http://agriprojournal.com

JOURNAL OF AGRICULTURAL PRODUCTION

ISSN: 2757-6620

https://prensip.gen.tr

RESEARCH ARTICLE

The Effect of Vitamin C and E Supplementation into Drinking Water on Carcass Characteristics, Meat Quality and Intestinal Microflora During Pre-Slaughter Feed Withdrawal in Broiler Chickens

Hacer Kaya✉

Gümüşhane University, Kelkit Aydın Doğan Vocational School, Department of Veterinary, Gümüşhane/Türkiye

ARTICLE INFO

Article History
Received: 09.04.2023
Accepted: 23.05.2023
First Published: 30.06.2023

Keywords
Broiler
Feed withdrawal period
Intestinal microflora
Meat quality
Slaughter characteristics

ABSTRACT

This study investigates the effects of adding vitamin C and E to the drinking water on carcass characteristics, meat quality and intestinal microflora populations in broiler chickens during the 10-h pre-slaughter feed withdrawal (FW) period. As study materials, forty male broilers at the age of 42 days were used. The broilers were randomly divided into four groups: Control (non-vitamin, NV), vitamin C (1000 mg/L, VC), vitamin E (500 mg/L, VE) and vitamin combination (1000 mg/L VC+500 mg/L VE, VCE). In the study, vitamin additions didn't affect carcass characteristics, visceral weights and the pH values of the digestive system (P>0.05). The addition of VC and VE increased the weight of the Bursa of Fabricius, and the addition of VE increased the weight of thymus (P<0.05). Additions of vitamin decreased tendency of carcass contamination (P<0.01) and increased pH_{45min} and pH_{24h} of thigh meat and pH_{24h} of breast meat (P<0.05, P<0.01, P<0.01, respectively). While a* color intensity of breast and thigh meat increased with all vitamin supplements, L* and b* values of thigh meat decreased (P<0.01). Vitamin supplements, especially VE, reduced the drip loss of breast and thigh meat (P<0.05) and the pathogenic microorganism populations of intestinal contents (P<0.01). As a result, it is thought that the addition of 500 mg/L vitamin E to the drinking water of broiler chickens exposed to the pre-slaughter fasting period will be beneficial to improve meat quality and reduce intestinal pathogenic microorganism load. However, more extensive experimental studies are needed.

Please cite this paper as follows:

Kaya, H. (2023). The effect of vitamin C and E supplementation into drinking water on carcass characteristics, meat quality and intestinal microflora during pre-slaughter feed withdrawal in broiler chickens. *Journal of Agricultural Production*, *4*(1), 47-55. https://doi.org/10.56430/japro.1280038

1. Introduction

The quality of chicken meat, which has an important place in meeting animal protein needs all over the world, is affected by pre-slaughter management. The pre-slaughter management, which covers all the activities and processes that the broilers are exposed to pre-slaughter, begins with the feed withdrawal (FW). The pre-slaughter FW is done to reduce the content of the digestive system and to prevent fecal contamination and carcass contamination during transport and slaughter (Petrolli et al., 2016). It has been reported that the 10 h pre-slaughter FW period is sufficient to maintain the balance between weight loss, meat quality and carcass contamination (Xue et al., 2021). However, taking longer pre-slaughter processes causes stress to the broiler chickens, negatively affecting both animal welfare and meat quality and causing economic losses (Pan et al., 2018). Oxidative stress occurring before slaughter can lead to poor meat quality by promoting oxidative reactions in meat after slaughter (Nawaz & Zhang, 2021), oxidizing lipids and proteins, causing undesirable changes in sensory properties such as color, taste, texture, and nutritional value (Attia et al.,

✉ Corresponding author
E-mail address: hacerkaya@gumushane.edu.tr

2017; Mazur-Kuśnirek et al., 2019). Therefore, the oxidative state of broiler chickens during slaughter is very important for quality poultry meat production (Zeferino et al., 2016; Pan et al., 2018).

There is a good balance in the intestinal bacterial flora of healthy animals (Yadav & Jha, 2019). However, it is reported that stress-causing factors such as transportation (Bello et al., 2018), stocking density (Yu et al., 2021), environmental temperature (Calik et al., 2022) and fasting (Kayan & Açıkgöz, 2020; Xue et al., 2021) can disrupt this balance (Wickramasuriya et al., 2022). Stress, which weakens immunity in chickens, can activate certain pathogenic microorganisms suppressed in the gut (Zhao et al., 2017; Mishra & Jha, 2019), causing a disruption of the floral balance (Burkholder et al., 2008) and a devastating effect on the intestinal immune barrier (Zhao et al., 2017). Burkholder et al. (2008) reported that broiler chickens exposed to pre-slaughter FW (24 hours) or heat stress (HS, 30 °C) increased their susceptibility to intestinal pathogens. Nawaz and Zhang (2021) pointed out that stress stimulates intestinal bacteria by affecting intestinal epithelial cells.

The fact that the maintenance and management conditions such as FW, catching and transportation applied to the broilers in the pre-slaughter period are suitable for animal welfare affect the taste and quality of the meat positively (Pan et al., 2018). Therefore, studies have recently focused on identifying effective ways to reduce the pre-slaughter stress response of broilers and improve post-slaughter meat quality and gut microbiota (Karacay et al., 2008; Kop-Bozbay & Ocak, 2015; Petrolli et al., 2016). It is well known that endogenous antioxidant factors such as vitamin C and vitamin E reduce some adverse effects caused by oxidative stress (Attia et al., 2017). It has been reported that vitamin C is effective in relieving multiple stress factors with its immunomodulatory, antimicrobial and strong antioxidant properties (Gan et al., 2020).

