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Sex and Tomato Flour Supplementation Influence Hepatic Function, Intestinal Morphometry, and Performance in Broilers

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ABSTRACT

Plant-derived supplements, such as tomato flour (TF), may enhance organ function and improve productive performance in poultry, but their effects under tropical conditions and between sexes remain unclear. This study assessed the effects of TF supplementation and sex on productive performance, hepatic function, and intestinal morphometry in broilers reared under tropical conditions. A total of 300 28-day-old Cobb-500 broilers were randomly allocated to six treatments: males and females fed a conventional diet or diets supplemented with 1% or 2% TF (T1-T6). Body weight, feed intake, and feed conversion ratio (FCR) were recorded weekly to evaluate productive performance. Intestinal morphometry (duodenum, jejunum, ileum), hepatic fatty infiltration (HFI), and liver function were analyzed. Supplementation with 1% TF improved final weight and FCR in male broilers (p<0.05). Supplementation with 2% TF reduced HFI in both sexes (p= 0.01) and lowered the albumin-to-globulin (A/G) ratio in males (p= 0.015). However, 2% TF impaired villus height and villus-to-crypt ratio in females, particularly in the duodenum and jejunum (p<0.05), while males were less affected. These findings indicate that TF enhances hepatic function and productive performance while reducing HFI in tropical-reared broilers. Its effects on intestinal morphometry are dose- and sex-dependent, with females being more negatively affected at higher doses, warranting further research into sex-specific dietary strategies.

Keywords: albumin-to-globulin ratio; hepatic fatty infiltration; growth performance; productive performance; sex-dependent effects; tropical conditions

INTRODUCTION

Broiler chicken meat is a major source of animal protein worldwide, with consumption increasing by over 300% since the 1960s (Farrell, 2016; Ahmad *et al*., 2022). Global production reached 139 million metric tons by 2023, with further growth expected in tropical regions (Dawson, 2023; Kpomasse *et al*., 2021). However, broilers are highly susceptible to heat stress, especially in hot and humid climates, leading to oxidative stress and inflammation, reducing villus height (VH), increasing the depth of Lieberkühn's crypts (CD), impairing nutrient absorption, and altering intestinal permeability, which facilitates the entry of pathogens and their toxins into the bloodstream. It also leads to hepatic lipid infiltration and compromises liver function. Alterations in intestinal microarchitecture and liver function impact poultry health and performance (Zaefarian *et al*., 2019; Kpomasse *et al*., 2021; Malila *et al*., 2022; Brugaletta *et al*., 2022).

Plant-based additives, such as tomato-derived compounds (*Solanum lycopersicum*), exhibit antimicrobial, antioxidant, and anti-inflammatory properties (Ali *et al*., 2021), which can support broiler health and growth performance. At the same time, their use contributes to the valorization of agro-industrial byproducts, thereby helping to reduce food waste (Hasted *et al*., 2021). This is particularly relevant given that post-harvest losses and food waste are a major concern, with estimates indicating that nearly 50% of global fruit and vegetable production is lost or discarded (Eslami *et al*., 2023). In particular, the industrial processing of tomatoes generates significant byproducts, mainly consisting of peels, seeds, and pulp residues, representing up to 5% of the total processed weight. Finding alternative uses for these byproducts could enhance sustainability in the food and feed industries (Coelho *et al*., 2023). If not repurposed, these byproducts contribute to environmental pollution; however, they are rich in bioactive compounds, particularly carotenoids (80%–90% lycopene), flavonoids, and dietary fiber, which may benefit poultry health (Borycka, 2017; Ferhoum & Benakmoum, 2021).

Unlike previous studies using high doses of tomato flour (TF), with controversial results (Hosseini-Vashan *et al*., 2016; Omar *et al*., 2019; Mohammed *et al*., 2021;

Reda *et al*., 2022), this study investigates 1% and 2% TF supplementation. These levels were selected to provide bioactive compounds while minimizing the potential negative impact of fiber on digestibility, as high-fiber diets can reduce nutrient absorption and feed efficiency in broilers (Tejeda & Kim, 2021; Jha & Mishra, 2021). Additionally, lower TF inclusion allows for better adaptation in commercial poultry diets, maintaining palatability and nutritional balance.

Given that heat stress alters the intestinal structure and liver function, we hypothesize that dietary TF supplementation will mitigate these effects by improving gut integrity, hepatic health, and enhancing productive performance. Additionally, as females are more vulnerable to intestinal disorders than males (Caekebeke *et al*., 2020; Müsse *et al*., 2022), this study explores whether TF supplementation provides sex-dependent benefits under heat-stress conditions. By addressing these aspects, this research provides novel insights into the potential of TF as a functional feed ingredient for broilers raised in tropical climates, promoting poultry health, valorizing tomato processing byproducts, and contributing to sustainable poultry farming.

MATERIALS AND METHODS

Ethical Approval

The study was conducted at the Experimental Centre of Veterinary Medicine at the Technical University of Manabí, Ecuador. This study was approved by the Bioethics Committee of the Technical University of Manabí, Manabí-Ecuador, on January 13, 2021 (021-1/21-1-1).

Tomato Flour Preparation

Tomatoes from the local market were selected based on their ripeness and color (Famuyini *et al*., 2020). They were dried using a hot air stream at 60 °C ± 2 to produce flour with a particle size of 0.02 mm, using an IKA MF10B mill. The flour was stored under dry conditions at temperatures below 30 °C, away from light, as reported by Saltos *et al*. (2021).

Broiler Management and Diet

A total of 300 Cobb 500 broiler chickens (48.8 ± 2.6 g) were reared in a conventional poultry house under natural environmental conditions. During the first week of life, the temperature was maintained at 32 °C using gas brooders and curtains. From day 21 onwards, fans were introduced to mitigate the impact of ambient temperature, which fluctuated in accordance with environmental conditions. Throughout the study, a 23:1 hour light-dark cycle was maintained. Temperature and humidity were recorded every two hours using a Boeco HTC-1 thermo-hygrometer.

