Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online) http://psj.gau.ac.ir
DOI: 10.22069/psj.2023.20607.1866

The Comparative Effects of Sweet Paprika, Hot Paprika, and Oxytetracycline Supplements on Broiler Growth Performance, Carcass traits, Meat quality, Intestinal Microbiota, Ileal morphology, and Immune Response

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Poultry Science Journal 2023, 11(2): 213-222

Keywords
Broiler
Sweet paprika
Hot paprika
Gut morphology
Lactic acid bacteria

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Article history
Received: September 23, 2022
Revised: May 26, 2023
Accepted: August 21, 2023

Abstract

This study examined the effects of oxytetracycline, sweet paprika, and hot paprika supplementation on broiler chicken growth performance, carcass characteristics, meat quality, intestinal microbiota, and immunological response. A total of 300 one-day-old broiler chickens were assigned to six groups, each with five replicates.The experimental treatments were as follows: 1) basal diet as control group, 2) a basal diet with oxytetracycline (0.05 percent), 3) a basal diet with hot paprika (0.75 percent), 4) a basal diet with hot paprika (1 percent), 5) a basal diet with sweet paprika (0.75 percent), and 6) a basal diet with sweet paprika (1 percent). The results showed that the dietary supplements had no effect on growth performance and carcass traits. Sweet and hot paprika positively affected lipid oxidation and cooking loss in breast meat. In the ileum, broiler chickens fed 1 percent sweet paprika had lower coliform counts and higher lactic acid bacteria/coliforms ratios than other treatments ($P < 0.05$). Furthermore, oxytetracycline in the diet significantly reduced the number of lactic acid bacteria compared to other treatments ($P < 0.05$). In addition, when compared to the control and antibiotic groups, birds fed 0.75 and 1 percent sweet paprika and 1 percent hot paprika had higher villus height, goblet cell density, and villus height/crypt depth ratio and lower crypt depth and epithelial cell layer thickness ($P < 0.05$). Dietary supplementation with 0.75 percent hot paprika or 1 percent sweet paprika increased total antibody response to sheep red blood cells and IgG compared to antibiotic and control groups ($P < 0.05$). Taken together, the findings of this study suggest that dietary inclusion of sweet paprika and hot paprika could improve meat quality, intestinal microbiota, intestinal morphology, and immune response in broiler chickens.

Introduction

For decades, antibiotic growth promoters such as oxytetracycline and virginiamycin have been widely used in broiler chicken farming to improve growth performance (Hernandez-Coronado *et al.*, 2019; Mysara *et al.*, 2021). Due to negative consequences such as the emergence of antimicrobial resistance and antibiotic residues in carcasses, the use of these additives in poultry nutrition was prohibited; however, they are still widely used outside the EU (Hernandez-Coronado *et al.*, 2019; Mysara *et al.*, 2021; Philpot *et al.*, 2021). This tragedy, combined with consumer preferences for products derived from antibiotic - free poultry, created an urgent need to investigate potential antibiotic alternatives (Seidavi *et al.*, 2021). Alternatives include probiotics, medicinal herbs, essential oils, and probiotic supplements (Hernandez-Coronado *et al.*, 2019; Vase-Khavari *et al.*, 2019). Recent research has focused on the efficacy of medicinal herbal plants in improving poultry performance (Abd El-Hack *et al.*, 2022). Medicinal herbs are easily mixed with other feed ingredients, leave no tissue residues, improve growth performance variables, have antimicrobial effects, reduce antibiotic usage, act as anti-inflammatory and antioxidants, and provide healthy products for human consumption (Abd El-Hack *et al.*, 2022).

Please cite this article as Masoumeh Monsefi, Mohsen Afsharmanesh, Mohammad Salarmoini & Mohammad Khajeh Bami. 2023. The Comparative Effects of Sweet Paprika, Hot Paprika, and Oxytetracycline Supplements on Broiler Growth Performance, Carcass traits, Meat quality, Intestinal Microbiota, Ileal morphology, and Immune Response. Poult. Sci. J. 11(2): 213-222.

