurant meal	0.00	3.11	1.56	0.00	2.81	1.41
Recycled meat and bone meal	0.00	8.30	4.15	0.00	8.00	4.00
Bakery meal	0.00	16.84	8.42	0.00	19.31	9.66
Oyster shell meal	0.00	1.28	0.64	0.00	6.16	3.08
Choline Cl 70%	0.061	0.268	0.165	0.066	0.268	0.167
L-lysine HCl	0.073	0.000	0.037	0.060	0.000	0.030
D,L-methionine	0.169	0.166	0.168	0.139	0.130	0.135
L-threonine	0.016	0.000	0.008	0.000	0.000	0.000
Xylanase ⁴	0.005	0.005	0.005	0.005	0.005	0.005
Phytase ⁵	0.006	0.006	0.006	0.006	0.006	0.006
Pigment jabiru red	0.004	0.004	0.004	0.004	0.004	0.004
Pigment jabiru yellow	0.003	0.003	0.003	0.003	0.003	0.003
Antioxidant	0.025	0.025	0.025	0.025	0.025	0.025
Vitamin–mineral premix ⁶	0.100	0.100	0.100	0.100	0.100	0.100
Calculated composition						
AMEn ⁷ , kcal/kg	2800	2800	2800	2700	2700	2700
Crude protein	17.80	25.57	21.69	17.00	24.10	20.55
Crude fat	5.28	13.44	9.36	3.05	12.83	7.94
Crude fiber	2.78	8.91	5.85	2.79	7.87	5.33
SID ⁸ arginine	0.945	0.962	0.954	0.872	0.913	0.893
SID lysine	0.780	0.808	0.794	0.700	0.761	0.731
SID methionine	0.420	0.445	0.433	0.380	0.398	0.389
SID cysteine	0.298	-	-	0.290	-	-
SID methionine + cysteine	0.719	0.670	0.695	0.671	0.600	0.636
SID tryptophan	0.213	0.193	0.203	0.198	0.183	0.191

Table A1. Cont.

Dietary Treatment	Weeks 24 to 37			Weeks 38 to 43		
	Control ¹	Food Waste ²	50:50 Blend	Control	Food Waste	50:50 Blend
Calculated composition						
SID histidine	0.378	-	-	0.352	-	-
SID phenylalanine	0.707	-	-	0.656	-	-
SID leucine	1.196	1.333	1.265	1.125	-	-
SID isoleucine	0.630	0.739	0.685	0.583	0.690	0.637
SID threonine	0.560	0.613	0.587	0.507	0.578	0.543
SID valine	0.733	0.962	0.848	0.691	0.894	0.793
Calcium	4.200	4.200	4.200	4.257	5.900	5.079
Available phosphorus	0.450	1.020	0.735	0.400	0.992	0.696
Sodium	0.180	0.450	0.315	0.170	0.480	0.325
Potassium	0.704	-	-	0.649	-	-
Chloride	0.222	-	-	0.201	-	-
Choline, mg/kg	1400	1400	1400	1400	1400	1400
Linoleic acid	1.582	-	-	1.000	-	-

The diets were formulated using feed formulation software (Concept 5 version 13.11.19, CFC Tech Services, Inc., Browerville, MN, USA). ¹ Control diet based on common feed ingredients to mimic commercial layer-hen feed. ² Food waste diet based on recycled food waste materials. A 50:50 treatment was made by blending the control diet and food waste diet together (50% each in weight). ³ Fish offal and spent brewers' grain blend was made by blending fish offal and spent brewers' grain together (50% each in volume). ⁴ Econase XT, 25, AB Vista. ⁵ Quantum Blue 5G Layers, AB Vista. ⁶ Vitamin-mineral premix provided the following per kilogram diet: vitamin A, 10,000 IU; vitamin D, 3000 IU; vitamin E, 20 mg; vitamin K, 3 mg; nicotinic acid (niacin), 35 mg; pantothenic acid, 12 mg; folic acid, 1 mg; riboflavin (B2), 6 mg; cyanocobalamin (B12), 0.02 mg; biotin, 0.1 mg; pyridoxine (B6), 5 mg; thiamine (B1), 2 mg; copper, 8 mg as copper sulphate pentahydrate; cobalt, 0.2 mg as cobalt sulphate 21%; molybdenum, 0.5 mg as sodium molybdate; iodine, 1 mg as potassium iodide 68%; selenium, 0.3 mg as selenium 2%; iron, 60 mg as iron sulphate 30%; zinc, 60 mg as zinc sulphate 35%; manganese, 90 mg as manganous oxide 60%; antioxidant, 20 mg. ⁷ AMEn: N-corrected apparent metabolizable energy. ⁸ SID: standardized ileal digestibility. Digestible amino acid coefficients of conventional feed ingredients were determined by near-infrared spectroscopy (Foss NIR 6500, Hillerød, Denmark) standardized with Evonik AMINONIR Advanced calibration.