Birds had *ad libitum* access to food and water from day 1 to 41. Table 1 details the diet formulation and composition. TF was added daily by premixing with 500 g of basal diet before homogenizing with the remaining feed (Saltos *et al*., 2021). On day 28, male birds were randomly assigned to three treatments (T1, T2, and T3) and female birds to three treatments (T4, T5, and T6) based on TF levels (0%, 1%, and 2%). The experimental treatments were: T1 (control, no TF supplementation, males), T2 and T3 (males with 1% and 2% TF, respectively), and T4, T5, and T6 (females with 0%, 1%, and 2% TF, respectively). Each treatment consisted of 5 replicates with 10 chickens per replicate, maintaining a density of six birds per m². Weekly records of the body weight of all birds and feed intake per replicate were taken using a CAMRY EK5055 digital scale with a capacity of 5000 g ± 1 g. These data allowed the calculation of weekly weight gain and the feed conversion ratio (FCR) for the treatments.

Sample Collection and Analysis

At 42 days of age, after a 10-hour overnight fast, two birds per replicate (10 per treatment) were randomly selected and euthanized by exsanguination. Manual selection within each replicate was conducted without subjective criteria such as size, weight, or appearance and was based on a uniform distribution of birds without following a predictable pattern. This approach guaranteed that all birds had an equal likelihood of selection, minimizing bias and controlling for confounding factors to ensure a fair representation of each group.

Exsanguination was performed with a single incision of the jugular vein using a sterile, straight-edged scalpel without compromising the spinal cord. To prevent involuntary movements, the birds were restrained in a holding cone, yielding approximately 8 mL of blood per bird. Blood samples were collected and centrifuged at 1700 g for 15 minutes. Serum samples were analyzed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, and albumin using a Paramedical PKL PPC 115 spectrophotometer, following the manufacturer's instructions (Spinreact, Spain). Globulin concentration was calculated by subtracting albumin from total protein concentration (Rezende *et al*., 2017), and the AST/ALT and albumin/globulin (A/G) ratios were also calculated.

Histological Analysis

For histological analysis, ten samples from the distal portion of the right hepatic lobe were collected per treatment, corresponding to the birds sacrificed in each experimental group. In each liver sample, measurements were taken from four distinct microscopic fields, counting microvacuoles (small cytoplasmic fat droplets without nuclear displacement) and macrovacuoles (larger droplets that displace the nucleus) in a 40× field (Takahashi & Fukusato, 2014).

Additionally, one sample from each section of the small intestine was collected per replicate, resulting in a total of five samples per treatment: duodenum (3 cm from its origin), jejunum (3 cm distal to the duodenal loop), and ileum (3 cm posterior to Meckel's diverticulum). Ten intact villi were randomly measured in each intestinal sample to assess VH, CD, and the VH:CD ratio.

Table 1. Composition and nutritional content of pre-experimental and experimental diets in the grower and finisher phases of male and female broilers supplemented with tomato flour

Items	GF	FF1	FF2 (Control)	FF2 (1% TF)	FF2 (2% TF)
Ingredients (%)					
Ground corn	63.25	68.65	72.45	70.15	68.45
Soybean meal	30.00	25.80	22.5	23.00	23.00
Palm oil	2.50	2.00	1.50	2.30	3.00
Calcium carbonate	1.20	1.00	1.00	1.00	1.00
Dicalcium phosphate	2.00	1.50	1.50	1.50	1.50
L-Lysine -HCl	0.20	0.20	0.20	0.20	0.20
DL-Methionine	0.15	0.15	0.15	0.15	0.15
Premix for broilers	0.20	0.20	0.20	0.20	0.20
Sodium chloride	0.30	0.30	0.30	0.30	0.30
Antimycotics	0.20	0.20	0.20	0.20	0.20
Tomato Flour	-	-	-	1.00	2.00
Total	100	100	100	100	100
Calculated nutritional content					
ME (Kcal/kg)	3074.80	3142.05	3151.78	3153.45	3155.46
Protein (%)	20.23	18.69	17.46	17.62	17.59
Moisture (%)	12.20	12.43	12.55	12.42	12.31
Crude fiber (%)	2.67	2.67	2.67	2.74	2.81
Ether extract (%)	5.36	5.01	4.61	5.53	6.01
Lysine (%)	1.24	1.12	1.03	1.04	1.04
Methionine (%)	0.49	0.47	0.46	0.46	0.45
Tryptophan (%)	0.27	0.25	0.22	0.23	0.23
Ash (%)	5.87	5.01	4.87	4.87	4.84
Calcium (%)	1.00	0.80	0.79	0.80	0.79
Available Phosphorus (%)	0.45	0.40	0.40	0.39	0.39

Note: Grower Feed (GF) was provided from day 1 to 14, Finisher Feed 1 (FF1) from day 14 to 28, and Finisher Feed 2 (FF2) from day 28 to 42. Tomato Flour (TF) was added at 1% and 2% in the FF2 phase. Treatments were assigned to male (M) and female (F) broilers as follows: T1: M-0% TF, T2: M-1% TF, T3: M-2% TF, T4: F-0% TF, T5: F-1% TF, T6: F-2% TF. Chemical composition was calculated based on data from the Spanish Foundation for the Development of Animal Nutrition (Fundación Española para el Desarrollo de la Nutrición Animal, FEDNA).

All samples were fixed in ethanol-formalin, embedded in paraffin, sectioned, and stained with Haematoxylin and Eosin (H&E) (Saltos *et al*., 2021). The analysis was carried out using an OPTIKA B-380Pli microscope, an AmScope MU 1000 camera, and AmScope 3.7 software.

Statistical Analysis

Normality and homogeneity of variance assumptions were assessed using the Ryan-Joiner and Bartlett tests, respectively. A two-way ANOVA with a 2 × 3 factorial arrangement was performed, considering sex (male and female) and TF dose (0%, 1%, and 2%) as factors. This analysis enabled the evaluation of the main effects of TF dose, sex, and their interaction (TF × sex). When significant effects were detected, Tukey's test was applied for multiple pairwise comparisons. For variables where the assumptions of normality and homogeneity were not met (VH:CD ratio of the duodenum, crypt depth of the jejunum and ileum, and A/G ratio), non-parametric methods were used: the Kruskal-Wallis test followed by Dunn's multiple pairwise comparisons. Statistical significance was set at $p<0.05$. All analyses were performed using Minitab 20 software, and GraphPad Prism 8 was employed for graphical presentation and Dunn's tests.