Paprika, a member of the Solanaceae family, is a popular spice in human food culture (Zhou *et al.*, 2021). Paprika is a natural food colorant with bioactive compounds with antioxidant, antiviral, antimicrobial, and anti-inflammatory properties (Molina *et al.*, 2022). There are two types of paprika based on their pungency: sweet (non-pungent) paprika products and hot (pungent) paprika products (Revilla and Quintana, 2005). Several studies have shown that peppers such as hot red pepper affect broiler chickens' performance (Abd El-Hack *et al.*, 2022). Recent research has found that hot red pepper improves broiler growth performance (Munglang *et al.*, 2019; Soliman and Al-Afifi, 2020), intestinal microbiota, and morphology (Soliman and Al-Afifi, 2020). Kishawy *et al.* (2022) found that supplementing broilers' diets with black pepper oil improves their growth performance, intestinal microbiota, and morphology. Although the effects of various peppers on broiler chickens have been studied, the impactof sweet or hot paprika compared to antibiotics are poorly understood. As a result, the current study aimed to assess the effects of sweet and hot paprika as a possible alternative to antibiotics on broiler chicken performance, carcass traits, meat quality, intestinal microbiota, ileal morphology, and immune response.

Materials and Methods

Birds, experimental design, and management

Three hundred one-day pld Ross 308 broiler chickens were randomly divided into six different treatment groups. The experimental treatments were: 1) basal diet as control group, 2) basal diet with oxytetracycline (0.05%, Rooyan Darou, Tehran, Iran), 3) basal diet with hot paprika (0.75%), 4) basal diet with hot paprika (1%), 5) basal diet with sweet paprika (0.75%), and 6) basal diet with sweet paprika (1%). There were 30-floor pens with five identical pens and 10 chicks per pen for each dietary treatment. The temperature was maintained at 32°C for the first three days while the birds were kept on wood shavings. Thereafter, it decreased by 3 °C per week until it reached 23 °C and remained steady. The relative humidity was kept between 50 and 70%, and the lights was kept on continuously during the rearing period. Throughout the 6-week experiment, the birds had unlimited access to food and water. The birds were fed mash diets as starters (1–21 days) and finishers (22–42 days) (Table 1). Fresh sweet and hot peppers were purchased from a farm and cleaned before being spread in a thin layer and dried to make paprika powder. The dried fruits were ground into powder via the attrition mill and was added to basal diet at certain doses. The experiment was conducted according to the animal welfare guidelines at the Veterinary Control and Research Institute of Kerman, Iran.

Growth performance

On days 1, 21, and 42, birds and feeds were weighed in pens, and the body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined. Birds were checked twice daily for mortality.

Carcass traits

Two broiler chickens were randomly chosen from each pen and killed by cervical dislocation at the end of the experiment (d 42). Carcass, breast, legs, liver, heart, pancreas, and abdominal fat were weighed and expressed relative to the live body weight. The quality of the meat was also assessed by separating the breastmuscle.

Meat quality variables

The antioxidant properties of meat were determined by analyzing the thiobarbituric acid reactive compounds (TBARS), as defined by Buege and Aust (1978),. The BioTek Epoch microplate microplate spectrophotometer was used to measure absorbance at 532 nm, and TBARS results were represented as mg of malondialdehyde per kilogram of meat. Jang *et al*. (2008) method was used to assess the pH of meat samples. A 5 g meat sample was homogenized for 1 minute in 25 mL of distilled water before the pH was measured with a digital pH meter. 1 g of meat was placed on filter paper inside a falcon tube and centrifuged at 1500 g for 4 min at 4 °C to assess the meat water-holding capacity (WHC) and the samples were then dried at 70°C. The WHC was calculated according to the method of Castellini *et al*. (2002). Using the Christensen (2003) method, the drip loss (DL) on meat samples after 24 hours at 4°C was reported as a percentage of weight loss. By calculating the weight difference between meat samples before and after being placed in a boiling water bath (85 C, 10 min), the cooking loss (CL) was determined and reported as a percentage of weight (Bertrama *et al*., 2003). Digital imaging and image analysis were used to determine the samples' color degree (L* = brightness; a* = redness; b* = yellowness) in the Lab mode of Photoshop CS6 version 13.0. (Khajeh Bami *et al*., 2021). According to Khajeh Bami *et al*. (2021) the hue angle and chroma were calculated as follows: hue angle = tan 1 (a/b) and chroma = [(a) 2 + (b) 2] ½.

Meat samples were analyzed for approximate compounds (moisture, crude fat, crude protein, and crude ash) using standardized procedures (AOAC, 2000). Moisture content was calculated as the percentage lost weight after drying the sample in a 105°C oven. Soxhlet extraction with anhydrous diethyl ether was used to determine the ether extract content. The Kjeldahl method was used to determine the crude protein content. Weighing samples after incineration at 525°C yielded the crude ash content.

