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Suitability of raw and heat-treated *Amaranthus spinosus* in broiler diets: Impact on performance, meat antioxidant capacity, and intestinal health

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ABSTRACT

We assessed the potential of *A. spinosus* as a feed ingredient for broiler chickens and evaluated the efficacy of heat treatment. A total of 210 male Ross 308 broiler chicks were randomly assigned to five dietary treatments, with six replicates and seven birds per replicate. The control group received a standard mix of maize and soybean meal, and the other groups received diets with varying proportions of raw and heat-treated amaranth, in which up to 10% and 20%, respectively, of the corn and soybean were replaced. Incorporating up to 20% *A. spinosus* into broiler diets did not have any adverse effects on animal health. We found no significant differences in body weight gain or ultimate body weight between amaranth-fed and control diet-fed birds. However, amaranth addition led to increased feed intake and therefore reduced feed efficiency. Amaranth feeding had no impact on carcass yield or organ weights but the amaranth-fed groups had heavier gizzards, reduced abdominal fat, improved meat antioxidant status, and no adverse effects on blood biochemical or hematological indices. Amaranth grain had no effect on cholesterol levels but decreased triglyceride levels. Heat treatment improved the favorable effects of amaranth on intestinal histology and increased immunoglobulin G levels. The caecal volatile fatty acid profile did not significantly change with amaranth incorporation, and heat-treated amaranth increased caecal branched-chain fatty acid levels and valerate

levels in broilers compared to those in raw plants. The data indicate that *A. spinosus* holds promise as an alternative to traditional grains; digestibility trials are necessary to clarify procedures for further use.

Keywords: *Amaranthus spinosus*; broiler performance; antioxidant potential; intestinal histomorphology; hemato-biochemical characteristics

1. Introduction

Cereals, legume seeds, and their byproducts make significant contributions as raw feed materials to all livestock enterprises, particularly poultry production. Not only are they important sources of energy and protein, but they are also valuable in providing elements with biological activity. However, the increasing demand for animal protein, combined with a scarcity of livestock feed resources and associated high feed costs, necessitates the investigation of alternatives that can provide at least the same nutrition as conventional feed materials. Natural remedies for sustainable agriculture have therefore brought renewed interest to useful but often neglected crops (Borelli *et al.*, 2020).

One such crop is amaranth (*Amaranthus spp.*), a pseudocereal widely grown in the tropics that has promising economic value due to its ability to withstand a wide range of climatic and agronomic conditions as well as its unique nutritional, functional, and technological capacities (Manyelo *et al.*, 2020). The Amaranth genus includes approximately 70 species of annual plants, some of which were domesticated primarily for edible grain (*A. cruentus*, *A. hypochondriacus*, *A. caudatus*), while some (*A. dubius*, *A. blitum* and *A. tricolor*) were cultivated for green vegetables (Das, 2012, Aderibigbe *et al.*, 2022); others are currently considered weeds until their potential was fully realized and more suitable use was discovered. From a nutritional standpoint, grain amaranth is a good source of lipids (linoleic and oleic acids), quality proteins, largely albumins with balanced amino acid composition (lysine and sulfur-containing amino acids), minerals (calcium, phosphorus, potassium, magnesium, iron) and vitamins (B-group, vitamins A, E, and C), when compared to conventional cereals. Additionally, amaranth plants are unique in terms of their health-beneficial phytochemicals, which include various phytosterols and tocopherols, as well as high levels of squalene (Gamel *et al.*, 2006, Baraniak and Kania-Dobrowolska, 2022).

In addition, the literature indicates that raw amaranth seeds do not naturally exhibit the protein quality suggested by their amino acid composition. The presence of various antinutrients in amaranth reduces the availability of essential amino acids, and it also interferes with the metabolic processes of other nutrients (Manyelo *et al.*, 2020). This is one of the primary reasons why livestock producers reject unconventional feed materials. To mitigate antinutrient effects and successfully incorporate amaranth into animal feeding programs while adhering to health recommendations, a variety of techniques have been proposed. Due to the heat-lability of most antinutrients, thermal processing techniques (roasting, flaking, popping, wet cooking, autoclaving, or extruding) are most effective at improving the nutritional value of feeds. In an experiment in which broilers were fed grain amaranth, heat treatment improved the metabolizable energy content of amaranth by 573 kcal/kg compared to that in untreated broilers; additionally, processed amaranth contains twice the crude protein and metabolizable energy value of corn grain (Janmohammadi *et al.*, 2022), thus making it a superior feed material with the potential to uphold performance targets in tandem with health, welfare, and economic aspects of animal production.

