

The growth performance and some carcass, gastrointestinal tract, meat quality, and blood biochemical properties of broiler chickens fed diets supplemented or not with tropical açai (*Euterpe oleracea*) fruit extract

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Research Article

Keywords: Anthocyanin, polyphenol, growth performance, intestinal growth, meat quality, blood biochemical

Posted Date: April 28th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6402004/v1>

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Version of Record: A version of this preprint was published at Tropical Animal Health and Production on July 7th, 2025. See the published version at <https://doi.org/10.1007/s11250-025-04545-x>.

Abstract

This study investigated the effects of lyophilised ethanolic açai (*Euterpe oleracea*) fruit extract (AFE) as a growth promoter on the carcass, gastrointestinal tract, meat quality, and blood biochemical properties in broilers. In total, 576 mixed-sex Ross 308 broilers were randomly assigned to four treatments, with six replications of 24 birds (12 females and 12 males) each. Control chickens were fed with an additive-free basic diet (AFE0). In contrast, test birds were fed one of the basic diets supplemented with 2.5 g (AFE2.5), 5 g (AFE5), and 10 g (AFE10) AFE/kg. Up to 42 days of age, the AFE2.5 and AFE5 diets increased body weight gain, while the AFE5 diet improved the feed conversion ratio, European broiler index, and carcass weight compared to the AFE0 diet. Dietary AFE did not affect dry matter, protein, and fat contents of breast and thigh meats but decreased their ash content. The AFE5 diet increased the thigh meat's pH and a* values, while the AFE10 diet decreased its L* value. While all AFE-supplemented diets decreased the serum aspartate aminotransferase level and the relative weight of the small intestine, the AFE5 and AFE10 diets decreased the serum glucose concentrations compared to the AFE0 diet. The AFE5 diet reduced the concentration of low-density lipoprotein and the relative length of the entire gastrointestinal tract and the small intestine. The dietary AFE at a 5 g/kg diet positively affected the growth performance of broiler chickens by modulating small intestine development and blood biochemicals.

Introduction

Polyphenols and anthocyanins, a class of nutritional phenolics, are valuable natural growth-promoting agents that positively impact nutrient intake, absorption and utilisation due to their high antioxidant and antimicrobial activities (Mahfuz et al., 2021; Wang et al., 2024). Therefore, there has been an increasing awareness that some phytogenic feed additives containing polyphenols and anthocyanins serve as growth-promoting agents in poultry nutrition, exhibiting multiple health effects and nutritional benefits (Lipiński et al., 2017; Basit et al., 2020; Wang et al., 2024). Indeed, in various studies, natural polyphenolic sources, such as grape seed (Abu Hafsa and Ibrahim, 2018), pomegranate peel (Gopi et al., 2020), cornelian cherry (Ibrahim et al., 2021), ginger root (Apalowo et al., 2024), bilberry (Wang et al., 2021) and turmeric extracts (Xu et al., 2024) have been improved growth performance, carcass weight, and meat quality by modifying some blood and gastrointestinal tract (GIT) indices and reducing feeding costs, morbidity, and mortality in broiler chickens (Gopi et al., 2020; Herrero-Encinas et al., 2023). However, there is evidence that dietary supplements with polyphenols and anthocyanins have not exerted a specific effect (positive, negative, or ineffective) on the growth of poultry due to ambiguous and sometimes detrimental impacts on GIT health, nutrient digestibility, and absorption (López de Dicastillo et al., 2019; Amer et al., 2022).

Açai (*Euterpe oleracea* Mart.), a tropical fruit, and its extracts (AFE) contain various polyphenols, including anthocyanins, proanthocyanidins, and flavonoids (Carvalho et al., 2017) with high antioxidant activity and antimicrobial properties (Hogan et al., 2010; Yamaguchi et al., 2015; López de Dicastillo et al., 2019). The total polyphenol content (TPC) and total anthocyanin content (TAC) of the AFE vary from

100 to 613 mg gallic acid equivalent (GAE) per 100 g dry weight (DW), respectively (Hogan et al., 2010; Kim et al., 2021). The AFE also contains major phytochemicals (Alqurashi et al., 2016), such as gallic acid (701.6 mg/100 g DW), cyanidin-3-O-glucoside (998.7 mg/100 g DW), peonidin-3-O-rutinoside (544.5 mg/100 g DW), and cyanidin-3-O-rutinoside (434.0 mg/100 g DW). Arruda et al. (2018) noted that dietary açai seed bran supplementation, up to 10% of the diet, did not impair the performance of slow-growth broilers at the early development stage. In contrast, dietary açai flour from ground seeds and residual dried fruits has enhanced growth performance and reduction of hepatic oxidative stress in chinks (Sousa et al., 2020). These impacts have been attributed to the bioactive compounds and their antioxidant properties in açai seeds and dried fruits (López de Dicastillo et al., 2019; Sousa et al., 2020).

Tropical regions are rich in promising alternative feed additives for animals, most of which have not been sufficiently studied. Based on its bioactive compounds, the AFE can be a substantial feed additive with desirable biological properties to improve broiler chickens' growth performance and meat quality. However, the productive and qualitative implications of AFE-supplemented diets for broiler chickens remain unclear. Therefore, the present study aimed to compare the effects of diets supplemented with or without different doses of polyphenol-rich AFE as a growth promoter on the growth performance, carcass, meat quality, gastrointestinal tract (GIT), and blood biochemical properties of broiler chickens.

