IJAS Iranian Journal of Applied Animal Science

Green Coffee Powder: Effect on Growth Performance, Biochemical Blood Indices, and Intestine Morphology in Broiler Chickens

Research Article

D. Manochehri[1], S.J. Hosseini-Vashan[1*], N. Afzali[1] and H. Naeemi Pour[1]

[1] Department of Animal Science, Faculty of Agriculture, University of Birjand, South Khorasan, Birjand, Iran

Received on: 6 Sep 2024
Revised on: 11 Mar 2025
Accepted on: 15 Apr 2025
Online Published on: Jun 2025

*Correspondence E-mail: jhosseiniv@birjand.ac.ir

Online version is available on: www.ijas.ir
https://doi.org/10.71798/ijas.2025.1214905

ABSTRACT

This research investigated the effect of different levels of green coffee powder (GCP) on growth performance, immune response, biochemical blood indices, and intestinal morphology in broiler chickens. A total of 300, one-day-old male Ross broilers were used in a completely randomized design with six treatments, five replicates, and 10 chicks each. The dietary treatment included six levels of coffee powder (0.0, 0.3, 0.6, 0.9, 1.2, and 1.5%). Compared to the control, GCP, at 0.3% and 1.5% increased the body weight gain and feed intake in broilers ($P<0.05$). Compared to the control, a lower feed conversion ratio was observed in broilers fed with 1.5% GCP ($P<0.05$). Adding 0.9% of GCP to the broiler diet increased relative weight of the breast, thigh, and bursa fabricius, compared to the control. The GCP at all levels reduced the relative weight of abdominal fat compared to control ($P<0.05$). The coffee powder did not affect the immunoglobulin M index against sheep red blood cell (SRBC); however, the total antibody index against SRBC increased in chickens fed GCP compared to the control ($P<0.05$). Coffee powder decreased blood cholesterol, low-density lipoprotein (LDL), and triglyceride concentration and increased blood high-density lipoprotein (HDL), albumin, glucose, and protein concentration compared to control ($P<0.05$). The villi height and crypt depth increased in broilers fed 1.2% GCP compared to control ($P<0.05$). It is concluded that using green coffee powder at the level of 0.3% in the broiler diet may improve growth performance and some biochemical indices; however, the levels of 1.2 and 1.5% GCP improved the intestinal morphology and immune response of broilers.

KEY WORDS abdominal fat, cholesterol, green coffee, SRBC, total antibody.

INTRODUCTION

The growth and development of extensive poultry farming have increased the demand for feed ingredients in the poultry industry. Therefore, among agricultural products and by-products, new sources should be sought to meet the needs of the livestock and poultry feed industry (Bouafou *et al*. 2011). Nutrition is the most important aspect of farming, which accounts for the highest cost of raising it. Today, poultry nutritionists try to meet all of a bird's requirements in a way that avoids nutrient deficiencies and excesses in the diet. This is critical because the nutritional requirements of broilers are changing with genetic advancements (Bedford, 2016).

Coffee is one of the most critical and valuable plants with nutrients and bioactive components. This plant is native to subtropical regions of Africa and some islands of South and Southeast Asia, and has spread from Africa to other parts of the world. Recently, it has been cultivated in more than 70 countries, primarily in the tropics of the United States, Southeast Asia, India, and Africa. As the fruit of the coffee plant ripens, the coffee beans are harvested, processed, and

finally dried (Feedipedia, 2011). Following water, coffee is the most popular beverage in the world. More than seventy countries in Asia, Africa, and South America cultivate it in latitudes 23 to 25 south. Countries such as Germany, the United States, Japan, and Italy have the highest per capita coffee consumption globally. Arabica and Canfora are the two main coffee varieties harvested around the world. The Canfora coffee is often referred to as Robusta. The other varieties of coffee are less popular and much less cultivated. Coffee peel contains 9.3% crude protein, 37.2% crude fiber, 6.5% ash, 0.44% calcium, 0.12% phosphorus, 31 mg zinc and 8 mg copper. The profile of amino acids in coffee based on the percentage of crude protein are lysine, threonine, methionine, cysteine, isoleucine, valine, leucine, phenylalanine, tyrosine, histidine, arginine, alanine, aspartate, glutamate, glycine, serine, and proline 3.4, 1.3, 0, 3, 3, 3, 7, 3, 7, 4, 3, 9, 1, 5, 2, 8, 5, 3, 7, 7, 7, 2 4, 3.3, and 7.3, respectively (FeediPedia, 2011).

