



Effects of Stevia Leaf Powder on Growth Performance, Intestinal Fluid Viscosity and Jejunal Histology in Broiler Chickens

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Abstract | Objective: this study aimed to assess the stevia leaf powder's impact on the efficiency, intestinal fluid viscosity, and histology of jejunal villi in broiler chickens. Materials and methods: Various quantities of Stevia leaf powder were administered to 240 Ross broiler chicks from December 21, 2023, to January 17, 2024. After 28 days of treatment. Four groups of chicks from Babylon's Modern Al-Bakri Hatchery were given various dose. The first group, which served as a control, had an ordinary diet. Groups 2, 3, and 4 were given meals supplemented with 10, 20, and 30 g/kg/diet of Stevia leaf powder, respectively. Dietary stevia, milled locally. Results: The first two weeks showed no notable changes. However, 10, 20, and 30g/kg/diet STV groups exhibited greater body weights, weight gain, and reduced feed consumption than the control group in the third and fourth weeks. Whereas had identical feed conversion ratios (FCR) and lowered FCRs in the subsequent weeks. The 20 and 30g/kg diet STV groups showed zero mortality and considerably greater production indexes. STV-treated groups had greater villus high although crypt depth did not change, led to a little higher villus/crypt ratio. The results also showed decreased in intestinal fluid viscosity. While, transverse jejunum sections showed longer villus length and lower crypt depth were seen in STV groups compared to controls. Conclusions: Stevia leaf powder at 20 and 30g/kg diet, improved development, feed efficiency, and intestinal health by the third and fourth weeks. These imply that Stevia supplementation may boost broiler production, especially at higher dosages.

Keywords | Stevia leaves powder, Broiler chickens, Growth performance, Intestinal fluid, Viscosity, Jejunal histology

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INTRODUCTION

The poultry industry has witnessed remarkable and rapid development in recent decades. The period of raising chicks until marketing as broilers has reached an unexpected level. This speed in the production cycle requires strict care and follow-up. Therefore, investors and farm owners have resorted to using several methods to reduce the period of raising and the cost of consumed feed in or-

der to achieve higher profits, especially with the increase in demand for poultry meat. Consequently, many countries have implemented regulations to restrict or prohibit the use of additives in animal feed like antibiotics (Casewell *et al.*, 2003). In response, researchers have turned to natural additives as alternative options to improve the growth and health of broiler chickens without contributing to antibiotic resistance or risk to public health (Windisch *et al.*, 2008). Various natural additives, including probiotics, prebiotics,

symbiotics, organic acids, and enzymes, have been investigated for their potential to enhance nutrient digestion and absorption, modulate the immune system, and inhibit the growth of harmful gut pathogens (Ricke, 2003). Recently, phytochemicals, which are derived from plants such as herbs, spices, or plant extracts, have garnered attention due to their beneficial effects on broiler chickens, including antioxidant, antimicrobial, coccidiostatic, anthelmintic, and immunostimulant properties (Gadde *et al.*, 2017).

Stevia, a plant which has gained recognition for its potential as a natural additive (Schiatti-Sisó *et al.*, 2023). The leaves of the stevia plant are containing high concentrations of glycosides, which provide a sweet taste without adding calories or affecting blood sugar levels (Singh *et al.*, 2019). Stevioside and rebaudioside A are the most abundant glycosides found in stevia leaves (Schiatti-Sisó *et al.*, 2023). Extensive research has been conducted on stevia and its extracts, establishing their safety and efficacy as a natural alternative to sugar (Singh *et al.*, 2019; Schiatti-Sisó *et al.*, 2023). Wide range of literature reported that stevia has the ability to regulate glucose levels (Li *et al.*, 2021), protection against cardiovascular diseases (Olas, 2022). Also, investigated that stevia could inhibit microbial growth, and use as anti-inflammatory, prevention of cancer, and other critical pharmacological industries (Basharat *et al.*, 2021). Although previously investigated that administration of stevia as feed additive enhances digestion, absorption and immunity of the animals (Molina-Barrios *et al.*, 2021). For instance, some research has demonstrated that stevia can improve intestinal morphology and barrier function in broiler chickens (Atteh *et al.*, 2008). However, the possibility of using stevia as a nutritional additive in broiler feeds and its impact on growth and studying its effect on the characteristics of the small intestine still requires further research and scrutiny.