Previously, in various metabolic and growth trials, grain amaranth was studied extensively as a feed ingredient for poultry. In recent years, concerted efforts have been made to further explore the incorporation of grain amaranth into avian diets as a strategy to replace traditional feed resources. A review of the literature revealed maintenance or improvement in performance indices (Popiela *et al.*, 2013, Hosseintabar-Ghasemabad *et al.*, 2022), hypocholesterolemic effects (Alizadeh-Ghamsari *et al.*, 2021), improved antioxidant status and atherogenic indices (Hosseintabar-Ghasemabad *et al.*, 2022), improved animal health and improved production of healthier products (Popiela *et al.*, 2013, Janmohammadi *et al.*, 2023) when amaranth was incorporated into poultry diets, either raw or processed, or with other additives. Furthermore, when different amaranth species were evaluated, records revealed significant variability in terms of essential nutrients (Szabóová *et al.*, 2020), phytochemical and antinutrient levels (Mlakar *et al.*, 2009), animal feed consumption and weight gain (Waldroup *et al.*, 1985, Pond *et al.*, 1991, Ravindran *et al.*, 1996). Research has also shown a greater hypocholesterolemic effect in chickens fed whole grain amaranth than in those fed popped or milled amaranth (Qureshi *et al.*, 1996). Thus, when formulating animal rations with amaranth grains, species- and milling-related differences in feed value should also be considered.

To date, animal studies have focused primarily on grain-type amaranth, specifically *A. cruentus* and *A. hypochondriacus*. There is hardly any information on the use of weed amaranths in poultry rations. *A. spinosus*,

commonly known as spiny amaranth or pigweed, was found to exhibit significant immunomodulatory, anti-inflammatory, antimicrobial, and antioxidant activities (Adegbola *et al.*, 2020). Therefore, the present study was initiated to shed light on the potential of using *A. spinosus* as a whole grain in broiler rations at a 10% or 20% level. This study aimed to investigate the nutritional potential of *A. spinosus* by determining the productive responses of the animals as well as their intestinal histomorphology, meat antioxidant capacity, and selected blood indices. The secondary objective was to gauge the effectiveness of heat treatment in modifying the effects of *A. spinosus* as a feed ingredient for broiler chickens.

2. Materials and methods

All animal care and use procedures were approved by The Animal Ethics Committee of Ankara University.

2.1. Procurement and heat treatment of amaranth (*A. spinosus*) grains

The amaranth seeds were procured from a local distributor, Yayla Agro Inc., which imported them from India. After species identification and confirmation with the Landscape Architecture Department, Faculty of Agriculture, Ankara University, the seeds were heat-treated to reduce the negative effects of antinutrients. A twin screw extruder (DG-85, Jinan, China) without a die was used to achieve controlled and uniform cooking. Following a few exploratory first experiments, the barrel temperature and seed flow rate were standardized to produce a final product with a temperature of 70–75°C. The seeds were subsequently introduced to a heated barrel with a temperature profile of 106°C, 122°C, and 180°C during treatment. The device was run at the slowest possible speed (20/50 Hz motor drive), and it took approximately 25-30 seconds for the seeds to travel from the entrance to the exit point. Heat-treated amaranth seeds were instantly spread out and cooled by convection before being properly packed. No popping was observed in the seeds, but they did have a slightly roasted color.

2.2. Birds, Diets, and Experimental Design

A total of 210, day-old male Ross 308, broiler chicks were obtained from a commercial hatchery (BeyPi Inc., Ankara, Turkey) and were randomly allocated to five experimental groups of six replicates each and 7 birds/replicate. Chicks were spray-vaccinated in the hatchery before arrival. The control group was fed a standard mix of maize and soybean meal (Table 1), which was prepared following Aviagen (2009) recommendations. The four other diets were obtained by substituting raw (**RW**) and heat-treated (**HT**) amaranth for portions of corn and

soybean up to 10 and 20% of the diet (RW₁₀, RW₂₀, HT₁₀, and HT₂₀). All the diets were isonitrogenous and isocaloric. Proximate analysis of amaranth seeds and experimental diets was performed according to the methods described in AOAC (2000).

In all feeding phases (starter, 1–10 days; grower, 11–24 days; and finisher, 25–39 days), the birds were given *ad libitum* access to feed (mash form) and water. Each pen was equipped with a plastic feeder and automatic nipple drinkers. The birds were reared in an environmentally controlled house at an initial temperature of 33°C, which gradually decreased to 22°C over a 3-week period; thereafter, by thermostatically controlled. Each pen (90 cm length×80 cm width×80 cm height) had wood shavings as litter. The relative humidity of the house during the experiment was 50±5%. The house was artificially ventilated, and the lighting schedule was 24L:0D for the first three days, reduced further to 23L:1D from d 4-7, and then kept constant at 20L:4D thereafter.

2.3. Data collection and sampling

On days 1, 10, 24, and 39 of the experiment, all the birds were individually weighed, and feed intake (**FI**) was recorded. Mortalities were monitored daily. Body weight gain (**BWG**) and the feed conversion ratio (**FCR**) were subsequently calculated to evaluate growth performance.

