ISSN: 2790-5306 (Print), 2790-5314 (Online)
IRAQI JOURNAL OF AGRICULTURAL RESEARCH - Ministry of Agriculture
Available online at: www.ijarmoa.gov.iq
VOL. 28 NO. (2) 2024
IJAR
IRAQI JOURNAL OF AGRICULTURAL RESEARCH

EFFECT OF ADDING DIETARY LOCAL GUJARAT (*Hibiscus sabdariffa* L.) POWDER ON SOME BIOCHEMICAL ATTRIBUTES, ANTIOXIDANTS AND VISCERAL MICROBAL CONTENTS OF BROILER*

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ABSTRACT

This study aimed to investigate the influence of dietary local Gujarat (*Hibiscus sabdariffa* L.) powder (LGP) supplementation on some biochemical attributes, antioxidants, and visceral bacterial contents of the broiler. This study was conducted at the Poultry Farm belonging to the Animal Production Department, College of Agriculture, Shoraw, University of Kirkuk. Three hundred unsexed one-day-old chicks of Ross 308 broiler were divided randomly into six treatments, with five duplicates per treatment and ten birds per replicate. The birds were fed on diets supplemented with LGP at 0.5, 1, and 2% for the treatments T3, T4, T5, and T6 respectively, while the T2 group was supplemented with BHT. The results showed a significant (P≤0.05) decrease in the concentration of LDL, VLDL cholesterol, triglycerides, MDH, and the kidney and E. coli bacterial count for the groups supplemented with Gujarat powder compared to the control group. A significant (P≤0.05) increase in HDL, GSH concentrations, and lactic acid bacteria count was observed for the sixth treatment compared with the control group. The differences in the total protein, albumin, and globulin concentrations for all treatments lacked significance. Moreover, a significant increase in glucose concentration was noticed in groups supplemented with LGP. In conclusion, LGP supplementation can improve some blood biochemical attributes and broiler chickens' antioxidant and visceral lactic acid bacterial contents.

Keywords: *Hibiscus sabdariffa*, Cholesterol, Malondialdehyde, Glutathione, Broiler chicken.

*** A part of Ph. D dissertation for the first author.**

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- **Received:** January 11, 2024.
- **Accepted:** February 14, 2024.
- **Available online:** December 25, 2024.

INTRODUCTION

The consumption of broiler meat has increased, both locally and internationally, due to the high demand for it [1]. Broiler production is considered one of the most crucial poultry industry sectors and has witnessed significant development compared to other animal products. A high volume of broiler productivity was found, regarded as one of the principal essential food sources used [2]. Medicinal plants have been used recently in consumers' consumption of animal protein because they contain effective compounds [3]. Additives are used to use natural materials instead of raw materials and chemicals to maintain consumer health, prevent accumulation in poultry meat, and improve production [4]. These natural products are also rich in antioxidants [5]. Therefore, the poultry meat industry is linked to plant production because it is involved in the composition of feed [6]. Hibiscus sabdariffa is a culinary and medicinal plant with healing qualities [7]. Local Gujarat (*Hibiscus sabdariffa*) powder (LGP) contains tartaric and citric acids, which are acids that enhance the absorption of nutrients and improve intestinal flora by increasing beneficial bacteria and inhibiting harmful bacteria, thus enhancing the availability of nutrients in the intestines [8]. It is also considered a powerful antioxidant because one of the essential functions of Hibiscus sabdariffa flowers is to protect the cells from destruction. After all, it contains good anthocyanin that reduces the incidence of cancerous diseases and increases the immunity of birds [9]. This study aimed to explore the effect of different levels of dietary LGP supplementation on some biochemical attributes, antioxidants, and visceral microbial contents of broiler chicken.

MATERIALS AND METHODS

Blood samples were taken via jugular venipuncture at the age of 42 days. The blood was collected from one bird from each replication. These tubes were placed in the refrigerator for 12 hours and then transported to the centrifuge (3000 rpm for 15 minutes). The serum was harvested and stored under-20 Celsius until assay.

