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Original Article

The Effects of Different Levels of Tomato Juice in Drinking Water on the Egg Quality, Performance, and Hematological Parameters of Babcock White Laying Hens

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ABSTRACT

This study investigates the use of tomato juice (TJ) to assess its effects on egg quality, laying hen performance, and various blood parameters. A total of 128 Babcock white laying hens, aged 50 weeks, were randomly divided into four groups (n=32), each consisting of four subgroups with eight hens. The experimental groups received tomato juice supplementation in their drinking water at concentrations of 0%, 1%, 2.5%, and 5% for four weeks. Fecal samples were collected on days 15 and 30 to evaluate total aerobic bacteria, *Lactobacillus* spp., *Enterococcus* spp., *Salmonella* spp., and *Clostridium perfringens* counts. The results showed that the 5% tomato juice group significantly increased egg weight (65.95 g/hen/day) and egg mass (56.52 g/hen/day) compared to the control group (62.63 g/hen/day and 45.35 g/hen/day, respectively). Additionally, the 5% TJ group showed improvements in egg quality, with a higher Haugh unit (80.77) and yolk color (11.21) compared to the control group (75.65 and 10.13, respectively). In conclusion, tomato juice supplementation positively affected laying hen performance, egg quality, and blood Vitamin E levels without adverse effects. Additionally, TJ supplementation appeared to enhance intestinal microbiota balance. These findings suggest that tomato juice, when added to drinking water at different concentrations, can improve both egg quality and intestinal health in older laying hens. Furthermore, the unchanged *Enterococcus* counts in the SBM agar indicate that tomato juice supplementation did not significantly impact this bacterial population, suggesting it may not affect all gut microbiota uniformly. However, the reduction in *Lactobacillus* spp. in the 5% TJ group suggests a selective influence on intestinal microbiota, which could be important for gut health management in laying hens.

INTRODUCTION

In the 19th century, synthetic antibiotics were widely used in poultry production to maintain health and productivity. However, recent discoveries have highlighted the potential of plant-based extracts as alternatives to synthetic antibiotics, offering benefits without compromising the performance of poultry farms (Gultepe *et al*., 2020). Numerous herbal and phytogenic products, including essential oils, powders, and extracts, have shown positive effects on the performance of laying hens (Ahmadi & Rahimi, 2011). These herbal extracts often possess antioxidant properties that help neutralize free radicals, protecting hens from stress-related health issues (Tatli *et al*., 2015). For example, peppermint oil has been shown to enhance the immune system and performance of laying hens, and to improve growth, immunity, and feed conversion ratios of broilers (Durrani & Chand, 2008).

Tomatoes (*Lycopersicon esculentum Mill*.) are particularly important vegetables, ranking second only to potatoes in global production. In 2022, global tomato production reached 186 million tons, with major producers including China, India, Turkey, the United States, Egypt, Italy, Mexico, Brazil, Nigeria, and Spain (FAOSTAT, 2023). Although most tomatoes are sold fresh, a significant portion is processed into products such as sauces, ketchup, tomato paste, canned tomatoes, and tomato juice. Tomato by-products, such as pomace, seeds, and peels, comprise 5% to 13% of the total fruit mass (Del Valle *et al*., 2007).

Tomatoes are rich in antioxidants, including carotenoids, vitamin C, and flavonoids, as well as phytochemicals like lycopene (Bohm *et al*., 1995). Lycopene, the red pigment in tomatoes, is a potent natural antioxidant capable of neutralizing free radicals. Its singlet oxygen quenching ability is ten times greater than that of α-tocopherol (Di Mascio *et al*., 1989). Consumption of tomato extracts has been linked to reductions in total cholesterol, triglycerides, and blood sugar levels, making it valuable for reducing the risk of cardiovascular conditions such as atherosclerosis (Duttaroy, 2011).

Tomato waste in the diets of laying hens has been shown to reduce egg yolk cholesterol and improve yolk color (Mahata *et al*., 2016). It is well known that the microorganisms in the digestive system are influenced by the consumption of herbal sources (Armstrong, 1996). In modern poultry production, vegetable-based additives are increasingly used to promote beneficial gut flora, replacing previously used antibiotics to boost yield (Markowiak & Slizewska, 2018). For instance, Mountzouris *et al*. (2007) demonstrated that probiotics delivered through drinking water were as effective as those included in feed at positively influencing broiler chicken intestinal flora.