At the end of the experiment (d 39), based on the group average weight, two birds from each replicate were randomly selected, and blood samples were obtained from the plain (blood serum antioxidant status and biochemical analysis) and EDTA-containing tubes (hematological parameters). Then, following slaughtering and proper evisceration, hot carcass weights were recorded, and carcass yield (relative to pre-slaughter live weight) was determined. Afterward, the weights of different organs (liver, heart, empty gizzard, spleen and bursa Fabricius) and abdominal fat were recorded and are expressed relative to the pre-slaughter live weight. The pH of the fresh breast meat samples was determined within 15 minutes after slaughter using a pH meter (Mettler Toledo, SevenGo™, pH meter SG2, puncture pH electrode LE427). Then, the breast meat was further excised, and samples were taken to determine the antioxidant status of the meat.

Furthermore, intestinal tissue samples (middle section, 1 cm in length) were obtained from the duodenum, jejunum, and ileum for histomorphological analysis. Additionally, cecum contents from each slaughtered bird were collected in suitable containers and stored at –18°C for VFA analysis.

2.4. Analyses of Blood Samples

Blood serum was removed from plain tubes using centrifugation ($1,690 \times g$ for 10 min at 4°C ; SL 16R centrifuge, Thermo Scientific, Osterode am Harz, Germany), and total antioxidant status (**TAS**), total oxidant status (**TOS**), and oxidative stress index (**OSI**) analyses were performed as described elsewhere (Ramay and Yalcin, 2020). Blood serum samples were also analyzed for total protein (**TP**), albumin (**ALB**), cholesterol (**CHL**), and triglyceride (TG) levels with an autoanalyzer (BT 3000, Biotechnica Instruments) using commercial kits from the Randox RX series (Randox Laboratories). The immunoglobulin IgG concentration was analyzed using an ELISA kit (Randox, IG3896) according to the manufacturer's protocol. The hematological parameters (red blood cells, **RBCs**; hematocrit, **HTC**; hemoglobin, **Hb**; white blood cells, **WBC**; platelets, **PLT**; mean corpuscular hemoglobin, **MCH**; mean corpuscular hemoglobin concentration, **MCHC**; mean corpuscular volume, **MCV**; red cell distribution width, **RDW**) were evaluated within two hours after blood sampling.

2.5. Breast Meat Antioxidant Status

The antioxidant status of the broiler breast meat was evaluated in terms of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (**DPPH**), TAS, TOS, and OSI (Ramay and Yalcin, 2020). Furthermore, catalase (**CAT**) and myeloperoxidase (**MPO**) enzyme activities were measured using commercially available kits (Rel Assay Diagnostic Kits) according to the manufacturer's protocol.

2.6. Histomorphological Measurements of the Intestinal Segments

The tissue samples were fixed in buffered formalin, dehydrated in a series of ethanol solutions, cleared with xylol, and finally embedded in paraffin wax for microscopic examination. Later, the intestinal segments were cut into sections measuring $5 \mu\text{m}$ in thickness and a total of 10 well-oriented villus-crypt units were randomly selected for histological measurements (Sakamoto et al., 2000).

2.7. Volatile Fatty Acid (VFA) Analysis of Cecum Contents

The cecum VFA profile was determined using a gas chromatograph (Shimadzu GC-2010, Shimadzu) coupled with a TRB-FFAP column (Teknokroma, $30\text{m} \times 0.53\text{mm}$ internal diameter) and flame ionization detector (Yalcin et al., 2023).

2.8. Statistical Analysis

The statistical analysis was conducted using SPSS 23.0 (SPSS Inc., Chicago, IL, USA). The normality of the data was evaluated using the Kolmogorov-Smirnov test. The means of the data were subjected to one-way ANOVA and significant means were separated using the Tukey test. Polynomial contrasts were also used to determine the linear and quadratic effects of amaranth incorporation into broiler diets. Moreover, the data were analyzed as a 2×2 factorial arrangement (excluding the control group), with the amaranth level, heat treatment, and their interactions as the main effects. The level of significance was set at $P \leq 0.05$.

3. Results

The chemical composition of the raw and heat-treated amaranth grain (*A. spinosus*) are presented in Table 2, indicating that there were no significant differences between the two plant types.

3.1. Performance traits of broiler chickens

Throughout the trial, all the birds showed good health signs, and no deaths occurred in either the control or amaranth-incorporated groups. The study's findings on performance traits are summarized in Table 3. The results demonstrated that the FI of all the amaranth-fed groups was comparable to that of the control group ($P > 0.05$) up to day 24 of the trial (starter and grower stages). However, during the finishing period (25-39d), broiler chickens fed 20% amaranth in their diets (either raw or heat-treated) consumed significantly more feed than did the control ($P = 0.002$). These variations were also reflected in the total FI (0-39d), with large differences ($P = 0.002$) between the control group and the 20% amaranth group. The polynomial contrast data showed that the inclusion of amaranth in broiler diets, both raw and heat-treated, also had a significant linear influence ($P < 0.05$) on total FI, particularly during the finishing phase. When the main effect of amaranth inclusion was compared, the feed consumption of the birds increased significantly ($P < 0.05$) when the amaranth concentration was raised in the diet from 10% to 20%, and these differences prevailed throughout almost the whole experiment.