AIM OF THE STUDY

This research aimed to evaluate the effects of stevia leaf powder on the viscosity of intestinal fluid, histological features of the jejunal villi, and the efficiency of broiler hens.

MATERIALS AND METHODS

ETHICAL APPROVAL

This research was conducted in one of the poultry halls in Babylon Governorate, affiliated with the College of Veterinary Medicine in the same governorate, Iraq. With the IACUC ethical approval for animal 45/37 in 2024.

BROILER MANAGEMENT AND NUTRITION

A total of 240 Ross broiler chicks, both male and female, weighing 42.6 grams at birth and one day old, were used in this investigation. A private hatchery in Babylon called the

Modern Al-Bakri sold the chicks. The chicks were raised on a floor divided by partitions of dimensions that were 160 x 150 x 90 cm, and the chicks were distributed at random on one level. There were four sets of sixty chicks, with twenty in each set. Crushed pellets were given to one brood per cage on 38 cm plastic plates throughout the first week of their lives. After that, throughout the first three weeks of rearing, 45 cm feeders were progressively added. Automatic feeds and water were supplied in the weeks that followed. Breeding was done under controlled conditions at temperature 22°C and relative humidity of 60 to 70%. It included 23 light hours and 1 dark hour to help the chicks adjust to darkness. Birds were given a low-calorie, high-crude-protein meal when they were two weeks old. During the last week of their study, animals ranging in age from 14 to 28 days were given a growth diet that was rich in calories but somewhat lacking in protein.

STUDY DESIGN

The two hundred and forty broiler chicks, split evenly between males and females, were subjected to four groups, with sixty birds per group and twenty birds each repetition: Ration was given to the chicks without any addition, which served as a control (G1). Second group (G2): chicks received STV leaves powder (10 g/kg of diets) for a duration of 28 days. Third group (G3) chicks in this group received STV leaf powder (20 g/kg of diets) for same duration. Fourth group (G4) chicks in this group fed with STV leaf powder (30g/kg of diets) for 28 days.

SOURCE AND PREPARATION OF STEVIA

Stevia leaves were purchased from a spice shop in Basra market in Basra Governorate. The plant leaves, which contained a small percentage of moisture, were ground and sifted using a sieve with small holes to obtain a fine powder that was packed in polyethylene bags until it was used in the experiment. Stevia leaves contain steviol, a water-soluble glycoside that is 200 times sweeter than sucrose.\

THE PREVENTIVE AND HEALTH PROGRAM

Half a gram of vitamins and minerals were added to each liter of water for the chicks. The Newcastle vaccine, which had been diluted with boiling and chilled water to remove chlorine while maintaining the viability of the vaccinia virus, was administered to the animals when they were 10 days old. The chicks didn't drink water for three hours prior to being vaccinated. Estimates of immunization dose were obtained by multiplying the water requirement by the quantity of chicks and their age.

BODY WEIGHT AND WEIGHT AGAIN

The chicks were weighed at the first day of age and then all birds were weighed weekly. The following formula was used to find out the average weight of the live bird within the

single repeater, as shown by *Al fayad et al. (2011)*. Average weight of the bird (g) = (total bird weights in the replicate) / (the total number of birds in the replicate in the end of week). The weight again achieved per week calculated according to the following equation: Weight again (g) = live bird weight at the end of the week (g) - live bird weight at the beginning of the week (g) (*Molina-Barrios et al., 2021*).

FEED INTAKE AND FEED CONVERSION RATIO

The amount of feed consumed per week was determined by subtracting the remaining amount of feed at the end of the week from the total quantity provided during beginning of the week, taking into account the feed consumed by dead birds in the calculation of the average weekly feed intake for birds. Feed intake (g) is calculated by subtracting the amount of feed remaining at the end of the period from the amount of feed provided to the chicks at the beginning of the same period (*Prakash et al., 2020*). Feed conversion ratio was calculated for each week according to (*Haladu and Abubakar 2020*) as the following equation: Feed conversion ratio = amount of feed consumed per week (g) / Weight again (g).

THE TOTAL FATALITY RATE AND PRODUCTION INDEX

The fatality was recorded for each replicate and calculated using the following formula: the following formula was used to calculate the percentage of fatality. The total fatality rate % = the number of fatality birds during the duration of the experiment / total number of birds × 100 (*Doering et al., 2020*). The production index was measured according to *Onawa (2022)*. Productivity index scale = average body weight (g) × vitality ratio / the number of training days × food conversion factor × 10. The vitality ratio = 100 - the percentage of fatalities.