Table 1. Ingredients and composition (as-fed basis) of the basal diet

Items [C]	Starter diet (d 1-21)			Finisher diet (d 22-42)		
	Control	Hot paprika or sweet paprika (0.75%) meal	Hot paprika or sweet paprika (1%) meal	Control	Hot paprika or sweet paprika (0.75%) meal	Hot paprika or sweet paprika (1%) meal
Ingredients (%)						
Corn (8%CP)	57.077	56.593	56.408	64.61	63.963	63.837
Soybean meal (44% CP)	36.76	36.46	36.36	29.23	29.09	28.93
Oil	2.2	2.2	2.2	2.89	2.89	2.89
Dicalcium phosphate	1.7	1.7	1.7	1.15	1.15	1.15
Calcium carbonate	0.82	0.82	0.82	0.76	0.76	0.76
DL-methionine	0.321	0.330	0.339	0.247	0.258	0.268
L- Lysine	0.244	0.259	0.275	0.266	0.281	0.297
Threonine	0.078	0.088	0.098	0.047	0.058	0.068
Vitamin premix[A]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[B]	0.25	0.25	0.25	0.25	0.25	0.25
Hot or sweet paprika (%)	0	0.75	1	0	0.75	1
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Calculated chemical composition						
Metabolizable energy (Kcal/kg)	2980	2980	2980	3125	3125	3125
Crude protein (%)	21.60	21.60	21.60	18.75	18.75	18.75
Calcium (%)	0.87	0.87	0.87	0.69	0.69	0.69
Available phosphorous (%)	0.43	0.43	0.43	0.32	0.32	0.32
Digestible methionine + cysteine (%)	0.90	0.90	0.90	0.76	0.76	0.76
Digestible lysine (%)	1.24	1.24	1.24	1.08	1.08	1.08
Digestible threonine (%)	0.81	0.81	0.81	0.68	0.68	0.68
Digestible Arginine	1.34	1.34	1.34	1.14	1.14	1.14
Sodium	0.15	0.15	0.15	0.16	0.16	0.16

[A] Supplied per kg of diet: vitamin A (retinol), 12000 IU; vitamin D3 (Cholecalciferol), 5000 IU; vitamin K3, 2.55 mg; thiamin, 3 mg; riboflavin, 7.5 mg; vitamin B6 (pyridoxine), 4.5 mg; vitamin B12 (cyanocobalamin), 0.02 mg; niacin, 51 mg; folic acid, 1.5 mg; biotin, 0.2 mg; pantothenic acid, 13.5 mg; choline chloride, 250 mg.
[B] Supplied per kg of diet: Mn, 120 mg; Cu, 16 mg; I, 1mg; Fe, 40 mg; Zn, 100 mg.
[C] The additives (oxytetracycline and sweet or hot paprika) were added to the basal diets at specific doses by replacement to form the experimental treatments.

Intestinal microbiota

At 42 days, one bird was chosen randomly from each replicate and euthanized via cervical dislocation. In preparation for the microbiological analysis, the ileal digesta was then collected and frozen at -80°C. The intestinal digesta were serially diluted in phosphate-buffered saline to count lactic acid bacteria (LAB) and coliforms (COL). Dilutions 10^{-3} to 10^{-6} were utilized to culture LAB, and 10^{-2} to 10^{-5} were used to culture COL. The COL was grown on MacConkey agar for 24 hours at 37°C, and the LAB was grown on MRS agar for 48 hours at that same temperature (Khajeh Bami *et al*., 2022).

Intestinal morphology

At 42 days of age, one bird from each pen was slaughtered to evaluate the structure of the ileum. After evacuation and washing, 1 cm ileal segments were fixed in 10% buffered formaldehyde. The samples were subsequently embedded in paraffin wax, and 5-m slices of each were stained with periodic Acid-Schiff for goblet cell density study and hematoxylin and eosin for histomorphological examination. The slides were examined using an optical microscope (Cat. No. 12-562-27, Micromaster, Fisher Scientific, Waltham, MA) and the Image Pro Plus v 4.5 software program (Media Cybernetics, Silver Spring, MD, USA) (Khajeh Bami *et al*., 2022). The measurements of the samples included goblet cell density (per 100 m), epithelial cell layer thickness, villus height, width, crypt depth, villus height to crypt depth ratio (VH/CD), villus surface area, and crypt depth.