The findings of the influence of amaranth inclusion on BWG showed that during the starter phase, BWG was significantly lower in the RW₂₀ group ($P = 0.029$) than in the control group. For the same period, the data also revealed a significant linear decrease in BWG as amaranth levels increased, whether raw or heat-treated ($P = 0.009$ and 0.006 , respectively). No significant differences in BWG were detected in subsequent stages or as a whole (0-39d) in control or amaranth-incorporated (either raw or heat-treated) groups ($P > 0.05$). Increasing the level of amaranth in broiler diets from 10 to 20% had no significant effect on BWG ($P > 0.05$).

The results for feed efficiency also varied considerably, with birds fed 20% amaranth (raw or heat-treated) having a poorer FCR than control birds during the starter and finishing periods ($P = 0.006$ and $P < 0.001$, respectively). For the entire trial (0-39d), birds fed control diets had better FCRs than did those in the amaranth-incorporated groups ($P < 0.001$). In addition, a significant linear trend in FCR degradation was observed with the introduction of amaranth to the broiler diets, both raw and heat-treated ($P < 0.05$). Furthermore, for both the starter and finisher periods as well as for the entire trial, an increase in amaranth level from 10 to 20% led to inferior FCR values.

Heat treatment of *A. spinosus* did not modify the performance indices of birds compared to those of raw amaranth-fed birds ($P > 0.05$).

3.2. Carcass yield and relative organ weights

Table 4 presents the data on key carcass features investigated in the study. The carcass yields from all the amaranth-incorporated groups were comparable to those of the control group fed a maize-soybean diet ($P > 0.05$). In addition, when increasing amounts of amaranth seeds (raw or heat-treated) were introduced to broiler diets, the data also showed a linear increase in carcass yield ($P = 0.050$ and 0.013 , respectively).

There were no significant differences ($P > 0.05$) among the control and amaranth-incorporated groups regarding relative organ weights (liver, heart, spleen, bursa of Fabricius) monitored on d 42. The gizzard was the lone exception, exhibiting linear increases in response to the addition of amaranth, both raw and heat-treated ($P < 0.001$). Increasing dietary amaranth levels from 10% to 20% had no significant influence on carcass yield or organ weights ($P > 0.05$), except in the gizzard, where it had an enhancing effect ($P < 0.001$). The data also indicated a significant interaction effect of level $\times$ heat treatment on gizzard weight ($P = 0.038$). Furthermore, heat-treated amaranth was found to reduce abdominal fat linearly ($P = 0.032$).

3.3. Meat pH and birds' antioxidant status

Changes in the meat pH and antioxidant status of birds fed amaranth and control diets are summarized in Table 5. The addition of raw amaranth to broiler diets resulted in a linear increase in the pH of the meat ($P = 0.031$). A similar trend was observed in the heat-treated group, but the difference was not statistically significant ($P = 0.068$). Compared to the control diet, the addition of amaranth to the broiler diet, whether raw or heat-treated, had a negative quadratic effect ($P < 0.05$) on the meat antioxidant status (TAS). The results also revealed a significant decrease in the antioxidant status of the meat when the amaranth concentration increased from 10 to 20% ($P < 0.001$). Moreover, adding raw amaranth to broiler diets linearly decreased the oxidation status (TOS) of the meat ($P = 0.016$), and when compared to the control, it also decreased the overall oxidative stress index (OSI) of the meat ($P = 0.008$). Heat treatment and amaranth levels tested had no significant effect on MPO, CAT, and DPPH levels in the meat ($P > 0.05$).

Furthermore, a linear decrease in blood serum TAS levels was observed when amaranth (raw or heat-treated) was incorporated into the broiler diet ($P < 0.05$). Additionally, increasing amaranth to 20% of the diet decreased blood TAS ($P < 0.001$) and increased OSI ($P = 0.013$) when compared to those fed 10% amaranth. The data also indicated a significant interaction effect of level $\times$ heat treatment on the blood OSI ($P = 0.05$).

3.4. Blood biochemical and hematological results

The levels of TP, ALB, and CHL in the blood serum were similar between the amaranth-fed groups and the control group (Table 6). A linear decrease in the serum TG concentration was observed when amaranth (raw or heat-treated) was incorporated into the broiler diet ($P < 0.05$). The HT₂₀ group presented with significantly lower serum TG levels than did the control group ($P = 0.001$). Data also showed lower serum TGs values for 20% amaranth level than 10% ($P < 0.001$). Furthermore, the addition of heat-treated amaranth to broiler diets resulted in a linear increase in the serum IgG concentration ($P = 0.001$). The main effects exhibited a significant positive effect of heat treatment on the serum IgG concentration ($P = 0.012$).

Amaranth incorporation into broiler diets had no significant effect ($P > 0.05$) on blood RBC, HTC, Hb, WBC, or PLT values (Table 7). Heat-treated amaranth significantly increased the MCH values ($P = 0.020$). Moreover, the MCHC linearly decreased when raw amaranth was used ($P = 0.022$). The raw and heat-treated amaranth groups

presented linear increases in the MCV and RDW ($P < 0.05$). Furthermore, heat treatment significantly increased the RDW ($P = 0.007$), while a significant interaction effect of level $\times$ heat treatment was noted for MCV ($P = 0.007$).