RESULTS

Temperature and Humidity Patterns

During weeks 5 and 6, the lowest temperature (23.41 ± 0.93 and 23.11 ± 0.76 °C) was recorded at 04:00, while the highest (31.24 ± 1.27 and 29.39 ± 1.81 °C) occurred at 14:00. Temperatures above 25 °C were observed between 08:00 and 20:00 in week 5. Maximum humidity during the fifth and sixth weeks (84.57 ± 8.06% and 90.57 ± 2.94%) was at 02:00 and 04:00, while minimum humidity (57.00 ± 8.42% and 70.00 ± 9.90%) occurred at 12:00.

Body Weight, Feed Intake, and Feed Conversion

Males had a higher initial body weight on day 28 (1773.7 ± 50.3 g) than females (1523.0 ± 38.2 g) ($p<0.001$). For males, the mean body weight per treatment was as follows: T1 (1755.4 ± 20.4 g), T2 (1811.8 ± 73.6 g), and T3 (1753.9 ± 17.6 g). For females, the mean body weight per treatment was as follows: T4 (1506.2 ± 49.4 g), T5 (1527.6 ± 35.5 g), and T6 (1535.2 ± 29.3 g), with no significant differences between treatments within each sex. At day 42, greater weight was observed in males ($p<0.001$) and in chickens supplemented with TF ($p = 0.017$), with a significant sex × TF interaction ($p=0.015$). Males receiving 1% TF achieved the highest final weight, whereas TF supplementation did not affect females. Between days 28 and 42, males receiving 1% TF experienced greater weight gain (p<0.05). Although weight gain declined between days 36 and 42, the reduction was less pronounced in this treatment. Feed intake was higher in males (p<0.001) but remained unaffected by TF. FCR decreased in males supplemented with 1% TF (p=0.004) but was unchanged in females. Table 2 shows the weight at day 42, cumulative feed intake and conversion during the experimental period, and the reduction in weight gain during the last week of the experiment.

Liver Function

The serum total protein concentration was higher in females and increased with TF dose (p<0.05), whereas albumin levels were unaffected by either sex or TF dose. Globulin concentration was higher in females (p=0.027) and increased with 2% TF supplementation (p=0.002), resulting in a decreased A/G ratio, which was more pronounced in males (p=0.015). AST levels were significantly higher in males and in broilers supplemented with TF (p<0.05), with a sex-TF interaction showing elevated levels in supplemented males but not in females (p<0.001). ALT levels and the AST/ALT ratio were un-affected by sex or TF dose, as shown in Table 3.

Liver Lipid Infiltration and Intestinal Morphology

A significant reduction in the count of macro- and microvacuoles was observed in hepatocytes from females and males supplemented with 2% TF (p<0.001; Figures 1 and 2). In the duodenum, 1% TF increased duodenal VH and the VH: CD ratio, while 2% TF reduced both, particularly in females with CD increasing with TF dose (p<0.001). An interaction was observed in VH and CD (p<0.05). In the jejunum, males exhibited taller villi and a higher VH: CD ratio (p<0.05). However, TF supplementation increased CD (p=0.001) and tended to decrease the VH: CD ratio, especially in females receiving 2% TF (p=0.071). Notably, males had greater VH and CD, whereas females had a lower VH: CD ratio (p=0.001). In the ileum, unsupplemented females or those receiving 2% TF had higher VH and VH:CD ratio, whereas 1% TF reduced both parameters (p<0.001), with a sex and TF dose interaction observed

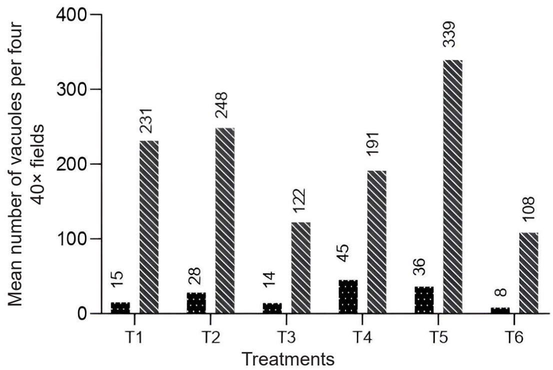

Figure 1. Mean number of hepatic vacuoles per sample (based on four 40× fields) in broiler chickens supplemented with tomato flour. The figure shows the mean number of macrovacuoles (■) and microvacuoles (▧) observed in hepatocytes, evaluated as an indicator of hepatic lipid infiltration in broiler chickens supplemented with 0%, 1%, and 2% tomato flour. Treatments were assigned to male (M) and female (F) broilers as follows: T1: M–0% TF, T2: M–1% TF, T3: M–2% TF, T4: F–0% TF, T5: F–1% TF, and T6: F–2% TF.

Table 2. Growth performance of broiler chickens supplemented with tomato flour

Variables	Treatments						*p*-value		
	T1	T2	T3	T4	T5	T6	Sex	TF	TF × sex
Body weight day 42 (g)	2931.4 ± 90.2[b]	3134.4 ± 166.8[a]	2928.6 ± 46.9[b]	2535.2 ± 37.9[c]	2536.8 ± 36.4[c]	2542.7 ± 36.4[c]	<0.001	0.017	0.015*
Cumulative feed intake (g)	2354.3 ± 185.1	2404 ± 313	2469 ± 275	1942.2 ± 110	1938.4 ± 152.1	2094.3 ± 155.5	<0.001	0.335	0.891
Cumulative feed conversion ratio	2.003 ± 0.07	1.812 ± 0.10	2.107 ± 0.23	1.888 ± 0.09	1.919 ± 0.12	2.082 ± 0.19	0.994	0.040	0.090
Reduction body weight 6th week (g)	-124.3 ± 108.5	-19.9 ± 106.5	- 104.7 ± 96.4	-48.2 ± 52.7	-95.9 ± 45.2	-94.4 ± 144.2	0.923	0.602	0.242

Note: Values are expressed as mean ± SD of the total number of broiler chickens per treatment (two-way ANOVA; Tukey's test). Treatments correspond to: T1 (M–0% TF), T2 (M–1% TF), T3 (M–2% TF), T4 (F–0% TF), T5 (F–1% TF), and T6 (F–2% TF), where M= males and F= females. Different superscripts indicate significant differences (p<0.05) between treatment groups. ANOVA: p-values for sex, TF, and TF × sex interaction (marked with *) are shown. Significant TF × sex interaction detected by two-way ANOVA (p<0.05).

