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Evaluation the Effect of Stevia Leaves Powder on Antioxidant, Digestive Enzyme and Meat Quality in Broiler Chickens

Zainab Khalil Ibrahim, Nameer A. Khudhair, Measem Hassan Ali

Department of Public Health , College of Veterinary Medicine, University of Basrah, Iraq.

Corresponding Author Email Address: nameer.khudhair@uobasrah.edu.iq

ORCID ID: https://orcid.org/0000-0001-8130-3254

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Abstract

Recently, there is a rising demand for poultry meat and egg products, Which prompted investors to developed large-scale manufacturing, which involves raising bird weights and productivity and commercial poultry farming is booming. This research aims to assess the effects of stevia leaf powder on oxidative status, digestion, and meat quality in broiler chickens. This study was conducted from December 21, 2023, to January 17, 2024. A total of 240 Ross broiler chicks were obtained from Babylon's Modern Al-Bakri Hatchery chicks reared along 28 days and divided into four underwent various treatments. Group 1 (control) had a regular diet, whereas Groups 2, 3, and 4 received 10, 20, and 30 (g/kg diet) Stevia leaf powder. Blood and meat samples were collected for further analysis. STV-treated groups exhibited substantially greater amylase levels ($p \leq 0.05$). The STV of 30g/kg diet group exhibited higher SOD, CAT, and GLUpx activity than other groups. STV addition lowered meat pH, improving tenderness and shelf life, but it increased cooking loss. The 30g/kg STV group had lower drip loss, suggesting better meat quality and water retention.
The results revealed that Stevia leaf powder improves broiler hens' digestive enzymes (amylase), antioxidants defense mechanism, and meat quality through improved beef tenderness and shelf life by reducing drip loss and pH. These data suggest broiler chickens should consume 30g/kg STV to boost antioxidant activity and meat quality.

Keywords: Stevia Leaves Powder, Broiler Chickens, Antioxidant, Digestive Enzyme.

Introduction

The poultry industry has relied on the development of large-scale production, which involves improving poultry weights and production in line with the volume of increasing demand, to meet the growing market demand for its meat and egg products. Commercial poultry production has become an accelerated industry nowadays (1). Given its numerous benefits, including a low-price tag, an easy digestive system, and a high protein content. Chicken is quickly becoming the most consumed meat in the world (2). One of the biggest obstacles in poultry production is finding ways to make broiler hens, that are primarily raised for their meat, behave better. Broiler chickens need a varied and balanced diet that includes all the nutrients they need to grow to their full potential. There are valid worries regarding the potential harmful impacts of antibiotics used as growth stimulants in chicken feed on human health, environment, and quality of the meat produced by the birds (3). antibiotics in feed have thus been subject to restrictions or outright bans in a number of nations (4). In light of this, scientists have been looking into natural additives as a way to boost broiler chicken health and growth without adding to antibiotic resistance or waste (5) Stevia, a South American natural plant, is one of the most promising plant extracts for broiler chickens (6, 7). Stevia contains steviol diterpene glycosides, which are sweeteners used in food (8,9). Sativoside and rebaudioside are examples of these molecules. In addition to the phenolic compounds, proteins, carbs, lipids, dietary fibers, oils, and vitamins found in stevia leaves and other components. There are approximately 11.2–16.0 grams of protein, 61.9 grams of carbs, 1.9–3.73 grams of lipids, and 6.8–15.2 grams of dietary fiber in 100 grams of dried Stevia leaves (10, 11). The growth performance, intestinal health, and digestive enzyme activity of broiler chickens can be enhanced by stevia extract, according to recent studies (12, 13, 14). The beneficial effects of stevia on gut health and nutrient absorption have been linked to increase feed conversion efficiency and a higher rate of body weight gain in broiler chickens supplemented with stevia leaf powder (15). Supplementing broiler chicks with stevia increased their breast meat yield and decreased their belly fat content, stevia may improve glucose and fat metabolism, which in turn promotes lean muscle gain and decreases fat storage (16). The antioxidant activities of stevia and its effect on digestive enzymes in broiler chickens do not previously discuss. Therefore, the present study aimed to evaluate the impact of stevia leaf powder on oxidative status, digestion and their reflection on meat quality parameters in broiler Chickens.