3.5. Intestinal histomorphology

The effects of amaranth incorporation into broiler diets on the histomorphological features of different intestinal sections are shown in Table 8 and Figure 1. The histomorphological changes (VH, VW, CD, VH:CD, and villus area) observed in the small intestine segments of the group fed amaranth were comparable to those in the control group. Furthermore, the VH of tissue sections from the duodenum and ileum increased linearly in the heat-treated amaranth groups ($P = 0.034$ and 0.050 , respectively). VH in the duodenum increased in response to both the amaranth level and heat treatment ($P = 0.024$ and 0.010 , respectively). Raw amaranth linearly decreased the VW in the ileum ($P = 0.005$). In contrast to a negative effect on the amaranth level ($P = 0.035$), heat treatment positively influenced the VW in the ileum ($P = 0.006$). A significant interaction effect of level $\times$ heat treatment was noted for VW in the ileum ($P < 0.001$). Furthermore, a shallower ileal CD was observed in the heat treatment group than in the untreated group ($P = 0.006$).

The inclusion of raw amaranth in broiler diets linearly decreased the VH:CD ratio in tissue sections from the jejunum and ileum ($P = 0.037$ and 0.016 , respectively). However, treatment with heat significantly improved the VH:CD ratio in the duodenum and ileum ($P = 0.020$ and $P < 0.001$, respectively). The data also showed a linear decrease in the villus area in the ileum with increasing levels of raw amaranth in birds' diets ($P = 0.007$). Increasing amaranth to 20% of the diet increased the villus area in the duodenum ($P = 0.048$) compared to that in the group fed 10% amaranth. Compared to those in the untreated group, the villus area in the duodenum and ileum was greater in the heat-treated amaranth group ($P = 0.017$ and 0.003 , respectively). A significant interaction effect of level $\times$ heat treatment was noted for the villus area in the ileum ($P < 0.001$).

3.6. Cecal VFA profile

Changes in the cecal VFA profiles of the birds are summarized in Table 9. There were no significant differences ($P > 0.05$) among the control and amaranth-incorporated groups regarding acetic, propionic, or butyric acid percentages. The inclusion of raw amaranth in broiler diets linearly decreased ($P = 0.005$) the isobutyric percentage; lower isobutyric acid was present in RW₂₀ broilers than in the control ($P = 0.030$). Compared to the control, the

addition of amaranth (raw or heat-treated) affected the cecal isovaleric percentage in a positive ($P = 0.001$) or negative ($P = 0.028$) quadratic fashion, respectively. The branched-chain fatty acid (BCFA) concentration decreased linearly with amaranth inclusion, whether raw or heat treated ($P = 0.004$ and 0.013 , respectively). The raw and heat-treated amaranth groups also presented linear decreases in the content of valeric acid ($P < 0.001$ and $P = 0.006$, respectively). Heat-treated amaranth linearly decreased the total VFA content in the birds' cecum ($P = 0.037$). Compared to those in the untreated groups, heat treatment of amaranth significantly increased the concentrations of isobutyric acid, isovaleric acid, and consequently BCFA ($P < 0.05$). A significant interaction effect of level $\times$ heat treatment was observed for isovaleric and valeric fatty acids ($P = 0.003$ and 0.006 , respectively).

4. Discussion

The inclusion of amaranth (*Amaranthus spp.*) in animal diets, whether as grain or leaf meal, has been shown to provide multiple advantages for animal health and performance. Nevertheless, there are various elements in amaranth that can impair its nutritive value and hinder growth in poultry. The current investigation aimed to determine the efficacy of heat treatment in modifying the impact of amaranth as a feed component for broiler chickens. While prior studies have focused predominantly on the use of grain-type amaranth, the present study sought to evaluate the potential of utilizing *A. spinosus* (pigweed) in broiler rations either in its raw form or after heat treatment.

The study revealed that the performance indices (FI, BWG, FCR) of birds fed heat-treated amaranth were similar to those of birds fed raw amaranth. Furthermore, compared to the control diet, the addition of amaranth to broiler diets had a negative impact on overall FCR. When the two levels of amaranth incorporation were evaluated, 20% of the dietary intake performed poorly compared to 10%. The deterioration in FCR was most likely caused by greater FI in birds fed amaranth rather than poor growth, given that no major changes were observed in BWG or final body weight (data not shown). The present study assumed the metabolizable energy (ME) of *A. spinosus* to be approximately 3200 kcal/kg, which was derived from previous research (Tillman and Waldroup, 1988a, Janmohammadi *et al.*, 2022). However, the ME for *A. spinosus* appeared to be overestimated. Thus, the amaranth inclusion levels in the study may not have been high enough to severely impair the birds' growth response, but they may have been insufficient to meet the birds' nutrient requirements, particularly in terms of energy. As a result, the birds may have increased their FI to compensate for the deficiency, which could have led to a poor FCR. To address

this issue, further digestibility studies are needed to explore species-specific variations in the metabolizable energy and nitrogen efficiency of amaranth for broilers. Additionally, although the inclusion of whole grains did not limit feed intake in the study, it is possible that this inclusion may also have impacted nutrient utilization, resulting in insufficient energy intake. However, this hypothesis requires further investigation.