Despite the known benefits of tomatoes and their by-products in poultry diets, the use of tomato juice in poultry drinking water has not been extensively studied, particularly in relation to its effects on egg production, egg quality, and gut health. While previous research has focused on tomato waste and extracts added to feeds, there is limited information on the impact of liquid tomato supplementation, such as juice, delivered via water. This method may offer enhanced bioavailability and rapid metabolism. This is a critical gap, as drinking water supplementation provides a more direct and efficient route for nutrient uptake compared to feed-based approaches, allowing for quicker physiological responses in hens.

This study aims to fill this research gap by exploring the effects of tomato juice supplementation through drinking water on the performance, egg characteristics, hematological parameters, and intestinal microbiota of laying hens. This method provides a unique approach to delivering antioxidants and other beneficial compounds present in tomatoes, with the potential to improve egg quality and hen health while enhancing intestinal microbiota balance. Furthermore, this research seeks to provide new insights into the bioavailability of tomato-derived nutrients when administered in liquid form, contributing to the growing body of literature on plant-based supplements as alternatives to synthetic antibiotics in poultry production.

MATERIALS AND METHODS

Ethical Approval

The entire experimental procedure was conducted at the Veterinary Research Laboratory of Afyon Kocatepe University, Turkey, with the approval of the ethics committee (Approval No: AKÜHADYEK-127-16, dated 08/11/2016).

Poultry Management and Experimental Design

A total of 128 fifty-week-old Babcock white laying hens were used in this study. The hens were randomly divided into four groups based on the level of tomato juice supplementation in their drinking water. Each group was further subdivided into 4 subgroups (n = 32), with each subgroup consisting of 8 hens. Freshly squeezed tomato juice was added to the drinking water at concentrations of 0%, 1%, 2.5%, and 5% for a duration of four weeks. The tomatoes were washed thoroughly, and the juice was extracted using a commercial juicer. The juice was then filtered to remove seeds and peels and stored in sterilized containers at 4°C to maintain freshness until use. Tomato juice was administered fresh every 24 hours to prevent spoilage or fermentation. A lighting schedule of 16 hours of light and 8 hours of darkness was maintained throughout the study. Feed was provided *ad libitum*, and all groups, including the control, received a basal diet formulated to meet the nutritional needs of laying hens, as specified by the NRC (1994) (Table 1).

Table 1 – Composition of basal diets.

FEEDSTUFFS	% (Fed Basis)
Corn	56.30
Sunflower Meal,28% CP	13.50
Soybean Meal 44%CP	10.00
Full-Fat Soybean	9.06
Dicalcium Phosphate	1.38
DL-Methionine	0.08
Limestone	9.00
L-Lysine Hydrochloride	0.03
Salt	0.40
Vitamin-Mineral Mix	0.25
Calculated values	
Dry matter,%	90.5
Crude protein,%	16.3
Metabolizable energy,kcal/kg	2739
Calcium,%	3.84
Available Phosphorus,%	0.32
Natrium,%	0.19
Methionine + Cystine,%	0.68
Lysine,%	0.79
Threonine,%	0.61
Tryptophan,%	0.21

Supplemented per kilogram of diet: Vitamin A: 12.000.000 IU; Vitamin D3: 3.000.000 IU; Vitamin E: 35.000 IU; Vitamin K3: 3.500 IU; Vitamin B1: 2.750 IU; Vitamin B2: 5.500 IU; Nicotinamide: 30.000 IU; Ca-D-Pantothenate: 10.000 IU; Vitamin B6: 4.000 IU; Vitamin B12: 15 IU; Folic acid: 1.000 IU; D-Biotin: 50 IU; Choline chloride: 150.000 IU; Manganese: 80.000 mg; Iron: 60.000 mg; Zinc: 60.000 mg; Copper: 5.000 mg; Iodine: 2.000 mg; Cobalt: 500 mg; Selenium: 150 mg; Antioxidant: 15.000 mg.