Serum cholesterol assay

Serum cholesterol concentration was assessed according to the enzymatic method described by Friedewald et al. [10] using a kit manufactured by Biolabo-France and an enzymatic process. Samples were read using a 500 nm wavelength spectrophotometer.

Serum high-density lipoprotein (HDL) assay

Serum HDL was assayed via the enzymatic analysis method reported by [11] using a kit manufactured by Biolabo-France. Samples were read using a 500 nm wavelength spectrophotometer.

Serum low-density lipoprotein (LDL) assay

Serum LDL concentration was determined using the following equation reported by Loans, C. [11].

LDL concentration (mg/dL) = total cholesterol concentration – (vLDL concentration + HDL concentration).

Serum triglycerides assay

Serum triglyceride concentrations were estimated based on the method described by Maggawa et al. [12] using a kit manufactured by Biolabo-France. Samples were read using a 546 nm wavelength spectrophotometer.

Table 1: Ingredient percentage of the experimental starter, growth and finisher diets

Ingredients	Starter (1-10 days)	Growth (11-24 days)	Finisher (25-42 days)
wheat	49	52.34	65.46
Yellow corn	10.30	10.00	0
Soybean meal	30.20	26.35	21.85
Salt	0.10	0.10	0.10
Sunflower oil	3.15	4.26	5.77
Limestone	1.93	1.7	1.80
Dicalcium Phosphate	0	0	0
Animal protein	5.00	5.00	5.00
Methionine	0.12	0.08	0.09
Lysine	0.10	0.07	0.08
Choline chloride	0.10	0.10	0.10
Total	100	100	100
Metabolic energy	3000	3100	3200
Calculated Crude Protein	23	21.51	20
calcium %	0.96	0.86	0.81
Methionine%	0.58	0.52	0.51
Lysine %	1.28	1.15	1.06

***According to the US National Research Council (Table 1) [17].**

Serum malondialdehyde assay

Serum malondialdehyde concentration as the final product of the lipid peroxidation process was assayed according to the method reported by Margesi et al. [13] using thiobarbituric acid (TBA). The reaction method for thiobarbituric acid (TBA). The measurement was based on the interaction between lipid peroxides represented by malondialdehyde with acid. Thiobarbituric in a medium that depends on the pH function.

Serum glutathione assay

Serum glutathione concentrations were determined using the Ellman according to the procedure described by Margesi *et al.* [13].

Estimation of kidney, colon and lactic acid bacterial count

The small intestine was cut from the jejunum area after slaughtering the birds, and 10 gm of its contents were taken and added to a 90 ml physiological saline solution under sterile conditions, the surface spreading method used, according to Chen et al. [8]. Through the solidified media, the numbers of total bacteria, Mac Con Key Agar, the quantities of lactic acid bacteria, MRS Agar, and coliform bacteria are estimated by transferring 0.1 ml of Decimal diluent through a micropipette to two Petri dishes prepared in advance for their culture medium. , and spread on the surface of the solidified medium through a sterile curved glass rod that resembles the letter. The unique dishes for total aerobic and coliform bacteria were incubated upside down at 37°C for 24 hours, and the special dishes for lactic acid bacteria were set upside down, away from the air, at 37°C for 48 hours. The growing colonies were observed in the three media, and the dish was chosen. Good cultivation in the growth of the number of colonies for each of the two layers of decimal dilution. Colony Foming Units (cfu) were calculated and multiplied by the reciprocal of the dilution to obtain the number of bacterial

colonies per gram of fasting sample. The bacterial concentrations were converted to logarithmic numbers to the base ten and expressed as log 10 and t m. /gloom.