INTESTINAL FLUID VISCOSITY

The intestinal content of 6 birds in each treatment was used to measure the viscosity. The jejunum and ileum material were collected in a glass container at the end of the experiment, and its viscosity was measured on the same day as described by (*Tejeda and Kim, 2021*). Where one gram of sample was taken, 5ml of distilled water was added to it, and it was placed in a water bath at 40°C for 15 minutes, while mixing the solution continuously. Once the extraction process was complete, the solution was washed with filter paper, and then the relative viscosity of the filtrate was determined using a viscometer, and the following formula was applied (*Li et al., 2016*).

$$\text{Viscosity (Pascals/sec)} = \frac{\text{flow time of aqueous extract}}{\text{flow time of distilled water}}$$

HISTOLOGICAL STUDY

Samples taken from the jejunum of the small intestine of birds were immediately placed in 10% formalin for 24

h, then dehydrated with a series of ethyl alcohol concentrations, immersed in paraffin wax, and cut with a rotary microtome to 4-6 um. Then the histological sections were stained with hematoxylin and eosin. Later the precise ocular scale on a microscope was compatible with the stage-mounted slide, and the constant value was recorded and raised. Then, a glass slide with the tissue was replaced, and the height of the villi and the depth of the crypts are measured in units (micron). Multiply the result by the constant to get microns. From the top to the attachment, the villi were long. The depth or distance between villi defines crypt depth.

A computerized camera photographed villi and grave histological sections. The equation extracts the constant: (*Naji et al., 2017*).

$$\text{Constant} = \frac{\text{Ocular lens measure} \times \text{lens power}}{\text{ouclometer}}$$

STATISTICAL ANALYSIS

The statistical significance between variables was determined using SPSS version 22. Analysis of variance testing was used to evaluate the results. It is deemed significant when the p-value is less than 0.05 and the confidence interval is 95%.

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

Weeks groups	1 st week	2 nd week	3 rd week	4 th week
Control	210.31 ± 3.37	479.31 ± 3.66	1044.08 ± 35.1 b	1460.67 ± 28.26 b
STV 10g/kg	224.33 ± 8.08	495.61 ± 8.26	1098 ± 21.50 b	1571.33 ± 22.45 a
STV 20g/kg	214.48 ± 2.74	476.12 ± 12.19	1110.33 ± 5.33 ab	1615 ± 27.53 a
STV 30g/kg	218.27 ± 4.45	482.57 ± 16.72	1154.67 ± 8.96 a	1647 ± 49.08 a
P ≤ 0.05	NS	NS	0.038	0.020

Small letter represents significant difference among group at (p ≤ 0.05); NS: non-significant.

RESULTS AND DISCUSSION

The effect of Stevia leaves powder on weekly live body weight (g), shown in *Table 1*. The data shows no statistically significant difference in live body weight between the different treatment groups (control, STV 10g/kg, STV 20g/kg, and STV 30g/kg) during the first two weeks. However, significant differences emerged in the third and fourth weeks. Treatment groups STV 10g/kg, STV 20g/kg, and STV 30g/kg consistently exhibited higher live body

weights compared to control. This indicates that, STV addition was associated with improved growth performance in broiler chickens over time after tow week of rearing. This is also what was shown in Table 2, which indicated that there were no significant differences in feed consumption during the first and second weeks, while significant differences appeared in feed consumption during the third and fourth weeks of broiler rearing, as Table 2 indicated a significant decrease in feed consumption for the groups that consumed stevia leaves (STV 10g/kg, STV 20g/kg, and STV 30g/kg) when compared with the control group. This suggests that STV addition may not have an immediate impact on feed consumption during the early stages and associated with reduced feed consumption in broiler chickens over time. The previous results in Tables 1 and 2 were reflected in the weight gain of broilers. The data in Table 3 showed no significant differences in the weight gain of broilers during the first and second weeks, while the results recorded a significant increase in the weight gain of broilers for the groups that consumed stevia leaves (STV 10g/kg, STV 20g/kg, and STV 30g/kg) when compared with the control group during the third and fourth weeks of the experiment. This indicates that STV addition on broiler feed was associated with improved growth performance throughout the rearing after the second week.

Table 2: Effects of different levels of STV addition on feed intake of broiler chicken over four weeks (Mean $\pm$ S.E.).