Materials and Methods

Animal care

The Experimental Animals Ethics Committee of Eskişehir Osmangazi University (Eskişehir, Turkey) reviewed and approved all experimental procedures used in the animal study. The committee also determined that this experiment was not an unnecessary repetition of previous studies (Approval number: 873/2021).

Experimental design, broiler husbandry and diets

One-day-old mixed-sex Ross 308 broiler chicks (n = 576; 288 females and 288 males) were transferred from a commercial hatchery (Hastavuk, Balıkesir, Türkiye) to the research unit of Eskişehir Osmangazi University. Upon arrival, they were vaccinated against diseases such as Newcastle, infectious bronchitis, and infectious bursal disease virus. Then, the chicks were weighed individually to obtain the initial body weight (BW) and allocated to one of 24 pens in a completely randomised design. Each pen with 24 (12 females and 12 males) birds was assigned to one of the four treatment groups described below.

The chickens were raised in an environmentally controlled house with floor pens (2.0 m × 1.2 m × 1.2 m) littered with wood shavings and equipped with a cylindrical hanging feeder and nipple drinker. The standard commercial practice (Aviagen, 2019) was followed in this study, which lasted 42 days. The facility temperature and relative humidity were 32 ± 1°C and 65% up to 5 days of age and were gradually decreased to 22 ± 1°C and 60% until 21 days of age and then were kept constant until 42 days. A lighting

schedule of 23 hours on and 1 hour off, 18 hours on and 6 hours off, and 23 hours on and 1 hour off was provided for the first week, the next four weeks, and the last week, respectively, using fluorescent lamps.

The study involved an experimental starter phase (1 to 21 days of age) and a finisher phase (22 to 42 days of age). The antibiotic- and coccidiostat-free basic diets, formulated using corn-soybean meal, were designed to meet or exceed the feeding standards for mixed-sex Ross 308 broilers (Aviagen, 2019) during these phases (Table 1). The experimental diets were supplemented with or without different doses of lyophilized ethanol AFE, as follows: 0 (AFE0), 2.5 (AFE2.5), 5 (AFE5), and 10 (AFE10) g/kg of feed. After the lyophilized AFE, measured by a measuring cylinder for each test treatment, was mixed with a small basic diet, this premix was supplemented into a sufficient amount of the relevant diet to achieve the desired final concentration. All AFE diets were prepared daily. The AFE0 chickens received the basic diet without an AFE supplement. In contrast, the test groups' birds received one of the AFE2.5, AFE5, and AFE10 diets. The chickens were fed the diets in a mash form with *ad libitum* access to feed and water. The general health of all birds was assessed twice daily.

Table 1
Ingredient and nutritional composition of the basic diets (as-fed).

Ingredients [g/kg]	1 to 21 days of age	22 to 42 days of age
Yellow corn	546.5	582.5
Soybean meal (48%)	360.5	308.8
Corn gluten meal (62%)	20.2	20.4
Dicalcium phosphate	15.0	10.5
Soybean oil	34.7	55.0
Ground limestone	13.0	10.0
L-lysine (79%)	1.6	2.5
DL-methionine (98%)	1.4	2.7
Threonine (98%)	0.1	0.1
Sodium chloride	2.5	2.5
Vitamin- Mineral premix ¹	4.5	5.0
Calculated nutritional value [g/kg, unless noted]		
Metabolizable energy [MJ/kg]	12.84	13.01
Crude protein	219.9	199.8
Lysine	11.6	11.5
Methionine + cysteine	10.2	9.7
Calcium	9.5	9.0
Available phosphorus	4.3	3.6
Threonine	8.1	8.2
¹ Contents per kg diet: 12000 I.U. Vit. A (retinyl acetate); 2850 I.U. Vit. D3 (cholecalciferol); 55.0 mg Vit. E (α-tocopherole acetate); 4.90 mg Vit. K3 (MNB); 5.00 mg Vit. B1 (mononitrate thamines); 6.00 mg Vit. B2 (cryst. riboflavin); 4.00 mg Vit. B6 (pyridoxine hydrochloride); 2.1 µg Vit. B12 (cyanocobalamin); 45 mg niacin (nicotinic acid); 210 µg biotin (commercial, feed grade); 2.50 mg folic acid (cryst., commercial, feed grade); 12 mg B5 (pantothenic acid); 600 mg choline (chloride); 100 mg iron (Sulfate(II) monohydrate); 95 mg zinc (zinc oxide); 80 mg manganese (manganese (II) oxide); 20 mg copper (copper oxide); 1.20 mg iodine (calcium iodate anhydrous granular); 1.2 mg cobalt; 0.35 mg selenium (coated granulated sodium selenium); 0.21 mg 25-hydroxy cholecalciferol; 0.09 mg propyl gallate; 0.083 mg butylated hydroxyanisole; 1.15 mg Butylated hydroxytoluene (BHT); 13.61 mg calcium D-pantothenate; carrier: calcium carbonate (calcium min 21.9%).		

Productive traits, sampling, and measurements

To calculate the productive traits, such as body weight gain (BWG, g/bird), feed intake (FI, g/bird), feed conversion ratio (FCR) and European broiler index (EBI), the body weight (BW) and feed intake (FI) were determined by pen at 21 and 42 days of age. The FCR was calculated as the FI divided by the BWG (g feed: g gain). Thus, except for the EBI, these variables were evaluated for the experimental starter, finisher, and overall period. The EBI was calculated using a formula that considered the survival rate (SR), BW, and FCR $((SR, \% \times BW, kg)/(FCR \times age, d) \times 100)$.