Data Collection and Analysis

At the beginning and end of the study, all hens were weighed to determine their live body weights. Daily records of total egg production and mortality were kept, while feed consumption was measured weekly. The feed conversion ratio (FCR) and egg mass were calculated based on total feed consumption and egg production. Eggs were weighed weekly, and after the 2nd and 4th weeks, eggs were taken to a laboratory to analyze quality parameters such as egg weight, yolk color index, albumen index, yolk index, eggshell thickness, and Haugh unit. The Haugh unit was calculated using albumen height (Digital Caliper, CD-15CP, Mitutoyo Ltd., UK), following the method of Haugh (1937). The color of the egg yolk was assessed using the Improved Roche Yolk Color Fan (Yolk-Fan™, DSM Nutritional Products AG, Kaiseraugst, Switzerland), which compares yolk color with a 15-band color scale. Albumen and yolk indices were calculated according to the methods described by Tilki & Saatci (2004).

At the end of the trial, blood samples were randomly collected from 3 hens per subgroup. Blood samples were drawn directly from the heart, and blood cell counts of full blood were performed using a compact blood analyzer. Parameters measured included lymphocytes (LC), neutrophils (NC), total leukocyte count (TLC), red blood cell count (RBC), and monocytes (MC). Additional hematological parameters included mean corpuscular volume (MCV), hemoglobin concentration (He), mean corpuscular hemoglobin concentration (MCHC), and mean platelet volume (MPV).

For serum analysis, samples were centrifuged, and the supernatants were stored under appropriate conditions until analysis. An ELISA analyzer was used to measure high density lipoprotein (HDL), low density lipoprotein (LDL), glucose, total protein (TPRO), alanine aminotransferase (ALT), total cholesterol (CHO), aspartate aminotransferase (AST), alkaline phosphatase (ALP), phosphorus, calcium, Vitamin E, total oxidant capacity (TOS), total antioxidant capacity (TAC), Gamma Glutamyl Transpeptidase (GGT), and Immunoglobulin G (IgG). Serum analyses were performed using colorimetric methods and relevant commercial kits, following the manufacturer's guidelines for each parameter.

Microbiological Analysis

Fecal samples were collected from four hens per subgroup on the 15th and 30th days of the trial. Samples were randomly selected and collected in sterile fecal containers before being transported to the diagnostic laboratory of Afyon Kocatepe University for microbiological analysis. On fecal sampling days, plastic covers were placed beneath each cage and monitored frequently. Observations continued until a sufficient quantity of sample material was collected from each representative group.

Fecal samples (1g) were weighed and diluted in sterile saline solution at a ratio of 1:10. A total of 12 sterile Falcon tubes (15 mL) were used to prepare the dilutions. The agars for microbiological culturing were prepared under standardized conditions to assess total aerobic bacteria, *Lactobacillus* spp., *Enterococcus* spp., and the presence of *Salmonella* spp. and *Clostridium perfringens*. Further details of the microbiological procedures are presented in Table 2.

Table 2 – Microbiology applications and methods of the study.

Microorganism	Media	Incubation conditions	Methods
Aerobic bacteria's total number	Plate Count Agar (PCA), (Oxoid CM 325)	30 °C 48-72 hours-aerobic	ISO 4833 (2013)
Lactobacillus spp.	MRS Agar (Oxoid CM 3611)	30 °C 24-72 hours-aerobic	Kneifel & Berger (1994)
Enterococcus spp.	Slanetz and Bartley medıum (SBM) (Oxoid Code: CM 377)	37 °C 24-48 hours-aerobic	Jackson *et al*. (2005)
Salmonella spp.	Brilliant Green Phenol Red Lactose Sucrose Agar (BPLS) (Merck 1.07237)	35-37 °C 24-48 hours-aerobic	Agay & Kimiran (2017)
Cl. Perfiringens	Perfiringens Agar Base (Oxoid CM 543)	37 °C 24-48 hours-anerobic	Petit *et al*. (1999)

To ensure accurate results, a pilot study determined the appropriate dilution rates for each bacterial sample based on their multiplication rates. Dilutions were as follows: for PCA, 10^{-8} and 10^{-10}; for MRS, 10^{-6} and 10^{-8}; for SBM, 10^{-6} and 10^{-8}; for PBLS, 10^{-6} and 10^{-8}; and for *Clostridium perfringens*, 10^{-6} and 10^{-8}, following the methodology of Siriken *et al*. (2003). Samples were incubated under either aerobic or anaerobic conditions, depending on the bacterial species, at appropriate environmental temperatures. Bacterial colonies were then counted, and the data were recorded digitally for further analysis.