Coffee waste and by-products generally contain 10% crude protein, 23% crude fat, 13% nitrogen-free extract, 37% crude fiber, and minerals such as calcium and phosphorus. Using coffee in animal nutrition was reported to be up 10-30, 10-24, 13-30, and 2.5-5% in ruminants, pigs, fish, and broilers, respectively, without adverse effects on animal physiology and health. However, some bioactive compounds such as alkaloids, caffeine, and tannins in coffee reduce the flavor of animal feed. If a higher percentage of coffee in feed is consumed, it may affect the animal´s health (Marcel *et al*. 2011). The endosperm tissue has the highest amount of storage polysaccharides. The plant cell wall is usually composed of cellulose and hemicellulose, and the cell wall of coffee beans is mainly composed of mannan-oligosaccharides. The most well-known compound in coffee beans is caffeine. The caffeine content of raw Arabica coffee is between 0.8% and 1.4%, while in Robusta it is 1.7 to 4%. However, coffee contains B-group vitamins such as niacin (b3) and ascorbic acid, which vary between 7 and 12 percent, equivalent to 3 to 5 times the caffeine found in coffee (Frances *et al*. 2023). Trigonelline is the most biologically important alkaloid in coffee, derived from the enzymatic methylation of nicotinic acid. Trigonelline, due to its potential biological activity, prevents cancer cell invasion and can regenerate dendrites and axons, possibly improving memory (Yi Fang Cho *et al*. 2012). The use of 25 g/kg coffee pulp in diets had no significant effect on growth performance and mortality of broiler chickens (Donkoh *et al*. 1988). Interestingly, in the composition of coffee minerals, potassium involves more than 40% of minerals, and sodium is in second place (Donkoh *et al*. 1988). Magnesium, phosphorus, calcium, sulfur, and trace elements such as zinc, strontium, silicon, manganese, iron, copper, barium, boron, and aluminum are also present in significant amounts in coffee (Arvay *et al*. 2018). In a study titled "Determining the nutritional value of dried coffee grounds in broiler chicken diets," it was reported that using dried coffee grounds did not affect the health and growth performance of broiler chickens (Donkoh *et al*. 1988). Coffee contains essential amino acids and significant amounts of erupins, pyridines, pyrazines, pyrroles, aldehydes, and melanin. Flavonoids are responsible for the color of coffee and, to some extent, their antioxidant activity (Yi-Fang Chu *et al*. 2012). Therefore, this research aimed to investigate the effect of different levels of green coffee powder (GCP) on growth performance, immune response, biochemical blood indices, and intestinal morphology in broiler chickens.

MATERIALS AND METHODS

Preparation of GCP

This research was done in the broiler research farm of the University of Birjand, Birjand, Iran. For dietary treatments, 10 kg of Robusta Vietnam coffee powder was prepared. The chemical composition of coffee powder (percentage of crude protein, crude fiber, ether extract, Ash, and nitrogen-free extracts) was determined in the Animal Nutrition Laboratory of the University of Birjand. Mineral elements such as calcium, phosphorus, potassium, magnesium, and sodium were also determined in coffee powder. The ash samples were determined by burning in an electric furnace at 530-550 °C. The nitrogen content of the samples was determined based on the AOAC method by a Kjeltec Analyzer instrument (1030 Foss Tecator, Sudan). The ether extract was measured using a Soxtec system (Soxtec system 2050 extraction unit, Tecator, Sudan). The phosphorus of samples was determined using a spectrophotometer (Unico 2150, USA) at a wavelength of 430 nm, and the amount of calcium was determined using the atomic absorption method. The chemical composition of the coffee powder is presented in Table 1.

Birds and diets

In this experiment, 300 one-day-old male Ross 308 broilers were used in a completely randomized design with six treatments, five replications, and ten chicks per replicate. Experimental treatments included five levels of 0.3, 0.6, 0.9, 1.2, and 1.5% coffee powder and a control group. The Robusta coffee used in this experiment was a Vietnamese sample. Throughout the experimental period, the breeding conditions were performed according to the recommendations of the Ross breeding handbook. Access to water and feed was free during the experiment. Diets were prepared in the form of three feeding periods involving starter (1-10 days), grower (11-24 days), and finisher (25-42 days). Ex-

perimental diets were iso-energetic and iso-nitrogenous. The feed ingredients and chemical composition are presented in Table 2.

Performance

The body weight and feed intake were recorded during the starter, grower, and finisher periods, then feed conversion ratio was calculated. At 42 days of age, two chicks from each replicate were weighed and slaughtered by decapitation. Carcass weight and weight of the various organs, including breast, thighs, and visceral organs such as liver, spleen, bursa of fabricius, heart, and abdominal fat were measured. The percentage weight as a relative weight of live weight was calculated.