At 42 days of age, four birds (two females and two males) per replicate, with body weight (BW) within one standard deviation of the mean, were selected to determine carcass and internal organ characteristics. After fasting for 10 hours, the selected birds were individually weighed and slaughtered. The carcass yield (dressing percentage) was determined by subtracting the eviscerated weight from the pre-slaughter body weight. The heart, liver, pancreas, empty GIT, and its segments, such as the proventriculus, gizzard, small intestine (duodenum, jejunum, and ileum), large intestine, and cecum, were individually weighed. The lengths of the entire GIT, the small intestine, and its segments were recorded. The relative weight and length of each part (if applicable) were calculated based on pre-slaughter body weight (g or cm/100 g BW).

To assay some serum parameters, blood samples (4 mL) were collected via the bronchial vein into Vacutainer heparinized sterile tubes (BD Biosciences, Franklin Lakes, NJ, USA) from two birds (one female and one male) for each replicate (i.e., 12 birds per treatment) slaughtered at 42 days. The blood samples were sent to a private laboratory (Simge Medical Laboratory, Eskişehir, Türkiye) and immediately centrifuged at $1792 \times g$ (4000 rpm) for 5 min at 23°C to isolate serum samples. The serum concentrations of aspartate aminotransferase (AST), glucose, total protein (TP), triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined using commercial diagnostic kits (Biotech Co., Ltd., Shanghai, China) validated for poultry serum in an automatic analyser (Airone-200, RA, Italy) based on the manufacturer's instructions.

The remaining two birds per replicate were used to assess the meat quality parameter and the liver colour traits. The colour of the liver and the right breast and thigh meats expressed in lightness (L^*), redness (a^*), and yellowness (b^*) in the Commission Internationale de l'Éclairage (CIELab) colour space were performed using a Minolta CR 300 Chroma Metre (Minolta Camera Co., Osaka, Japan) at immediately and 12 h post-mortem after slaughter. Then, the pH of both muscles was measured using a digital portable pH meter (Model PC 510; Cyberscan, Singapore) with an insertion electrode calibrated with pH 4.01 and 7.01 buffers (S175CD Spear Tip; Sensorex, Garden Grove, CA, USA) at ambient temperature. Averaging three readings per sample on ventral (anterior) aspects and inner space to represent the whole surface of both muscles and the liver was performed (Kop-Bozbay et al., 2021). The meat's water-holding capacity (WHC) was measured according to the procedures described by Kop-Bozbay et al. (2021). The nutrient content of the breast and thigh meats was analysed by AOAC-approved methods (AOAC, 2005) in duplicate.

The total phenolic (TPC) and anthocyanin (TAC) contents and antioxidant activity lyophilized ethanol and aqueous AFE provided by a commercial enterprise (TALYA Herbal LLC, Antalya, Turkiye) were determined as described by Aksu et al. (2020), who used the same procedures, chemicals, and equipment as employed in the present study.

Statistics

The experimental units for data analysis were replicate pens (six pens per treatment). To confirm the homogeneity of data variance, normality distribution was checked using Shapiro–Wilk and Kolmogorov–Smirnov tests and Levene’s test. The measured parameters were analysed using a one-way ANOVA (SPSS 16; SPSS Inc., Chicago, IL). Orthogonal polynomial contrasts were used to assess the effects of graded levels of AFE (0, 2.5, 5, and 10 g/kg). The means were subjected to the Duncan multiple range test, where differences were considered significant at $p < 0.05$.

Results

Total phenolic and anthocyanin compounds

The TPC, TAC and antioxidant activity of the lyophilised ethanol AFE and the aqueous AFE are presented in Table 2. The lyophilised ethanol AFE, in terms of these parameters, had numerically higher values than the aqueous AFE.

Table 2
Total phenolic and total anthocyanin contents and antioxidant activity of lyophilized açai (*Euterpe oleracea*) fruit extract

Item	Extract	
	Ethanol	Aqueous
Total polyphenolic content, mg GAE/100 g dry weight	209.9	149.2
Total anthocyanin content, mg C3G/100 g dry weight	125.2	107.7
Antioxidant activity, mmol TE/100 g dry weight		
FRAP, ferric reducing antioxidant power	4.12	3.76
Free radical-scavenging activity		
DPPH (2,2-Diphenyl-1-picrylhydrazyl)	0.41	0.23
ABTS (2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid))	0.22	0.35
GAE: gallic acid equivalent, C3G: cyanidin 3-glucoside, TE: Trolox equivalent.		