Statistical Analysis

All data were analyzed using a randomized block cage design, with each cage treated as the experimental unit to account for variability between cages. The PROC GLIMMIX procedure was used to analyze performance parameters, such as feed consumption, hen day egg production (HDEP), feed conversion ratio (FCR), egg weight, and egg mass, with repeated measures over time. Tomato juice supplementation, sampling week, and their interactions were considered as fixed effects, while the cage was treated as a random effect. Different covariance structures were tested, and the best structure for each parameter was selected based on Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). Tukey-Kramer adjustments were applied to control for Type I errors in *post-hoc* comparisons.

For single-point data such as egg traits, serum biochemical parameters, and fecal microbial counts, the same PROC GLIMMIX model was applied without considering time. Linear, quadratic, and cubic contrasts were used to explore trends across the different tomato juice supplementation levels (0%, 1%, 2.5%, and 5%). SAS PROC IML was used to calculate contrast coefficients, allowing for comparisons between treatment levels.

Data are presented as pooled SEM ± least-square means, with statistical significance set at $p \leq 0.05$. Tendencies for significance were noted for *p*-values between 0.05 and 0.15.

RESULTS AND DISCUSSION

In the present study, feed consumption increased linearly with the dose of tomato juice supplementation. Across the experimental groups, egg production rose over time compared to the control group. The experimental groups exhibited significantly higher ($p<0.05$) egg weight and egg mass values as compared to the control group. However, no significant differences were observed in feed conversion ratio (FCR) and body weight among the groups (Table 3).

Table 3 – Effects of tomato juice supplementation through drinking water on different parameters of performance in older laying hens (50 weeks of age) for four weeks.

Items	Initial body weight, g	Final body weight, g	Feed consumption, g/hen/day	HDEP[2], %	FCR[3]	Egg weight, g/hen/day	Egg mass, g/hen/day
Control	1518.50	1589.21	117.50	72.29	2.68	62.63	45.35^{b}
%1 TJ	1544.67	1602.03	113.12	74.40	2.43	64.22	47.75^{ab}
%2.5 TJ	1583.15	1624.34	123.75	85.27	2.28	65.26	55.77^{ab}
%5 TJ	1580.99	1618.92	126.25	85.71	2.29	65.95	56.52^{a}
SEM[4]	31.43	31.31	4.69	4.54	0.19	1.04	3.04
p-values							
Main effects							
TJ	0.41	0.85	0.23	0.12	0.45	0.18	0.05
Time	n.a.	n.a.	0.08	0.01	0.17	0.44	0.07
TJ×time	n.a.	n.a.	0.75	0.32	0.96	0.37	0.35
Contrasts[5]							
Cont *vs*. TJ	0.16	0.48	0.49	0.10	0.14	0.05	0.04
Linear	0.16	0.48	0.09	0.03	0.20	0.05	0.01
Quadratic	0.40	0.61	0.99	0.37	0.34	0.41	0.28
Cubic	0.85	0.88	0.26	0.45	0.87	0.83	0.47

Data are shown as least squares means. Values represent the average of four replicate cages per ration, each containing eight hens (n=32). Control*: no supplementation/plain drinking water;* %1 TJ*: 1% tomato juice supplementation in drinking water;* %2.5 TJ*: 2.5% tomato juice supplementation in drinking water;* %5 TJ*: 5% tomato juice supplementation in drinking water.* [2]*Hen-day egg production;* [3]*Feed conversion ratio: feed consumption (g)/egg mass (g);* [4]*Standard error of means;* [5]*Coefficients of contrast for tomato juice supplementation levels were calculated using PROC IML of SAS.* [a,b,c] *Means within the same column with different superscripts differ significantly (p<0.05). Means sharing a common superscript are not significantly different. Differences at 0.05<p<0.15 are indicated as tendencies.*

Water consumption was highest in the group supplemented with 2.5% tomato juice (Table 4). However, these observations only reflect group-level records, since statistical analysis was not feasible, as individual subgroups could not be recorded.