Table A2. Diet composition for experimental treatments during the second phase of the study from 44 to 63 weeks of age (as-is basis, %, otherwise as indicated).

Dietary Treatment	Weeks 44 To 58			Weeks 59 to 63		
	Control ¹	Food Waste ²	50:50 Blend	Control	Food Waste	50:50 Blend
Ingredients						
Wheat	46.35	0.00	23.18	45.78	0.00	22.89
Sorghum	20.00	0.00	10.00	20.00	0.00	10.00
Soybean meal	8.71	0.00	4.36	8.84	0.00	4.42
Canola meal	10.00	0.00	5.00	10.00	0.00	5.00
Commercial meat and bone meal	4.00	0.00	2.00	4.00	0.00	2.00
Canola oil	0.53	0.00	0.27	0.48	0.00	0.24
Limestone	9.80	0.00	4.90	9.80	0.00	4.90
Salt	0.19	0.00	0.10	0.19	0.00	0.10
Vegetable and fruit meal	0.00	5.00	2.50	0.00	2.50	1.25
Spent brewers' grain	0.00	16.44	8.22	0.00	4.00	2.00
Fish offal and spent brewers' grain blend ³	0.00	23.00	11.50	0.00	33.00	16.50
Hospital and nursing home meal	0.00	15.00	7.50	0.00	2.90	1.45
Pub and restaurant meal	0.00	5.00	2.50	0.00	10.00	5.00
Recycled meat and bone meal	0.00	12.00	6.00	0.00	14.00	7.00
Bakery meal	0.00	18.76	9.38	0.00	27.07	13.54
Oyster shell meal	0.00	4.23	2.12	0.00	5.50	2.75
Choline Cl 70%	0.068	0.268	0.168	0.069	0.268	0.169
Titanium dioxide	0.00	0.00	0.000	0.500	0.500	0.500
L-lysine HCl	0.070	0.00	0.035	0.068	0.00	0.034

Table A2. Cont.

Dietary Treatment	Weeks 44 To 58			Weeks 59 to 63		
	Control ¹	Food Waste ²	50:50 Blend	Control	Food Waste	50:50 Blend
Ingredients						
D,L-methionine	0.141	0.155	0.148	0.141	0.117	0.129
Xylanase ⁴	0.005	0.005	0.005	0.005	0.005	0.005
Phytase ⁵	0.006	0.006	0.006	0.006	0.006	0.006
Pigment jabiru red	0.004	0.004	0.004	0.004	0.004	0.004
Pigment jabiru yellow	0.003	0.003	0.003	0.003	0.003	0.003
Antioxidant	0.025	0.025	0.025	0.025	0.025	0.025
Vitamin–mineral premix ⁶	0.100	0.100	0.100	0.100	0.100	0.100
Calculated composition						
AMEn ⁷ , kcal/kg	2720	2720	2720	2700	2700	2700
Crude protein	17.00	21.62	19.31	17.00	22.43	19.72
Crude fat	3.05	15.05	9.05	2.99	15.92	9.46
Crude fiber	2.77	6.52	4.65	2.76	4.81	3.79
SID ⁸ arginine	0.862	0.889	0.876	0.864	0.922	0.893
SID lysine	0.700	0.728	0.714	0.700	0.764	0.732
SID methionine	0.380	0.421	0.401	0.380	0.413	0.397
SID cysteine	0.284	0.179	0.232	0.283	-	-
SID methionine + cysteine	0.668	0.600	0.634	0.667	0.600	0.634
SID tryptophan	0.194	0.160	0.177	0.194	0.169	0.182
SID isoleucine	0.581	0.593	0.587	0.582	0.612	0.597
SID threonine	0.508	0.518	0.513	0.508	0.549	0.529
SID valine	0.691	0.756	0.724	0.691	0.774	0.733
Calcium	4.325	4.325	4.325	4.325	4.320	4.323
Available phosphorus	0.440	0.978	0.709	0.439	1.094	0.767
Sodium	0.170	0.403	0.287	0.170	0.453	0.312
Chloride	0.200	-	-	0.200	-	-
Choline, mg/kg	1400	1400	1400	1400	1400	1400
Linoleic acid	0.991	-	-	0.974	-	-