Animal performance

In all treatments, the mortality rate was 0%, and no abnormal signs were observed during the experiment. Up to 21 days of age (Table 3), the dietary AFE linearly affected FI ($p < 0.001$) and FCR ($p = 0.017$). The FI of the AFE10 chickens was lower than that of the AFE2.5 and AFE5 birds ($p < 0.05$). The FCR of the AFE5 broilers was higher (poorer) than that of the AFE0 and AFE10 birds ($p < 0.05$). During the finisher period (22 to 42 days), BWG and FCR were linearly ($p = 0.006$) and quadratically ($p = 0.019$) affected by the dietary AFE, respectively. The AFE5 broilers had a higher BWG than the AFE0 and AFE10 birds ($p < 0.05$). The AFE2.5 diet enhanced BWG compared with the AFE10 diet ($p < 0.05$). From day 1 to day 42, the dietary AFE linearly increased the BWG ($p = 0.001$) and FI ($p = 0.031$) of broilers. The AFE2.5 and AFE5 chickens had a higher BWG than the AFE0 and AFE10 birds ($p < 0.05$). The AFE2.5 and AFE5 diets increased the FI of birds compared to the AFE0 diet ($p < 0.05$). The effect of the dietary AFE on the FCR ($p = 0.045$) and EBI ($p = 0.025$) exhibited a quadratic trend. The FCR was better in the AFE5 group than in the AFE0 and AFE10 groups ($p < 0.05$). The EBI of the AFE5 group was higher than that of the AFE0 and AFE10 groups ($p < 0.05$).

Table 3
Body weight, body weight gain, feed intake, and feed conversion ratio of chickens fed diets supplemented or not with different doses of açai (*Euterpe oleracea*) fruit extract

Item ¹	Dietary supplementation of AFE				SEM	p-value		
	AFE0	AFE2.5	AFE5	AFE10		AFE	L	Q
BW								
at 1 day	45.6	45.8	45.8	45.6	0.15	0.949	0.846	0.911
at 21 days	714.1	773.0	744.1	759.3	9.27	0.123	0.125	0.098
at 42 days	2776 ^b	2881 ^a	2950 ^a	2732 ^b	26.7	0.002	0.001	0.123
1 to 21 days								
BWG	668.5	727.3	698.2	713.7	9.30	0.127	0.128	0.099
FI	1038 ^c	1180 ^a	1165 ^a	1104 ^b	16.7	0.001	< 0.001	0.212
FCR	1.45 ^b	1.53 ^{ab}	1.56 ^a	1.46 ^b	0.018	0.037	0.017	0.372
22 to 42 days								
BWG	2062 ^{bc}	2108 ^{ab}	2205 ^a	1973 ^c	28.3	0.012	0.006	0.059
FI	3799	3854	3861	3861	28.3	0.742	0.741	0.799
FCR	1.87 ^{ab}	1.83 ^b	1.75 ^c	1.93 ^a	0.020	0.002	0.001	0.019
1 to 42 days								
BWG	2730 ^b	2835 ^a	2904 ^a	2686 ^b	26.7	0.002	0.001	0.123
FI	4893 ^b	5041 ^a	5026 ^a	4904 ^{ab}	26.5	0.046	0.031	0.779
FCR	1.76 ^a	1.75 ^{ab}	1.71 ^b	1.79 ^a	0.011	0.018	0.009	0.045
AFE = açai fruit extract; AFE0 = AFE-free basic diet; AFE2.5 = basic diet supplemented with 2.5 g AFE/kg diet; AFE5 = basic diet supplemented with 5 g AFE/kg diet; AFE10 = basic diet supplemented with 10 g AFE/kg diet; BW = body weight (g); BWG = body weight gain (g); FI = feed intake (g); FCR = feed conversion ratio (g of feed / g of weight gain); EBI = European broiler index ((survival rate, % × BW, kg) / (FCR × age, d) × 100); SEM = standard error of mean; L = linear; Q = quadratic.								
^{a,b,c} Means in the same row not sharing a common superscript differ for p ≤ 0.05.								
¹ Values are means of six replicate-pens with 24 birds per treatment.								

Item ¹	Dietary supplementation of AFE				SEM	p-value		
	AFE0	AFE2.5	AFE5	AFE10		AFE	L	Q
EBI	375.1 ^{bc}	392.1 ^{ab}	412.4 ^a	362.5 ^c	5.87	0.003	0.001	0.025
AFE = açai fruit extract; AFE0 = AFE-free basic diet; AFE2.5 = basic diet supplemented with 2.5 g AFE/kg diet; AFE5 = basic diet supplemented with 5 g AFE/kg diet; AFE10 = basic diet supplemented with 10 g AFE/kg diet; BW = body weight (g); BWG = body weight gain (g); FI = feed intake (g); FCR = feed conversion ratio (g of feed / g of weight gain); EBI = European broiler index ((survival rate, % × BW, kg) / (FCR × age, d) × 100); SEM = standard error of mean; L = linear; Q = quadratic.								
^{a,b,c} Means in the same row not sharing a common superscript differ for $p \leq 0.05$.								
¹ Values are means of six replicate-pens with 24 birds per treatment.								

Carcass and organ traits

The AFE addition had a quadratic effect (Table 4) on the carcass weight ($p = 0.006$) and the relative pancreas weight ($p = 0.037$), whereas a linear impact on the relative heart ($p = 0.030$), gizzard ($p = 0.029$), small intestine ($p = 0.022$), jejunum ($p = 0.016$), ileum ($p = 0.022$) weights. While the relative whole GIT ($p = 0.019$) and ileum ($p = 0.002$) lengths were linearly affected by the dietary AFE, small intestine length ($p = 0.038$) was quadratically influenced. The carcass weight of the AFE5 broilers was higher than those of other treatment birds ($p < 0.05$). The AFE10 diet increased the heart weight compared to other diets ($p < 0.05$). The gizzard weights of AFE2.5 and AFE5 broilers were lower than those of the AFE0 and AFE10 birds ($p < 0.05$). The AFE5 diet decreased the pancreas weight compared to the AFE0 and AFE10 diets ($p < 0.05$). The AFE0 broilers had a higher weight of the small intestine, jejunum, and ileum than the AFE2.5, AFE5, and AFE10 birds ($p < 0.05$). The AFE0 and AFE2.5 broilers had higher GIT length than the AFE5 birds ($p < 0.05$). The small intestine and ileum lengths of the AFE5 birds were lower than those of other treatment birds ($p < 0.05$). Compared to the AFE0 diets, the AFE2.5 and AFE10 diets decreased ileum length ($p < 0.05$).