Immune response

At days 21 and 35 of the experiment, 1 mL of the 0.5 percent sheep red blood cells (SRBC) suspension was injected into the breast muscle of 2 birds per replicate. Seven days after the immune challenge, blood samples were collected and sera were frozen. The sera were then heat-inactivated for 30 minutes at 56°C. Hemagglutination tests were used to measure total and IgG anti-SRBC antibodies (mercaptoethanol-resistant antibody against SRBC) in a 96-well microplate with a U-bottom (Wegmann and Smithies, 1966; Khajeh Bami *et al*., 2022). The difference between total and IgG antibody titers was used to calculate the amount of IgM antibody titer.

Statistical analysis

All data were analyzed as a completely randomized design arrangement using the General Linear Model procedure of SAS software (SAS Institute, Cary, NC). The means of the treatments were compared using Tukey's test, and differences were preposition at $P < 0.05$. The standard error of the means was shown for the means (*SEM*).

Results

Growth performance

Table 2 shows the effects of dietary treatments on broiler chicken growth performance. At 22 to 42 days, the birds fed oxytetracycline had higher FI than those provided sweet paprika ($P < 0.05$). There was no significant effect on growth performance variables (BWG, FI, and FCR) over the period of the experiment (1–42 days).

Table 2. Effects of experimental treatments on the growth performance of broilers.

Items	Body weight gain (g/bird/d)			Feed intake (g/ bird/d)			Feed conversion ratio (g/g)		
	d1-21	d22-42	d1-42	d1-21	d22-42	d1-42	d1-21	d22-42	d1-42
Control	33.33	83.56	57.68	43.73	140.66^{ab}	90.13	1.315	1.685	1.563
Oxytetracycline (%0.05)	32.48	83.74	57.83	43.05	141.59^{a}	91.69	1.326	1.706	1.589
Hot paprika (0.75%)	32.71	81.79	56.64	43.84	138.70^{ab}	91.27	1.340	1.696	1.612
Hot paprika (%1)	33.77	80.04	55.95	43.14	138.19^{ab}	89.02	1.277	1.728	1.592
Sweet paprika (0.75%)	31.86	76.12	53.21	42.97	135.28^{b}	88.75	1.349	1.777	1.651
Sweet paprika (%1)	32.81	81.83	57.11	43.98	137.35^{ab}	90.25	1.341	1.678	1.581
SEM	0.541	1.756	1.251	0.503	1.323	1.204	0.023	0.036	0.023
P-value	0.236	0.060	0.151	0.572	0.040	0.474	0.316	0.437	0.172

$^{a-b}$ Different letters in the same column indicate significant differences ($P < 0.05$).

Carcass traits

Table 3 shows the effects of dietary treatment on carcass traits of broiler chickens. Treatments did not affect the relative weights of the carcass, breast, legs, liver, heart, pancreas, or abdominal fat.

Meat quality measures

Table 4 shows the effects of dietary treatments on the meat quality of broiler chickens. The meat TBARS value of the birds fed sweet paprika 0.75% was lower than that of the other birds ($P < 0.05$). Furthermore, the CL of meat from broilers fed sweet paprika 1% was significantly lower than that of oxytetracycline-fed birds ($P < 0.05$). When compared to the sweet paprika 1% group, birds fed diets containing supplemental hot paprika 0.75% had higher WHC values in meat ($P < 0.05$). There was no significant difference in pH, DL, or proximate composition (moisture, crude protein, crude fat, and crude ash) of meat samples. Table 5 shows the effects of dietary treatments on the meat color of broiler chickens. Treatments had no effect on the color of the breast muscle.

Table 3. Effects of experimental treatments on carcass characteristics (% of body weight) of broilers

Items	Carcass	Breast	Legs	Liver	Heart	Pancreas	Abdominal fat
Control	64.87	24.58	18.58	2.09	0.537	0.236	1.092
Oxytetracycline (%0.05)	4.99	24.62	17.51	2.15	0.526	0.291	1.447
Hot paprika (%75)	63.78	23.72	19.21	2.25	0.561	0.285	1.034
Hot paprika (%1)	64.69	23.72	19.26	2.31	0.598	0.284	1.606
Sweet paprika (%75)	64.81	24.77	18.97	2.37	0.602	0.261	1.087
Sweet paprika (%1)	65.53	25.09	18.15	2.38	0.586	0.265	0.965
SEM	0.789	0.694	0.529	0.142	0.034	0.022	0.153
P-value	0.763	0.646	0.195	0.644	0.506	0.529	0.050

SEM, standard error of the means.