Intestinal morphology

To study intestinal morphology, a piece of 1 cm of jejunum was removed and, after washing with saline, placed in formalin (10%). After 24 hours, the formalin was replaced. After fixing the tissue in alcohol and making a suitable incision with a microtome (Pouyan MK1110 model, made in Iran), it was stained with hematoxylin-eosin dye, and the indices of villi height, villi width, and crypt depth were determined using a camera attached to Olympus microscope.

$$\text{Absorption area} = (\text{villus height}) \times (\text{villus width}/2) \times \pi^{2}$$

Blood indices and immune system

At 42 days of age, blood samples were taken from two broiler chicks of each replicate. Blood samples were taken from each bird in two test tubes without anticoagulant and test tubes containing ethylene diamine tetraacetic acid. Then, blood samples were centrifuged at 3000 rpm for 15 minutes, and serum and plasma were separated. Blood serum was used to study antibody titer against sheep erythrocytes, and plasma was used to measure other blood indices, including triglyceride, cholesterol, glucose, albumin, high-density lipoprotein, low-density lipoprotein concentration, and enzyme activity of aspartate aminotransferase and alanine aminotransferase. The laboratory kits of Pars Azmoon Company and an automatic optical spectroscopy device were used (spectrophotometer model Jessan Cham 200, Italy).

Statistical analysis

This experiment was performed in a completely randomized design. All the data were analyzed using a general linear model by SAS software (SAS, 2004). Differences among treatment means were detected using Tukey's test. Statements of significance were based on $P \leq 0.05$.

The model was used as:

$$Y_{ij} = \mu + T_j + e_{ij}$$

Where:
μ: overall mean.
T_i:: treatment effect.
e_{ij}: experimental error.

RESULTS AND DISCUSSION

The results showed that including of coffee powder in broiler diets did not influence the growth performance indices (Table 3). The results related to weight gain showed a significant difference with other treatments in the starter period among treatments ($P<0.05$). Throughout the growth period, chickens receiving 0.3% coffee powder had a higher body weight gain than treatment containing zero and 1.2% coffee powder ($P<0.05$). At the end of the experiment, no significant difference was observed in the weight gain of birds fed with different treatments. Coffee powder has been reported to have a stimulating effect on water intake, leading to increased feed intake and weight gain (Skinner-Noble and Titter, 2004). On the other hand, the caffeine in coffee has a fat-burning effect and therefore reduces fat retention in tissues, thus reducing weight gain with increased feed intake (Garg, 2016). The results of feed consumption showed that in the finisher and whole period, feed intake in broilers fed with 1.5% coffee powder was not different from the control; however, the level of 0.3% coffee powder reduced feed intake compared to the control ($P<0.05$). Increasing the feed intake in birds fed diets containing 1.5% coffee powder may be due to higher amino acids in feed, which has been reported to improve feed consumption. Coffee has diuretic properties that stimulate feed consumption and increase appetite (Rang *et al*. 2001). Similarly, water-stimulating drinking agents in birds have also been reported to boost feed intake (Skinner Nobel and Titre, 2004). Another study reported that coffee powder did not affect feed intake (Mendes *et al*. 2013). The feed conversion ratio is one of the most important growth performance indicators for assessing production performance. The smaller this indicator, the more economically viable it will be. The feed conversion ratio results showed that there was a difference between the coffee treatments and the control treatment ($P<0.05$. Throughout the entire period, the lowest feed conversion ratio (FCR) was for chickens fed with 0.3 and 1.5% coffee powder levels, indicating that adding coffee at 0.3 and 1.5% levels improves FCR. This finding indicated that using coffee powder at the level of 0.3% causes more weight gain and decreases FCR.

Table 1 Chemical composition of green coffee powder

	Moisture	Crude protein	Ether extract	Ash	K	Na	Ca	Mg	P
Green coffee powdwr	5.4	17.14	6.97	4.38	0.92	0.07	0.3	0.27	0.32

Table 2 The ingredients and chemical composition of diets contained different levels of green coffee