Table 4

The characteristics of carcass and internal organs of broiler chickens fed diets supplemented or not with different doses of açai (*Euterpe oleracea*) fruit extract

Item ¹	Dietary supplementation of AFE				SEM	p-value		
	AFE0	AFE2.5	AFE5	AFE10		AFE	L	Q
CW	1666 ^b	1720 ^b	1886 ^a	1611 ^b	26.8	< 0.001	< 0.001	0.006
CY	74.2	74.6	75.4	72.7	0.65	0.495	0.390	0.560
Internal organ weight [g / 100 g BW]								
Heart	0.51 ^b	0.46 ^b	0.49 ^b	0.60 ^a	0.038	0.008	0.030	0.973
Liver	1.62	1.51	1.40	1.41	0.071	0.160	0.680	0.743
Proventriculus	0.45	0.47	0.52	0.46	0.015	0.402	0.274	0.382
Gizzard	1.79 ^a	1.58 ^b	1.65 ^b	1.80 ^a	0.123	0.023	0.029	0.993
Pancreas	0.17 ^a	0.16 ^{ab}	0.14 ^b	0.18 ^a	0.012	0.087	0.040	0.037
GIT	8.12	7.35	7.98	7.51	0.159	0.425	0.324	0.150
Small intestine	3.88 ^a	3.31 ^b	3.15 ^b	3.23 ^b	0.088	0.009	0.022	0.795
Duodenum	0.68	0.72	0.77	0.77	0.056	0.680	0.917	0.846
Jejunum	1.66 ^a	1.39 ^b	1.41 ^b	1.38 ^b	0.039	0.031	0.016	0.321
Ileum	1.54 ^a	1.19 ^b	0.97 ^b	1.09 ^b	0.052	< 0.001	0.022	0.559
Large intestine	0.22	0.16	0.13	0.16	0.011	0.056	0.113	0.940
Cecum	0.44	0.46	0.44	0.38	0.026	0.748	0.755	0.890
Internal organ length [cm / 100 g BW]								
GIT	10.62 ^a	10.31 ^a	9.25 ^b	10.15 ^{ab}	0.153	0.049	0.045	0.086
Small intestine	9.78 ^a	9.55 ^a	8.52 ^b	9.45 ^a	0.157	0.021	0.019	0.038
AFE = açai fruit extract; AFE0 = AFE-free basic diet; AFE2.5 = basic diet supplemented with 2.5 g AFE/kg diet; AFE5 = basic diet supplemented with 5 g AFE/kg diet; AFE10 = basic diet supplemented with 10 g AFE/kg diet; CW, carcass weight (g); CY, carcass yield (% of slaughter BW); BW, body weight; GIT, empty gastrointestinal tract; SEM = standard error of mean; L = linear; Q = quadratic.								
^{a,b,c} Means in the same row not sharing a common superscript differ for $p \leq 0.05$.								
¹ Values are means of six replicates with four birds per treatment.								

Item ¹	Dietary supplementation of AFE				SEM	p-value		
	AFE0	AFE2.5	AFE5	AFE10		AFE	L	Q
Duodenum	1.49	1.57	1.47	1.57	0.040	0.775	0.619	0.336
Jejunum	3.67	3.79	3.43	3.75	0.063	0.172	0.087	0.040
Ileum	4.61 ^a	4.19 ^b	3.62 ^c	4.13 ^b	0.090	0.001	0.002	0.063
Large intestine	0.49	0.51	0.40	0.46	0.015	0.078	0.091	0.036
Cecum	1.85	1.77	1.66	1.69	0.036	0.246	0.635	0.589
AFE = açai fruit extract; AFE0 = AFE-free basic diet; AFE2.5 = basic diet supplemented with 2.5 g AFE/kg diet; AFE5 = basic diet supplemented with 5 g AFE/kg diet; AFE10 = basic diet supplemented with 10 g AFE/kg diet; CW, carcass weight (g); CY, carcass yield (% of slaughter BW); BW, body weight; GIT, empty gastrointestinal tract; SEM = standard error of mean; L = linear; Q = quadratic.								
^{a,b,c} Means in the same row not sharing a common superscript differ for $p \leq 0.05$.								
¹ Values are means of six replicates with four birds per treatment.								