It has been reported that the addition of vitamin E, which is a powerful antioxidant, antimicrobial and immune system supporter (Dalia et al., 2018; Calik et al., 2022), to broiler diets slows down oxidative stress, reduces drip loss, and improves meat quality (Souza et al., 2007; Attia et al., 2017; Ghasemi-Sadabadi et al., 2022). Vitamin C and vitamin E, which have strong antioxidant properties, are reported to have a synergistic effect in reducing the negative effects of stress (Attia et al., 2017). There are limited studies evaluating the effects of adding vitamin C and vitamin E alone or in combination to chicken diets on pre-slaughter multiple stress. This study was carried out to investigate the effects of 1000 mg/L vitamin C (VC), 500 mg/L vitamin E (VE) and 1000 mg/L vitamin C +500 mg/L vitamin E (VCE) supplementations into drinking water during the 10 h pre-slaughter feed withdrawal period on slaughter characteristics, meat quality and intestinal microflora populations of male broiler chickens.

2. Materials and Methods

2.1. Animals and Experimental Design

40 healthy male broiler chicks, aged 42 days, obtained from a commercial enterprise, were randomly divided into 4 treatment groups, each of which was housed in an individual compartment. These are follows; NV (non-vitamin): birds were given drinking water during the 10 h fasting period, VC: birds were given drinking water with 1000 mg/L VC during the 10 h FW period, VE: birds were given drinking water with 500 mg/L VE during the 10 h FW period, and VCE: birds were given drinking water with 1000 mg/L VC+ 500 mg/L VE during the 10 h fasting period. In the study, artificial light and ad-libitum water were provided to the chickens during the 10-hour fasting period (from 22:00 in the evening to 08:00 the next day). Feed withdrawal time was planned individually for each broiler so that the pre-slaughter fasting period did not exceed 10 hours (Xue et al., 2021). The determined amounts of vitamins obtained from a commercial company were added to the water that the broilers would drink in 10 hours (Aviagen, 2009).

2.2. Sample Collection and Determination of Parameters

In broiler chickens sacrificed by cervical dislocation method, the weights of carcass, thigh meat, breast meat and visceral organs were relatively calculated by dividing live weight. pH values of the crop, proventriculus, gizzard, meat and intestine were measured with a calibrated pH meter (MARTINI Mi 150 pH / Temperature Laboratory Bench Meter) with buffer solutions of 4.00, 7.00 and 10.00 (Ohkawa et al., 1979). Two different pH values for breast and thigh meat, 45 minutes (pH_{45min}) and 24 h after death (pH_{24h}), were measured 3 times in 3 locations, and the average values were taken as the final result. The pH decline within 24 h after slaughter was calculated as a percentage (Pan et al., 2018).

$$\text{pH decline (\%)} = [(pH_{45min} - pH_{24h})/pH_{45min}] \times 100 \quad (1)$$

To detect drip loss, breast and thigh fillets (approximately 5 cm×3 cm×2 cm) were weighed after cutting (W_1), hung in a 50 mL cold storage tube at 4°C for 24 h, and the surface liquids were wiped off and reweighed (W_2). Drip loss was calculated as a percentage of weight loss during storage (Xue et al., 2021).

$$\text{Drip loss (\%)} = [(W_1 - W_2)/W_1] \times 100 \quad (2)$$

Color intensities (L*, a* and b*) in breast and thigh meats were determined using a Konica Minolta colorimeter (Chroma Meter, CR-400, Minolta Konica, Japan). Color intensities were determined according to the specifications given by the International Commission on Illumination CIELAB (Commission Internationale de I'e Clairage) based on three-dimensional color measurement (CIE, 1986). According to

these criteria; L* (=0 black, =100 white (darkness/lightness)), a* (= +60 red, = -60 green) and b* (= +60 yellow, = -60 blue) indicate different color intensities. The colorimeter was calibrated using a special whiteboard before measurement, and the tip of the measuring head was placed flat on the middle surface. Measurements from 3 different sessions were averaged for each sample. For the microbiological analysis of the contents of the small intestine, the samples taken from 4 animals from each group in sterile conditions were stored at –80ºC until the day of analysis. Total mesophilic aerobic bacteria (*TMAB*) and *Coliform* (Harrigan, 1998), *E. coli* (ISO 16649-2, 2001) and *Salmonella spp*. (Andrews & Hammack, 2011) populations were determined in the microbiology laboratory.

Table 1. Contamination tendency scale (Xue et al., 2021).

Grade	Volume of feces excretion (ml)
0	0
1	0-2
2	2-4
3	4-6
4	6-8
5	>8

Contamination tendency was assessed and subjectively recorded in slaughtered animals after peeling and plucking, before dissection. After the chickens were pressed 3 times in a row by applying a uniform force on the abdomen, faecal shedding was observed by three observers, with a value of 0 indicating no faecal discharge, and a value of 5 denoting maximum faecal discharge, evaluated with a scale numbered from 0 to 5 (Table 1). The mean of these observations was determined as the contamination tendency.

2.3. Statistical Analysis

The data obtained from the study were analyzed with the "Statistical Package for Social Sciences" (IBM SPSS Statistic 25) statistical program. One-way analysis of variance (ANOVA) was applied to determine the statistical calculations of carcass characteristics, meat quality, digestive system pH and intestinal microflora and the importance of the difference between the mean values of the groups. Duncan's multiple comparison test was used to control the significance of the difference between the groups. Differences between groups in intestinal *Salmonella spp.* were evaluated with the "chi-square test" and a $P<0.05$ level was considered statistically significant.

3. Results and Discussion

3.1. Carcass Characteristics and Visceral Weights

Table 2 shows that the addition of vitamins to the drinking water of broilers exposed to pre-slaughter FW period has no effect on carcass characteristics and some visceral weights. The results of previous studies (Rathgeber et al., 2007; Karacay et al., 2008; Kop-Bozbay & Ocak, 2015; Petrolli et al., 2016; Güler et al., 2019) and current study results were similar. On the other hand, Kayan and Açıkgöz (2020) reported that the addition of 0.1% mixed organic acid into drinking water during the 6 or 12 h pre slaughter FW period reduced carcass and breast meat yield and thigh weight. It has been reported that the addition of VC (200 mg/kg), VE (100 mg/kg) or VCE to HS-administered chicken diets did not affect liver and heart weights, but the addition of VE increased the weight of carcass and abdominal fat (Attia et al., 2017). Zeferino et al. (2016) reported that the addition of VC (257 to 288 mg/kg) and VE (93 to 109 mg/kg) did not affect carcass, thigh and breast yields and abdominal fat, heart and liver weights in chickens under HS, similar to the results of the present study. Also, Yu et al. (2021) determined that the addition of 200 mg/kg VC to chicken diets under high stocking density (HSD) stress did not affect breast meat yield. It has been reported that the addition of 250, 500 and 1000 g/ton VC + citric flavonoids did not affect the yield of carcass, breast, thigh and abdominal fat in chicken diets under HS (Peña et al., 2008), and that the addition of 100 and 200 mg/kg VE to carcass, heart, liver and does not affect abdominal fat yield (Mazur-Kuśnirek et al., 2019).