Ingredient	Starter (1-10 days)						Grower (11-24 days)						Finisher (25-42 days)					
	0%	0.3%	0.6%	0.9%	1.2%	1.5%	0%	0.3%	0.6%	0.9%	1.2%	1.5%	0%	0.3%	0.6%	0.9%	1.2%	1.5%
Corn	51.23	50.80	50.47	50.14	49.82	49.49	54.99	54.75	54.51	54.28	54.04	53.08	60.12	59.78	59.41	89.04	58.66	58.29
Soybean meal	40.25	40.32	40.44	40.52	40.62	40.73	36.59	36.57	36.54	36.52	36.49	36.47	30.37	30.51	30.66	30.80	30.95	31.09
Fish meal	1.90	1.94	1.86	1.78	1.70	1.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Coffee	0.00	0.30	0.60	0.90	1.20	1.50	0.00	0.30	0.60	0.90	1.20	1.50	0.00	0.30	0.60	0.90	1.20	1.50
Oil	3.00	3.00	3.00	3.00	3.00	3.00	4.40	4.40	4.40	4.37	4.32	4.31	5.50	5.50	5.50	5.50	5.50	5.50
DCP	1.28	1.27	1.27	1.27	1.27	1.27	1.34	1.33	1.33	1.33	1.32	1.34	1.23	1.23	1.23	1.23	1.23	1.23
Calcium carbonate	1.58	1.57	1.57	1.57	1.59	1.60	1.81	1.81	1.81	1.81	1.81	1.81	1.60	1.60	1.60	1.60	1.59	1.59
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Min premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vit premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.05	0.05	0.05	0.05	0.05	0.05	0.09	0.09	0.09	0.09	0.09	0.09	0.14	0.10	0.10	0.10	0.10	0.10
The calculated nutrients																		
Metabolizable energy (kcal/kg)	2950	2950	2950	2950	2950	2950	3050	3050	3050	3050	3050	3050	3180	3180	3180	3180	3180	3180
Crude protein (%)	23.00	23.00	23.00	23.00	23.00	23.00	20.5	20.5	20.5	20.5	20.5	20.5	18.50	18.50	18.50	18.50	18.50	18.50
Fat (%)	5.24	5.31	5.35	5.41	5.45	4.49	6.58	6.60	6.63	6.66	6.68	6.72	7.75	7.72	7.87	7.92	7.98	8.04
Crude fiber (%)	3.89	4.00	4.11	4.23	4.33	4.44	3.66	3.76	3.87	3.97	4.09	4.18	3.33	3.43	3.56	3.67	3.78	3.90
Ca (%)	1.00	1.00	1.00	1.00	1.00	1.00	0.95	0.95	0.95	0.95	0.95	0.95	0.90	0.90	0.90	0.90	0.90	0.90
Available P (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.45	0.45	0.45	0.45	0.45	0.43	0.43	0.43	0.43	0.43	0.43
Na (%)	0.29	0.30	0.296	0.289	0.282	0.280	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
K (%)	0.82	0.826	0.830	0.831	0.833	0.834	0.739	0.739	0.739	0.739	0.739	0.739	0.618	0.621	0.624	0.627	0.631	0.634
Cl (%)	0.041	0.041	0.041	0.041	0.041	0.041	0.029	0.029	0.029	0.029	0.029	0.029	0.03	0.03	0.03	0.03	0.03	0.03
Arg (%)	1.68	1.703	1.71	1.718	1.726	1.733	1.468	1.473	1.481	1.490	1.499	1.508	1.273	1.287	1.301	1.315	1.329	1.342
Lys (%)	1.42	1.338	1.345	1.353	1.360	1.367	1.112	1.129	1.137	1.146	1.156	1.164	1.088	1.085	1.048	1.02	1.07	1.04
Met + Cys (%)	0.77	0.78	0.78	0.78	0.78	0.78	0.676	0.676	0.676	0.676	0.676	0.676	0.686	0.614	0.616	0.619	0.612	0.624
Thr (%)	0.95	0.969	0.975	0.975	0.969	0.969	0.836	0.844	0.852	0.860	0.868	0.876	0.754	0.761	0.771	0.782	0.792	0.802
Trp (%)	0.266	0.266	0.266	0.266	0.266	0.266	0.232	0.232	0.232	0.232	0.236	0.239	0.206	0.207	0209	0.211	0.212	0.214

* Supplied the following per kilogram of diet: vitamin A: 440000 IU; vitamin D: 72000 IU; vitamin E: 14400 mg; vitamin K: 2000 mg; vitamin B_1: 140 mg; vitamin B_2: 3000 mg; vitamin B_6: 612 mg; vitamin B_{12}: 0.015 mg; Folic acid: 0.025 mg; Nicotinic acid: 12160 mg; Calcium pantothenate: 4896 mg; Fe: 80 mg; Cu: 8 mg; Mn: 64.5 mg; Se: 8 mg; I: 640 mg; Zn: 33.8 g and Co: 190 mg.

Although the FCR was reduced in diets containing 1.5% coffee powder, it has been shown to reduce weight gain due to the lipolytic effects of caffeine (Fischer *et al*. 2014). In another study, coffee powder did not alter FCR and other performance indicators in laying hens.

The data relating to the effect of coffee powder on carcass efficiency and relative weight of carcass components involving breast, thigh, abdominal fat, heart, and pancreas are presented in Table 4.

At the end of the experiment, carcass efficiency and relative thigh weight were not affected by treatments receiving different levels of coffee powder. However, the relative weight of the breast in the control treatment was higher than the level of 0.9% coffee powder. The highest and lowest breast relative weights were observed in birds fed 0.9% and 0.3 % coffee powder, respectively ($P<0.05$). The relative weight of the heart and pancreas in control chickens was lower than those fed with coffee powder.