Materials and Methods

This research took place at a chicken-specific home on Al-Hilla / Street 80. Research began on December 21, 2023, and ended on January 17, 2024. This experiment examines the effects of different Stevia leaf powder dosages over 28 days. In present study, 240 male and female Ross broiler chicks, one day old and 40 to 46 grams at

birth were used. Chicks were bought from private Babylon's Modern Al-Bakri Hatchery and reared in cutter (Measurements for each cutter: 160 x 150 x 90 cm for length, breadth, and height). The chicks were divided into four groups equally (60 chick for each) and each group distributed into 3 replicates (20 chicks for each replicate). All birds were fed on pellets obtained from Al Ghadeer Feed Factory's. Growth and starter diets are the main types of pellets introduced to chicks depending on its age. First week of life optimum temperature was 33°C with 60–70% relative humidity, then temperature fell two degrees per week till 20-22°C. To acclimate the chicks to darkness, it provided 23 hours of light and 1 hour of darkness. All birds supplemented with vitamins and minerals depending on its need along the study period, and provided with vaccination program to ensure healthy chicks.

This study designed into, first group (control) chicks received ordinary ration, While the second group, STV (10 g/kg diet), chicks were fed standard ration and Stevia leaves powder at 10 g/kg diet for 28 days. In the third treatment, STV 20 g/kg, chicks were fed a conventional diet with 20 g/kg diet Stevia leaf powder for 28 days. However, the fourth treatment (STV 30g/kg diet) chicks received regular ration and Stevia leaves powder 30g/kg diet for 28 days. Natural Stevia leaves were brought from the local markets in the province of Basrah, they were grounded and added to the diet.

At the end of experiment (after 28 day), blood and meat sample were extracted from the chicks for further analysis and measurement.

Blood was separated into serum by centrifuged into 4000rpm/minute for 10 minutes, then amylase and lipase enzymes were measured by using Biolabo Assay Kit for spectropyrometer at 405nm and 550nm, respectively (19) (20). Antioxidant activity was evaluated for the birds represented by Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione (GSH) enzymes. The measurement of antioxidant enzymes activity was done by using special ELISA kit (hen SOD ELISA kit, chicken catalase (CAT) ELISA kit, quantitative chicken glutathione peroxidase (GSH)). These ELISA kits purchased from sun long, biotech/China. This kit employed sandwich-ELISA kit and measured at 440nm, 450nm and 412nm, respectively.

After 28 days, each group selected 20 broiler chicks weighing an average of 2 kg. meat quality was evaluated by bloodlet, scalded, plucked, eviscerated, and chilled. Measurements included cooking loss depend on Rasmussen and Mast methods estimated cooking loss (17). Drip loss was done through weighted samples and refrigerated at 5ºC for 24 hours. After that, they were weighed again and depend on formula according to (18). PH of meat was assessed through homogenizing 10 g of samples with 50 ml of distilled water for 60 seconds and two measurements were taken for each sample. Statistical analysis was done through application of SPSS program virsion22 to determine statistical significance between variables. Results were assessed using analysis of variance

(ANOVA) testing. A p-value <0.05 with 95% confidence interval is considered significant.

Results

The measurement of digestive enzymes levels is an indicator to the ability of digestion process and benefit from the feed consumed from the broiler chickens. As illustrated in table (1), STV treated group's recorded significant elevation (p≤ 0.05) in the level of amylase enzyme when compared with control group. In contrast, there were no significant effect among groups treated with STV under different concentration While results showed non-significant (p≤ 0.05) difference between STV treated groups and control group in the level of lipase enzyme although there was slightly increase in fourth group that had 3% of STV.

Table (1): Effects of different levels of STV addition on digestive enzymes of broiler chicken over four weeks (Mean ± S.E.).

Treatments	amylase (U/L)	lipase (U/L)
Control	260.2 ± 2.81 b	4.4 ± 0.23 b
STV 10g/kg	342.8 ± 14.79 a	5.12 ± 0.26 ab
STV 20g/kg	354.4 ± 9.11 a	5.38 ± 0.44 ab
STV 30g/kg	353 ± 9.88 a	6 ± 0.37 a
Significant	S	S

Small letter represents significant difference among group at (p ≤ 0.05), S: mean significant