The diets were formulated using feed formulation software (Concept 5 version 13.11.19, CFC Tech Services, Inc., Browerville, MN, USA). ¹ Control diet based on common feed ingredients to mimic commercial layer-hen feed. ² Food waste diet based on recycled food waste materials. A 50:50 treatment was made by blending the control diet and food waste diet together (50% each in weight). ³ Fish offal and spent brewers' grain blend was made by blending fish offal and spent brewers' grain together (50% each in volume). ⁴ Econase XT, 25, AB Vista. ⁵ Quantum Blue 5G Layers, AB Vista. ⁶ Vitamin–mineral premix provided the following per kilogram diet: vitamin A, 10,000 IU; vitamin D, 3000 IU; vitamin E, 20 mg; vitamin K, 3 mg; nicotinic acid (niacin), 35 mg; pantothenic acid, 12 mg; folic acid, 1 mg; riboflavin (B2), 6 mg; cyanocobalamin (B12), 0.02 mg; biotin, 0.1 mg; pyridoxine (B6), 5 mg; thiamine (B1), 2 mg; copper, 8 mg as copper sulphate pentahydrate; cobalt, 0.2 mg as cobalt sulphate 21%; molybdenum, 0.5 mg as sodium molybdate; iodine, 1 mg as potassium iodide 68%; selenium, 0.3 mg as selenium 2%; iron, 60 mg as iron sulphate 30%; zinc, 60 mg as zinc sulphate 35%; manganese, 90 mg as manganous oxide 60%; antioxidant, 20 mg. ⁷ AMEn: N-corrected apparent metabolizable energy. ⁸ SID: standardized ileal digestibility. Digestible amino acid coefficients of conventional feed ingredients were determined by near-infrared spectroscopy (Foss NIR 6500, Hillerød, Denmark) standardized with Evonik AMINONIR Advanced calibration.

Table A3. Analyzed nutrient values of experimental diets during the first phase of the study from 24 to 43 weeks of age (as-is basis, %, otherwise as indicated).

Dietary Treatment	Weeks 24 to 37			Weeks 38 to 43		
	Control ¹	Food Waste ²	50:50 Blend ³	Control	Food Waste	50:50 Blend
Dry matter	91.16	93.00	91.80	91.02	91.24	91.00
Gross energy, kcal/kg	3717	4501	4001	3523	4175	3748
Crude protein	17.93	22.93	20.12	17.40	19.60	17.96
Crude fat	4.38	9.57	7.35	5.19	6.76	6.06
Crude fiber	8.72	12.99	10.62	9.00	9.54	9.49
Ash	13.51	9.93	11.16	15.22	11.28	12.80
Calcium	4.99	3.13	3.96	5.71	4.04	5.38

Table A3. Cont.