Table 3 Effect of green coffee powder (GCP) on growth performance

GCP % of diet	Body weight gain (g)				Feed intake (g)				Feed conversion ratio			
	1-10 days	11-24 days	25-42 days	1-42 days	1-10 days	11-24 days	25-42 days	1-42 days	1-10 days	11-24 days	25-42 days	1-42 days
Zero	145.0^{b}	545.5^{bc}	1049.7	1740.2	244.1	1006.7	2120.4^{a}	3371.2^{a}	1.68^{a}	1.82^{a}	1.95^{a}	1.84^{a}
0.3	167.5^{a}	636.7^{a}	1007	1811.3	242.2	977.7	1688.9^{c}	2929.0^{c}	1.45^{b}	1.57^{c}	1.68^{b}	1.57^{c}
0.6	156.2^{ab}	604.6^{ab}	1047	1807.6	244.0	997.2	1804.5^{bc}	3045.8^{bc}	1.57^{ab}	1.65^{bc}	1.72^{b}	1.65^{bc}
0.9	155.3^{b}	558.1^{bc}	1124	1837.9	238.0	1005.4	2031.4^{ab}	3275.0^{ab}	1.53^{ab}	1.80^{ab}	1.80^{ab}	1.74^{ab}
1.2	169.5^{a}	530.1^{c}	1078	1777.6	241.9	941.4	1809.3^{bc}	2992.8^{bc}	1.43^{b}	1.77^{ab}	1.67^{b}	1.64^{bc}
1.5	157.7^{ab}	573.6^{abc}	1123	1854.3	243.2	982.6	1845.4^{abc}	3071.7^{abc}	1.54^{ab}	1.71^{abc}	1.64^{b}	1.61^{c}
SEM	5.09	16.97	41.41	63.21	2.43	17.03	67.67	71.80	0.04	0.03	0.05	0.02
P-value	0.0270	0.0025	0.3427	0.5443	0.5333	0.1020	0.0020	0.0013	0.013	0.006	0.0031	0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effect of green coffee powder (GCP) on carcass characteristics (% of live body weight)

GCP, % of diet	Carcass	Breast	Thigh	Heart	Abdominal fat	Gall bladder	Pancrease
Zero	69.43	21.70^{a}	18.53	1.12^{d}	2.10^{a}	0.11^{b}	0.35^{b}
0.3	68.48	20.94^{ab}	18.54	1.24^{bcd}	1.31^{b}	0.26^{a}	0.53^{a}
0.6	69.62	20.71^{ab}	19.06	1.15^{cd}	1.31^{b}	0.14^{b}	0.40^{b}
0.9	67.60	19.60^{b}	18.58	1.26^{bc}	1.16^{b}	0.15^{b}	0.41^{b}
1.2	68.90	20.20^{ab}	18.80	1.42^{ab}	1.1^{b}	0.13^{b}	0.33^{b}
1.5	68.82	20.15^{ab}	18.47	1.54^{a}	1.23^{b}	0.15^{b}	0.37^{b}
SEM	0.61	0.50	0.44	0.0557	0.1186	0.0143	0.258
P-value	0.2489	0.0735	0.9373	0.0001	0.0001	0.0002	0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effect of green coffee powder (GCP) on antibody against sheep red blood cells (SRBC, $\log_2$) and lymphoid organs (% of carcass weight) of broiler

GCP, % of diet	SRBC	IgM	IgG	Spleen	Bursa of fabricius	Liver
Zero	4.20^{b}	1.70	3.50	0.22^{b}	0.10^{b}	4.18
0.3	5.60^{a}	2.30	3.30	0.34^{a}	0.19^{a}	4.15
0.6	5.80^{a}	2.10	3.70	0.26^{b}	0.11^{b}	3.99
0.9	5.30^{ab}	2.20	3.10	0.23^{b}	0.12^{b}	3.85
1.2	5.60^{a}	1.70	3.90	0.24^{b}	0.11^{b}	3.99
1.5	5.90^{a}	1.90	4.0	0.25^{b}	0.11^{b}	3.85
SEM	0.2687	0.2498	0.2531	0.0174	0.0095	0.1844
P-value	0.0004	0.3970	0.1243	0.0002	0.0001	0.7277

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effect of green coffee powder (GCP) on plasma lipid in broiler (mg/dL)

GCP, % of diet	Triglyceride	Cholesterol	LDL	HDL
Zero	109.7^{a}	188.46^{a}	164.62^{a}	63.68^{d}
0.3	107.3^{a}	168.78^{a}	143.49^{a}	71.27^{cd}
0.6	87.83^{bc}	137.45^{b}	99.55^{b}	80.84^{abc}
0.9	81.91^{c}	137.26^{b}	100.38^{b}	75.49^{cd}
1.2	78.97^{c}	132.41^{b}	81.65^{b}	92.54^{a}
1.5	100.14^{ab}	141.07^{b}	104.19^{b}	87.17^{ab}
SEM	3.914	5.158	5.682	3.405
P-value	0.0001	0.0001	0.0001	0.0001

LDL: low-density lipoprotein and HDL: high-density lipoprotein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The highest relative weight of the pancreas was observed in the treatment of 0.3% coffee powder, which probably increases the activity of the pancreas to supply digestive enzymes due to increased feed intake (Hosseini-Vashan *et al*. 2016).