Weeks Groups	1 st week	2 nd week	3 rd week	4 th week	Cumulative
Control	310.52 $\pm$ 5.26	362.28 $\pm$ 6.14	931.57 $\pm$ 15.78a	1034.67 $\pm$ 17.33a	2639.04 $\pm$ 44.52a
STV 10g/kg	305.26 $\pm$ 5.26	356.14 $\pm$ 6.14	763.16 $\pm$ 13.15b	763.16 $\pm$ 13.15b	2187.71 $\pm$ 37.71b
STV 20g/kg	305.26 $\pm$ 5.26	356.14 $\pm$ 6.14	763.16 $\pm$ 13.15b	763.16 $\pm$ 13.15b	2187.71 $\pm$ 37.71b
STV 30g/kg	300 $\pm$ 5.26	350 $\pm$ 6.14	750 $\pm$ 13.14 b	850 $\pm$ 15.12 b	2250 $\pm$ 35.41b
P $\leq$ 0.05	NS	NS	0.085	0.052	0.082

Small letter represents significant difference among group at ($p \leq 0.05$); NS: non-significant.

In Table 4, the data indicates that there is no statistically significant difference in feed conversion ratio (FCR) among the different treatment groups (control, STV 10g/kg, STV 20g/kg, and STV 30g/kg) during the first two weeks. This suggests that the addition of STV may not have an immediate effect on feed efficiency in the early stages. However, significant differences were observed in the third and fourth weeks. The treatment groups receiving STV at 10g/kg, 20g/kg, and 30g/kg consistently showed a lower FCR compared to the control group. This indicates that adding STV is associated with improved feed efficiency in broiler chickens throughout the trail period.

Table 3: Effects of different levels of STV addition on weight gain of broiler chicken over four weeks (Mean $\pm$ S.E.).

Weeks Groups	1 st week	2 nd week	3 rd week	4 th week	Cumulative
Control	167.71 $\pm$ 3.37	268.99 $\pm$ 6.77	564.78 $\pm$ 38.44 b	416.58 $\pm$ 63.05	1418.07 $\pm$ 28.26 b
STV 10g/kg	181.73 $\pm$ 8.08	271.27 $\pm$ 1.44	614.73 $\pm$ 3.90 ab	461 $\pm$ 17.15	1528.73 $\pm$ 22.45 a
STV 20g/kg	171.88 $\pm$ 2.74	261.64 $\pm$ 14.81	615.43 $\pm$ 29.99 ab	460.33 $\pm$ 34.18	1572.4 $\pm$ 27.53 a
STV 30g/kg	175.67 $\pm$ 4.45	264.3 $\pm$ 12.31	678.54 $\pm$ 17.52 a	549 $\pm$ 48.50	1604.4 $\pm$ 49.08 a
P $\leq$ 0.05	NS	NS	0.083	NS	0.020

Small letter represents significant difference among group at ($p \leq 0.05$); NS: non-significant.

Table 4: Effects of different levels of STV addition on FCR of broiler chicken over four weeks (Mean $\pm$ S.E.).

Weeks Groups	1 st week	2 nd week	3 rd week	4 th week	Cumulative
Control	1.85 $\pm$ 0.04	1.35 $\pm$ 0.04	1.66 $\pm$ 0.08 a	2.61 $\pm$ 0.41 a	1.86 $\pm$ 0.06 a
STV 10g/kg	1.69 $\pm$ 0.10	1.31 $\pm$ 0.02	1.24 $\pm$ 0.01 b	1.66 $\pm$ 0.08 b	1.43 $\pm$ 0.04 b
STV 20g/kg	1.78 $\pm$ 0.03	1.37 $\pm$ 0.08	1.13 $\pm$ 0.02 b	1.68 $\pm$ 0.13 b	1.39 $\pm$ 0.04 b
STV 30g/kg	1.71 $\pm$ 0.04	1.33 $\pm$ 0.05	1.22 $\pm$ 0.05 b	1.57 $\pm$ 0.14 b	1.40 $\pm$ 0.04 b
P $\leq$ 0.05	NS	NS	0.001	0.027	0.008

Small letter represents significant difference among group at ($p \leq 0.05$); NS: non-significant.

Table 5: Effects of different levels of STV addition on mortality and production index of broiler chicken over four weeks (Mean $\pm$ S.E.).