The antioxidant activities of superoxide dismutase (SOD), catalyze (CAT), and glutathione peroxidase (GLUpx) were measured to evaluate the antioxidant activities in broiler chicken fed on standard ration and supplemented with STV 10g/kg, STV 20g/kg, and STV 30g/kg of stevia leaves powder. The results in table (2) revealed significant increase (p ≤ 0.05) in antioxidant enzymes activities (SOD, CAT, GLUpx) in chicken supplemented with 20g/kg of STV compared with other studied groups. While the second treatment that supplemented with 10g/kg of STV showed significant elevation (p ≤ 0.05) in their antioxidant enzymes activities compared with broiler chickens fed with 10g/kg STV supplement group and control group. In contrast, the first treatment that broiler chickens fed with 10g/kg STV appeared non-significant difference (p ≤ 0.05) for studied antioxidant enzymes values (SOD, CAT, GLUpx). STV-treated groups (STV 10g/kg, STV 20g/kg, and STV 30g/kg) exhibited significantly lower pH values in their meat compared to the control group. A lower pH can contribute to improve meat tenderness and shelf life. STV supplementation was associated with slightly increased cooking loss, but the differences were not as pronounced as for pH. The STV-treated groups, particularly STV 20g/kg, showed significantly lower drip loss compared to the control. Drip loss is a measure of water retention in meat, and lower drip loss can indicate better meat quality. The results were shown in Table (3).

Table (2): Effects of different levels of STV addition on antioxidant enzymes activities of broiler chicken (Mean ± S.E.).

Treatments	SOD (pg/ml)	CAT (pg/ml)	Glutathione (ng/ml)
Control	877.2 ± 35.49 d	24.8 ± 0.40 c	2.43 ± 0.11 c
STV 10g/kg	1027.2 ± 34.54 c	26.8 ± 0.88 bc	2.86 ± 0.09 bc
STV 20g/kg	1206.2 ± 52.75 b	30.89 ± 1.62 b	3.22 ± 0.12 ab
STV 30g/kg	1421.6 ± 55.99 a	40.18 ± 2.08 a	3.6 ± 0.27 a
significant	S	S	S

Small letter represents significant difference among group at ($p \leq 0.05$), S: mean significant

Table (3): Effects of different levels of STV addition on meat quality of broiler chicken (Mean ± S.E.)

Treatments	ph meat	cooking loss	Drip loss
Control	6.57 ± 0.03 a	4.1 ± 0.05 b	5.13 ± 0.23 a
STV 10g/kg	6.1 ± 0.05 b	4.23 ± 0.08 ab	5.22 ± 0.31 a
STV 20g/kg	6.1 ± 0.05 b	4.2 ± 0.05 b	4.54 ± 0.56 c
STV 30g/kg	5.6 ± 0.05 c	4.43 ± 0.03 a	4.9 ± 0.74 b
Significant	S	S	S

Small letter represents significant difference among group at ($p \leq 0.05$), S: mean significant

Discussion

Table (1) shows that adding Stevia to broiler chicken feeds enhances digestion, (21) that showed greater amylase enzyme levels in STV-treated groups ($p \leq 0.05$) compared to the control group. Amylase converted carbohydrates into faster-absorbing sugars. (22) said amylase digested starches and complex carbs. Stevia supplementation increased amylase levels in STV-treated pigeons, improving starch digestion and feed utilization. Increasing energy available would help birds to grow and perform. High amylase levels facilitated small intestine carb breakdown and sugar absorption. STV increased feed conversion efficiency and weight gain, indicating these animals used their diet of nutrients better (23). Stevia may promote digestive health by boosting gut flora or anti-inflammatory properties, as seen by amylase levels (24). Broilers' stomachs must be healthy to absorb nutrients and produce enzymes for digestion and growth. Higher amylase levels gave broiler chicks more energy from the same meal, which was crucial because carbohydrates were their major energy source. Metabolic efficiency may explain where the STV groups gained weight and consumed less feed to obtain the same or higher energy levels (25). Amylase may be affected by stevia. Stevia's bioactive components may boost intestinal enzyme function or pancreatic enzyme secretion (26). The control and STV groups had similar lipase enzyme levels, indicating that stevia supplementation did not impact broiler chickens' fat digestion. The 3% STV group rose somewhat. Lipases convert dietary lipids into glycerol and fatty acids for energy or storage (27). Due to its greater amylase levels, stevia affects carbohydrate