Table 3. Liver function of broiler chickens supplemented with tomato flour

Biochemical variables	Treatments						*p*-value		
	T1	T2	T3	T4	T5	T6	Sex	TF	TC
Total protein	2.18 ± 0.40	2.22 ± 0.38	2.62 ± 0.28	2.56 ± 0.16	2.96 ± 0.21	2.95 ± 0.41	<0.001	0.026	0.31
Albumin	1.21 ± 0.26	0.94 ± 0.24	0.79 ± 0.14	1.21 ± 0.27	1.09 ± 0.27	1.12 ± 0.27	0.088	0.072	0.34
Globulin	0.97 ± 0.48	1.28 ± 0.40	1.83 ± 0.35	1.350 ± 0.38	1.87 ± 0.09	1.83 ± 0.42	0.027	0.002	0.225
A/G †	1.47[a]	0.63[ab]	0.44[b]	0.77[ab]	0.54[ab]	0.54[ab]	0.803	0.003	0.015
AST	137.1 ± 30.8[b]	212.9 ± 13.94[a]	210.25 ± 14.27 [a]	181.9 ± 38.3 [ab]	147.8 ± 27.5[b]	170.25 ± 20.16[ab]	0.043	0.040	<0.001*
ALT	19.39 ± 3.5	23.29 ± 5.56	21.47 ± 2.97	21.48 ± 3.52	19.6 ± 3.92	18.25 ± 2.33	0.481	0.665	0.457
AST/ALT	7.11 ± 1.45	9.67 ± 2.80	9.95 ± 1.6	8.73 ± 2.09	8.08 ± 3.65	9.37 ± 0.88	0.191	0.207	0.622

Note: Values are expressed as mean ± SD (two-way ANOVA; Tukey's test) or median (Kruskal-Wallis; Dunn's comparisons). Each group (n= 10 broilers/treatment) corresponded to: T1 (M–0% TF), T2 (M–1% TF), T3 (M–2% TF), T4 (F–0% TF), T5 (F–1% TF), and T6 (F–2% TF), where M= males and F= females. Different superscripts indicate significant differences (p<0.05). ANOVA: p-values for Sex, TF and TF × sex interaction (marked with *) are shown. Kruskal-Wallis (†): p-values for sex, TF, and treatment comparisons (TC) are shown. *Significant TF × sex interaction detected by two-way ANOVA (p<0.05). † Variable analyzed with Kruskal-Wallis test.

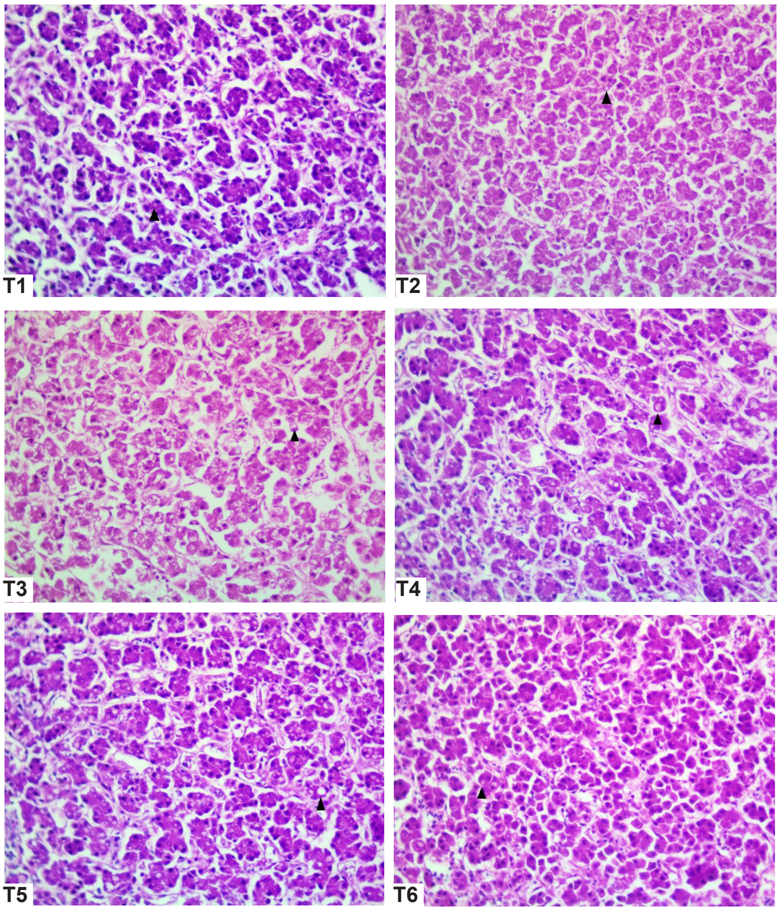

Figure 2. Liver micrographs of lipid infiltration in hepatocytes (H&E; 40×) in broiler chickens supplemented with tomato flour. Treatments were assigned to male (M) and female (F) broiler chickens as follows: T1: M-0% TF, T2: M-1% TF, T3: M-2% TF, T4: F-0% TF, T5: F-1% TF, T6: F-2% TF. Triangles indicate lipid infiltration in hepatocyte vacuoles.

Table 4. Intestinal morphometry of broiler chickens supplemented with tomato flour