Groups	Mortality	production index	Rank of production index
Control	3.33 $\pm$ 1.66a	272.71 $\pm$ 20.17d	4
STV 10g/kg	1.67 $\pm$ 1.66b	388.15 $\pm$ 21.64c	3
STV 20g/kg	0.00 $\pm$ 0.00c	416 $\pm$ 18.67b	2
STV 30g/kg	0.00 $\pm$ 0.00c	441.69 $\pm$ 26.11a	1
P $\leq$ 0.05	0.097	0.003	

Small letter represents significant difference among group at ($p \leq 0.05$); NS: non-significant.

In Table 5, mortality rate appeared significantly higher in control group compared with other STV treated groups. Nevertheless, STV 20g/kg and STV 30g/kg groups recorded zero mortality rates and appeared significantly lower mortality rate compared to control and STV 10g/kg. These in turn, showed significant elevation in production index for the STV-treated groups (STV 20g/kg and STV 30g/kg)

compared to control and 10g/kg groups. This indicates that STV addition is associated with better overall performance, likely due to a combination of factors such as increased weight gain, reduced feed consumption, and lower mortality.

Small intestinal characteristics was exhibiting significant elevation in villus height for STV-treated groups (STV 10g/kg, STV 20g/kg, and STV 30g/kg) compared to the control group. Whereas, STV addition on feed of broiler had no effect on crypt depth, and this reflected on the villus to crypt ratio (Table 6). This suggests that STV supplementation may promote intestinal development and nutrient absorption through the increase of villus to crypt ratio although statistical difference absent, but there was an increase in ratio for the STV treated groups compared with control group. In the other hand, STV-treated groups showed significantly lower intestinal fluid viscosity compared to the control, this reduction in viscosity could improve nutrient absorption and digestion for the broiler that fed on STV.

Table 6: Effects of different levels of STV addition on small intestine and intestinal fluid viscosity of broiler chicken (Mean $\pm$ S.E.).

Groups	Villus height	Crypt depth	Villus/crypt ratio	Viscosity (pas-cal / second)
Control	60.13 $\pm$ 1.00 b	13.87 $\pm$ 3.75	4.33 $\pm$ 0.26	3.56 $\pm$ 0.60 a
STV 10g/kg	74 $\pm$ 15.10 ab	15.67 $\pm$ 2.40	4.72 $\pm$ 6.29	3.16 $\pm$ 0.53 b
STV 20g/kg	88.87 $\pm$ 0.75 a	19.4 $\pm$ 4.64	4.58 $\pm$ 0.16	3.12 $\pm$ 0.50 b
STV 30g/kg	98.93 $\pm$ 7.09 a	19.8 $\pm$ 2.90	4.99 $\pm$ 2.44	3.10 $\pm$ 0.47 b
P $\leq$ 0.05	0.048	N. S	N. S	0.006

Small letter represents significant difference among group at ($p \leq 0.05$); NS: non-significant.

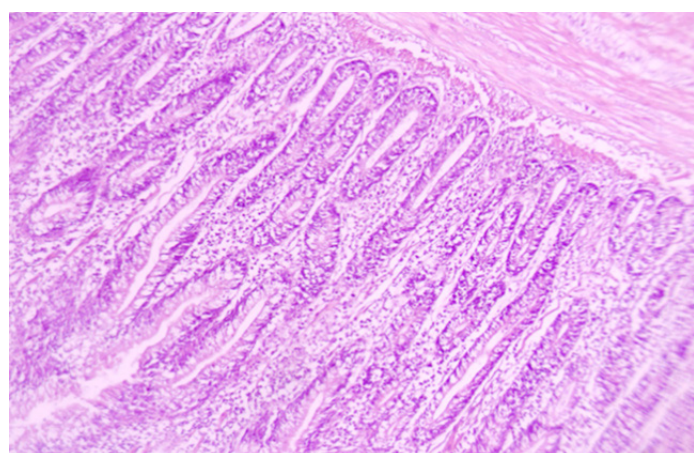


Figure 1: Photomicrograph of transverse section of jejunum from control group of broilers showed the villus height 60.13 micrometer while the crypt depth is 13.87 micrometer (HandE stain. 10X).

In Figure 1, transverse section of jejunum for control group of broiler chicken was showed that, the villus height was 60.13 micrometer while the crypt depth was 13.87 micrometer. while in feed supplemented with STV 10g/kg the villus height was 74 micrometer and the crypt depth was 15.67 micrometer (Figure 2). The transverse section of jejunum in broiler fed STV 20g/kg diet was 88.87 micrometer for the villus height and 19.4 micrometer for the crypt depth as shown in Figure 3. In Figure 4, transverse section of jejunum in STV 30g/kg broiler chicken was studied, and it was found that, the villus height was 98.93 micrometer while the crypt depth was 19.8 micrometer.