Table 4 – Effects of tomato juice water supplementation on water intake (ml/hen/day) of laying hens for 4 weeks.

Experimental Groups	Day 7	Day 14	Day 21	Day 28
Control	398	370	366	378
%1 TJ	298	345	321	321
%2.5 TJ	452	405	393	417
%5 TJ	357	339	363	353

Day 7, Day 14, Day 21 *and* Day 28 *indicate the measurement days.* Control, %1 TJ, %2.5 TJ, *and* %5 TJ *represent the experimental groups receiving different levels of tomato juice supplementation.*

Previous studies have reported mixed results regarding the effects of tomato-based supplements on egg production and feed intake. Akdemir *et al*. (2012) found that increasing tomato powder concentration in hen diets led to greater egg production, feed consumption, and egg weight, along with improved FCR. In contrast, Roberta *et al*. (2007) noted a decrease in egg yield with more than 5% tomato meal in the diet. Salajegheh *et al*. (2014) observed that up to 15% tomato powder could be included in laying hen diets, with 5% and 10% concentrations enhancing egg yield and mass, though FCR worsened at 15%.

The increased feed consumption in our study with tomato juice supplementation might be attributed to the high glutamic acid content in tomatoes. Glutamic acid, an excitatory neurotransmitter, enhances appetite by improving palatability. Safamehr *et al*. (2011) reported glutamic acid levels in tomatoes ranging from 282 to 3632 mg/L, with red tomatoes containing the highest concentrations.

Higher egg weight and mass in the experimental groups may be related to the elevated lycopene content in tomato juice. Nguyen and Schwartz (1998) found that tomato juice contains 5.0-11.6 mg/100g of lycopene, compared to 0.9-4.2 mg/100g in raw tomatoes. Lycopene's strong antioxidant properties could contribute to improved egg quality.

Regarding egg quality parameters, the Haugh unit and yolk color values significantly increased (p<0.05) in the 5% tomato juice group, though eggshell thickness was reduced. The enhanced yolk color is likely due to the lycopene content. Yilmaz *et al*. (2010) similarly observed that tomato powder darkened the yolk color. The reduced eggshell thickness in the 5% group might be attributed to the relatively low calcium content in tomato juice. Coïc *et al*. (1961) highlighted the importance of calcium for eggshell formation, suggesting that sodium and potassium in tomato juice might impair calcium absorption.

Table 5 – Effects of tomato juice supplementation through drinking water on different egg quality parameters of old laying hens (50 weeks age) throughout 4 weeks.

Items	Shell thickness mm		Yolk color		Albumen index		Yolk index		Haugh unit	
	Mid	Final	Mid	Final	Mid	Final	Mid	Final	Mid	Final
Control	0.3871	0.4248^{a}	10.06	10.13^{a}	5.48	5.06	41.41	41.21^{a}	79.87	75.65^{a}
%1 TJ	0.3866	0.4044^{ab}	10.33	10.17^{a}	5.62	5.35	42.79	44.54^{b}	79.99	79.33^{ab}
%2.5 TJ	0.3879	0.4015^{ab}	10.14	10.63^{a}	5.73	5.40	44.12	44.91^{b}	80.87	79.01^{ab}
%5 TJ	0.3833	0.3911^{b}	10.17	11.21^{b}	5.37	5.70	42.89	44.96^{b}	81.00	80.77^{b}
SEM[3]	0.0107	0.0075	0.13	0.18	0.24	0.28	0.69	0.80	0.88	1.26
p-values	0.99	0.02	0.50	0.0003	0.74	0.47	0.07	0.004	0.75	0.04
Contrasts[4]										
Cont *vs*. Juice	0.92	0.005	0.30	0.01	0.73	0.21	0.03	0.0003	0.47	0.008
Linear	0.80	0.007	0.94	<.0001	0.69	0.13	0.16	0.008	0.32	0.02
Quadratic	0.85	0.31	0.54	0.70	0.30	0.87	0.02	0.02	0.76	0.35
Cubic	0.91	0.33	0.16	0.53	0.91	0.69	0.84	0.18	0.76	0.19