Table 4. Effects of experimental treatments on breast meat quality of broilers

Items	TBARS (ppm DA)	pH	WHC (%)	CL (%)	DL (%)	CP (%)	Moisture (%)	Ash (%)	FAT (%)
Control	0.391^{a}	6.00	60.44^{ab}	29.51^{ab}	7.25	21.41	76.81	0.77	7.67
Oxytetracycline(%0.05)	0.368^{a}	5.95	57.58^{ab}	31.37^{a}	6.25	21.87	77.04	0.97	7.35
Hot paprika (%75)	0.327^{a}	6.01	61.67^{a}	27.63^{ab}	6.62	21.95	76.49	1.55	6.81
Hot paprika (%1)	0.366^{a}	6.05	58.05^{ab}	28.08^{ab}	7.27	21.57	77.40	0.96	6.39
Sweet paprika (%75)	0.212^{b}	6.12	59.80^{ab}	27.04^{ab}	6.43	21.62	77.70	0.94	6.05
Sweet paprika (%1)	0.378^{a}	6.00	56.20^{b}	24.97^{b}	8.00	20.93	78.29	1.16	7.89
SEM	0.018	0.045	0.988	1.299	0.537	0.262	0.920	0.198	0.511
P-value	0.001	0.172	0.001	0.046	0.370	0.136	0.777	0.148	0.117

$^{a-b}$ Different letters in the same column indicate significant differences ($P < 0.05$), and the same letters mean no significant difference ($P > 0.05$). TBARS, thiobarbituric acid; WHC, water holding capacity; CL, cooking loss; DL, dripping loss; CP, crude protein; Ash, crude ash; FAT, crude fat; *SEM*, standard error of the means

Table 5. Effects of experimental diets on the meat color of broilers

Item	Lightness (L*)	Redness (a*)	Yellowness (b*)	Hue angle (degrees)	Chroma
Control	45.46	22.67	18.68	39.475	29.39
Oxytetracycline (%0.05)	45.31	22.80	18.79	39.53	29.55
Hot paprika (%75)	45.50	22.46	18.73	39.85	29.25
Hot paprika (%1)	45.99	23.85	18.69	38.11	30.31
Sweet paprika (%75)	44.78	22.60	18.72	39.67	29.36
Sweet paprika (%1)	46.96	22.33	17.89	38.71	28.63
SEM	0.881	0.536	0.366	0.839	0.490
P-value	0.623	0.642	0.521	0.674	0.337

SEM, standard error of the means.

Intestinal microbiota

Table 6 shows the effects of dietary treatments on intestinal microbiota. The use of oxytetracycline in the diet significantly reduced the number of LAB. Also, the birds fed sweet paprika dietary supplements at a level 1% had lower COL counts in the ileum ($P < 0.05$). Furthermore, using sweet paprika at a percent level 1% resulted in higher LAB/COL ratios in the ileum ($P < 0.05$).

Table 6. Effects of experimental treatments on the ileal microbiota (log cfu/g) of broilers

Items	Lactic acid bacteria	Coliforms	Lactic acid bacteria / Coliforms
Control	5.411^{a}	3.348^{a}	1.598^{bc}
Oxytetracycline (0.05%)	3.098^{b}	3.143^{a}	0.990^{c}
Hot paprika (0.75%)	5.184^{a}	3.403^{a}	1.521^{bc}
Hot paprika (1%)	5.125^{a}	2.994^{a}	1.794^{b}
Sweet paprika (0.75%)	6.458^{a}	3.566^{a}	1.840^{b}
Sweet paprika (1%)	6.808^{a}	1.534^{b}	4.472^{a}
SEM	0.419	0.238	0.158
P-value	0.001	0.001	0.001

$^{a-c}$ Within a column, means followed by the same letter are not significantly different ($P< 0.05$). *SEM*, standard error of the means.

Intestinal morphology

Table 7 and Figure 1 show the effects of dietary treatments on ileal morphology. When compared to the control and antibiotic groups, birds fed sweet paprika at a percentage of 1 and 0.75 and hot paprika at a percentage of 1 had higher villus height, VH/CD,

and goblet cell density and lower epithelial cell layer thickness ($P < 0.05$). Nonetheless, antibiotic treatment had no effect on variables such as villus height, villus width, VH/CD ratio, or villus surface area when compared to the control group.