Variables	Treatments						*p*-value		
	T1	T2	T3	T4	T5	T6	Sex	TF	TC
Duodenal measurements									
Height villi (μm)	2187.7 ± 82.6^{c}	2516.8 ± 101^{b}	2192.0 ± 107.3^{c}	2253.7 ± 44.2^{c}	2881.1 ± 179.7^{a}	1785.6 ± 113.3^{d}	0.833	<0.001	<0.001*
Crypt depth (μm)	446.7 ± 17.67^{ab}	420.98 ± 9.7^{bc}	431.72 ± 12.2^{abc}	406.77 ± 21.7^{c}	423.61 ± 11.3^{abc}	458.7 ± 36.5^{a}	0.616	0.023	0.001*
VH:CD ratio †	4.96 ± 0.31^{c}	6.1 ± 0.4^{ab}	5.17 ± 0.2^{b}	5.66 ± 0.4 ^{ab}	6.84 ± 0.4^{a}	4.22 ± 0.3^{d}	0.658	<0.001	<0.001
Jejunal measurements									
Height villi (μm)	1769.0 ± 87.6	1791.3 ± 107	1819.5 ± 35.2	1642.1 ± 139.6	1640 ± 99.6	1539.9 ± 56.7	<0.001	0.631	0.119
Crypt depth (μm) †	281.58^{ab}	318.64^{a}	315.34^{a}	270.42^{b}	286.38^{ab}	321.7^{a}	0.164	<0.001	0.002
VH:CD ratio	6.27 ± 0.50	5.71 ± 0.7	5.81 ± 0.5	6.18 ± 0.29	5.62 ± 0.48	4.96 ± 0.37	0.039	0.001	0.071
Ileal measurements									
Height villi (μm)	890.7 ± 60.7^{a}	821.1 ± 31.4^{a}	825.4 ± 59.1^{a}	901.4 ± 58.7^{a}	629.2 ± 78.1^{b}	874.8 ± 62.2^{a}	0.036	<0.001	<0.001*
Crypt depth (μm) †	240.64^{ab}	242.87^{a}	237.73^{ab}	225.4^{ab}	190.17^{b}	201.15^{b}	<0.001	0.679	0.005
VH:CD ratio	3.78 ± 0.31^{bc}	3.44 ± 0.2^{c}	3.50 ± 0.13^{c}	4.12 ± 0.23^{ab}	3.37 ± 0.34^{c}	4.49 ± 0.31^{a}	<0.001	<0.001	<0.001*

Note: Values are expressed as mean ± SD (two-way ANOVA; Tukey's test) or median (Kruskal-Wallis; Dunn's comparisons). Each group (n= 5 broilers/treatment) corresponded to: T1 (M–0% TF), T2 (M–1% TF), T3 (M–2% TF), T4 (F–0% TF), T5 (F–1% TF), and T6 (F–2% TF), where M= males and F= females. Different superscripts indicate significant differences ($p<0.05$). ANOVA: p-values for Sex, TF and TF × sex interaction (marked with *) are shown. Kruskal-Wallis (†): p-values for, sex, TF and treatment comparisons (TC) are shown. *Significant TF × sex interaction detected by two-way ANOVA ($p<0.05$). † Variable analyzed with Kruskal-Wallis test.

in both cases. Conversely, TF supplementation did not affect CD (p=0.679; Table 4).

DISCUSSION

Temperatures above 25 °C induce heat stress in poultry, reducing gastrointestinal tract (GIT) blood flow and compromising intestinal integrity, which negatively affects health and productivity (Kpomasse *et al*., 2021; Brugaletta *et al*., 2022; Liu *et al*., 2022). This study used a density of 6 birds/m² to minimize the impact of overcrowding, compared to conventional rearing with 10 chickens/m² (Asaniyan & Akinduro, 2020).

Productivity Performance

Consistent with the findings of Madilindi *et al*. (2018) and Da Costa *et al*. (2017), feed intake, weight gain, final body weight, and carcass weight were higher in male chickens, which can be attributed to sexual dimorphism (M**ü**sse *et al*., 2022). Feed intake was higher in males receiving TF, while in females, it remained similar across treatments. This partially aligns with previous studies in quails and broilers supplemented with tomato byproducts. The improvement in feed intake could be related to the increased palatability conferred by TF, as well as the alleviation of heat stress, possibly due to the antioxidant and antimicrobial properties of the bioactive compounds in tomatoes. Additionally, TF's potential to enhance digestive enzyme activity may have contributed to this effect (Olugbenga *et al*., 2022; Alagawany *et al*., 2022).

In broilers, the antioxidant activity of bioactive such as lycopene (Selim *et al*., 2013; Arain *et al*., 2018; Wang *et al*., 2020) could explain the attenuation of the reduction in weight gain observed in male chickens supplemented with 1% TF towards the end of the production cycle, a period when birds are particularly susceptible to heat stress (Saltos *et al*., 2021; Malila *et al*., 2022). In contrast, increasing the TF dose to 2% under oxidative stress conditions may stimulate the pro-oxidant activity of carotenoids, leading to cellular damage (Ribeiro *et al*., 2018; Shin *et al*., 2020). Furthermore, Selim *et al*. (2013) observed a lower FCR in broilers supplemented with 0.5% tomato puree but not with 1%, compared to the control group. In the present study, the better performance observed in broilers supplemented with 1% TF resulted in a lower FCR at this dose compared to supplementation with 2% TF.

Intestinal Morphology

The effect of the TF dose on intestinal morphology was influenced by the sex of the bird, with a significant statistical interaction observed, resulting in a more pronounced effect in females. Supplementation with 1% TF increased duodenal VH, suggesting improved intestinal integrity (Ducatelle *et al*., 2018; Alagawany *et al*., 2022; Hidayat *et al*., 2023). However, 2% TF led to a marked reduction in VH, especially in females, likely due to their higher susceptibility to intestinal dysbiosis, oxidative stress, and inflammation, where carotenoids may act as pro-oxidants (Ducatelle *et al*., 2018; Shin *et al*., 2020; Caekebeke *et al*., 2020). This interaction also explains the increased duodenal CD in females at higher TF doses, indicating a greater proliferative response. In contrast, males showed stable CD, possibly due to their better ability to maintain intestinal homeostasis under heat stress (Caekebeke *et al*., 2020). The best VH: CD ratio was achieved with 1% TF, highlighting that females respond more favorably to this dose but are more vulnerable to the adverse effects of 2% TF.

In the ileum, unsupplemented females or those supplemented with 2% TF had greater VH than those receiving 1% TF, while males exhibited similar VH across treatments. This suggests, in females, a response dependent on the impact of TF on the morphology of the cranial sections of the small intestine, which could induce compensatory growth of the ileal villi (Yamauchi, 2002). The VH: CD ratio in the ileum was primarily influenced by the reduction in VH in females supplemented with 1% TF.

Hepatic Function and Serum Parameters

Although heat stress can induce hepatic steatosis (Simon *et al*., 2020; Emami *et al*., 2021), the decreased presence of vacuoles in hepatocytes with 2% TF suggests a protective effect, likely due to the anti-adipogenic properties of carotenoids (Li *et al*., 2018; Wang *et al*., 2020; Wan *et al*., 2021). Additionally, ALT levels remained unchanged, indicating that TF supplementation does not induce hepatocellular damage (Pozzo *et al*., 2013; Senanayake *et al*., 2015). On the other hand, AST is widely distributed in tissues; it is less specific as a marker for liver injury (York, 2017). The increase in AST levels in males supplemented with 1% and 2% TF may be linked to their greater weight gain, final weight, and feed intake, factors associated with heat stress and rapid growth (Senanayake *et al*., 2015; Tang *et al*., 2022).