Dietary Treatment	Weeks 24 to 37			Weeks 38 to 43		
	Control ¹	Food Waste ²	50:50 Blend ³	Control	Food Waste	50:50 Blend
Total phosphorus	0.56	1.31	0.91	0.58	0.92	0.78
Sodium	0.14	0.38	0.28	0.14	0.32	0.26
Potassium	0.74	0.41	0.53	0.66	0.43	0.58
Arginine	1.006	1.165	1.056	0.969	0.987	0.983
Lysine	0.867	0.935	0.911	0.829	0.786	0.805
Methionine	0.393	0.395	0.393	0.394	0.410	0.399
Histidine	0.452	0.458	0.453	0.425	0.402	0.410
Phenylalanine	0.823	1.018	0.882	0.764	0.827	0.825
Leucine	1.399	1.524	1.480	1.309	1.266	1.290
Isoleucine	0.725	0.849	0.732	0.675	0.695	0.677
Threonine	0.658	0.771	0.708	0.616	0.635	0.634
Valine	0.851	1.074	0.971	0.810	0.866	0.846
Glycine	0.851	1.632	1.057	0.966	1.376	0.969
Serine	0.808	0.915	0.829	0.769	0.763	0.764
Glutamic acid	3.641	4.151	3.814	3.487	3.595	3.499
Proline	1.232	1.906	1.472	1.257	1.590	1.319
Alanine	0.846	1.209	0.993	0.841	1.003	0.887
Tyrosine	0.451	0.514	0.487	0.412	0.469	0.456
Aspartic acid	1.388	1.548	1.435	1.261	1.295	1.269

Values of all the amino acids presented refer to total amino acids (measured on an as-is basis). ¹ Control diet based on common feed ingredients to mimic commercial layer-hen feed. ² Food waste diet based on recycled food waste materials. ³ The 50:50 blend diet was made by blending the control diet and food waste diet together (50% each in weight).

Table A4. Analyzed free-sugar and non-starch-polysaccharide (NSP) content of experimental diets from weeks 24 to 37 (as-is basis, g/kg).

Nutrients	Control ¹				Food Waste ²				50:50 Blend ³			
	Free Sugars	SNSP ⁴	INSP ⁵	Total NSP	Free Sugars	SNSP	INSP	Total NSP	Free Sugars	SNSP	INSP	Total NSP
Rhamnose	0.00	0.00	0.00	0.00	0.00	0.16	0.00	0.16	0.00	0.06	0.00	0.06
Fucose	0.00	0.00	0.64	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.25
Ribose	0.00	0.44	0.00	0.44	0.00	0.19	0.00	0.19	0.00	0.28	0.00	0.28
Arabinose	0.53	3.52	18.25	21.77	0.81	3.28	36.42	39.70	0.60	3.31	25.41	28.72
Xylose	0.00	3.52	14.87	18.38	0.99	3.87	71.28	75.15	0.49	3.63	41.21	44.84
Mannose	4.65	1.13	1.33	2.45	3.15	1.34	1.76	3.10	4.57	1.21	1.68	2.89
Galactose	6.44	1.80	8.90	10.69	1.68	1.89	6.83	8.72	3.07	1.83	6.74	8.57
Glucose	18.24	1.55	22.99	24.54	16.77	1.75	16.96	18.72	17.35	1.62	19.21	20.83
Total	29.85	10.61	59.31	69.92	23.39	11.09	117.78	128.87	26.09	10.66	102.32	112.98
Starch (%)	35.66				14.49				25.14			

¹ Control diet based on common feed ingredients to mimic commercial layer-hen feed. ² Food waste diet based on recycled food waste materials. ³ The 50:50 blend diet was made by blending the control diet and food waste diet together (50% each in weight). ⁴ SNSP: soluble NSP. ⁵ INSP: insoluble NSP.

Table A5. Analyzed free-sugar and non-starch-polysaccharide (NSP) content of experimental diets from weeks 38 to 43 (as-is basis, g/kg).