Table 7. Effects of experimental treatments on the ileal morphology[1] of broilers

Item	VH (µm)	VW (µm)	CD (µm)	VH:CD (µm)	VS (mm^2)	ECT (µm)	GD
Control	968^{dc}	129.69^{a}	124.54^{a}	7.813^{b}	0.394^{b}	35.23^{c}	6.15^{d}
Oxytetracycline (0.05%)	931^{d}	137.72^{a}	113.85^{b}	8.186^{b}	0.403^{b}	51.77^{a}	5.07^{e}
Hot paprika (0.75%)	1,138^{bc}	140.79^{a}	127.36^{a}	8.946^{b}	0.503^{a}	40.97^{b}	7.60^{c}
Hot paprika (1%)	1,434^{a}	103.31^{b}	112.78^{b}	12.722^{a}	0.465^{ab}	30.45^{d}	7.97^{c}
Sweet paprika (0.75%)	1,341^{ab}	102.89^{b}	110.90^{b}	12.091^{a}	0.405^{b}	28.48^{d}	8.60^{b}
Sweet paprika (1%)	1,346^{a}	102.48^{b}	113.30^{b}	11.877^{a}	0.433^{ab}	26.59^{d}	10.37^{a}
SEM	45	2.94	1.69	0.464	0.017	0.91	0.09
P-value	0.001	0.001	0.001	0.001	0.001	0.001	0.001

[a-d] Within a column, means followed by the same letter are not significantly different ($P < 0.05$). *SEM*, standard error of the means.
[1]VH= Villus height; VW= Villus width; CD= Crypt depth; VH:CD= Villus height to crypt depth ratio; VS= Villus surface area; ECT= Epithelial cell layer thickness; GD= Goblet cell density

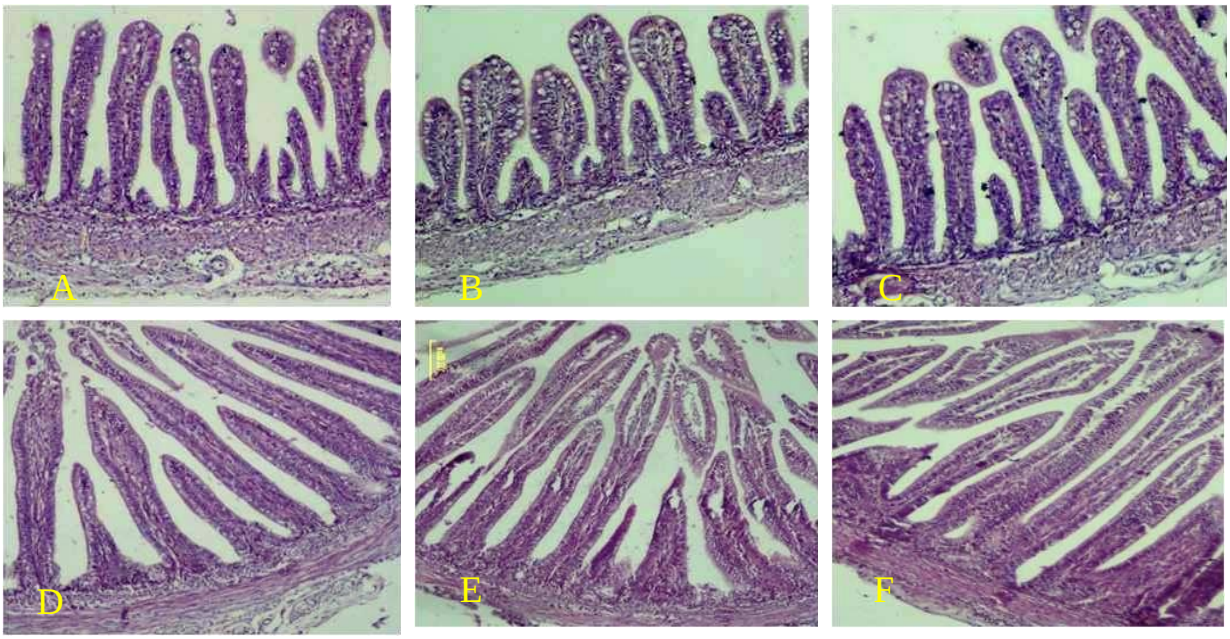

Figure 1. A: Control, B: Oxytetracycline (0.05%), C: Hot paprika (0.75%), D: Hot paprika (1%), E: Sweet paprika (0.75%), F: Sweet paprika (1%).