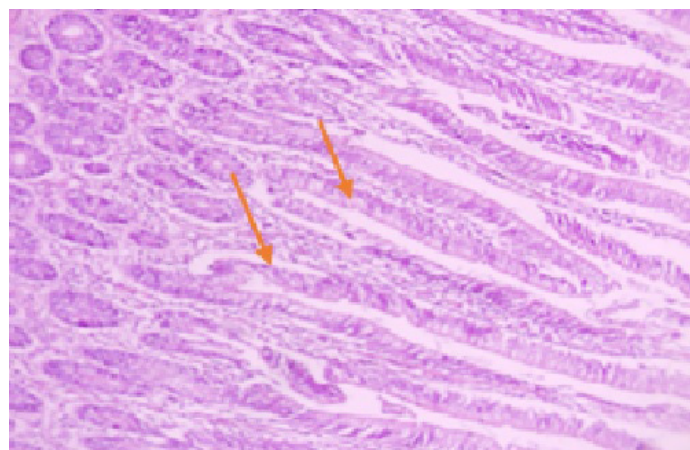


Figure 2: Photomicrograph of transverse section of jejunum in STV 10g/kg broiler showed the villus height was 74 micrometers while the crypt depth was 15.67 micrometer (HandE stain. 10X).

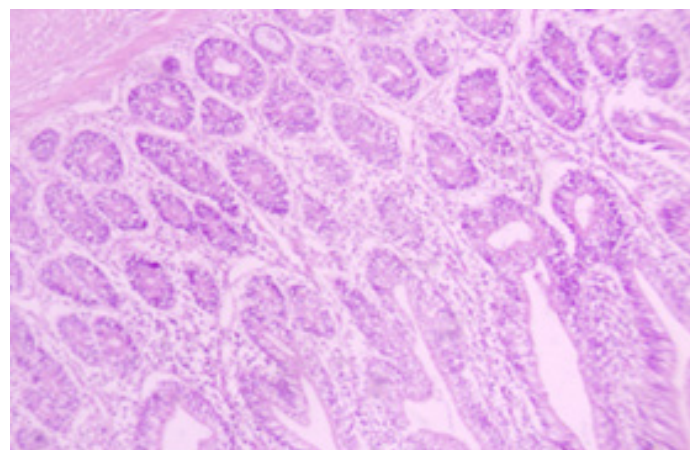


Figure 3: Photomicrograph of transverse section of jejunum in STV 20g/kg broiler showed the villus height was 88.87 micrometer while the crypt depth was 19.4 micrometer (HandE stain. 10X).

The results of the present study referred to non-significantly difference in live body weights, feed consumption and weight gain across treatment groups during the first and second week of the experiment. These may be belonged to several causes, like due to confounding circumstances; a limited sample size may limit study findings and others.

According to Pirgozliev *et al.* (2021) said that two-week trials may not show weight changes and results may take longer and this mean weight measuring results depend on timing and procedure. Wu *et al.* (2019) found starting weight and growth rate disguise treatment benefits. The control and treatment groups ate similar meals (except Stevia), therefore their growth rates may have been similar. Khalifah *et al.* (2021) suggested low-dose stevia may not lose weight. Stevia may increase hen growth for weeks (Gao *et al.*, 2021). Broiler chicks fed on stevia may have needed time to acclimate, and metabolic changes may increase weight after 2-3 weeks (Saleh *et al.*, 2023). For the first two weeks, feed intake was similar for the control, STV 10g/kg, 20g/kg, and 30g/kg groups (Table 2). Stevia (STV) did not instantly impact chicken appetites or eating behavior. The early weeks' Stevia doses (10g/kg, 20g/kg, 30g/kg) were too low to impact diets or appetites. Initial dosages may alter metabolism or development but not feed consumption (Pirgozliev *et al.*, 2021). For the first few weeks, Stevia-containing broiler chicken feed won't affect growth, say Peralta *et al.* (2020). Early weeks may maintain feed intake due to growth delay. In the third and fourth weeks, STV 10g/kg, 20g/kg, and 30g/kg broiler hens consumed less feed than the control group, suggesting stevia supplementation improved feed efficiency or metabolism and have helped chicken nutrition or digestion and learning to get nutrients and energy reduces their feeding needs. Sun *et al.* (2022) suggest stevia helps birds gain or maintain weight with less food. This reveal treated groups grew quicker with less food as shown in Table 3. Becker *et al.* (2020) say hens require longer time to gain from Stevia's metabolism or nutrition, because all chickens eat the same quantity, Stevia's first nutritional boost may not help weight. Stevia boosts metabolism, nutrition absorption, and intestinal health, but weight gain takes weeks. Broiler fed on stevia in the diet appeared rapid development in weeks 3 and 4. The STV 10g/kg, 20g/kg, and 30g/kg groups gained more than the control group. These agreed with study found that stevia may help third- and fourth-week chicks eat, develop, and metabolically improve (Saleh *et al.*, 2023). The feed conversion ratio (FCR) did not differ between the control, 10g/kg Stevia (STV), 20g/kg STV, and 30g/kg STV treatments in the first two weeks. According to Peralta *et al.* (2020), genes and diet enable broiler chicks grow swiftly in the first two weeks and stevia may not boost FCR since genetic growth potential impacts feed efficiency (Omar *et al.*, 2022). The first two weeks may have been too short for hens to undergo metabolic changes needed for considerable FCR gains and stevia's supplementation have cumulative effects may take longer (Gungor and Erener, 2019). The hens' digestive processes may not have changed enough to affect FCR before feeding, nutrition and digestion may be unaffected (González-Ortiz *et al.*, 2019). The third and fourth weeks showed STV 10g/kg, 20g/kg, and 30g/kg groups consistently had lower