Statistical analysis

Data were statistically analyzed via the SAS program [8] using a completely randomized design to study the effect of LGP and BHT on different parameters. Significant means were compared using the Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Table 2 revealed a significant (P≤0.05) decrease in cholesterol (135.9 ± 1.2 mg/dl), LDL (79.25 ± 0.6 mg /dl), and triglycerides (39.35 ±0.2 mg /dl) concentrations of the T6 treatment compared to the control group. Concomitantly, a significant (P≤0.05) increase in HDL of the T6 treatment (81.35 ± 1.1 mg /dl) was observed compared to the control group. The reason behind lowering cholesterol concentration is attributed to the use of the LGP, which uses cholesterol to manufacture bile acids in liver cells because it contains an active ingredient that leads to lowering cholesterol in the blood [7] and also reducing cholesterol levels [16] due to the presence of active ingredient cocosides, hepsin hydrochloride, and phenolic substances in its leaves and containing essential fatty acids, including urinary palmitic and oleic [17]. It helps reduce fat and prevent serious diseases, including high blood cholesterol and cardiovascular disease [5]. Also, Sadeq, M. and M. AL-Neemi [18] found in their study that adding different levels of Gujarat did not affect, significantly the lipids profile.

Table 2: Effect of different levels of dietary local Gujarat powder supplementation on serum lipid profile of broiler chicken (Mean ± SE)

Parameter	Treatments					
	T1	T2	T3	T4	T5	T6
Cholesterol (mg/dL)	214.9±0.4a	192.1±5.9b	189.6±1.4b	176.7±0.9c	168.1±1.2c	135.9±1.2d
HDL (mg/dl)	54.5±1.3d	61.8±4.9c	66.1±0.8bc	68.15±.3b	71.7±0.3b	81.35±1.1a
LDL (mg/dl)	127.7±0.6a	100.7±0.4b	94.2±1.0c	87.7±1.0d	83.1±0.05e	79.25±0.6f
Triglycerides (mg/d	66.95±0.4a	61.7±0.8b	51.85±0.4c	42.65±2.5d	41.1±4.0d	39.35±0.2d
vLDL(mg/dl)	37.79±1.41a	36.95±1.22ab	35.88±1.22b	34.12±1.25b	32.71±0.71c	32.37±0.71c
Significance level	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05

Means with different superscripts within each row indicated significant differences (P≤0.05) among treatments.

T1: control treatment (Concentrate diet); T2: 0.02 mg BHT; T3: 2% local Gujarat powder (LGP); T4: 1.5% LGP; T5: 1% LGP; T6: 0.05% LGP.

Table 3 showed non-significant differences in total protein, albumin, and globulin concentrations for all treated groups. A significantly (P≤0.05) higher glucose concentration in the T3, T4, T5, and T6 treated groups than in the control group. The LGP contains essential fatty acids, the most important of which are oleic acid, linolenic acid, and palmitic acid, as well as phenolic compounds and flavonoids. It has a role in protecting meat proteins and preserving the nutritional value of the meat. These fatty acids are also beneficial for chickens. Consequently, a prominent effect of the extracts on the juiciness of the birds' muscles was observed [19]. Gujarat contains a percentage of beta-carotene and good levels of glucose, which are essential elements in maintaining the value of meat [20]. The

LGP contains juices that activate the juices within the digestive system by secreting digestive enzymes, which help with metabolism [8]. The secretion of digestive enzymes breaks down fats and proteins, especially pancreatic enzymes, and inhibits fat oxidation [9].

Table 3: Effect of different levels of dietary local Gujarat powder supplementation on serum proteins and glucose concentrations of broiler chickens (Mean ± SE)

Parameters	Treatments					
	T1	T2	T3	T4	T5	T6
Total protein (g/dl)	2.88±0.9	2.87±1.7	2.71±1.2	2.53±3.4	2.41±1.7	2.38±3.6
Albumin (g/dl)	1.220±0.4	1.126±0.7	1.120±0.9	1.030±0.7	1.026±2.1	1.013±2.0
Globulin (g/dl)	1.613±2.0	1.613±1.2	1.596±0.4	1.503±1.2	1.386±2.8	1.373±5.1
Glucose (mmol/l)	200.8±0.2b	207.3±0.1b	234.1±0.4a	238.6±0.5a	239.2±16.8a	238.8±4.3a
Level of significance	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05

Means with different superscripts within each row indicated significant differences (P≤0.05) among treatments.