Table 8. Effects of experimental treatments on the immune response (log2) of broilers at 28 and 42 d

Item	Total antibody		IgG		IgM	
	28 d	42 d	28 d	42d	28 d	42 d
Control	2.4^{b}	3.3^{b}	1.0^{b}	1.2^{b}	1.4	2.0^{b}
Oxytetracycline (0.05%)	2.4^{b}	3.2^{b}	1.0^{b}	1.0^{b}	1.4	2.2^{ab}
Hot paprika (0.75%)	4.4^{a}	7.2^{a}	1.7^{ab}	3.0^{a}	2.6	4.2^{ab}
Hot paprika (1%)	4.7^{a}	4.1^{b}	2.3^{a}	1.7^{b}	2.1	2.3^{ab}
Sweet paprika (0.75%)	3.6^{ab}	4.5^{b}	1.6^{ab}	1.7^{b}	2.0	2.7^{ab}
Sweet paprika (1%)	2.9^{ab}	8.0^{a}	1.2^{b}	3.5^{a}	1.6	4.5^{a}
SEM[1]	0.45	0.57	0.19	0.27	0.39	0.53
P-value	0.001	0.001	0.001	0.001	0.198	0.009

[a-b] Within a column, means followed by the same letter are not significantly different ($P < 0.05$).
[1] *SEM*, standard error of the means.

Immune response

The results of dietary treatment on the immunological response in birds at 28 and 42 days are shown in Table 8. After 28 days, the experimental treatment substantially impacted the overall antibody response to SRBC and IgG. When compared to the antibiotic

and control groups, dietary supplementation with hot paprika at a level of percent 1 increased total antibody response to SRBC and IgG ($P < 0.05$). Birds fed a diet containing 0.75 percent hot paprika or 1 percent sweet paprika had a higher total antibody response to SRBC and IgG at 42 days than the antibiotic and control groups ($P < 0.05$). Furthermore, dietary supplementation with sweet paprika percent 1 significantly increased IgM at 42 days.

Discussion

According to the findings of this study, different levels of sweet and hot paprika had no significant effect on broiler BWG and FCR. In contrast to the current study, Al-Kassie *et al.* (2012) found that a 0.75 percent and 1 percent mixture of black and hot red pepper improved broiler growth performance. Furthermore, Kishawy *et al.* (2022) found that dietary supplementation with black pepper oil significantly improves broiler growth performance. Shahverdi *et al.* (2013) found that adding red and black pepper to broiler diets improved BWG, FI, and FCR. Soliman and Al-Afifi (2020) observed that supplementing broiler diets with different graded concentrations of hot red pepper improves growth performance. However, dietary supplementation with capsaicin (one of the compounds found in red pepper) had no effect on broiler growth performance in one study (Platel and Srinivasan, 2000). According to Williams and Kienholz (1974), different concentrations of pepper chili (1.5 percent, 3 percent, 6 percent, and 12 percent) had little effect on broiler growth performance. Dougnon *et al.* (2014) found no difference in the growth performance of broilers fed diets supplemented with hot red pepper at 0 percent, 0.5 percent, and 1 percent concentrations. Differences between studies could be attributed to differences in the tested material's level, breeding season, hygienic conditions, or chicken breeds used.

Treatments did not affect the relative weights of carcass, breast, legs, liver, heart, pancreas, and abdominal fat, which are consistent with unaffected growth performance. According to Shahverdi *et al.* (2013), supplementing the diet of broiler chickens with hot red pepper (0.02 percent) alone or in combination with black pepper (0.01 percent + 0.01 percent) significantly increased the relative weights of the liver, breast, gizzard, heart, and spleen compared to the control group. El-Deek *et al*. (2012), on the other hand, found that supplementing broiler chickens with hot red pepper had no effect on the relative weight of the liver, heart, pancreas, kidney, and spleen. According to Al-Kassie *et al*. (2011), a pepper mixture (black and red) had no effect on the heart, gizzard, or liver weights of broilers.

The TBARS value in the meat of birds fed sweet paprika % 0.75 was lower in this study than in other birds. Furthermore, the CL of meat in broilers fed sweet paprika % 1 diet was significantly lower than in birds fed oxytetracycline diets. Lipid peroxidation, which causes the production of unpleasant tastes, rancidity, and harmful substances such as peroxides, is a significant factor in meat deterioration (Khajeh Bami *et al*., 2021; Pitargue *et al*., 2019). The susceptibility of chicken meat to the development of lipid oxidation is increased when the meat is richer in polyunsaturated fatty acids (Leskovec *et al*., 2019). Lipid peroxidation concentration has typically been determined using the TBARS value, reported as malondialdehyde concentration. It has been suggested that proteolysis, a crucial component in establishing the DL and CL, two measures commonly used to estimate the WHC of meat, might be impacted by variations in the antioxidant classification system between birds (Cai *et al*., 2012). As a result, the decrease in TBARS values of meat in broiler chickens fed with 1% sweet paprika may have contributed to the significantly lower CL in the current study. El-Deek *et al*. (2012) reported that supplementation with hot red pepper improved the meat quality of broiler chickens, which is consistent with the current study's findings.