According to research by Orczewska-Dudek *et al.* (2018), feeding broilers with a diet containing 8% raw amaranth led to reduced FI and BWG but did not significantly alter the FCR when compared to a maize soybean-based diet. Alizadeh-Ghamsari *et al.* (2021) reported that when broilers were fed amaranth (*A. hybridus*) supplemented with up to 6% of the pelleted diet, there was no effect on FI, but there was a linear decline in BWG and FCR. Additionally, other studies have reported that feeding broilers amaranth up to 8% of their diet, whether raw or heat-treated, had no effect on final body weight, FI, or FCR (Roučková *et al.*, 2004, Pisarikova *et al.*, 2006). Experiments with laying hens revealed that, compared with raw amaranth, autoclaved amaranth (*A. hybridus chlorostachys*) produced better outcomes in terms of FI and FCR (Hosseintabar-Ghasemabad *et al.*, 2022, Janmohammadi *et al.*, 2023). The discrepancies among studies could be attributed to several factors, particularly amaranth variety, processing methods, inclusion levels, and bird species.

The current findings on carcass yield were consistent with previous research showing no significant difference in carcass yield between control groups and broilers fed either raw amaranth (Orczewska-Dudek *et al.*, 2018) or raw and popped amaranth (Pisarikova *et al.*, 2006) at an 8% dietary inclusion level. Feeding broilers a diet that contained 40% extruded amaranth (*A. cruentus*) also had no effect on dressing percentage (Tillman and Waldrup, 1988b). However, Alizadeh-Ghamsari *et al.* (2021) reported that the inclusion of 6% amaranth (*A. hybridus*) in a pelleted diet significantly decreased the carcass yield of broilers, while 2% and 4% amaranth inclusion did not significantly differ from the control. According to Roučková *et al.* (2004), a 7% dietary inclusion level of raw amaranth reduced carcass yield, whereas heat-treated amaranth had no significant effect when compared to the control. Additionally, the present study revealed no difference between the effects of raw and heat-treated amaranth. Interestingly, the current study revealed a linear increase in carcass yield with increasing dietary level of both raw and heat-treated amaranth, although the main effects of amaranth level and heat treatment were not significant. Orczewska-Dudek *et al.* (2018) reported an increase in breast muscle percentage with amaranth feeding; however, this needs to be investigated further.

Like in the present study, Alizadeh-Ghamsari *et al.* (2021) reported no significant differences in the relative weights of the heart, spleen, or bursa of Fabricius in broilers fed amaranth (*A. hybridus*) as part of a pelleted diet. Similarly, Pisarikova *et al.* (2006) reported no effect on liver development in broilers fed raw or heat-treated amaranth. However, some studies have reported negative effects on liver weight with the inclusion of 6% amaranth (*A. hybridus*) (Alizadeh-Ghamsari *et al.*, 2021), while others have shown a positive response with the inclusion of 8% raw amaranth in broiler diets (Orczewska-Dudek *et al.*, 2018). The inclusion of amaranth in broiler diets has also been shown to reduce abdominal fat (Orczewska-Dudek *et al.*, 2018, Alizadeh-Ghamsari *et al.*, 2021), which is partly in agreement with the findings of the present study, as heat-treated amaranth was found to reduce abdominal fat in a linear pattern. However, Pisarikova *et al.* (2006) reported no difference in abdominal fat between control, raw and popped amaranth-fed broilers. Although statistically insignificant, the decrease in abdominal fat with amaranth incorporation, especially at the 20% level, may also reflect an overestimation of the ME value of amaranth in the present study.

Another significant aspect of amaranth inclusion (whether raw or heat-treated) in broiler diets was the notable increase in gizzard weight with increasing amaranth level. Although heat treatment of amaranth did not affect gizzard development, raw amaranth resulted in a more substantial increase in gizzard weight than heat-treated amaranth (15% versus 5%) when the weight was raised from 10 to 20% of the diet. Alizadeh-Ghamsari *et al.* (2021) reported no effect on gizzard weight when broilers were fed a pelleted diet containing 6% amaranth (*A. hybridus*). The gizzard is an important component of avian digestion, and it tends to increase in size to accommodate fiber in the diet. Thus, the increased gizzard weight observed in this study could be related to the increased dietary fiber content associated with amaranth. Moreover, heat treatment of amaranth grain may have enhanced its digestive efficiency. A high lignin content in the amaranth cortex was found to be negatively correlated with energy and digestibility, and popping or toasting *Amaranthus caudatus* increased both soluble fiber concentration and fiber digestibility (Pedersen *et al.*, 1990).