Lower globulin levels in unsupplemented chickens are consistent with studies linking heat stress to reduced serum globulin concentrations (Senanayake *et al*., 2015; Siddiqui *et al*., 2022). TF supplementation increased globulin levels, suggesting a positive effect on the immune response of birds under heat stress (Siddiqui *et al*., 2022). On the other hand, higher serum total protein levels in females align with the findings of Rezende *et al*. (2017) and are likely associated with elevated estrogen levels (Scholtz *et al*., 2009).

The tendency for lower albumin concentrations in males may reflect their greater body development and amino acid requirements (Rezende *et al*., 2017; Awad *et al*., 2017; England *et al*., 2023). In broiler chickens, oxidative stress induced by heat stress increases non-esterified fatty acid levels, requiring the transport of albumin (Ward *et al*., 2022; Teyssier *et al*., 2023). The tendency for lower albumin concentrations following TF supplementation suggests a potential antioxidant effect similar to that observed in broiler chickens supplemented with lycopene (Arain *et al*., 2018; Wang *et al*., 2020; Hidayat *et al*., 2023). These findings highlight the potential of TF to improve the health and performance of broilers under heat-stress conditions.

CONCLUSION

Supplementation with 1% TF enhanced final weight, feed efficiency, and intestinal health, particularly in males, while 2% TF reduced hepatic lipid infiltration in both sexes but negatively affected intestinal morphology in females. These findings suggest a dose- and sex-dependent response to TF supplementation. Further research is needed to clarify the mechanisms underlying these effects, particularly regarding potential interactions with oxidative stress regulation, nutrient metabolism, and intestinal health. Additionally, optimizing the dosage to enhance its benefits while minimizing adverse effects is essential, especially in heat-stressed conditions and different production stages.

CONFLICT OF INTEREST

The authors declare that this study was conducted without any commercial or financial relationships that could be perceived as a potential conflict of interest.

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REFERENCES

Ahmad, R., Yu, Y. H., Hsiao, F. S., Su, C. H., Liu, H. C., Tobin, I., Zhang, G., & Cheng, Y. H. (2022). Influence of heat stress on poultry growth performance, intestinal inflammation, and immune function and potential mitigation by probiotics. Animals, 12(17), 1-17. https://doi.org/10.3390/ani12172297

Alagawany, M., El-Saadony, M. T., El-Rayes, T. K., Madkour, M., Loschi, A. R., Di Cerbo, A., & Reda, F. M. (2022). Evaluation of dried tomato pomace as a non-conventional feed: its effect on growth, nutrients digestibility, digestive enzyme, blood chemistry and intestinal microbiota of growing quails. Food Energy Security, 11(4), 1–15. https://doi.org/10.1002/fes3.373

Ali, M. Y., Sina, A. A. I., Khandker, S. S., Neesa, L., Tanvir, E. M., Kabir, A., Khalil, M. I., & Gan, S. H. (2021). Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: A Review. Foods, 10(1), 1-32. https://doi.org/10.3390/foods10010045

Arain, M. A., Mei, Z., Hassan, F. U., Saeed, M., Alagawany, M., Shar, A. H., & Rajput, I. R. (2018). Lycopene: a natural antioxidant for prevention of heat-induced oxidative stress in poultry. World's Poultry Science Journal, 74(1), 89–100. https://doi.org/10.1017/S0043933917001040

Asaniyan, E. K., & Akinduro, V. O. (2020). Impact of stocking density on the rearing environment and growth performance of broiler chicken. Nigerian Journal of Animal Production, 47(6), 65–81. https://doi.org/10.51791/njap.v47i6.2875

Awad, E. A., Zulkifli, I., Soleimani, A. F., & Aljuobori, A. (2017). Effects of feeding male and female broiler chickens on low-protein diets fortified with different dietary glycine levels under the hot and humid tropical climate. Italian Journal of Animal Science, 16(3), 453–461. https://doi.org/10.1080/1828051X.2017.1291288

Borycka, B. (2017). Tomato fibre as potential functional food ingredients. Polish Journal of Natural Science, 32(1), 121–130. https://www.uwm.edu.pl/polish-journal/sites/default/files/issues/articles/borycka_2017.pdf

Brugaletta, G., Teyssier, J.-R., Rochell, S. J., Dridi, S., & Sirri, F. (2022). A review of heat stress in chickens. Part I: Insights into physiology and gut health. Frontiers in Physiology, 13, 1-15. https://doi.org/10.3389/fphys.2022.934381

Caekebeke, N., Ringenier, M., De Meyer, F., Ducatelle, R., Ongena, N., Van Immerseel, F., & Dewulf, J. (2020). A study on risk factors for macroscopic gut abnormalities in intensively reared broiler chickens. Avian Pathology, 49(2), 193–201. https://doi.org/10.1080/03079457.2019.1711019

Coelho, M. C., Rodrigues, A. S., Teixeira, J. A., & Pintado, M. E. (2023). Integral valorisation of tomato byproducts towards bioactive compounds recovery: Human health benefits. Food Chemistry, 410, 1-15. https://doi.org/10.1016/j.foodchem.2022.135319

Da Costa, M. J., Zaragoza-Santacruz, S., Frost, T. J., Halley, J., & Pesti, G. M. (2017). Straight-run vs. sex separate rearing for 2 broiler genetic lines Part 1: Live production parameters, carcass yield, and feeding behavior. Poultry Science, 96(8), 2641-2661. https://doi.org/10.3382/ps/pex035

Dawson, M. (2023). Poultry to comprise 40% global meat production in 2023. WATTAgNet. https://www.wattagnet.com/broilers-turkeys/broilers/article/15635594/worldwide-poultry-meat-production-outpaces-production-of-other-meat-sources

Ducatelle, R., Goossens, E., De Meyer, F., Eeckhaut, V., Antonissen, G., Haesebrouck, F., & Van Immerseel, F. (2018). Biomarkers for monitoring intestinal health in poultry: Present status and future perspectives. Veterinary Research, 49(43), 1-9. https://doi.org/10.1186/s13567-018-0538-6