FCR than the control group. Stevia may have aided hens by boosting intestinal flora and healthy digestive tracts use nutrients to lower FCR (Jiang *et al.*, 2020). Greater Stevia dosages may increase bird health and illness resistance due to the antioxidants stevioside and rebaudioside A in Stevia may reduce chicken oxidative stress according to Paredes-López *et al.* (2019). Stevia's 20g/kg and 30g/kg antioxidants may have protected avian cells and reduced mortality these may be due to the ameliorative role of stevia that improve bird health (Nettleton *et al.*, 2019). Stevia's anti-inflammatory and immune-boosting effects may help hens' survival period and this occurred when administration of STV 20g/kg and 30g/kg in groups and there is no bird die, probably because Galamatis *et al.* (2021) showed greater effects at higher doses and mentioned that changes in nutrition, processing, and stress are the main causes that kill birds. Stevia may reduce stress through antioxidant or metabolic balance. STV 20g/kg and STV 30g/kg may have survived because lower stress promotes immunity and disease resistance (Forouzi *et al.*, 2020). Antibacterial stevia may protect broiler chicks from infections and other issues, according to one study. Higher Stevia doses may have reduced fatal infections due to antibacterial properties and reduced mortality rate (Zhao *et al.*, 2019). Production index measures avian health and efficiency by weight gain, feed consumption, and mortality (Park *et al.*, 2018). Third and fourth weeks showed significant weight gain in STV treated groups, these in turn increased productivity since bigger birds produce more. Stevia may boost growth by improving digestion and metabolism of the nutrients (Gao *et al.*, 2021). These birds produced more on less feed due to lower cost and volume (Pompeu *et al.*, 2018). STV 20g/kg and 30g/kg broiler chicken production was likely cheaper than the control group due to feed efficiency and mortality reduction. Lower mortality and feed efficiency cut manufacturing expenses. Production index efficiency and performance improvement (Farahat *et al.*, 2020).

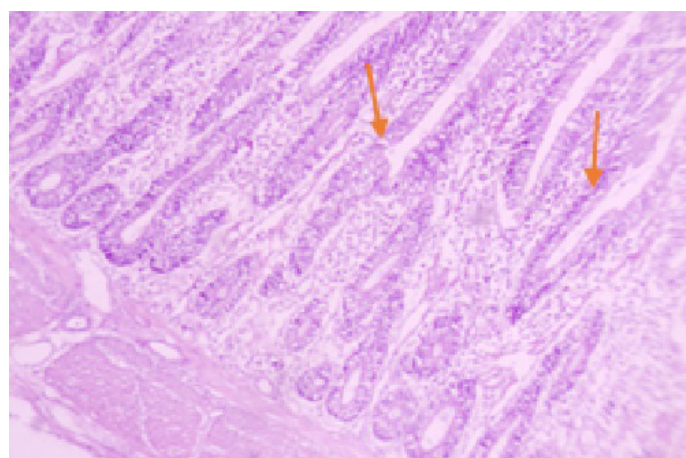


Figure 4: Photomicrograph of transverse section of jejunum in STV 30g/kg broiler showed the villus height was 98.93 micrometer while the crypt depth was 19.8 micrometer (HandE stain. 10X).