Table 2. The effect of adding vitamin to the drinking water during the pre-slaughter FW period on carcass characteristics and visceral weights of broiler chickens (% of live body weight).

Parameters (%, BW)	Treatment Groups					
	NV	VC	VE	VCE	Average	p-*value*
Hot dressing	71.58±0.31	70.98±0.29	71.67±0.24	71.67±0.53	71.47±0.18	0.470
Cold dressing	71.00±0.33	70.61±0.31	71.27±0.24	71.37±0.50	71.06±0.18	0.449
Thigh dressing	26.64±0.42	26.44±0.58	26.06±0.21	26.90±0.39	26.51±0.21	0.553
Breast dressing	22.67±0.39	22.48±0.62	21.93±0.47	23.31±0.66	22.59±0.275	0.371
Heart	0.43±0.00	0.44±0.01	0.44±0.01	0.44±0.01	0.44±0.06	0.667
Kidney	0.03±0.00	0.02±0.00	0.02±0.00	0.03±0.00	0.03±0.00	0.224
Abdominal fat	1.41±0.06	1.47±0.05	1.43±0.06	1.53±0.08	1.46±0.03	0.610
Liver	1.82±0.03	1.72±0.06	1.81±0.06	1.76±0.06	1.78±0.03	0.547
Spleen	0.103±0.00	0.105±0.01	0.106±0.00	0.114±0.01	0.107±0.00	0.556
Bursa fabricus	$0.40±0.00^{c}$	$0.50±0.00^{b}$	$0.62±0.00^{a}$	$0.45±0.00^{bc}$	0.49±0.02	0.000
Thymus	$0.05±0.00^{b}$	$0.05±0.00^{b}$	$0.07±0.00^{a}$	$0.06±0.01^{b}$	0.06±0.00	0.020

NV, Non vitamin-Control; VC, 1000 mg/L water Vitamin C; VE, 500 mg/L water Vitamin E; VCE, Combination of VC (1000 mg/L water) and VE (500 mg/L water). $^{a-c}$; Means in the same row with different superscripts differ significantly ($P < 0.05$). Values are given as mean±standard deviation.

In this study, bursa weight increased with both VC and VE additions (P<0.01), while thymus weight increased only with VE addition (P<0.05) (Table 2). It was reported that adding 200 mg/kg Vit C or 300 mg/kg Vit E to the broilers diets reared under HS (Gharieb & Moursi, 2013), 125 mg/kg Vit E (Habibian et al., 2014) increased the weights of thymus and bursa, similar to current study results. In another study, it was reported that VE supplementation at 100 and 200 mg/kg did not affect the weight of the thymus but increased the weights of the bursa and spleen in chickens reared under thermoneutral (TN) conditions (Singh et al., 2006). On the other hand, Niu et al. (2009), Dalia et al. (2018) and Attia et al. (2017) reported that the addition of different doses of VC or VE to broiler diets did not affect thymus and bursa weights, contrary to the current study. Regarding the effects of VC and VE additions on the weights of the lymphoid organ thymus and bursa, the reason for the discrepancy between the results of the present study and other studies is not yet fully understood. However, it has been reported that lymphoid organ weights can be a reliable indicator of stress (Rosales, 1994) and that stress can cause lymphoid organ atrophy (Moberg, 2000). In this context, it is thought that the addition of VE and VC with antioxidant effects to the drinking water of chickens found in the pre-slaughter FW period may be effective in suppressing stress by affecting lymphoid organ weights. It has been reported that the addition of VE in poultry increases the T-helper/T-cytotoxic lymphocyte ratio and the percentages of T-helper lymphocytes in the spleen and thymus (Erf et al., 1998) and improves the immune response in rats (Moriguchi et al., 1993).

3.2. Meat Quality

The effects of VC, VE or VCE additions to drinking water during the 10 h pre-slaughter FW period on thigh and breast meat quality characteristics are shown in Table 3. The additions of vitamin increased the pH_{45min} and pH_{24h} values of thigh meat (P<0.05, P<0.01, respectively) but did not affect the pH decline rate (P>0.05). Addition of VC, VE or VCE to the drinking water of broilers during pre-slaughter FW period resulted in decreased thigh meat L* and b* color intensities and drip loss, and increased a* color intensity (P<0.01). With the results of this study, Lin et al. (2007), Serdaroğlu and Öztürk (2011), Güler et al. (2019) and Pan et al. (2018) reports are compatible. In poultry production, stress caused by various environmental factors during pre-slaughter accelerates post-slaughter glycolysis and leads to lactic acid accumulation, thereby reducing meat pH (Lin et al., 2007). The decrease in meat pH initiates protein denaturation, which has an effect on the color and water holding capacity of the meat, resulting in pale and low water holding capacity meat production (Serdaroğlu & Öztürk, 2011). This condition is called pale, soft and juicy (pale, soft and exudative; PSE) meat (Barbut, 1998).

Table 3. The effect of adding vitamin to drinking water during the pre-slaughter FW period on meat quality of broiler chickens.