digestion more than fat digestion. STV 3% exhibited a modest lipase rise, but not enough to warrant further investigation. Stevia's bioactive components may impact carbohydrate metabolism more than fat digestion (25). Stevia supplementation had less effect on lipase enzyme development and modulation in broiler diets with lower fat-to-carbohydrate ratios. (27) found that low-fat broiler chicks' lipase activity and production may not have altered after Stevia introduction. The higher amylase than lipase increase may be because broiler chicks ate largely carbohydrates. Most broiler diets are carb-based, therefore Stevia may increase carbohydrate metabolism more than lipase (28). STV 3% increases lipase somewhat but not dramatically. This suggests larger Stevia dosages work. Stevia may influence lipase synthesis slowly or at greater amounts (29). Stevia may indirectly alter fat metabolism but somewhat boost lipase production, resulting in non-significant differences between treatment and control groups (30). Broiler chickens' lipase activity was high enough to break down dietary fat (31). Table (2) reveals that broiler chicks given 20g/kg Stevia (STV) had higher SOD, CAT, and GLUpx levels than other groups. The dietary supplement Stevia may reduce oxidative stress (32). Additionally, hens fed 20g/kg STV exhibited considerably greater SOD, CAT, and GLUpx activity, showing Stevia may improve antioxidant defenses. These enzymes eliminate ROS to protect cells from oxidative damage. The group with the greatest antioxidant enzyme activity was 20g/kg STV, indicating this may be the best dosage for antioxidant responses. Stevia's bioactive components were more accessible at this concentration, which may boost enzyme activity or synthesis (33). The 10g/kg STV group had considerably greater antioxidant enzyme activity than the control group, but not as much as the 20g/kg group. This suggests that Stevia's antioxidant capacity-boosting action may be dose-dependent, with larger doses having a greater effect at lower concentrations (34). The lack of significant differences in SOD, CAT, and GLUpx activities between the first treatment group (probably the control group or another low STV concentration) and itself and the control group suggests that Stevia's antioxidant effects may not be apparent at lower concentrations or may take time to appear Stevia raises antioxidant enzyme activity till a threshold (35). Some stevia bioactives are antioxidants. Flavonoids and phenolic acids may directly scavenge free radicals or boost antioxidant enzymes (29). Compounds may minimize oxidative stress and enhance poultry health and performance (36). (27) suggest that oxidative stress may influence broiler chicken growth, immunological function, and health. Stevia may relieve stress by increasing antioxidant enzymes. Stevia supplements may boost antioxidant protection and development. Studies might examine Stevia's antioxidant bioactive components. The long-term impact of Stevia supplementation on broiler chicken health and output may be interesting (37). Table (3) shows that Stevia (STV) (10g/kg, 20g/kg, and 30g/kg) supplementation increased cooking loss and lowered meat pH relative to the control group. STV-fed meat had lower pH values, suggesting Stevia supplementation affected muscle tissue

metabolic processes after slaughter (38). Lower pH increases protein denaturation, softening meat, according to Stamataki et al. Quality of meat and customer service rely on this. Reducing pH, which inhibits microbial development, may improve meat quality and shelf life. Fresher meat may appeal to customers (39). Adding STV somewhat increases cooking loss, suggesting the pH increased softness and moisture loss (40).Water evaporates naturally when cooking, but too much diminishes meat juiciness and taste. (41) said balancing softness and water retention was difficult. The lower pH softens the meat, but the increased cooking loss makes it less juicy. Tender beef was vital while shopping. Consumers may prefer STV-treated birds' meat because lower pH softens it. To achieve softness and moisture preservation, meat processors may need to account cooking loss (42). Stevia influences post-slaughter muscle metabolism and glycogen breakdown, lowering meat pH. Stevia's bioactive antioxidants may alter meat quality after death (43). Stevia (STV), especially 20g/kg STV, decreased drip loss compared to the control group. This amazing finding affects meat quality greatly. (44) found drip loss in beef throughout storage, heating, and processing. Key indicator of meat quality and water retention. Because meat with reduced drip loss retains water, premium cuts are tender and juicy (45). Stevia may help beef retain water, since STV-treated groups showed decreased drip loss. Many advantages may ensue. Reduced drip loss while cooking preserved meat moisture, making it tastier (46). Retained moisture preserves flavor, enhancing meals. Effective water retention improves beef shelf life and decreases degradation, improving marketability and lowering economic losses. Stevia may improve muscle structure and connections, making meat more water-retentive and minimizing drip loss (47). By enhancing protein denaturation during cooking, stevia may retain moisture. STV-treated beef may retain more water owing to lower pH (48). Lower pH may increase protein binding and minimize drip loss by affecting protein solubility and water retention. Demand was greater for beef with less drip loss and more juice, which may please buyers. Chicken sales and quality may suffer (49).