The abdominal fat percentage in the control treatment was higher than in birds fed with coffee powder (P<0.05). Caffeine has been reported to be a possible cause of decreased abdominal fat. The chlorogenic acid of coffee reduces appetite, food intake, and daily caloric intake in humans. This in turn reduces the heat generated in the body. By acting as a peroxisome proliferator-activated receptor α (PPAR) agonist, just like statins, which are used to reduce cholesterol and triglyceride disorders. Green coffee prepares the liver to produce more bile, increasing metabolism and also releasing glucose into the bloodstream (Garg, 2016).

The effect of adding coffee powder on the internal organs of broilers fed with coffee powder shows that the mean liver weight did not show a statistical difference with the control group (Table 5). The mean relative weight of the spleen and bursa of fabricius increased compared to the control (P<0.05). The relative weight of the spleen and bursa of Fabricius was higher at the level of 0.3% coffee powder than other treatments (P<0.05). Increased fat-burning activity of the liver requires more liver activity and bile secretion. Therefore, at 0.3% of coffee powder, bile secretion and relative bile weight have increased (Garg, 2016). On the other hand, the relative weight of Fabricius spleen and spleen, which are involved in the immune response, increased in chickens fed at the level of 0.3%, which may increase the absorption of polyphenolic compounds and their positive effects on the immune response. Likewise, Hosseini-Vashan *et al*. reported that increasing the amount of polyphenolic compounds received by broilers increases the relative weight of lymphatic organs (Hosseini-Vashan *et al*. 2020).

The results related to sheep red blood cell (SRBC) antibody titer showed that the lowest and highest response to SRBC was observed in control and birds fed diets containing 0.3 and 0.6% coffee powder, respectively (P<0.05). The addition of coffee powder did not affect the immunoglobulin M titer against SRBC. However, it increased the immunoglobulin G titer against SRBC so that the control treatment had the lowest immunoglobulin G antibody titer (P<0.05). The antioxidant activity of coffee is related to bioactive compounds, including phenolic and non-phenolic compounds such as chlorogenic, ferulic, caffeine, and coumaric acids (Garg, 2016). Green coffee beans are a good source of polyphenols including chlorogenic acid, 5-caffeoquinacid, and 3,5-dicafuiloquinic acid with multifaceted antioxidant activity. GCBE extracts, 5-caffeic acid and 3,5-decafolic acid, greatly reduced ROS production and restored glutathione concentration and antioxidant enzyme activity in HepG2 cells (Garg, 2016).

High levels of senna coffee in broiler diets cause the production of toxins and reduced performance and immune response and should be detoxified before supplementation with broiler diets (Kontan *et al*. 2019). Previous research on blood biochemical indices and gut microbial populations in broiler chickens has shown that consumption of less than 5% coffee powder can have beneficial effects on liver and health function without producing toxins or adverse effects. (Hyunko *et al*. 2014).

Serum cholesterol, triglyceride, HDL, and LDL concentrations in dietary treatments decreased compared to control treatment (P<0.05). The lowest plasma concentration of cholesterol, triglyceride, and LDL in blood was observed in the treatment of 1.2% coffee powder compared to the control (Table 6). The highest concentration of HDL in blood was observed in chickens fed with the level of 1.2% coffee powder compared to the control (P<0.05). Duskaev *et al*. (2021) reported a reduction in blood cholesterol and LDL concentration in broiler chickens fed plant extract. Chlorogenic acid in coffee reduces the amount of liver triglycerides (Garg, 2016). Other phenolic acids in green coffee extract, namely neochlorogenic acid and ferroluconic acid, increase hepatic carnitine.

Green coffee extract alters the secretion of the hormone insulin and increases glucose tolerance in humans by facilitating glucose uptake from the distal gastrointestinal tract. The anti-obesity effects are caused by changes in plasma adipokine levels, body fat distribution, improper regulation of fatty acids and cholesterol production, and re-regulation of fatty acid oxidation and PPARα expression in the liver, which affects the rate of body fat retention. In addition, it causes the elimination of hepatic triglycerides (Garg, 2016).

Based on these findings, the concentration of albumin, total protein, and glucose were affected by dietary treatments (P<0.05). Plasma glucose and protein concentrations increased in chickens fed coffee powder, compared to the control (Table 7).

Coffee consumption reduces the risk of developing diabetes, and there is an inverse relationship between drinking coffee and the risk of developing diabetes, which is a positive relationship between drinking coffee and lowering blood insulin (Garg, 2016). Green coffee beans reduce the risk of diabetes; However, the exact mechanism of action has not yet been determined. Chlorogenic acid is slowly absorbed from the intestinal wall and acts as an alpha-glucosidase inhibitor to prevent glucose uptake from the human intestine.