Data are shown as least squares means. The values represent the average of four replicate cages per diet, each containing eight hens (n=32). Control*: no water supplementation/plain water;* 1% TJ*: 1% tomato juice supplementation in drinking water;* 2.5% TJ*: 2.5% tomato juice supplementation in drinking water;* 5% TJ*: 5% tomato juice supplementation in drinking water.* Mid*: represents the middle of the study;* Final*: denotes the end of the study.* [3]*Standard error of means;* [4]*Coefficients of contrast for tomato juice supplementation levels were calculated using SAS PROC IML.* [a,b,c]*: Means within the same column with different superscripts differ significantly (p<0.05). Means sharing a common superscript are not significantly different. Differences at 0.05<p<0.15 are indicated as tendencies.*

Biochemical parameters revealed a linear increase in total antioxidant capacity (TAC) and calcium levels in the experimental groups, while total protein values increased quadratically. Vitamin E levels were significantly higher in the 1% tomato juice group ($p<0.05$), and total oxidative status (TOS) values increased cubically. Other parameters, including IgG, HDL, GGT, cholesterol, and glucose, were not significantly affected ($p>0.05$) (Table 6). The observed increase in serum calcium levels in the experimental groups may indicate reduced calcium absorption, potentially due to high sodium and potassium levels in tomato juice, as noted by Kemi *et al*. (2007) and Sellmeyer *et al*. (2002).

The study's findings align with previous research on the antioxidant effects of lycopene, which may enhance egg quality and biochemical parameters by scavenging free radicals and reducing oxidative stress.

Table 6 – Effects of TJ supplementation through drinking water on some biochemical parameters of laying hens (50 weeks age) for 4 weeks.

Items	AST (U/L)	ALT (U/L)	GGT (U/L)	ALP (U/L)	HDL (mg/dL)	LDL (mg/dL)	CHO (mg/dL)	GLU (mg/dL)	TP (mg/dL)	Ca/p	PHOSP (mg/dL)	IGG (mg/dL)	VITE (µg/mL)	TAC (mmol/L)	TOS (µmol/L)
Control	132.13	0.825	21.75	233.25	13.50	35.38	83.38	185.62	4.65	19.58	3.79	150.62	3.96[a]	0.79	23.31
%1 TJ	164.25	0.803	21.63	226.50	14.13	44.13	112.12	177.87	6.03	20.71	3.98	186.12	4.58[b]	0.83	30.42
%2.5 TJ	173.29	0.817	24.29	210.71	14.29	40.57	93.57	186.86	6.00	22.63	4.50	208.57	3.92[a]	0.94	21.84
%5 TJ	131.75	0.809	21.25	230.25	13.38	37.25	84.88	188.38	5.20	25.20	4.80	209.75	4.32[ab]	1.00	36.25
SEM[9]	15.21	0.047	2.26	49.32	0.50	6.80	18.12	13.25	0.41	2.20	0.51	32.54	0.13	0.08	4.10
p-values	0.15	0.99	0.80	0.99	0.52	0.81	0.66	0.95	0.18	0.50	0.65	0.56	0.008	0.32	0.23
Contrasts[10]															
Cont *vs*. Juice	0.18	0.78	0.81	0.86	0.47	0.51	0.53	0.94	0.06	0.25	0.33	0.20	0.057	.0.145	0.24
Linear	0.71	0.89	0.97	0.97	0.69	0.92	0.73	0.73	0.79	0.14	0.23	0.23	0.60	.0.08	0.16
Quadratic	0.03	0.93	0.41	0.77	0.16	0.49	0.48	0.88	0.07	0.95	0.83	0.45	0.89	.0.77	0.41
Cubic	0.78	0.77	0.55	0.91	0.87	0.53	0.35	0.64	0.43	0.97	0.84	0.90	0.001	.0.81	0.13