Parameters	Treatment Groups					
	NV	VC	VE	VCE	Average	p-*value*
Thigh meat						
pH_{45min}	6.16±0.06^{b}	6.28±0.03^{a}	6.32±0.06^{a}	6.31±0.02^{a}	6.27±0.02	0.016
pH_{24h}	5.67±0.03^{b}	5.84±0.04^{a}	5.89±0.03^{a}	5.88±0.02^{a}	5.82±0.02	0.000
pH decline (%)	7.90±0.61	7.01±0.76	6.77±0.37	6.88±0.43	7.14±0.28	0.483
L*	50.49±0.25^{a}	48.84±0.31^{b}	48.41±0.87^{b}	46.65±0.27^{c}	48.60±0.32	0.000
a*	4.01±0.10^{c}	4.71±0.12^{b}	5.49±0.22^{a}	5.62±0.08^{a}	4.96±0.12	0.000
b*	7.98±0.18^{a}	6.15±0.20^{b}	5.54±0.30^{b}	5.67±0.24^{b}	6.33±0.19	0.000
Drip loss (%)	7.67±0.27^{a}	6.53±0.47^{b}	5.76±0.29^{b}	6.44±0.44^{b}	6.60±0.21	0.010
Breast meat						
pH_{45min}	6.10±0.03	6.18±0.04	6.21±0.02	6.19±0.03	6.17±0.02	0.057
pH_{24h}	5.76±0.03^{b}	5.86±0.03^{a}	5.91±0.03^{a}	5.86±0.03^{a}	5.85±0.02	0.007
pH decline (%)	5.63±0.37	5.14±0.49	4.93±0.26	5.27±0.48	5.24±0.20	0.677
L*	47.74±0.56	48.01±0.52	48.65±0.18	48.07±0.61	48.12±0.25	0.628
a*	2.56±0.18^{b}	4.02±0.23^{a}	3.82±0.17^{a}	3.53±0.39^{a}	3.48±0.15	0.001
b*	6.22±0.26^{a}	5.48±0.18^{b}	5.68±0.10^{b}	6.27±0.18^{a}	5.91±0.11	0.010
Drip loss (%)	6.26±0.30^{a}	5.34±0.41^{b}	4.57±0.26^{b}	4.91±0.27^{b}	5.27±0.18	0.04

NV, Non vitamin-Control; VC, 1000 mg/L water Vitamin C; VE, 500 mg/L water Vitamin E; VCE, Combination of VC (1000 mg/L water) and VE (500 mg/L water). $^{a-d}$; Means in the same row with different superscripts differ significantly (P<0.05). Values are given as mean±standard error.

In this study, it was found that vitamin supplements increase the pH values of thigh meat, decrease drip loss and improve the a* color parameter, which is consistent with the reports of Souza et al. (2007) and Serdaroğlu and Öztürk (2011). On the other hand, the results of the current study on the color density and pH values of thigh meat differ from the results of Imik et al. (2012), Kop-Bozbay and Ocak (2015) and Karacay et al. (2008). It is thought that the reason for this situation is due to the different types and doses of additives added to the drinking water of chickens.

Additions of vitamin to drinking waters of broilers during the 10 h pre-slaughter FW period did not affect breast meat pH_{45min}, pH decrease rate and L* color intensity (P>0.05), but pH_{24h} and drip loss decreased (P<0.01, P<0.01, respectively) (Table 3). Breast meat's a* color intensity increased with all vitamin supplements (P<0.01), while b* color intensity decreased with VC and VE (P<0.01). These results are consistent with the results of Petrolli et al. (2016) and Kop-Bozbay and Ocak (2015). While Petrolli et al. (2016) reported that the addition of 200 mg/L VE to the water of broilers during the 12 h pre-slaughter FW period did not affect the L* value of breast meat, Kop-Bozbay and Ocak (2015) reported that addition of 3 g/L glucose, sucrose to the drinking water during the 10 h pre-slaughter FW period did not affect the pH_{1h} and L* values of breast meat, but the a* color intensity increased by addition of starch. On the other hand, the results of the present study are partially compatible with the results of Zhang et al. (2022) and Karacay et al. (2008). It is reported that the addition of guanidineacetic acid to the drinking water of broilers exposed to transportation stress for 3 hours after the 8-hour pre-slaughter FW period did not affect the breast meat's pH_{45min} and a* and b* color densities, but increased pH_{24h} and decreased L* color density and drip loss (Zhang et al., 2022), and the addition of sucrose to drinking water during the 10 h pre-slaughter FW period did not affect the breast meat's L* and b* color intensities, but increased the a* value (Karacay et al., 2008). In addition, there are studies reporting that the addition of different levels of VC, VE or VCE to chicken diets under stress has no effect on breast meat's pH and color parameters (Peña et al., 2008; Zeferino et al., 2016; Attia et al., 2017; Mazur-Kuśnirek et al., 2019; Yu et al., 2021). However, Imik et al. (2012) reported that VC addition decreased breast meat's pH and a* value and increased L* and b* values, while Zhang et al. (2013) reported that VE addition decreased L* and b* color intensity and increased a* value. It was reported that the quality defect PSE can be determined by examining pH_{24h} values lower than 5.8 and L* values higher than 52 together (Barbut, 1998). Accordingly, it can be said that the incidence of PSE meat is low since the pH_{24h} values of thigh and breast meat detected in all vitamin supplemented groups in the current study were above 5.8 and L* values were below 52. The results of the current study, which found that breast meat's drip loss was reduced, were similar with the results of Mazur-Kuśnirek et al. (2019), but different from the results of Peña et al. (2008), Zeferino et al. (2016) and Zhang et al. (2013).