This study showed that birds fed sweet paprika dietary supplements at a level of 1% had lower COL counts and higher LAB/COL ratios in the ileum. In addition, when compared to other treatments, the addition of antibiotics inhibited the growth of LAB. Capsaicin, a terpenoid compound found in paprika, has antibacterial properties (Abd El-Hack *et al.*, 2022). Studies have shown capsaicin-containing herbs to be effective against a wide range of pathogens (Amad *et al.*, 2011; Kim *et al.*, 2009). Furthermore, some herbal antioxidants have been shown in studies to increase the counts of beneficial bacteria while suppressing pathogenic bacteria colonization (Dieumou *et al.*, 2009; Kırkpınar *et al.*, 2011). According to Abdul Aziz (2010), the effects of pepper on bacterial strains are related to the bacteriostatic and bactericidal activities of the capsaicin derivatives t-cinnamic and caffeic acids. According to one study, using hot pepper reduces the population of pathogenic bacteria such as *E. coli* and *Enterobacteriaceae* (Soliman and Al-Afifi, 2020). Kishawy *et al.* (2022) found that dietary supplementation with black pepper oil increased LAB and decreased COL counts in broiler cecal. There is no research on the effects of paprika on the intestinal microbiota of broiler chickens that we are aware of. The findings of this study demonstrated that paprika has the potential to modulate intestinal microbiota, which, as evidenced by an immune response and intestinal morphology data from the current experiment, can improve intestinal health and immune response in broiler chickens.

In the current study, birds fed sweet paprika at a percentage of 0.75 and a percentage of 1 and hot

paprika at a percentage of 1 had higher villus height, goblet cell density, and VH/CD and lower crypt depth and epithelial cell layer thickness compared to the control and antibiotic groups. These findings indicated that paprika supplementation improved intestinal structure. According to the study, changes in intestinal morphology have been associated with immunological and disease resistance, the number of secretory cells in the gut, and functional development of the gut (Tang *et al*., 2020). These findings are consistent with the results of Soliman and Al-Afifi (2020), who found that adding hot red pepper to diets at 0.5 and 1% increased villus height significantly. It has been reported that red pepper improves intestinal morphology by reducing the growth of pathogenic intestinal bacteria and that this reduction in pathogenic bacteria reduces inflammatory reactions in the intestinal mucosa, resulting in a significant increase in villus area (Abd El-Hack *et al.*, 2022). As a result, the mechanism by which paprika improves intestinal morphology may be due to decreased COL counts, which reduces inflammatory processes in the intestinal mucosa and thus improves intestine morphology.

In this study, using 0.75 percent hot paprika or 1 percent sweet paprika resulted in a higher total antibody response to SRBC and IgG than the antibiotic and control groups. Furthermore, dietary supplementation with 1% sweet paprika significantly increased IgM. Valizadeh *et al.* (2018) found that using red pepper at 2% improved immune response. According to the findings of one study, adding 0.5 percent red pepper powder to broiler diets improved antibody titers against Newcastle virus (Aghayarifar *et al.*, 2015). Kishawy *et al.* (2022) found that supplementing broilers' diets with black pepper oil increases serum IgG and IgM concentrations. Flavonoids and polyphenolic chemicals found in herbal plants have antioxidant characteristics that, in accordance with Amresh *et al*. (2007)'s findings, can improve the immune system. On the other hand, prior studies have demonstrated that an enhanced immunological status is linked to an altered intestinal microbiota and improved intestinal morphology, as seen in the current study (Tang *et al*., 2020). Thus, a better microbial population and gut morphology may be what helps the humoral immune system function.

Conclusion

It can be concluded that dietary inclusion of 1% sweet and hot paprika improved meat quality, intestinal microbiota, intestinal morphology, and immune response in broilers. Furthermore, our findings suggest that paprika could be a viable alternative to antibiotics in broiler feed.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

We would like to thank the Shahid Bahonar University of Kerman for help during the experiment.

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