Conclusions

According to the research, broiler chickens' digestive enzyme activity, antioxidant levels, and meat quality can be improved by supplementing with Stevia leaf powder (STV). Groups treated with STV had higher amylase activity, suggesting improved digesting capability; nevertheless, there were only small variations between STV dosages. Antioxidant activity was best enhanced at a dosage of 20g/kg STV, with levels of SOD, CAT, and GLUpx all showing notable increases at this concentration, indicating enhanced oxidative defense. Additionally, 20g/kg STV significantly improved water retention, as shown by lower drip loss, and STV supplementation decreased meat pH, which can increase softness and shelf life. According to these findings, the ideal dosage of STV to promote antioxidant activity and meat quality in broiler chickens is 20g/kg.

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Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

The Research Ethical Committee approves this work.

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تقييم تأثير مسحوق أوراق ستيفيا على مضادات الأكسدة وأنزيمات الهضم وجودة اللحوم في الدجاج اللاحم

زينب خليل إبراهيم, نمير عبد الكريم خضير, ميسم حسن علي.

فرع الصحة العامة، كلية الطب البيطري، جامعة البصرة، العراق.

الخلاصة

في الآونة الأخيرة، هناك طلب متزايد على لحوم الدواجن ومنتجات البيض، مما دفع المستثمرين إلى تطوير صناعات انتاجية واسعة النطاق ، تشمل زيادة أوزان الطيور والإنتاجية، مما أدى إلى ازدهار تربية الدواجن التجارية. تهدف هذه الدراسة إلى تقييم تأثير مسحوق أوراق ستيفيا على الحالة التأكسدية، والهضم، وجودة اللحوم في دجاج اللحم. أُجريت الدراسة في الفترة من 21 ديسمبر 2023 إلى 17 يناير 2024. تم الحصول على 240 فرخ دجاج من نوع روس من مفقس "البكري الحديث" في محافظة بابل، حيث تم تربية الطيور لمدة 28 يومًا، وتم تقسيمها إلى أربع مجموعات خضعت لعلاجات مختلفة. تناولت المجموعة 1 (مجموعة السيطرة) نظامًا غذائيًا عاديًا، في حين تلقت المجموعات 2 و 3 و 4 مساحيق أوراق ستيفيا بتركيزات 10 و 20 و 30 جرام/كجم من العلف على التوالي. تم جمع عينات من الدم واللحوم لإجراء التحاليل اللازمة. أظهرت المجموعات المعالجة بالستيفيا مستويات أكبر بشكل ملحوظ من الأميلاز ($p\leq 0.05$). كما أظهرت مجموعة 30جرام/كجم من الستيفيا نشاطًا أعلى من إنزيمات SOD و CAT و GLUpx مقارنة ببقية المجموعات. أدى إضافة الستيفيا إلى انخفاض درجة حموضة اللحوم، مما ساهم في تحسين الطراوة ومدة الصلاحية، لكنه أدى إلى زيادة في الفقد عند الطهي. كانت مجموعة الستيفيا بتركيز 30 جرام/كجم أكثر انخفاضًا في فقدان العصارة، مما يشير إلى جودة أفضل في اللحوم واحتفاظ أفضل بالماء. يمكن أن تستنتج النتائج أن مسحوق أوراق ستيفيا يحسن إنزيمات الجهاز الهضمي (الأميليز) وآلية الدفاع عن مضادات الأكسدة وجودة اللحوم من خلال تحسين طراوة اللحم ومدة الصلاحية عن طريق تقليل فقدان التنقيط ودرجة الحموضة. تشير هذه البيانات إلى أن دجاج التسمين يجب أن يستهلك 30 جرامًا/كجم من STV لتعزيز نشاط مضادات الأكسدة وجودة اللحوم.

الكلمات المفتاحية: مسحوق أوراق ستيفيا, الدجاج اللاحم, مضادات الاكسدة, انزيمات الهضم.