Nutrients	Control ¹				Food Waste ²				50:50 Blend ³			
	Free Sugars	SNSP ⁴	INSP ⁵	Total NSP	Free Sugars	SNSP	INSP	Total NSP	Free Sugars	SNSP	INSP	Total NSP
Rhamnose	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.13	0.00	0.05	0.00	0.05
Fucose	0.00	0.00	0.58	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.22
Ribose	0.00	0.42	0.00	0.42	0.00	0.19	0.00	0.19	0.00	0.26	0.00	0.26
Arabinose	0.44	3.62	17.76	21.38	0.46	2.77	17.69	20.46	0.42	2.91	17.69	20.60
Xylose	0.00	3.37	14.74	18.11	0.51	3.08	37.59	40.67	0.20	3.15	24.67	27.81
Mannose	3.88	1.25	1.33	2.59	3.73	2.05	1.79	3.84	3.81	1.44	1.82	3.25

Table A5. *Cont.*

Nutrients	Control ¹				Food Waste ²				50:50 Blend ³			
	Free Sugars	SNSP ⁴	INSP ⁵	Total NSP	Free Sugars	SNSP	INSP	Total NSP	Free Sugars	SNSP	INSP	Total NSP
Galactose	5.95	1.74	7.48	9.23	1.91	1.41	3.58	5.00	2.89	1.53	4.43	5.96
Glucose	17.65	1.59	23.71	25.30	19.56	6.20	39.06	45.26	18.38	3.74	34.09	37.83
Total	27.93	10.65	58.14	68.78	26.17	14.12	88.64	102.76	25.71	11.77	75.65	87.43
Starch (%)	37.42				15.33				26.29			

¹ Control diet based on common feed ingredients to mimic commercial layer-hen feed. ² Food waste diet based on recycled food waste materials. ³ The 50:50 blend diet was made by blending the control diet and food waste diet together (50% each in weight). ⁴ SNSP: soluble NSP. ⁵ INSP: insoluble NSP.

Table A6. Analyzed nutrient values of experimental diets during the second phase of the study from 44 to 63 weeks of age (as-is basis, %, otherwise as indicated).

Dietary Treatment	Weeks 44 to 58			Weeks 59 to 63		
	Control ¹	Food Waste ²	50:50 Blend	Control	Food Waste	50:50 Blend
Dry matter	91.13	90.84	91.00	91.46	89.84	90.60
Gross energy, kcal/kg	3534	4252	3857	3550	4093	3851
Crude protein	16.64	20.98	18.56	17.13	18.71	17.56
Crude fat	4.32	12.12	7.19	3.03	12.91	6.85
Crude fiber	10.03	7.35	9.30	10.19	5.11	7.44
Ash	14.70	11.03	12.85	15.10	12.12	13.40
Calcium	5.73	3.94	4.92	5.36	4.33	4.23
Total phosphorus	0.57	1.05	0.82	0.50	1.10	0.72
Sodium	0.13	0.32	0.25	0.12	0.41	0.28
Potassium	0.64	0.44	0.53	0.64	0.46	0.52
Arginine	0.956	1.102	0.985	0.946	1.029	0.975
Lysine	0.795	0.915	0.838	0.849	0.850	0.854
Methionine	0.368	0.473	0.436	0.377	0.455	0.404
Histidine	0.417	0.453	0.426	0.428	0.394	0.408
Phenylalanine	0.758	0.863	0.794	0.764	0.756	0.762
Leucine	1.288	1.356	1.340	1.311	1.167	1.228
Isoleucine	0.655	0.747	0.736	0.673	0.637	0.649
Threonine	0.598	0.702	0.652	0.612	0.615	0.613
Valine	0.793	0.925	0.878	0.811	0.802	0.804
Glycine	0.984	1.571	1.041	0.902	1.599	1.060
Serine	0.760	0.824	0.783	0.761	0.748	0.752
Glutamic acid	3.485	3.712	3.544	3.494	3.427	3.443
Proline	1.277	1.632	1.446	1.237	1.600	1.448
Alanine	0.842	1.123	0.939	0.827	1.015	0.877
Tyrosine	0.411	0.488	0.443	0.416	0.409	0.413
Aspartic acid	1.234	1.435	1.252	1.243	1.245	1.246

Values of all the amino acids presented refer to total amino acids (measured on an as-is basis). ¹ Control diet based on common feed ingredients to mimic commercial layer-hen feed. ² Food waste diet based on recycled food waste materials. The 50:50 blend diet was made by blending the control diet and food waste diet together (50% each in weight).