Meat quality

The dietary AFE had a linear effect on the WHC ($p = 0.014$) and ash content ($p = 0.027$) of the breast meat (Table 5) and a quadratic effect on the pH ($p < 0.001$), L^* ($p = 0.041$) and a^* ($p = 0.007$) values as well as a linear effect on the ash content ($p = 0.015$) of the thigh meat. The WHC of breast meat in AFE2.5 broilers was lower than that in AFE0 and AFE10 birds ($p < 0.05$). All AFE diets decreased the ash content of the breast meat compared to the AFE0 diet ($p < 0.05$). The liver a^* and b^* values were affected linearly ($p = 0.025$) and quadratically ($p = 0.011$), respectively, by the dietary AFE treatments. Compared to the AFE5 diet, other diets resulted in a lower pH in the thigh meat ($p < 0.05$). The AFE10 and AFE5 diets decreased the thigh meat L^* and a^* values compared to the other diets, respectively ($p < 0.05$). All AFE-supplemented diets decreased the ash content of thigh meat compared to the AFE0 diet ($p < 0.05$). The thigh meat a^* value of the AFE5 decreased compared to the other diets ($p < 0.05$). The liver a^* value of AFE5 and AFE10 broilers was higher than that of the AFE2.5 birds ($p < 0.05$). The liver b^* value was higher in the AFE5 treatment than in the other treatments and the AFE2.5 group compared to the AFE0 and AFE10 groups ($p < 0.05$).

Table 5

The quality traits of the breast and thigh meats and liver colour values of broiler chickens fed diets supplemented or not with different doses of açai (*Euterpe oleracea*) fruit extract

Item ¹	Dietary supplementation of AFE				SEM	p-value		
	AFE0	AFE2.5	AFE5	AFE10		AFE	L	Q
Breast meat								
pH	5.75	5.77	5.72	5.81	0.020	0.460	0.374	0.271
Colour								
L*	57.08	56.68	58.21	54.60	0.052	0.076	0.083	0.117
a*	2.09	1.87	2.11	1.85	0.011	0.781	0.659	0.366
b*	0.44	0.54	0.53	0.41	0.108	0.313	0.421	0.679
WHC	75.72 ^a	69.38 ^b	73.94 ^{ab}	74.68 ^a	1.011	0.018	0.014	0.159
Nutrient composition								
DM	24.97	24.98	25.40	24.94	0.132	0.582	0.394	0.298
Protein	21.71	21.98	22.24	22.23	0.107	0.247	0.765	0.795
Fat	1.57	1.48	1.53	1.22	1.333	0.071	0.306	0.282
Ash	1.29 ^a	1.19 ^b	1.20 ^b	1.12 ^b	0.017	0.001	0.027	0.116
Thigh meat								
pH	6.24 ^b	6.17 ^b	6.42 ^a	6.21 ^b	0.026	0.001	< 0.001	< 0.001
Colour								
L*	60.06 ^a	59.38 ^a	60.29 ^a	56.68 ^b	0.397	0.001	0.013	0.041
a*	2.97 ^a	2.97 ^a	2.06 ^b	2.64 ^a	0.107	0.004	0.009	0.007
b*	0.09	0.75	1.38	0.87	0.178	0.082	0.176	0.467
WHC	80.08	82.44	83.22	85.68	0.841	0.116	0.903	0.655
AFE = açai fruit extract; AFE0 = AFE-free basic diet; AFE2.5 = basic diet supplemented with 2.5 g AFE/kg diet; AFE5 = basic diet supplemented with 5 g AFE/kg diet; AFE10 = basic diet supplemented with 10 g AFE/kg diet; WHC = water-holding capacity; DM = dry matter; SEM = standard error of mean; L = linear; Q = quadratic.								
^{a,b,c} Means in the same row not sharing a common superscript differ for p ≤ 0.05.								
¹ Values are means of six replicates with two birds per treatment.								

Item ¹	Dietary supplementation of AFE				SEM	p-value		
	AFE0	AFE2.5	AFE5	AFE10		AFE	L	Q
Nutrient composition								
DM	23.76	23.49	23.17	22.84	0.139	0.093	0.352	0.352
Protein	20.10	20.69	20.46	19.91	0.120	0.081	0.056	0.625
Fat	1.74	1.59	1.25	1.54	0.098	0.378	0.356	0.359
Ash	1.23 ^a	1.11 ^b	1.12 ^b	1.09 ^b	0.167	0.012	0.015	0.203
Liver colour								
L	34.10	34.30	31.83	32.71	0.377	0.060	0.160	0.066
a*	15.09 ^c	16.34 ^b	17.62 ^a	17.67 ^a	0.264	< 0.001	0.250	0.478
b*	11.11 ^c	14.68 ^b	17.61 ^a	12.68 ^c	0.507	< 0.001	< 0.001	0.011
AFE = açai fruit extract; AFE0 = AFE-free basic diet; AFE2.5 = basic diet supplemented with 2.5 g AFE/kg diet; AFE5 = basic diet supplemented with 5 g AFE/kg diet; AFE10 = basic diet supplemented with 10 g AFE/kg diet; WHC = water-holding capacity; DM = dry matter; SEM = standard error of mean; L = linear; Q = quadratic.								
a,b,cMeans in the same row not sharing a common superscript differ for p ≤ 0.05.								
¹ Values are means of six replicates with two birds per treatment.								

Serum biochemicals

The serum AST ($p = 0.036$) and LDL-C ($p = 0.006$) levels exhibited quadratic responses to the dietary AFE levels (Table 6), whereas the glucose concentration displayed a linear response ($p < 0.001$). The AFE-supplemented diets decreased the serum AST level compared to the AFE0 diet ($p < 0.05$). The AFE5 and AFE10 diets resulted in lower serum glucose concentrations than the AFE0 and AFE2.5 diets ($p < 0.05$). The serum LDL-C level in the AFE5 chickens was lower than that of the AFE0 and AFE2.5 birds ($p < 0.05$).