AST: *Aspartate aminotransferase;* ALT: *Alanine aminotransferase;* GGT: *Gama-glutamyl transferase;* ALP: *Alkaline phosphatase;* HD: *high density lipoprotein;* LDL: *low density lipoprotein;* CHO: *Cholesterol;* GLU: *Glucose;* TP: *Total protein;* PHOSP: *Phosphorus;* IGG: *Immunoglobulin G;* VIT E: *Vitamin E;* TAC: *Total antioxidant capacity;* TOS: *Total oxidant status.*

This study investigated the effects of tomato juice supplementation on the hematological parameters of older laying hens (Table 7). Compared to the control group, hens receiving 1%, 2.5%, and 5% tomato juice supplementation showed significant differences in lymphocyte counts ($p<0.05$) and some other hematological parameters. Notably, 2.5% tomato juice supplementation positively influenced mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). The results suggest that tomato juice supplementation may have limited but potentially beneficial effects on specific hematological parameters.

Table 7 – Effects of tomato juice supplementation through drinking water on some hematological parameters of older laying hens (50 weeks age) for 4 weeks.

Items	TLC $\times10^9$/L	LC $\times10^9$/L	NC $\times10^9$/L	MC $\times10^8$/L	RBC $\times10^{12}$/L	He g/dL	Ht %	MCV fL	MCH pg	MCHC g/dL	PLT 10^{10}/L	MPV fL
Control	2.56	1.75[ab]	0.80	0.0441	2.675	10.48	34.07	108.29	29.35[a]	30.94	27.60	6.40
%1 TJ	2.32	1.80[ab]	0.70	0.0428	2.609	10.67	34.98	107.91	31.44[b]	30.36	27.81	6.50
%2.5 TJ	2.08	1.87[a]	0.72	0.0440	2.659	10.27	35.17	108.90	31.28[b]	30.98	26.67	6.56
%5 TJ	2.46	1.72[b]	0.67	0.0429	2.678	10.92	34.14	108.90	30.73[ab]	31.71	27.04	6.41
SEM[9]	0.26	0.04	0.04	0.0007	0.045	0.25	0.43	0.56	0.46	0.49	0.54	0.08
p-values	0.58	0.048	0.20	0.47	0.68	0.33	0.17	0.64	0.010	0.30	0.43	0.47
Contrasts[10]												
Cont *vs*. Juice	0.35	0.31	0.046	0.33	0.62	0.63	0.17	0.97	0.001	0.89	0.50	0.35
Linear	0.84	0.52	0.09	0.48	0.65	0.31	0.80	0.91	0.18	0.13	0.29	0.95
Quadratic	0.17	0.008	0.53	0.88	0.54	0.28	0.03	0.49	0.007	0.40	0.49	0.12
Cubic	0.88	0.51	0.23	0.16	0.35	0.26	0.70	0.28	0.09	0.42	0.29	0.98
Control	2.56	1.75[ab]	0.80	0.0441	2.675	10.48	34.07	108.29	29.35[a]	30.94	27.60	6.40

Control = *no water supplementation/plain water;* 1% TJ *: 1% tomato juice supplementation to drinking water;* 2.5% TJ*: 2.5% tomato juice supplementation to drinking water;* 5% TJ*: 5% tomato juice supplementation to drinking water;* MCV*: Mean corpuscular volume;* Ht*: Hematocrit;* He*: Hemoglobin;* RBC*: Red blood cell count;* MC*: Monocyte count;* NC*: Neutrophil count;* LC*: Lymphocyte count;* TLC*: Total leukocyte count;* MPV*: Mean platelet volume;* PLT*: Platelet;* MCHC*: Mean corpuscular hemoglobin concentration;* MCH*: Mean corpuscular hemoglobin.* [a,b,c:] *Means within the same column with different superscripts differ significantly (p<0.05). Means sharing a common superscript are not significantly different. Differences at 0.05<p<0.15 are indicated as tendencies.*

Olugbenga *et al*. (2022) found that most hematological parameters and serum enzymes were unaffected by tomato supplementation. Similarly, our study showed limited but positive effects on lymphocyte counts, MCV, and MCHC, without significant changes in other blood markers. This suggests that while tomato supplementation can enhance certain blood parameters, its overall impact on poultry health is beneficial and safe, with no observed adverse effects.