3.3. Digestive System pH, Intestinal Microflora and Contamination Tendency

It was determined that the addition of VC, VE and VCE to the drinking water of broilers during the pre-slaughter FW period did not affect the pH values of crop, proventriculus, gizzard and intestinal contents (P>0.05), but reduced the tendency of carcass contamination (P<0.01) (Table 4). Kayan and Açıkgöz (2020) reported that the addition of organic acid to the drinking water during the 6 or 12 hour pre- slaughter FW period did not affect the pH values of crops, gizzards and proventriculus. On the other hand, Hinton et al. (2000) reported that the feed material in the crop was depleted 6 hours after the chickens were prevented from accessing the food, while Kayan and Açıkgöz (2020) reported that the pH of the gizzard and bezel stomach decreased as the FW extended from 6 hours to 12 hours. Depletion of feed material in the crop lowers the lactic acid concentration, leading to an increase in pH and thus a decrease in its ability to inhibit the growth of enteropathogens (Hinton et al., 2002). The main purpose of the pre-slaughter FW period in of broiler chickens production is to prevent carcass contamination (*Salmonella*, *Campylobacter*, etc.) and to produce hygienic chicken meat by ensuring that the digestive system is empty. It is reported that pre-slaughter fasting period and transportation time are associated with the contamination tendency, which is one of the indicators of carcass pollution, and the increasing contamination tendency increases processing costs and reduces profitability (Menconi et al., 2014). In addition, it is important to control the rate of carcass contamination during slaughter since carcass contamination is a serious public health problem and adversely affects carcass production and meat quality (Xue et al., 2021). In this respect, the fact that the addition of VC, VE or VCE to the water during the 10 h pre-slaughter FW period reduces the tendency of carcass contamination (P<0.01), is seen as an important result in terms of both hygienic chicken meat production and processing cost.

All of the vitamin additions to the drinking water of broilers during the 10-hour pre-slaughter FW period decreased the pathogenic microorganism population (P<0.01) in the intestinal contents (Table 4). The addition of VE was more effective at attenuating *TMAB*, *Coliform* and *E. coli* populations than other groups, while the addition of VCE was more effective than the control and VC additions (P<0.01). Oxidative stress damages the intestinal microflora and leads to an increase in pathogenic bacteria (Mishra & Jha, 2019). Dietary antioxidants can help alleviate the negative effects of various stress factors on microbiota by regulating the intestinal microflora (Yang et al., 2020). It is reported that the addition of VE (250 mg/kg) to broilers' diets housed under HS improves antioxidant status, alters intestinal microorganism population and functions, and alleviates the negative effects of stress (Calik et al., 2022). However, there are also studies reporting that the addition of VE at different doses (up to 200 mg/kg) to chickens' diets does not affect the intestinal pathogenic microorganism (*Coliform*, *TMAB*, *E. coli*) population (Scocco et al., 2017; Dalia et al., 2018; Ghasemi-Sadabadi et al., 2022). Mandal et al. (2005) reported that the intestinal *Coliform* count was reduced by supplementation of 150 mg/kg of VC or 300 mg/kg of VE in broilers during the 8 h pre-slaughter FW period. It has been observed that the addition of 50 ml/L VC reduces the intestinal *E. coli* count (Nosrati et al., 2017) and the addition of 300

mg/kg VC reduces both the intestinal *Coliform* and *E. coli* counts (Hajati et al., 2015). On the other hand, during 6 h pre-slaughter FW period the addition of maltodextrin did not affect the *TMAB* count of the crop content, but weakened the *E. coli* and *Coliform* bacteria population (Rathgeber et al., 2007), the addition of 0.1% organic acid during the 6 or 12 h pre-slaughter FW period did not affect the intestinal *Coliform* count (Kayan & Açıkgöz, 2020).

Table 4. The effect of adding vitamin to drinking water during the pre-slaughter FW period on the pH values of the digestive system and intestinal microflora of broiler chickens.

Parameters	Treatment Groups					
	NV	**VC**	**VE**	**VCE**	**Average**	**p-*value***
Crop pH	5.05±0.27	5.52±0.09	5.70±0.17	5.21±0.11	5.37±0.09	0.057
Proventriculus pH	3.54±0.85	3.74±0.09	3.63±0.12	3.78±0.06	3.67±0.05	0.556
Gizzard pH	3.01±0.05	3.21±0.06	2.93±0.06	3.17±0.24	3.08±0.06	0.363
Intestine pH	6.45±0.09	6.47±0.12	6.53±0.08	6.65±0.05	6.52±0.05	0.382
Contamination tendency (ml)	$1.63±0.29^{a}$	$0.80±0.19^{b}$	$0.90±0.17^{b}$	$0.40±0.10^{b}$	0.93±0.12	0.001
***TMAB* (log kob/gr)**	$7.03±0.02^{a}$	$6.85±0.02^{b}$	$4.58±0.04^{d}$	$4.90±0.01^{c}$	5.84±0.29	0.000
***Coliform* (log kob/gr)**	$7.13±0.01^{a}$	$6.94±0.02^{b}$	$4.32±0.05^{d}$	$6.71±0.02^{c}$	6.27±0.29	0.000
***E. coli* (log kob/gr)**	$7.02±0.01^{a}$	$6.80±0.02^{b}$	$4.21±0.03^{d}$	$4.99±0.01^{c}$	5.76±0.31	0.000

NV, Non vitamin-Control; VC, 1000 mg/L water Vitamin C; VE, 500 mg/L water Vitamin E; VCE, Combination of VC (1000 mg/L water) and VE (500 mg/L water); *TMAB*, Total Mesophilic Aerobic Bacteria. $^{a-d}$; Means in the same row with different superscripts differ significantly (P<0.05). Values are given as mean±standard error.

Table 5. The effect of adding vitamin to drinking water during the pre-slaughter FW period on intestine *Salmonella spp*. populations of broiler chickens.

Groups	Positive	Negative	Total
NV[a]	4	0	4
VC[a]	4	0	4
VE[b]	0	4	4
VCE[a]	3	1	4
Total	11	5	16

(X^2)=12.509 P=0.006. NV, Non vitamin-Control; VC, 1000 mg/L water Vitamin C; VE, 500 mg/L water Vitamin E; VCE, Combination of VC (1000 mg/L water) and VE (500 mg/L water). $^{a-b}$; Differences between the groups shown with different superscripts are statistically significant (P < 0.05).