Emami, N. K., Jung, U., Voy, B., & Dridi, S. (2021). Radical response: Effects of heat stress-induced oxidative stress on lipid metabolism in the avian liver. Antioxidants, 10(1), 1-15. https://doi.org/10.3390/antiox10010035

England, A., Gharib-Naseri, K., Kheravii, S. K., & Wu, S. B. (2023). Influence of sex and rearing method on performance and flock uniformity in broilers—Implications for research settings. Animal Nutrition, 12, 276–283. https://doi.org/10.1016/j.aninu.2022.12.003

Eslami, E., Carpentieri, S., Pataro, G., & Ferrari, G. (2023). A comprehensive overview of tomato processing byproduct valorization by conventional methods versus emerging technologies. Foods, 12(1), 1-33. https://doi.org/10.3390/foods12010166

Famuyini, J., Ayoola P O., & Sedara, A. (2020). Effect of maturity stage on quality and shelf life of tomato (*Lycopersicon esculentum* mill) using refrigerator storage system. Eurasian Journal of Agricultural Research, 4(1), 23-44. https://dergipark.org.tr/en/pub/ejar/issue/56788/766785

Farrell, D. (2016). The role of poultry in human nutrition. Food and Agriculture Organization of the United Nations. https://openknowledge.fao.org/handle/20.500.14283/AL709E

Ferhoum, F., & Benakmoum, A. (2021). Effects of different levels of dried tomato corps byproducts supplementation on carcass characteristics and fatty acid composition of broiler meat. Algerian Journal of Environmental Science and Technology, 7(1), 1769–1776. https://www.aljest.net/index.php/aljest/article/view/308

Hasted, T., Sharif, S., Boerlin, P., & Diarra, M. S. (2021). Immunostimulatory potential of fruits and their extracts in poultry. Frontiers in Immunology, 12, 1-11. https://doi.org/10.3389/fimmu.2021.641696

Hidayat, D. F., Mahendra, M. Y. N., Kamaludeen, J., & Pertiwi, H. (2023). Lycopene in feed as antioxidant and immuno-modulator improves broiler chicken's performance under heat-stress conditions. Veterinary Medicine International, 2023, 1-7. https://doi.org/10.1155/2023/5418081

Hosseini-Vashan, S. J., Golian, A., & Yaghobfar, A. (2016). Growth, immune, antioxidant, and bone responses of heat stress-exposed broilers fed diets supplemented with tomato pomace. International Journal of Biometeorology, 60(8), 1183-1192. https://doi.org/10.1007/s00484-015-1112-9

Jha, R., & Mishra, P. (2021). Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: a review. Journal of Animal Science and Biotechnology, 12(1), 1-16. https://doi.org/10.1186/s40104-021-00576-0

Kpomasse, C. C., Oke, O. E., Houndonougbo, F. M., & Tona, K. (2021). Broiler production challenges in the tropics: A review. Veterinary Medical Science, 7(3), 831–842. https://doi.org/10.1002/vms3.435

Li, C. C., Liu, C., Fu, M., Hu, K.-Q., Aizawa, K., Takahashi, S., Hiroyuki, S., Cheng, J., von Lintig, J., & Wang, X. D. (2018). Tomato powder inhibits hepatic steatosis and inflammation potentially through restoring SIRT1 activity and adiponectin function independent of carotenoid cleavage enzymes in mice. Molecular Nutrition & Food Research, 62(8), 1-51. https://doi.org/10.1002/mnfr.201700738

Liu, L., Sui, W., Yang, Y., Liu, L., Li, Q., & Guo, A. (2022).

Establishment of an enteric inflammation model in broiler chickens by oral administration with dextran sulfate sodium. Animals, 12(24), 1-12. https://doi.org/10.3390/ani12243552

Madilindi, M. A., Mokobane, A., Letwaba, P. B., Tshilate, T. S., Banga, C. B., Rambau, M. D., Bhebhe, E., & Benyi, K. (2018). Effects of sex and stocking density on the performance of broiler chickens in a subtropical environment. South African Journal of Animal Science, 48(3), 459-468. https://doi.org/10.4314/sajas.v48i3.6

Malila, Y., Sanpinit, P., Thongda, W., Jandamook, A., Srimarut, Y., Phasuk, Y., & Kunhareang, S. (2022). Influences of thermal stress during three weeks before market age on histology and expression of genes associated with adipose infiltration and inflammation in commercial broilers, native chickens, and crossbreeds. Frontiers in Physiology, 13, 1-14. https://doi.org/10.3389/fphys.2022.858735

Mohammed, L. S., Sallam, E. A., Edris, S. N., Khalifa, O. A., Soliman, M. M., & Shehata, S. F. (2021). Growth performance, economic efficiency, meat quality, and gene expression in two broiler breeds fed different levels of tomato pomace. Veterinary Research Communications, 45(4), 381-397. https://doi.org/10.1007/s11259-021-09819-x

Müsse, J., Louton, H., Spindler, B., & Stracke, J. (2022). Sexual dimorphism in bone quality and performance of conventional broilers at different growth phases. Agriculture, 12(8), 1-15. https://doi.org/10.3390/agriculture12081109

Olugbenga, O. O., Adebola, S. S., Friday, A. D., Mercy, A. T., & Keniokpo, O. S. (2022). Effect of dietary tomato powder on growth performance and blood characteristics of heat-stressed broiler chickens. Tropical Animal Health and Production, 54(37), https://doi.org/10.1007/s11250-021-03041-2

Omar, M., Abdel-hamid, T., Esam, S., & Omar, A. (2019). Growth and economic performance of using dried tomato pomace for mallard ducks. Slovenian Veterinary Research, 56(22), 699–706. https://doi.org/10.26873/SVR-810-2019

Pozzo, L., Tarantola, M., Biasibetti, E., Capucchio, M. T., Pagella, M., Mellia, E., Bergagna, S., Gennero, M. S., Strazzullo, G., & Schiavone, A. (2013). Adverse effects in broiler chickens fed a high lycopene concentration supplemented diet. Canadian Journal of Animal Science, 93(2), 231–241. https://doi.org/10.4141/cjas2012-081