T1: control treatment (Concentrate diet); T2: 0.02 mg BHT; T3: 2% local Gujarat powder (LGP); T4: 1.5% LGP; T5: 1% LGP; T6: 0.05% LGP.

The LGP contains juices that activate the juices within the digestive system by secreting digestive enzymes, which help with metabolism [8]. The secretion of digestive enzymes breaks down fats and proteins, especially pancreatic enzymes, and inhibits fat oxidation [9]. MDA and chlorination are considered an indicator of antioxidants in the first and second indicators of lipid peroxidation. The reason behind that may be attributed to the flowers of the plant Hibiscus sabdariffa, which contain phenolic compounds that work to inhibit lipid oxidation [9]. The LGP contains high percentages of the phenolic compound. This leads to the protection of liver cells [21] because the active ingredient cocosides, hepsin hydrochloride, and phenolic substances found in its leaves, which stop the activity of free radical formation and slow down the formation of peroxides, and hydroperoxides reduce the formation of malondialdehyde [15]. Moreover [22] found that adding Gujarat with different levels did not significantly affect the albumin, total protein, and cholesterol.

Table 4: Effect of different levels of dietary local Gujarat powder supplementation on serum malondialdehyde and glutathione concentrations of broiler chickens (Mean ± SE)

Parameter	Treatments					
	T1	T2	T3	T4	T5	T6
MDA (mmol/l)	0.908±0.02a	0.841±0.03b	0.756±0.02c	0.728±0.01c	0.718±0.02cd	0.708±3.6d
GSH (mmol/l)	27.63±3.5c	30.52±7.3b	30.67±5.2b	30.72±4.2b	32.04±4.2ab	32.51±8.5a

Means with different superscripts within each row indicated significant differences (P≤0.05) among treatments.

T1: control treatment (Concentrate diet); T2: 0.02 mg BHT; T3: 2% local Gujarat powder (LGP); T4: 1.5% LGP; T5: 1% LGP; T6: 0.05% LGP.

Table 5 revealed a significant (P≤0.05) decrease in the kidney and E. coli bacterial count of the T6 treatment compared to the control group. Higher (P≤0.05) increase in the lactic acid bacterial count of the T6-treated group than the control group. Gujarat extract consists of active compounds such as saponins, glycosides, alkaloids, and flavonoids. It also represents antibacterial activities against Bacillus stearothermophilus, Clostridium sporogenes, and Klebsiella. This is the reason behind reducing the aerobic bacterial count in poultry meat treated with Gujarat powder. It works as an antibacterial agent against pneumonia, Staphylococcus aureus, Escherichia coli, and Bacillus bacteria, the protein that breaks down the cell wall. The effect of currants is to inhibit bacterial growth and protect the cell wall, thus reducing microbial growth [23]. Phenolic compounds facilitate digestion processes, activate beneficial bacteria in the intestines, and inhibit the proliferation of harmful bacteria [9]. The LGP stimulates the digestive system by increasing the secretion of digestive enzymes. The calyx leaves of Gujarat contain 3-4% tartaric acid and citric acid, which increase the absorption of nutrients, increase beneficial bacteria in the intestines, and improve the intestinal flora of the stomach [8].