In order to determine the statistical effects of vitamin additions to the drinking water of broilers during the pre-slaughter FW period on *Salmonella spp.* in the intestinal content, the groups were compared according to the X^2 test and a significant difference was observed (P<0.05). It was determined that the difference between the groups was due to the VE group (P<0.05). Accordingly, it can be said that the addition of VE to the drinking water of broilers during the pre-slaughter FW period reduces the risk of *Salmonella spp.* compared to other groups. It is thought that the addition of vitamin E, which has a strong antioxidant effect (Dalia et al., 2018; Calik et al., 2022), to the drinking water of broiler chickens in the pre-slaughter FW period may have affected the intestinal microflora by reducing intestinal oxidation stress (Attia et al., 2017; Ghasemi-Sadabadi et al., 2022). It has been emphasized that adding 30 IU/kg VE to the diets of layer chickens exposed to Salmonella Enteritidis (SE) reduces the stress symptoms caused by SE and can be recommended to improve poultry health and production performance by controlling Salmonella infection (Liu et al., 2019). Also, Dalia et al. (2018) observed that adding 100 mg/kg VE to chickens' diets reduced the number of *Salmonella spp.*

4. Conclusion

The results of this study show that the addition of VC or VCE, especially 500 mg/L VE, to the drinking water of broilers during the pre-slaughter FW period may be a good alternative, as it improves meat quality and reduces intestinal pathogenic microorganism populations. It is thought that more studies with more different doses are needed to fully determine the effects of vitamin C and E supplementation in the drinking water of broiler chickens during the pre-slaughter FW period.

Acknowledgment

The author would like to thank Şeyda Merve KARATAŞ (Asst.) for helping at different stages of the project.

Compliance with Ethical Standards

The study was carried out after the approval of the Animal Experiments Local Ethics Committee of Kafkas University (KAÜ-HADYEK/ 2023-040).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Andrews, W. H., & Hammack, T. S. (2011). *Bacteriological analytical manual (BAM), Chapter 5: Salmonella*. U.S. Food & Drug Administration.

Attia, Y. A., Al-Harthi, M. A., El-Shafey, A. S., Rehab, Y. A., & Kim, W. K. (2017). Enhancing tolerance of broiler chickens to heat stress by supplementation with vitamin E, vitamin C and/or probiotics. *Annals of Animal Science*, *17*(4), 1155-1169. https://doi.org/10.1515/aoas-2017-0012

Aviagen. (2009). *Ross broiler management manual, aviagen broiler breeders*. www.aviagen.com

Barbut, S. (1998). Estimating the magnitude of the PSE problem in poultry. *Journal of Muscle Foods*, *9*(1), 35-49. https://doi.org/10.1111/j.1745-4573.1998.tb00642.x

Bello, A. U., Idrus, Z., Meng, G. Y., Award, E. A., & Farjam, A. S. (2018). Gut microbiota and transportation stress response affected by tryptophan supplementation in broiler chickens. *Italian Journal of Animal Science*, *17*(1), 107–113. https://doi.org/10.1080/1828051X.2017.1340814

Burkholder, K. M., Thompson, K. L., Einstein, M. E., Applegate, T. J., & Patterson, J. A. (2008). Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to Salmonella enteritidis colonization in broilers. *Poultry science*, *87*(9), 1734-1741. https://doi.org/10.3382/ps.2008-00107

Calik, A., Emami, N. K., Schyns, G., White, M. B., Walsh, M. C., Romero, L. F., & Dalloul, R. A. (2022). Influence of dietary vitamin E and selenium supplementation on broilers subjected to heat stress, Part II: oxidative stress, immune response, gut integrity, and intestinal microbiota. *Poultry Science*, *101*(6), 101858. https://doi.org/10.1016/j.psj.2022.101858

CIE. (1986). *Colorimetry (2nd Ed)*. CIE Publication.

Dalia, A. M., Loh, T. C., Sazili, A. Q., Jahromi, M. F., & Samsudin, A. A. (2018). Effects of vitamin E, inorganic selenium, bacterial organic selenium, and their combinations on immunity response in broiler chickens. *BMC Veterinary Research*, *14*(1), 1-10. https://doi.org/10.1186/s12917-018-1578-x

Erf, G. F., Bottje, W. G., Bersi, T. K., Headrick, M. D., & Fritts, C. A. (1998). Effects of dietary vitamin E on the immune system in broilers: altered proportions of CD4 T cells in the thymus and spleen. *Poultry Science*, *77*(4), 529-537. https://doi.org/10.1093/ps/77.4.529

Gan, L., Fan, H., Mahmood, T., & Guo, Y. (2020). Dietary supplementation with vitamin C ameliorates the adverse effects of Salmonella Enteritidis-challenge in broilers by shaping intestinal microbiota. *Poultry Science*, *99*(7), 3663-3674. https://doi.org/10.1016/j.psj.2020.03.062

Gharieb, M. M., & Moursi, M. K. (2013). Comparative efficacy of different supplement used to reduce heat stress and their impact on performance, immunity and some biochemical parameters in broilers chickens. *Egyptian Journal of Veterinary Sciences*, *44*(1), 49-67. https://doi.org/10.21608/ejvs.2013.329

Ghasemi-Sadabadi, M., Ebrahimnezhad, Y., Maheri-Sis, N., Shaddel-Teli, A., Ghalehkandi, J. G., & Veldkamp, T. (2022). Effects of supplementation of pomegranate processing by-products and waste cooking oils as alternative feed resources in broiler nutrition. *Scientific Reports*, *12*(1), 21216. https://doi.org/10.1038/s41598-022-25761-7

Güler, H. C., Demir, N., KurbaL, Ö. F., & Babacanoğlu, E. (2019). The effect of dietary humic acid on some carcass traits, meat quality and blood parameters of Japanese quails subjected to pre-slaughter feed withdrawal stress. *Journal of Animal Production*, *60*(1), 15-23. https://doi.org/10.29185/hayuretim.551705

Habibian, M., Ghazi, S., Moeini, M. M., & Abdolmohammadi, A. (2014). Effects of dietary selenium and vitamin E on immune response and biological blood parameters of broilers reared under thermoneutral or heat stress conditions. *International Journal of Biometeorology*, *58*, 741-752. https://doi.org/10.1007/s00484-013-0654-y

Hajati, H., Hassanabadi, A., Golian, A. G., Nassiri, M. H., & Nassiri, M. R. (2015). The effect of grape seed extract and vitamin C feed supplements carcass characteristics, gut morphology and ileal microflora in broiler chickens exposed to chronic heat stress. *Iranian Journal of Applied Animal Science*, *5*(1), 155-165.