Stevia supplementation affects broiler chicken intestine growth and health, as villus heights are larger in the STV 10g/kg, 20g/kg, and 30g/kg treatment groups than in the control group (Table 6). Villi height increases absorption surface area that allow the nutrients to take enough time for enzymes mixing and absorption, which may boost broiler development and feed efficiency (Jiang *et al.*, 2021). Cell turnover is reflected by intestinal epithelial villi crypt depth, while an unchanged crypt depth suggests STV therapy does not affect epithelial cell proliferation and renewal (Jiang *et al.*, 2019). Stevia may improve villus height and nutrition absorption but not intestinal cell turnover since crypt depth did not alter. Pirgozliev *et al.* (2021) discovered that Stevia mostly increases intestinal epithelium absorptive surface area without affecting self-renewal by promoting beneficial bacteria and minimizing oxidative stress, stevia's flavonoids and phenolics may increase villus height. Higher villus heights improve nutrient absorption, health, and growth, hence intestinal morphology affected poultry nutrition and production (Amer *et al.*, 2021). Photomicrographs of control broiler chicken transverse jejunum sections show important histological features (Figure 1). The finger-like villi increase absorption surface area, according to Dameanti *et al.* (2023), also mentioned that nutrients absorption structures are well-developed with 60.1 micrometer villous length and Lieberkühn's tubular crypts at villi bases produced intestinal juices and regenerated enterocytes. With 13.87-millimeter crypt depth, a healthy intestinal lining supports gut integrity and cell turnover (Vimon *et al.*, 2023). Good jejunal architecture in the control group ensured broiler chicken digestion and nutrient absorption. villus height was 74 micrometers, compared to 15.67 in the control group (Figure 2). This decrease in absorptive surface area may affect nutrient absorption. Villi shorten from nutrition, stress, and sickness. Crypt depth was 19.8 micrometers, deeper than the control group (13.86). Greater crypt depth may indicate higher cell turnover because enterocytes must replace damaged or inflamed ones (Song *et al.*, 2022). This may compensate for a less efficient absorptive surface (Campos *et al.*, 2023). Figure 3 shows a 20 g/kg STV-treated broiler chicken transverse jejunum photomicrograph, when compared with previous figures for 10 g/kg and control groups (13.86 and 19.8 micrometers), villus height was 88.87 micrometers. the lower dosage group had longer villi. This may indicate partial recovery or less absorptive surface area damage than the lower dosage (Olyayee *et al.*, 2022). Intermediate measures included 15.67 micrometer crypt depth. Shallower than 10 g/kg (98.9 micrometers) but deeper than control (60.1). Song *et al.* (2022) found that treatment-induced nutrition absorption changes may impair cell turnover and regeneration. In contrast, the transverse jejunum photomicrograph in Figure 4 of broiler chickens given 30 g/kg STV showed villus height was 19.4 micrometers, matching the control group (19.8), demonstrating a typical absorptive surface

area. Enhancing STV dosage can preserve or restore villous structure, crucial for nutrition absorption (Novotný *et al.*, 2023). The crypt depth was 98.93 micrometers, deeper than the control group (60.1) and other treated groups, but shallower than the 10 g/kg group (13.87). Therapy may enhance enterocyte turnover due to nutrient intake, gut lining damage, or stress adjustment (Han *et al.*, 2023).

CONCLUSIONS AND RECOMMENDATIONS

The growth performance, feed efficiency, and intestinal health of broiler chickens were positively affected in the third and fourth weeks when they were fed diets that included Stevia leaf powder, particularly at doses of 20g/kg and 30g/kg. These results suggest that supplementing broiler diets with Stevia may enhance their productivity, with higher doses producing a more significant impact.

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NOVELTY STATEMENTS

With these factors, the study's goal was to evaluate out how adding stevia leaves powder to broiler chicken feed could improve their health and output by looking at their growth, gut enzyme activity, and meat quality.

AUTHOR'S CONTRIBUTIONS

Dr. Zainab K Ibrahim in poultry farming as well as in sample collection.

Dr. Maysam H. Ali contributed to the statistical analysis.

Dr. Nameer A. Khader contributed to laboratory procedures and blood tests.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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