Table 5: Effect of different levels of dietary local Gujarat powder supplementation on some blood bacterial contents of broiler chickens (Mean ± SE)

Parameter	Treatments					
	T1	T2	T3	T4	T5	T6
Preparation of total bacteria	8.89±0.11a	7.44±0.10b	7.12±0.12a	7.11±0.13a	6.65±0.11c	6.22±0.18e
E. coli	6.32±0.18a	6.32±0.17a	6.23±5.2ab	5.59±0.11b	5.33±0.15b	5.28±0.11c
Lactic acid	5.23±0.11	5.61±0.15a	6.34±0.18c	6.67±0.19b	6.92±0.15a	6.98±0.11
Level of significance	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05	P≤0.05

Means with different superscripts within each row indicated significant differences (P≤0.05) among treatments.

T1: control treatment (Concentrate diet); T2: 0.02 mg BHT; T3: 2% local Gujarat powder (LGP); T4: 1.5% LGP; T5: 1% LGP; T6: 0.05% LGP.

Conclusions

Applying dietary LGP decreases the serum cholesterol, LDL, and e triglycerides, and increases serum HDL, glucose, glutathione, and lactic acid bacterial count. A reduction in MDE, and the kidney and E. coli bacterial count was noticed, thus raising the bird's body immunity.

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ISSN: 2790-5306 (Print), 2790-5314(Online)
مجلة الزراعة العراقية البحثية - وزارة الزراعة
متاح على الانترنت: www.ijarmoa.gov.iq
مجلد 28 العدد (2) 2024
IJAR
IRAQI JOURNAL OF AGRICULTURAL RESEARCH

تأثـــير اضافة مستويات مختلفة مـــن مسحـــوق الكجرات (*Hibiscus sabdariffa* L.) الى العليقة في بعض الصفات الكيماحيوية ومضادات الاكسدة والمحتوى المايكروبي لأحشاء فروج اللحم*

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المخلص

أجريت هذه الدراسة بهدف بيان تأثير اضافة مستويات مختلفة من مسحوق الكجرات (*Hibiscus sabdariffa* L.) الى العليقة في بعض الصفات الكيماحيوية ومضادات الاكسدة والمحتوى البكتيري لأحشاء فروج اللحم. نفذت هذه الدراسة في حقول الطيور الداجنة التابعة لبحوث الانتاج الحيواني في كلية الزراعة/ شوراو- جامعة كركوك. تم استخدام **300** فروج بعمر يوم واحد غير المجنس لمدة **42** يوماً مكون من **6** معاملات مقسمة الى **5** مكررات لكل مكرر **10** طيور تشمل المعاملة الاولى السيطرة والثانية تحتوي على **0.05** ملغم من مادة **BHT** (**Butylated Hydroxyl Toluene**) والمعاملات الثالثة والرابعة والخامسة والسادسة تحتوي على **0.5**، **1**، **1.5**، **2%** من *Hibisscus Sabdariffa* L. على التوالي. أظهرت النتائج وجود فروق معنوية على مستوى ($p\leq0.05$). وجد انخفاضاً معنوياً في تراكيز الكوليسترول والكليسيريدات الثلاثية و**MDE** و**VLDL** و**LDL** واعداد البكتيريا الكلية وبكتيريا القولون في المعاملة السادسة مقارنة مع معاملة السيطرة وحصول ارتفاع معنوي في تراكيز **HDL** والكلوكوز و**GSH** وبكتيريا الحامض وبكتيريا اللاكتيك في المعاملة السادسة مقارنة مع مجموعة السيطرة، وعدم وجود اي فروق معنوية في البروتين الكلي والالبومين والكلوبيولين للدم. يمكن الاستنتاج بأن إضافة مسحوق الكجرات المحلي الى عليقة فروج اللحم حسن من بعض معايير الدم الكيمياحيوية ومضادات الأكسدة ومحتوى الاحشاء الداخلية من البكتيريا.

الكلمات الدالة: النبات الكجرات، الكوليسترول، المالونوالديهايد، الكلوتاثيون، دجاج فروج اللحم.

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- تاريخ تسلم البحث: 2024/1/11.
- تاريخ قبول البحث: 2024/2/14.
- متاح على الانترنت :25/كانون اول/2024.