Table A7. Analyzed free-sugar and non-starch-polysaccharide (NSP) content of experimental diets from weeks 44 to 58 (as-is basis, g/kg).

Nutrients	Control ¹				Food Waste ²				50:50 Blend			
	Free Sugars	SNSP ³	INSP ⁴	Total NSP	Free Sugars	SNSP	INSP	Total NSP	Free Sugars	SNSP	INSP	Total NSP
Rhamnose	0.00	0.00	0.00	0.00	0.00	0.12	0.00	0.12	0.00	0.05	0.00	0.05
Fucose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ribose	0.00	0.35	0.00	0.35	0.00	0.21	0.00	0.21	0.00	0.26	0.00	0.26
Arabinose	0.43	2.59	18.53	21.13	0.49	2.96	17.67	20.62	0.46	2.63	18.28	20.91
Xylose	0.00	2.46	18.11	20.57	0.53	3.88	34.18	38.06	0.36	2.91	29.84	32.75

Table A7. Cont.

Nutrients	Control ¹				Food Waste ²				50:50 Blend			
	Free Sugars	SNSP ³	INSP ⁴	Total NSP	Free Sugars	SNSP	INSP	Total NSP	Free Sugars	SNSP	INSP	Total NSP
Mannose	4.20	1.89	1.26	3.14	3.62	1.37	1.68	3.05	4.13	1.44	1.59	3.03
Galactose	5.31	1.76	6.98	8.74	1.76	1.45	3.64	5.09	2.95	1.64	4.60	6.25
Glucose	16.90	1.66	22.61	24.27	20.66	3.78	35.03	38.81	18.92	2.78	28.89	31.67
Total	26.85	9.53	60.00	69.54	27.06	12.20	81.93	94.14	26.81	10.69	76.41	87.10
Starch (%)	27.99				15.20				20.85			

¹ Control diet based on common feed ingredients to mimic commercial layer-hen feed. ² Food waste diet based on recycled food waste materials. The 50:50 blend diet was made by blending the control diet and food waste diet together (50% each in weight). ³ SNSP: soluble NSP. ⁴ INSP: insoluble NSP.

Table A8. Analyzed free-sugar and non-starch-polysaccharide (NSP) content of experimental diets from weeks 59 to 63 (as-is basis, g/kg).

Nutrients	Control ¹				Food Waste ²				50:50 Blend			
	Free Sugars	SNSP ³	INSP ⁴	Total NSP	Free Sugars	SNSP	INSP	Total NSP	Free Sugars	SNSP	INSP	Total NSP
Rhamnose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Fucose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ribose	0.00	0.40	0.00	0.40	0.00	0.17	0.00	0.17	0.00	0.25	0.00	0.25
Arabinose	0.37	3.33	17.59	20.92	0.00	2.82	8.68	11.50	0.14	2.94	14.26	17.20
Xylose	0.00	3.35	15.09	18.44	0.00	3.46	16.53	19.99	0.00	3.38	16.26	19.64
Mannose	4.59	0.97	1.29	2.26	4.02	1.70	1.25	2.95	4.14	1.20	1.26	2.47
Galactose	5.34	1.63	7.08	8.72	1.77	1.27	0.00	1.27	2.72	1.49	3.60	5.09
Glucose	17.15	1.24	22.92	24.17	18.31	8.94	19.44	28.38	17.36	4.88	22.16	27.03
Total	27.45	9.69	56.35	66.04	24.09	16.40	40.80	57.20	24.37	12.91	49.89	62.80
Starch (%)	28.88				20.46				24.26			

¹ Control diet based on common feed ingredients to mimic commercial layer-hen feed. ² Food waste diet based on recycled food waste materials. The 50:50 blend diet was made by blending the control diet and food waste diet together (50% each in weight). ³ SNSP: soluble NSP. ⁴ INSP: insoluble NSP.

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