Harrigan, W. F. (1998). *Laboratory methods in food microbiology*. Academic Press.

Hinton Jr, A., Buhr, R. J., & Ingram, K. D. (2000). Physical, chemical, and microbiological changes in the crop of broiler chickens subjected to incremental feed withdrawal. *Poultry Science*, *79*(2), 212-218. https://doi.org/10.1093/ps/79.2.212

Hinton Jr, A., Buhr, R. J., & Ingram, K. D. (2002). Carbohydrate-based cocktails that decrease the population of Salmonella and Campylobacter in the crop of broiler chickens subjected to feed withdrawal. *Poultry Science*, *81*(6), 780-784. https://doi.org/10.1093/ps/81.6.780

Imik, H., Ozlu, H., Gumus, R., Atasever, M. A., Urcar, S., & Atasever, M. (2012). Effects of ascorbic acid and α-lipoic acid on performance and meat quality of broilers subjected to heat stress. *British Poultry Science*, *53*(6), 800-808. https://doi.org/10.1080/00071668.2012.740615

ISO. (2001). *Horizontal method for the enumeration of β-glucuronidase-positive Escherichia coli-Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide*. https://www.iso.org/obp/ui/#iso:std:iso:16649:-2:ed-1:v1:en

Karacay, N., Ocak, N., Sarica, M., & Erener, G. (2008). Effect of carbohydrate supplementation provided through drinking water during feed withdrawal on meat and liver colors in broilers. *Journal of Science of Food and Agriculture*, *88*(3), 479–484. https://doi.org/10.1002/jsfa.3110

Kayan, H., & Açıkgöz, Z. (2020). The effects of pre-slaughter feed withdrawal period and organic acid supplementation into drinking water on slaughter yield, meat quality, intestinal microflora and some blood traits in broiler. *Journal of Agriculture Faculty of Ege University*, Özel Sayı, 131-142. https://doi.org/10.20289/zfdergi.826655

Kop-Bozbay, C., & Ocak, N. (2015). Body weight, meat quality and blood metabolite responses to carbohydrate administration in the drinking water during pre-slaughter feed withdrawal in broilers. *Journal of Animal Physiology and Animal Nutrition*, *99*(2), 290-298. https://doi.org/10.1111/jpn.12194

Lin, H., Sui, S. J., Jiao, H. C., Jiang, K. J., Zhao, J. P., & Dong, H. (2007). Effects of diet and stress mimicked by corticosterone administration on early postmortem muscle metabolism of broiler chickens. *Poultry Science*, *86*(3), 545-554. https://doi.org/10.1093/ps/86.3.545

Liu, Y. J., Zhao, L. H., Mosenthin, R., Zhang, J. Y., Ji, C., & Ma, Q. G. (2019). Protective effect of vitamin E on laying performance, antioxidant capacity, and immunity in laying hens challenged with Salmonella Enteritidis. *Poultry Science*, *98*(11), 5847-5854. https://doi.org/10.3382/ps/pez227

Mandal, A. B., Yadav, A. S., Elangovan, A. V., Tyagi, P. K., Tyagi, P. K., & Toppo, S. (2005). Effect of pre-slaughter feed withdrawal and dietary supplementation of vitamin E and ascorbic acid on microbiological and keeping quality of broiler chicken meat. *Indian Journal of Poultry Science*, *40*(3), 285-287.

Mazur-Kuśnirek, M., Antoszkiewicz, Z., Lipiński, K., Kaliniewicz, J., Kotlarczyk, S., & Żukowski, P. (2019). The effect of polyphenols and vitamin E on the antioxidant status and meat quality of broiler chickens exposed to high temperature. *Archives of Animal Nutrition*, *73*(2), 111-126. https://doi.org/10.1080/1745039X.2019.1572342

Menconi, A., Kuttappan, V. A., Hernandez-Velasco, X., Urbano, T., Matté, F., Layton, S., & Tellez, G. (2014). Evaluation of a commercially available organic acid product on body weight loss, carcass yield, and meat quality during preslaughter feed withdrawal in broiler chickens: a poultry welfare and economic perspective. *Poultry Science*, *93*(2), 448-455. https://doi.org/10.3382/ps.2013-03444

Mishra, B., & Jha, R. (2019). Oxidative stress in the poultry gut: potential challenges and interventions. *Frontiers in Veterinary Science*, *6*, 60. https://doi.org/10.3389/fvets.2019.00060

Moberg, G. P., (2000). Biological response to stress: implications for animal welfare. In G. P. Moberg & J. A. Mench (Eds.), *The biology of animal stress: Basic principals and implications for animal welfare* (pp. 1–22). CABI Publishing. https://doi.org/10.1079/9780851993591.0001

Moriguchi, S., Mıwa, H., Okamura, M., Maekawa, K., Kıshıno, Y., & Maeda, K. (1993). Vitamin E is an important factor in T cell differentiation in thymus of F344 rats. *Journal of Nutritional Science and Vitaminology*, *39*(5), 451-463. https://doi.org/10.3177/jnsv.39.451

Nawaz, A. H., & Zhang, L. (2021). Oxidative stress in broiler chicken and its consequences on meat quality. *International Journal of Life Science Research Archive*, *01*(01), 045-054. https://doi.org/10.53771/ijlsra.2021.1.1.0054

Niu, Z. Y., Liu, F. Z., Yan, Q. L., & Li, W. C. (2009). Effects of different levels of vitamin E on growth performance and immune responses of broilers under heat stress. *Poultry Science*, *88*(10), 2101-2107. https://doi.org/10.3382/ps.2009-00220

Nosrati, M., Javandel, F., Camacho, L. M., Khusro, A. M. E. E. R., Cipriano, M., Seidavi, A., & Salem, A. Z. M. (2017). The effects of antibiotic, probiotic, organic acid, vitamin C, and Echinacea purpurea extract on performance, carcass characteristics, blood chemistry, microbiota, and immunity of broiler chickens. *Journal of Applied Poultry Research*, *26*(2), 295-306. https://doi.org/10.3382/japr/pfw073