Microbiological results indicated no significant effect of tomato juice supplementation on *Enterococcus* spp. levels in SBM agar. However, significant changes were observed in MRS and PCA counts. At the second measurement, PCA values were significantly lower in the 1% and 2.5% tomato juice groups compared to the control group ($p<0.01$), while MRS counts were lower in the 2.5% group ($p<0.05$), but increased in the 5% group ($p<0.05$) (Table 8). The reduction in PCA values may be attributed to the antioxidant properties of lycopene, which can inhibit aerobic bacterial growth.

Table 8 – Effects of tomato juice supplementation through drinking water on the bacterial populations in feces of older laying hens (50 weeks age) for 4 weeks ($\log_{10}$ cfu/g).

Items	PCA	MRS	SBM	PBLS	PERF
Control	1.72[a]	1.14[ab]	0.64[b]	n.d.	n.d.
%1 TJ	1.08[b]	1.35[a]	1.42[a]	n.d.	n.d.
%2.5 TJ	0.97[b]	0.44[b]	1.10[ab]	n.d.	n.d.
%5 TJ	1.41[ab]	1.20[ab]	1.02[ab]	n.d.	n.d.
SEM[7]	0.14	0.22	0.15	-	-
p-values	0.002	0.04	0.05	-	-
Contrasts[8]					
Cont *vs*. Juice	0.001	0.59	0.01	-	-
Linear	0.40	0.73	0.53	-	-
Quadratic	0.0003	0.05	0.03	-	-
Cubic	0.29	0.03	0.01	-	-

Values shown as square least means. The data are an average of four replica cages in one diet with eight hens (n=32). Control= *no water supplementation/plain water;* %1 TJ*: %1 tomato juice supplementation to drinking water;* %2.5 TJ*: %2.5 tomato juice supplementation to drinking water;* %5 TJ*: %5 tomato juice supplementation to drinking water.* PCA*: Total aerobic plate count;* MRS*: Lactobacillus spp.;* SBM*: Enterococcus spp.;* PBLS*: Salmonella spp.;* PERF*: Clostridium perfiringens; The standard error of mean, n.d.: Not detected; Coefficients of contrast for tomato juice supplementation amount were measured using SAS PROC IML.* [a,b,c] *Means within the same column with different superscripts differ significantly (p<0.05). Means sharing a common superscript are not significantly different. Differences at 0.05<p<0.15 are indicated as tendencies.*

The suppression of *Salmonella* spp. in this study may be related to the low pH of tomato juice (4.10-4.60), which could inhibit bacterial growth. McClane *et al*. (2013) also noted that tomato products could reduce the growth of harmful bacteria such as *Cl. perfringens*. At the study's end, the control group had the highest levels of *Cl. perfringens*, while the 5% tomato juice group had the lowest levels.

CONCLUSION

In conclusion, tomato juice supplementation in the drinking water of laying hens may lead to improvements in performance parameters such as egg production and quality without negatively affecting egg characteristics. While positive effects were observed with lower doses, higher doses did not show the same level of benefit, particularly with respect to eggshell thickness and microbial counts. These findings suggest the possible application of tomato juice as a dietary supplement in older laying hens and highlight the need for further research to elucidate its underlying mechanisms and long-term effects on production and gut health.

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AUTHOR CONTRIBUTIONS

Conceptualization; UO, AI, CU, ISÇ and IB, methodology ISÇ and IB, software; UO, AI, EEG and CU, validation AI, OKT, BK and IB, formal analysis; UO, AI, CU, EEG and BK, investigation; UO, AI, CU, EEG and OKT, resources; UO, AI, EEG and OKT, data curation; UO, AI, CU, ISÇ, RUK and IB, writing-original draft preparation; UO, EEG and OKT, writing-review and editing; UO and OKT, visualization; RUK, IB, supervision; ISÇ and IB, project administration; UO, ISÇ and IB, funding acquisition; ISÇ, BK and IB.

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DATA AVAILABILITY STATEMENT

Data is available upon request.

CONFLICT OF INTEREST

No conflicts of interest exist among the authors regarding the publication of this data.

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