Muscle acidity has a significant impact on meat quality, where a decrease in pH can negatively affect certain attributes, particularly the ability of meat to hold water. The current study revealed an increase in breast meat pH with the addition of amaranth to broiler diets. These findings align with a previous study that also reported an increase in the pH of both breast and thigh meat when *Amaranthus cruentus* leaf meal was added to broiler diets

(Manyelo *et al.*, 2022). In contrast, one study showed that adding amaranth grain (8%) to broiler diets had no effect on the pH of breast muscle (Orczewska-Dudek *et al.*, 2018). Similarly, when broiler chickens were fed *Amaranthus caudatus* grain at concentrations of 0, 5, or 10% of their diet, there were no changes in the pH of their breast meat (Longato *et al.*, 2017). The quantities of bioactive chemicals in amaranth, which can be impacted by both heat treatment and amaranth, may be responsible for changes in meat pH.

Meat quality and the body's antioxidant status are also closely linked. Several studies have indicated that incorporating amaranth into the diet of birds can enhance their antioxidant capacity. Longato *et al.* (2017) reported a significant decrease in the serum lipid peroxidation level when broiler chickens were fed *Amaranthus caudatus* grain up to 10% of their diet. Similarly, Orczewska-Dudek *et al.* (2018) demonstrated the strong antioxidant effect of amaranth supplementation on broilers fed diets enriched with polyunsaturated fats. However, Janmohammadi *et al.* (2023) reported no change in blood antioxidant status (MDA, total antioxidant capacity) in laying hens fed raw *Amaranthus hybridus chlorostachys* (up to 40% of the diet). On the other hand, Hosseintabar-Ghasemabad *et al.* (2022) reported that feeding laying hens processed with (autoclaved) *Amaranthus hybridus chlorostachys* (up to 40% of the diet) improved their total antioxidant capacity but did not affect MDA levels in the blood. The findings of the present study indicated that heat treatment had no adverse effect on the antioxidant activity of amaranth. Additionally, when compared to the control diet, the inclusion of amaranth in broiler diets was found to enhance the antioxidant status of meat; however, the improvement was not proportional to the increase in amaranth level. The study also revealed a similar trend, although not statistically significant, for other oxidation-related parameters (MPO, CAT, and DPPH), indicating better results with 10% amaranth than with 20% amaranth. Furthermore, the TAS and OSI values of blood serum indicated better outcomes with 10% amaranth inclusion in broiler diets. Amaranth seeds contain various phytochemicals, including polyphenols, tocopherols, squalene, and proteins or peptides with antioxidant activity, which contribute to their high antioxidant potential (Tironi and Añón, 2010). However, a higher inclusion of amaranth in broiler diets may lead to inefficient digestion, thus, decreasing the potential release of antioxidant compounds in the animal body. The use of exogenous enzymes in combination with heat treatment may be beneficial for improving the digestion process and, subsequently the antioxidant activity of amaranth grains when fed to broilers.

Blood biochemical analysis is useful for linking animal diets to their internal environment. The incorporation of amaranth into poultry diets has been shown to have a hypocholesterolemic effect in various studies (Longato *et al.*, 2017, Alizadeh-Ghamsari *et al.*, 2021, Hosseintabar-Ghasemabad *et al.*, 2022, Janmohammadi *et al.*, 2023). Interestingly, the current study revealed no impact on cholesterol levels or protein or albumin concentrations in blood when amaranth was added to broiler diets, whether raw or heat-treated. Similarly, Popiela *et al.* (2013) reported no cholesterol-lowering effect or changes in total protein or albumin levels when extruded amaranth grains were added to the diet of laying hens. Studies conducted on broiler chickens have also shown that adding amaranth to their diet does not significantly impact cholesterol (Roučková *et al.*, 2004, Orczewska-Dudek *et al.*, 2018), total protein (Roučková *et al.*, 2004, Alizadeh-Ghamsari *et al.*, 2021) or albumin levels (Longato *et al.*, 2017) in the blood. Furthermore, the present study revealed reduced blood triglycerides following the incorporation of amaranth (either raw or heat-treated) into broiler diets. This could suggest a low energy intake by birds fed amaranth in their diets, and this effect was more pronounced at higher inclusion levels. Other studies also reported decreased blood triglycerides (Longato *et al.*, 2017, Orczewska-Dudek *et al.*, 2018), but some reported no significant changes (Popiela *et al.*, 2013, Alizadeh-Ghamsari *et al.*, 2021, Janmohammadi *et al.*, 2023). Amaranth contains several bioactive compounds, such as squalene, phytosterols, and tocopherols, which contribute to its hypocholesterolemic and antioxidant effects. The composition of fatty acids in the diet may also play a role in these effects (Janmohammadi *et al.*, 2023). These inconsistent results could be due to differences in amaranth species, supplementation levels, processing methods, diet compositions, and animal types. Albumin and globulin are key blood protein markers. Amaranth feeding had no adverse impact on protein metabolism in birds, as demonstrated by unaltered blood levels of total protein and albumin. Furthermore, this study revealed that amaranth feeding did not impair bird immunity, as measured by IgG. In contrast, the NPQ significantly improved when heat-treated amaranth was added to the broiler diet.