Table 6

Some serum parameters of chickens fed diets supplemented or not with different doses of açai (*Euterpe oleracea*) fruit extract

Item ¹	Dietary supplementation of AFE				SEM	p-value		
	AFE0	AFE2.5	AFE5	AFE10		AFE	L	Q
AST	319.9 ^a	222.6 ^b	236.1 ^b	222.8 ^b	9.86	< 0.001	< 0.001	0.036
Glucose	234.8 ^a	236.5 ^a	215.9 ^b	207.5 ^b	3.09	< 0.001	< 0.001	0.098
TP	2.28	2.21	2.15	2.24	0.033	0.625	0.493	0.627
Triglyceride	26.50	24.00	21.50	21.25	1.125	0.320	0.859	0.823
HDL-C	90.13	86.25	82.63	81.38	1.332	0.079	0.186	0.124
LDL-C	22.13 ^{ab}	24.50 ^a	18.00 ^c	20.13 ^{bc}	0.751	0.008	0.020	0.006
AFE = açai fruit extract; AFE0 = AFE-free basic diet; AFE2.5 = basic diet supplemented with 2.5 g AFE/kg diet; AFE5 = basic diet supplemented with 5 g AFE/kg diet; AFE10 = basic diet supplemented with 10 g AFE/kg diet; AST, aspartate aminotransferase; TP, total protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SEM = standard error of mean; L = linear; Q = quadratic.								
^{a,b,c} Means in the same row not sharing a common superscript differ for $p \leq 0.05$.								
¹ Values are means of six replicates with two birds per treatment.								

Discussion

The TPC, TAC, and antioxidant activity values were among the ranges reported for the AFE, especially ethanolic AFE (Hogan et al., 2010; Maria do Socorro et al., 2010; Kim et al., 2021). A previous study (López de Dicastillo et al., 2019) reported that the ethanolic AFE had desirable biological properties compared to the aqueous AFE, as reported herein. Therefore, based on our TPC and TAC results, the ethanolic AFE contained bioactive phytochemicals (López de Dicastillo et al., 2019) and exhibited antioxidant activity (Carvalho et al., 2017; Sousa et al., 2020), as assessed by FRAP, DPPH, and ABTS (Alqurashi et al., 2016).

To the best of our knowledge, the present study is the first attempt to use lyophilized ethanolic AFE as a growth promoter in broiler diets, thereby investigating and developing its functional value. Our results indicate that the dietary AFE, especially the AFE5 diet, is a potential candidate for improving the growth performance of broiler chickens during both the finisher and overall periods, as well as their carcass weight and meat quality, through the modulation of gut development and blood biochemicals. As reported previously for the açai seed and fruits (Carvalho et al., 2017; Sousa et al., 2020), these results may be associated with the efficient utilization of nutrients and increased protein biosynthesis (Sousa et

al., 2020; da Silveira et al., 2023; Herrero-Encinas et al., 2023) due to possibly the biological function of phenolic compounds in AFE. In contrast, the finding that worsened FCR by AFE-supplemented diets during the starter period contradicts that of a previous study in which broilers were fed a diet supplemented with açai flour (Sousa et al., 2020). Considering gallic acid, one of the main active agents or the TAC of AFE, as an external standard, it can be explained why dietary AFE has shown no beneficial effect on broiler performance up to 21 days of age, as previously reported (Csernus et al., 2020). This result supports the notion that polyphenols have ambiguous and sometimes detrimental effects on gut health and nutrient digestibility and absorption (Lipiński et al., 2017; Amer et al., 2022). The inconsistent results between the experimental phases may be attributed to the specific effect depending on supplementation doses, diet composition, and bird age (Abu Hafsa and Ibrahim, 2018). Thus, the use of these plant products grown in tropical regions as sustainable additives in animal nutrition is supported.

Although the carcass weight of AFE5 birds increased, dietary AFE levels did not affect carcass yield, as indicated in previous studies that used extracts with high polyphenol content (Lipiński et al., 2017; Mazur-Kuśnirek et al., 2019). These findings suggest that the impacts of phytogetic feed additives on carcass weight and yield depend on the amount and type of their polyphenolic compounds (Mazur-Kuśnirek et al., 2019).

Changes in the GIT in the present study may be related to increased muscular development, which is needed to cope with higher levels of indigestible material, increased muscle thickness to assist in the expulsion of bulky faeces, and increased microbial activity to stimulate intestinal growth (Wu et al., 2004). Accordingly, the AFE2.5 and AFE5 diets during the grower period may be associated with increased secretion and activity of endogenous enzymes and eventually increased nutrient utilization rate (Lipiński et al., 2017). In addition, the evident effect of the AFE5 diet on the small intestine and its segments suggests that its TPC and TAC are associated with stimulating nutrient digestibility and absorption (Lipiński et al., 2017; Sousa et al., 2020). Indeed, polyphenol-rich feed additives have altered intestinal surface area, digestive enzyme activities, microbial composition, and bacteria-derived metabolites of polyphenols, which can improve GIT growth parameters, chicken health and productivity (Lipiński et al., 2017; Wang et al., 2024). Nevertheless, growth performance results indicate that in the current study, the high polyphenols provided by the AFE10 diet likely led to adverse metabolic effects by inhibiting digestive enzyme secretion, as stated by Amer et al. (2022), and thus did not positively affect BWG, FRC, and EBI.