Reda, F. M., Madkour, M., El-Azeem, N. A., Aboelazab, O., Ahmed, S. Y. A., & Alagawany, M. (2022). Tomato pomace as a nontraditional feedstuff: productive and reproductive performance, digestive enzymes, blood metabolites, and the deposition of carotenoids into egg yolk in quail breeders. Poultry Science, 101(4), 1-10. https://doi.org/10.1016/j.psj.2022.101730

Rezende, M., Mundim, A., Fonseca, B., Miranda, R., Oliveira, Jr., Lellis, C. (2017). Profile of serum metabolites and proteins of broiler breeders in rearing age. Brazilian Journal of Poultry Science, 19(04), 583–586. https://doi.org/10.1590/1806-9061-2016-0338

Ribeiro, D., Freitas, M., Silva, A. M. S., Carvalho, F., & Fernandes, E. (2018). Antioxidant and pro-oxidant activities of carotenoids and their oxidation products. Food and Chemical Toxicology, 120, 681-699. https://doi.org/10.1016/j.fct.2018.07.060

Saltos, D., Sancán, M., Arteaga, J., Gonzáles, C., Bulnes, C., Villanueva, M. E., & Reyna Gallegos, S. L. (2021). Dietary supplementation with an extract of purple corn (*Zea mays* L.) reduces vascular risk and hepatic steatosis in broilers. Revista de Investigaciones Veterinarias del Perú, 32(1), 1-15. https://doi.org/10.15381/rivep.v32i1.17634

Scholtz, N., Halle, I., Flachowsky, G., & Sauerwein, H. (2009). Serum chemistry reference values in adult Japanese quail (*Coturnix coturnix japonica*) including sex-related differences. Poultry Science, 88(6), 1186–1190. https://doi.org/10.3382/ps.2008-00546

Selim, N., Youssef, S., Abdel-Salam, A., & Nada, Sh. A.. (2013). Evaluations of some natural antioxidant sources in broiler diets: 1-effect on growth, physiological and immunological performance of broiler chicks. International Journal of Poultry Science, 12(10), 561-571. https://doi.org/10.3923/ijps.2013.561.571

Senanayake, S. S. H. M. M. L., Ranasinghe, J. G. S., Waduge, R., Nizanantha, K., & Alexander, P. A. B. D. (2015). Changes in the serum enzyme levels and liver lesions of broiler birds reared under different management conditions. Tropical Agricultural Research, 26(4), 584–595. https://doi.org/10.4038/tar.v26i4.8121

Shin, J., Song, M.-H., Oh, J.-W., Keum, Y.-S., & Saini, R. K. (2020). Pro-oxidant actions of carotenoids in triggering apoptosis of cancer cells: A review of emerging evidence. Antioxidants, 9(6), 1-16. https://doi.org/10.3390/antiox9060532

Siddiqui, S. H., Khan, M., Kang, D., Choi, H. W., & Shim, K. (2022). Meta-analysis and systematic review of the thermal stress response: *Gallus gallus domesticus* show low immune responses during heat stress. Frontiers in Physiology, 13, 1-11. https://doi.org/10.3389/fphys.2022.809648

Simon, G., Heckmann, V., Tóth, D., Pauka, D., Petrus, K., & Molnár, T. F. (2020). The effect of hepatic steatosis and fibrosis on liver weight and dimensions. Legal Medicine, 47, 1-7. https://doi.org/10.1016/j.legalmed.2020.101781

Takahashi, Y., & Fukusato, T. (2014). Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World Journal of Gastroenterology, 20(42), 15539–15548. https://doi.org/10.3748/wjg.v20.i42.15539

Tang, L.-P., Liu, Y.-L., Zhang, J.-X., Ding, K.-N., Lu, M.-H., & He, Y.-M. (2022). Heat stress in broilers of liver injury effects of heat stress on oxidative stress and autophagy in liver of broilers. Poultry Science, 101(10), 1-8. https://doi.org/10.1016/j.psj.2022.102085

Tejeda, O. J., & K. Kim, W. (2021). Role of dietary fiber in poultry nutrition. Animals, 11(2), 1-16. https://doi.org/10.3390/ani11020461

Teyssier, J. R., Cozannet, P., Greene, E., Dridi, S., & Rochell, S. J. (2023). Influence of different heat stress models on nutrient digestibility and markers of stress, inflammation, lipid, and protein metabolism in broilers. Poultry Science, 102(11), 1-16. https://doi.org/10.1016/j.psj.2023.103048

Wan, X., Yang, Z., Ji, H., Li, N., Yang, Z., Xu, L., Yang, H., & Wang, Z. (2021). Effects of lycopene on abdominal fat deposition, serum lipids levels and hepatic lipid metabolism-related enzymes in broiler chickens. Animal Bioscience 34(3), 385–392. https://doi.org/10.5713/ajas.20.0432

Wang, J., Geng, T., Zou, Q., Yang, N., Zhao, W., Li, Y., Tan, X., Yuan, T., Liu, X., & Liu, Z. (2020). Lycopene prevents lipid accumulation in hepatocytes by stimulating PPARα and improving mitochondrial function. Journal of Functional Foods, 67, 1-10. https://doi.org/10.1016/j.jff.2020.103857

Ward, E. S., Gelinas, D., Dreesen, E., Van Santbergen, J., Andersen, J. T., Silvestri, N. J., Kiss, J. E., Sleep, D., Rader, D. J., Kastelein, J. J. P., Louagie, E., Vidarsson, G., & Spriet, I. (2022). Clinical significance of serum albumin and implications of FcRn inhibitor treatment in IgG-mediated autoimmune disorders. Frontiers in Immunology, 13, 1-13. https://doi.org/10.3389/fimmu.2022.892534

Yamauchi, K. (2002). Review on chicken intestinal villus histological alterations related with intestinal function. Poultry Science Journal, 39(4), 229–242. https://doi.org/10.2141/jpsa.39.229

York, M. J. (2017). Clinical Pathology. In A. S. Faqi (Ed.), A comprehensive guide to toxicology in nonclinical drug development (2nd ed., pp. 325–374). Elsevier. https://doi.org/10.1016/B978-0-12-803620-4.00014-1

Zaefarian, F., Abdollahi, M. R., Cowieson, A., & Ravindran, V. (2019). Avian liver: The forgotten organ. Animals, 9(2), 1-23. https://doi.org/10.3390/ani9020063