Table 7 Effect of green coffee powder on plasma glucose and protein in broiler

Green coffee powder, % of diet	Glucose (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
Zero	234.8^{d}	3.631^{b}	1.340
0.3	248.9^{cd}	4.175^{a}	1.717
0.6	274.9^{cd}	4.232^{a}	1.530
0.9	294.5^{cd}	4.110^{a}	1.630
1.2	288.5^{abc}	4.187^{a}	1.480
1.5	324.8^{a}	4.177^{a}	1.696
SEM	10.65	0.1122	0.0915
P-value	0.0001	0.0034	0.0437

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 8 Effect of green coffee powder on jejunal morphology (µm) in broilers

Green coffee powder, % 0f diet	Villus height	Villus width	Crypt depth	Villus height/crypt depth
Zero	1196^{c}	125.5^{bc}	133.1^{ab}	8.99^{b}
0.3	1277^{a}	120.5^{c}	132.6^{ab}	9.63^{a}
0.6	1294^{a}	119.0^{c}	140.8^{a}	9.19^{ab}
0.9	1198^{bc}	132.0^{ab}	120.7^{b}	9.93^{a}
1.2	1250^{ab}	118.0^{c}	137.1^{a}	9.12^{ab}
1.5	1203^{bc}	136.0^{c}	131.6^{ab}	9.15^{ab}
SEM	11.82	2.062	19.39	8.896
P-value	0.0001	0.0001	0.0191	0.0114

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Glucogenic acid inhibits the enzyme glucose-6-phosphatase, which is involved in gluconeogenesis and glycogenolysis. In addition to being a specific competitive inhibitor of glucose-6-phosphate translocase, when glycogenolysis is inhibited, the body receives energy from fat cells, and the superficial blood decreases with weight loss. Coffee compounds also reduce glucose absorption from the intestine by regulating the concentration of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide I (GLP-I), increasing insulin secretion after oral administration of glucose. In addition, chlorogenic acid increases glucose utilization by muscle by stimulating the enzyme AMPK, specifically, subunit A2, where glucose uptake into the muscle to supply insulin-independent energy. Chlorogenic acid also stimulates glucose-carrying protein type 4 (glut4), which helps insulin transport glucose to the cell. GLUT4 protein is stimulated by phosphorylation of AKT type 2 protein kinase B. Chlorogenic acid and magnesium in green coffee reduce insulin resistance (Garg, 2016). In poultry, blood sugar is regulated by glucagon. Coffee consumption increases blood sugar, which is likely due to differences in its regulatory mechanism compared to humans (Pourreza, 2013).

The data relating to the jejunal morphology were presented in Table 6. Based on these findings, the villi height in the control group was statistically different from chickens that received 1.2% of coffee powder (P<0.05), the highest villi height in birds fed with 0.6% coffee powder, and the lowest was observed in birds fed the control diet (Table 8). Birds fed the 0.3, 0.6, and 0.9% coffee powder had a lower villi width index than the 1.5% coffee powder (P<0.05). The jejunal ratio of villi height to crypt depth was the highest in broilers fed with 1.5% coffee powder (P<0.05). Youssef *et al.* (2021) reported improvements in intestinal morphology in broiler chickens fed probiotics. Bioactive compounds of coffee involving chlorogenic acid and caffeic acid have stimulating effects on the growth of intestinal villi. On the other hand, the proper combination of amino acids and fatty acids in coffee can be another compelling factor in improving the intestinal villi characteristics of broilers fed with coffee powder (Rang *et al.* 2001; Garg, 2016). Polyphenolic compounds have also been reported to play an effective role in developing intestinal villi (Sharifian *et al.* 2019; Hosseini Vashan *et al.* 2020). Polyphenol compounds escape digestion and absorption in the proventriculus and small intestine and accumulate in the hindgut, so they may act as prebiotic substances (Gessner *et al.* 2017), which increase the abundance of the desirable bacteria while reducing that of pathogenic bacteria (Rezvani *et al.* 2016).

CONCLUSION

This research showed that the use of coffee powder at a level of 0.3% in broiler diet had a positive effect on growth performance, reduced plasma lipid, and improved antibody

titers and immune response of broilers. Up to 1.2% Coffee powder improved jejunal morphology, and the highest jejunal morphology was observed in birds fed 1.2% coffee powder. Finally, coffee powder can be used at 0.3% in broiler diets without compromising performance.

ACKNOWLEDGEMENT

This work was supported by the University of Birjand under contract number 53534.

REFERENCES

Árvay J., Šnirc M., Hauptvogl M., Bilčíková J., Bobková A. and Demková L. (2018). Concentration of micro- and macro-elements in green and roasted coffee: Influence of roasting degree and risk assessment for the consumers. *Bio. Trace Elem. Res.* **190,** 226-233.

Bouafou K.J.M., Konan B.A., Zannou T.V. and Kati C.S. (2011). Potential food waste and by-products of coffee in animal feed. *Elec. J. Bio.* **7(4),** 74-80.

Donkoh A., Atuahene C.C., Kese A.G. and Mensah-Asante B. (1988). The nutritional value of dried coffee pulp (DCP)in broiler chickens' diets. *Anim. Feed Sci. Technol.* **22,** 139-146.

Duskaev G., Kvan O., Vershinina I. and Rakhmatullin S. (2021). Assessment of lipid metabolism in broilers against plant extract and probiotic substance or their combined use. *Iranian J. Appl. Anim. Sci.* **11(1),** 161-168.