The dietary AFE did not consistently affect the chemical composition of breast and thigh meats, independent of growth performance results. Phenolic compounds could directly or indirectly impact meat quality (Mahfuz et al., 2021). Our results on meat quality align with previous findings (Amer et al., 2022), indicating that dietary phytogetic additives in broiler diets did not affect broiler meat's dry matter, protein, and fat contents. Furthermore, although dietary antioxidants did not influence the pH of broiler meat (Mazur-Kuśnirek et al., 2019), they increased the ash content. They improved the WHC (Li et al., 2020), in contrast to the findings reported for breast meat. However, dietary AFE had a similar impact on the ash content of the thigh meat. The AFE5 diet increased the thigh meat's pH and L* values but

decreased its a^* values, a crucial factor for controlling meat freshness for human consumption (Kop-Bozbay and Ocak, 2015; Li et al., 2020). Mazur-Kuśnerek et al. (2019) found that antioxidants did not affect breast meat's L^* and a^* values. In contrast, Li et al. (2020) noted that eucalyptus leaf polyphenols improved the a^* value due to increased myoglobin content in the breast muscle, which agrees with our study. These results suggest that AFE polyphenols, which exhibit high antioxidant activity, may not significantly affect other meat quality traits or that polyphenols do not have such an effect.

Although the size and colour of the liver depend on body weight (Zaefarian et al., 2019), the changes in body weight by dietary AFE were not reflected in the relative liver weight and the liver CIELab values, which highlighted that feeding AFE had no unpleasant effects on meat quality in broilers (Liu et al., 2020). The results of the liver a^* and b^* values may be relatively explained by high feed intake in the AFE2.5 and AFE5 birds because feed restriction, anti-nutritional factors, structural components, and feed additives affect liver colourations, one critical indication of the physiological state in poultry (Kop-Bozbay and Ocak, 2015; François et al., 2022). Because the mention of liver colour is not common in available literature regarding phytogetic feed additives, the impact of the dietary AFE on the liver a^* and b^* values remained to be explained.

In the present study, the results on serum biochemicals may be attributed to polyphenols acting as glucose absorption inhibitors and high antioxidant substances decreasing the LDL-C level, suggesting that the AFE supplement may have a hypocholesterolemic impact (Ibrahim et al., 2021; Mahfuz et al., 2021). Indeed, a significant reduction has been observed in birds fed diets with dietary polyphenol-rich curcumin (Rajput et al., 2013), grape seed (Abu Hafsa and Ibrahim, 2018), and ginger (Shewita and Taha, 2018), regarding blood glucose, total lipids, triglycerides, LHDLC, and total cholesterol. Some contradictions between the blood biochemistry results of the present study and previous studies may be due to differences in the relative amount of each polyphenol in total polyphenolic compounds of phytogetic feed additives (Colombino et al., 2020; Ibrahim et al., 2021). In conclusion, the inclusion of AFE, a tropical fruit, in broiler diets showed a regulatory effect on lipid metabolism by reducing blood LDL-C levels. These findings support the potential of evaluating tropical plant sources as natural and functional feed additives in poultry nutrition.

Conclusions

The present results indicate that the lyophilized aqueous and, mainly, ethanolic AFE, a tropical fruit, is rich in phenolic and anthocyanin antioxidants with potent free radical scavenging activity and may be a suitable feed additive for broiler chickens. Ethanolic AFE can be included at the level of 5 g/kg in diets for broilers until 42 days of age because it enhances growth performance by improving feed utilization and modulating the development of the small intestine and concentration of serum biochemicals. Dietary AFE supplementation at levels 2.5 and 10 g/kg showed no potential beneficial effects on some studied parameters. Dietary AFE did not consistently affect the chemical composition of breast and thigh meats, in contrast to growth performance results under present experimental conditions. These findings reveal the potential of tropical plant sources as natural and functional additives in animal

nutrition and provide scientific contributions to studies on sustainable, plant-based feed additives. Further studies are needed to determine whether the changes observed in the GIT, especially in the small intestine and serum biochemicals and subsequent growth performance and meat quality, are related to the specific action mechanisms of the AFE polyphenols and anthocyanins.

Declarations

Acknowledgments

The authors thank the staff and facilities of the Animal Science Department, Eskişehir Osmangazi University, for their invaluable support. They would also like to congratulate Emre Turan for analysing the bioactive components.

Author contributions

Conceptualization, CKB, EB, GYA; project administration, CKB; data collection, CKB, EB, GYA; laboratory studies, EB and GYA; methodology; CKB and NO; software: CKB and NO; writing - original draft; CKB and NO; writing - review and editing: CKB, EB, GYA, NO.

Data availability

The data for this study are available from the corresponding author upon reasonable request.

Ethics approval

The Experimental Animals Ethics Committee of Eskişehir Osmangazi University (Eskişehir, Turkey) reviewed and approved all the experimental procedures of the animal study. The committee also determined that this experiment was not an unnecessary repetition of previous studies (Approval number: 873/2021).

Funding

The author(s) reported that no funding is associated with the work featured in this article.

Conflict of interest

The authors have no relevant financial or nonfinancial interests to disclose. The authors have no competing interests to declare that are relevant to the content of this article.

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