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351-358. https://doi.org/10.1016/0003-2697(79)90738-3

Pan, L., Zhao, P. F., Ma, X. K., Shang, Q. H., Long, S. F., Wu, Y., & Piao, X. S. (2018). Forsythia suspensa extract protects broilers against breast muscle oxidative injury induced by corticosterone mimicked pre-slaughter acute stress. *PoultrySscience*, *97*(6), 2095-2105. https://doi.org/10.3382/ps/pey046

Peña, J. E. M., Vieira, S. L., López, J., Reis, R. N., Barros, R., Furtado, F. V. F., & Silva, P. X. (2008). Ascorbic acid and citric flavonoids for broilers under heat stress: Effects on performance and meat quality. *Brazilian Journal of Poultry Science*, *10*, 125-130. https://doi.org/10.1590/S1516-635X2008000200008

Petrolli, T. G., Junqueira, O. M., Domingues, C. H. F., Pereira, A. S. C., Santos, E. T., & Rocha, R. X. (2016). Effects of the addition of glucose, sodium bicarbonate, and vitamin E to the drinking water of pre-slaughter broiler chickens on carcass yield, gastric emptying and meat quality. *Brazilian Journal of Poultry Science*, *18*, 29-34. https://doi.org/10.1590/1516-635x1801029-034

Rathgeber, B. M., MacIsaac, J. L., & MacKenzie, M. E. (2007). Feeding turkeys a highly digestible supplement during preslaughter feed withdrawal. *Poultry Science*, *86*(9), 2029-2033. https://doi.org/10.1093/ps/86.9.2029

Rosales, A. G. (1994). Managing stress in broiler breeders: A review. *The Journal of Applied Poultry Research, 3*, 199–207. https://doi.org/10.1093/japr/3.2.199

Scocco, P., Forte, C., Franciosini, M. P., Mercati, F., Casagrande-Proietti, P., Dall'Aglio, C., & Trabalza-Marinucci, M. (2017). Gut complex carbohydrates and intestinal microflora in broiler chickens fed with oregano (*Origanum vulgare* L.) aqueous extract and vitamin E. *Journal of Animal Physiology and Animal Nutrition*, *101*(4), 676-684. https://doi.org/10.1111/jpn.12588

Serdaroğlu, M., & Öztürk, B. (2011). Pale poultry muscle syndrome in broilers and Turkeys. *Journal of Animal Production*, *52*(1). https://dergipark.org.tr/en/download/article-file/85021

Singh, H., Sodhi, S., & Kaur, R. (2006). Effects of dietary supplements of selenium, vitamin E or combinations of the two on antibody responses of broilers. *British Poultry Science*, *47*(6), 714-719. https://doi.org/10.1080/00071660601040079

Souza, V. L. F., Silva, R. S. F., & Silva, C. A. (2007). Vitamina E no desempenho, características de carcaça e qualidade do presunto cozido de suínos. *Pesquisa Agropecuária Brasileira*, *42*(4), 581-587. https://doi.org/10.1590/S0100-204X2007000400017

Wickramasuriya, S. S., Park, I., Lee, K., Lee, Y., Kim, W. H., Nam, H., & Lillehoj, H. S. (2022). Role of physiology, immunity, microbiota, and infectious diseases in the gut health of poultry. *Vaccines*, *10*(2), 172. https://www.mdpi.com/2076-393X/10/2/172

Xue, G., Cheng, S., Yin, J., Zhang, R., Su, Y., Li, X., & Bao, J. (2021). Influence of pre-slaughter FW on weight loss, meat quality and carcass contamination in broilers. *Animal Bioscience*, *34*(6), 1070-1077. https://doi.org/10.5713/ajas.20.0560

Yadav, S., & Jha, R. (2019). Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *Journal of Animal Science and Biotechnology*, *10*(1), 1-11. https://doi.org/10.1186/s40104-018-0310-9

Yang, Q., Liang, Q., Balakrishnan, B., Belobrajdic, D. P., Feng, Q. J., & Zhang, W. (2020). Role of dietary nutrients in the modulation of gut microbiota: A narrative review. *Nutrients*, *12*(2), 381. https://doi.org/10.3390/nu12020381

Yu, D. G., Namgung, N., Kim, J. H., Won, S. Y., Choi, W. J., & Kil, D. Y. (2021). Effects of stocking density and dietary vitamin C on performance, meat quality, intestinal permeability, and stress indicators in broiler chickens. *Journal of Animal Science and Technology*, *63*(4), 815. https://doi.org/10.5187%2Fjast.2021.e77

Zeferino, C. P., Komiyama, C. M., Pelícia, V. C., Fascina, V. B., Aoyagi, M. M., Coutinho, L. L., & Moura, A. S. A. M. T. (2016). Carcass and meat quality traits of chickens fed diets concurrently supplemented with vitamins C and E under constant heat stress. *Animal*, *10*(1), 163-171. https://doi.org/10.1017/S1751731115001998

Zhang, B., Liu, N., Kang, K., Zhang, R., Hao, M., Song, P., & Li, C. (2022). Dietary guanidineacetic acid supplementation ameliorated meat quality and regulated muscle metabolism of broilers subjected to pre-slaughter transport stress by metabolomics analysis. *Poultry Science*, *101*(4), 101739. https://doi.org/10.1016/j.psj.2022.101739

Zhang, Y., Shan, A., Jiang, W., Bi, C., & Li, Z. (2013). The effect of vitamin E on growth performance and meat quality in broilers given diets containing distillers' dried grain with solubles (DDGS). *British Poultry Science*, *54*(1), 138-143. https://doi.org/10.1080/00071668.2012.757578

Zhao, H., He, Y., Li, S., Sun, X., Wang, Y., Shao, Y., & Xing, M. (2017). Subchronic arsenism-induced oxidative stress and inflammation contribute to apoptosis through mitochondrial and death receptor dependent pathways in chicken immune organs. *Oncotarget*, *8*(25), 40327. https://doi.org/10.18632%2Foncotarget.16960