Feedipedia (2011). Coffee hulls, fruit pulp, and by-products. Tables regions chaudes. A project bu Inra, Cirad and afz with support of FAO, Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Fischer T.W., Herczeg-Lisztes E., Funk W., Zillikens D., Bíró T. and Paus R. (2014). Differential effects of caffeine on hair shaft elongation, matrix and outer root sheath keratinocyte proliferation, and transforming growth factor-β2/insulin-like growth factor-1-mediated regulation of the hair cycle in male and female human hair follicles *in vitro*. *Br. J. Dermatol.* **171(5),** 1031-43.

Frances C., Nnamdi Enoch E., Odir Johnson N., Onyinye Ann O. and Eziamaka A. (2023). Estimation of caffeine and vitamin B- complex (vitamin B2, B3, B5 and B6) constituents of selected energy drinks. *J. Appl. Chem. Sci. Inter.* **14(1),** 42-48.

Garg S.K. (2016). In Nutraceuticals: Efficacy, Safety and Toxicity. Elsevier Inc., London, United Kingdom.

Gessner D., Ringseis R. and Eder K. (2017). Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. *J. Anim. Physiol. Anim. Nutr.* **101,** 605-628.

Hosseini-Vashan S.J., Golian A. and Yaghobfar A. (2016). Growth, immune, antioxidant, and bone responses of heat stress-exposed broilers fed diets supplemented with tomato pomace. *Int. J. Biometr.* **60,** 1183-1192.

Hosseini-Vashan S.J., Sharifian M., Piray A.H. and Fathi Nasri M.H. (2020). Growth performance, carcass and blood traits, immunity, jejunal morphology, and meat quality of heat-stressed broiler chickens fed urea-treated pomegranate (*Punica granatum L.)* peel. *Anim. Feed Sci. Technol.* **267,** 114553-114563.

Hyun Ko Y., Hyun Yun S., Hae Song M., Yun Kim M., Sun Kim J., Wook Kim H. and Surk Jang I. (2014). Effects of dietary supplementation of coffee meal on intestinal enzyme activity, biochemical profiles and microbial population in broiler chicks. *Korean J. Poult. Sci.* **41,** 105-113.

Kontan D.D., Yusuf H.B., Shaahu D.T., Nyameh J. and Antyev M. (2019). Nutritional evaluation and histopathological assessment of unprocessed Senna Occidentalis (*Coffee Senna*) seed meal in broiler chicks. *Nigerian J. Anim. Sci.* **21(1),** 72-79.

Marcel B.K.M., André K.B., Zannou-Tchoko V. and Séraphin K.C. (2011). Potential food waste and by-products of coffee in animal feed. *Elec. J. Bio.* **7(4),** 74-80.

Mendes L.R., Silva R.B., Bueno C.F.D., Couto F.A.P., Dias A.N., Fernandes V. and Faria F.D.E. (2013). The inclusion of coffee in commercial layer diets. *Brazilian J. Poult. Sci.* **15,** 141-144.

Pourreza J. (2013). Scientific and Practical Principles of Poultry Production. Arkan Danesh, Isfahan, Iran.

Rang H.P., Dale M.M. and Ritter J.M. (2001). Farmacologia. Guanabara Koogan, Rio de Janeiro, Brazil.

Rezvani M.R., Rahimi S. and Dadpasand M. (2016). Effect of adding pomegranate peel powder to fat-containing diets on performance of broilers. *Anim. Prod.* **18,** 335-346.

SAS Institute. (2004). SAS®/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.

Sharifian M., Hosseini-Vashan S.J., Fathi Nasri M.H. and Perai A.H. (2019). Pomegranate peel extract for broiler chickens under heat stress: Its influence on growth performance, carcass traits, blood metabolites, immunity, jejunal morphology, and meat quality. *Lives. Sci.* **227,** 22-28.

Skinner-Noble D.O. and Teeter R.G. (2004). Components of feed efficiency in broiler breeding stock: the use of fasted body temperature as an indicator trait for feed conversion in broiler chickens. *Poult. Sci.* **83(4),** 515-520.

Yi-Fang C., Wen-Han C., Richard M.B., Jia-Ren L., Pradoldej S., Yumin C., Huilin W., Qiuyan Z. and Irene H.C. (2012). Crude caffeine reduces memory impairment and amyloid β1–42 levels in an Alzheimer's mouse model. *Food Chem.* **135(3),** 2095-2102.

Youssef I.M.I., Abdel-Razik A.H., Aboelhadid S.M., Arafa W.M., Shany S.A. and Abdel-Daim A.S.A. (2021). Comparative effects of dietary saponin and probiotic supplementation on performance, carcass traits and intestinal histomorphology of broilers challenged with *Eimeria tenella*. *Iranian J. Appl. Anim. Sci.* **11(1),** 147-159.