MCV and RDW were greater in the amaranth-fed group (either raw or heat-treated) than in the control group, although they were still within the normal range. In the case of raw amaranth, a decrease in MCHC was observed, while heat-treated amaranth improved MCH, which may suggest improved iron availability in processed amaranth. Popiela *et al.* (2013), however, reported a decrease in blood iron concentration in laying hens when extruded

amaranth was increased from 0 to 10% in their diets. More research is needed to understand the effects of processing and amaranth supplementation on hematological indices in broiler chickens.

The small intestine plays a crucial role in the digestion of feed and absorption of nutrients, making it an essential organ in the body. By evaluating the mucosal structure of the small intestine, it is possible to estimate the animal's gut health, digestive capacity, and growth potential. The present study revealed that the incorporation of amaranth into broiler diets, whether raw or heat-treated, had similar impacts on the morphology of the duodenum, jejunum, and ileum as that in the control group. Additionally, the heat-treated amaranth plants had greater benefits than the raw amaranth plants since heat treatment improved the VH:CD ratio and villus surface area in the duodenum and ileum segments. The jejunum was the least affected segment by the addition of amaranth to the broiler diet, suggesting that amaranth feeding had no or little effect on the absorptive capacity of the jejunum. Furthermore, increasing the level of amaranth had a positive impact on the villus surface area of the duodenum, which could be attributed to an increase in villus height. On the other hand, incorporating amaranth at a higher level (20%) negatively affected the villus width in the ileum compared to 10%, although it had no significant effect on the surface area of the villi. Research examining the effects of amaranth feeding on intestinal morphology is limited. In one previous study involving broilers, Alizadeh-Ghamsari *et al.* (2021) reported results similar to those of the present study, indicating that amaranth (*A. hybridus*) feeding had no adverse impact on the histomorphological characteristics of the small intestine compared to those of the control group. The changes in intestinal morphology observed in the present study following amaranth feeding, whether raw or heat-treated, and at different levels may be associated with changes in the activity of digestive enzymes and modulation of the cecal microbiota. However, further extensive research is needed to better comprehend the influence of amaranth feeding on the health of the intestine.

VFAs play an essential role by contributing to host nutrition and promoting gut health. The main byproducts of carbohydrate fermentation are typically acetic, propionic, and butyric acids, whereas isobutyric and isovaleric acids have been proposed as markers of protein degradation (Elling-Staats *et al.*, 2022). The present study revealed no significant changes in the levels of major VFAs (acetic, propionic, or butyric) in broilers fed diets supplemented with amaranth compared to those in the control group. However, feeding heat-treated amaranth resulted in increased

branched-chain fatty acids and valerate in the ceca of broilers, which may suggest a negative effect of heat on the protein quality of the grain. Despite these changes, the overall concentration of VFAs remained similar across all groups.

5. Conclusions

The substitution of *A. spinosus* for conventional grains in broiler diets revealed that while amaranth had no detrimental impact on body weight gain or the ultimate body weight of the birds, its presence in the diet had a negative influence on total feed efficiency, owing to greater feed intake. Feeding amaranth to broiler chicks had no effect on carcass yield or organ weight, except for the gizzard, which was heavier in the amaranth-fed groups. Furthermore, amaranth feeding reduced abdominal fat content and demonstrated the ability to withstand changes in meat pH while improving meat antioxidant status. The addition of amaranth to broiler diets had no adverse effects on blood biochemical or hematological indices. Although heat treatment of amaranth did not significantly affect the performance of broiler chickens, it improved the beneficial effects of amaranth feeding on the histological features of the duodenum and ileum and strengthened immunity by boosting immunoglobulin G levels. Overall, the findings of this study contribute to the growing body of knowledge on utilizing sustainable feed sources, such as amaranth, for poultry production, ultimately promoting more sustainable and environmentally conscious agricultural practices. More research, including different processing methods and enzyme supplementation methods, is needed to determine the impact of various amaranth species on poultry nutrition.

Declaration of competing interest

All the authors declare that they have no conflicts of interest.

Submission declaration

This work is Dr. Emre Sunay Gebes's PhD thesis and has not been published elsewhere. This work is not under consideration for publication elsewhere, is approved by all the authors and is tacitly or explicitly approved by the responsible authorities where the work was carried out, and if accepted, will not be published elsewhere in the same

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Author's contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Emre Sunay Gebes, Sakine Yalçın, Muhammad Shazaib Ramay, Akın Ünal. Intestine samples were evaluated by Kürşat Filikçi. The first draft of the manuscript was written by Emre Sunay Gebes and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

The data generated and analyzed during the current study are not publicly available due to privacy reasons. However, upon reasonable request, the data can be made available by contacting Dr. Emre Sunay GEBES at esunayg@gmail.com. We are committed to facilitating scientific reproducibility and transparency and will provide access to the data in accordance with applicable privacy and confidentiality regulations.

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