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Effects of Dietary Supplementation of Barley Malt Extract and Malt Vinegar on Growth Performance, Jejunal Morphology and Meat Quality of Broiler Chickens

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Abstract

The objective of this study was to evaluate the effects of dietary supplementation of barley malt extract and barley malt vinegar on growth performance, jejunal morphology and meat quality of broilers. A total of 600 day-old chicks were allocated to four dietary treatments to evaluate the effect of dietary inclusion of 0.2% barley malt extract alone or along with 0.4 or 0.8% of malt vinegar as well as the control group. The results of the experiment have shown that dietary supplementation of 0.2% malt extract along with 0.4% malt vinegar resulted in a higher average daily gain (P = 0.007) and European production efficiency factor (P = 0.028) concomitantly lower feed conversion ratio (P = 0.047) compared to the birds were fed the control diet or supplemented by 0.2% malt extract along with 0.8% malt vinegar. Dietary supplementation of 0.2% malt extract increased the apparent absorption of surface area in the jejunum (P = 0.024). Moreover, dietary supplementation of malt extracts and malt vinegar alleviated malondialdehyde formation in the breast and thigh muscles (P < 0.05). Ceca microflora enumeration did not differ among the dietary treatments. In conclusion, dietary supplementation of 0.2% malt extract along with 0.4% malt vinegar may improve broiler growth performance and alleviate lipid oxidation.

Introduction

Malting is the process of cereal grains germination that have been dried. The germination starts by soaking barley grains in water. Once sprouting starts, it should be stopped by drying step. Extractable part of barely starch content is converted into other carbohydrates by malting. In the next step, malt is cracked for extracting its sugars and then is soaked in temperature-modulated water (Biagi *et al*., 2007). Using low temperature in malt production process enables enzymes to remain active. These enzymes are necessary for altering the starch content of malt into sugars such as isomaltose. Other enzymes, such as protease, which decompose protein of grain into the usable forms for yeasts, are expanded during grains malting (Qingming *et al*., 2010). Furthermore, malting has a considerable effect on the phenolic contents and their antioxidant activity. Antioxidant activity of some phenolic compounds could be increased by germination and subsequent, kilning. One form of barley malt extract is liquid malt extract, which is thick syrup and used for several applications, such as brewing and baking or as an appetizer (Qingming *et al*., 2010).

The barley malt vinegar is produced by converting grain starch to maltose in the malting process. Then maltose is brewed to ale and subsequently turns into vinegar, which is then aged and usually light-brown in color. The procedure of vinegar production includes a

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double fermentation, in which the alcohols that gained from the first fermentation are exposed to acidification (Jones and Greenshields, 1969). Acetic acid (CH3COOH) as a primary component of malt vinegar is considered as an organic acid with a long history of consumption as a food additive for increasing the shelf life of perishable food ingredients. Since ancient times it has been utilized for treating fever, ulcers, pleurisy, and constipation (Myers, 2007). Furthermore, vinegar has indicated anti-bactericidal activity against *Escherichia coli (E.Coli)*, *Staphylococcus aureus*, *Salmonella Enteritidis*, *Shigella sonnei* and *Listeria monocytogenes* due to its acidity (Medina *et al*., 2007). Vinegar contains 5% acetic acid and its phenolic content consists of gallic acid, catechin, caffeic acid, vanillic acid, m-coumaric acid, 4-hydroxybenzaldehyde, vanillin, and etc. (Gálvez *et al*., 1994).

There is a lack of information about the effects of dietary supplementation of barley malt extract and barley malt vinegar on broiler performance; Thus, this experiment aimed to evaluate the effects of the different combination of malt extract and malt vinegar on growth performance, jejunal morphology, meat quality, and ceca microflora enumeration in broilers chickens.

Materials and Methods

Birds and Management

A total of 600 day-old broiler chicks (Ross × Ross 308, unsexed) were used and allocated to four treatments and six replications of 25 chicks each. Four dietary treatments included a basal corn-soybean meal diet without malt extract and vinegar extraction (Control) and basal diet supplemented with 0.2% (of kg diet) malt extract (ME2), or 0.2% malt extract along with 0.4% (of kg diet) malt vinegar (ME2MV4) and 0.2% (of kg diet) malt extract along with 0.8% (of kg diet) malt vinegar (ME2MV8). These diets were given to birds from day 1 to 43.

Diets

The pelleted dietary regimes were composed of a starter (1 to 10 d), grower (11 to 24 d), and finisher diets (25 to the end of the experiment). The iso-nutrient diets which were formulated to meet the nutrient requirements according to the Ross-308 guideline are shown in Table 1. Feed and water were supplied for *ad libitum* consumption with nipple drinkers and tube feeders. The temperature of poultry house was set at 32°C on day one and decreased by 1°C every other day to the temperature of 21°C. The lighting cycle of rearing house was 24 h lighting during the first two days and a 23: 1 h light: dark cycle until the end of the experiment.

To prepare the dietary treatments, at first, a single batch of control diet was divided into four parts according to the experimental treatments and then malt extract was added on top to the prepared diets in the form of pellet in feed factory. Malt vinegar was carefully sprayed on diets at the certain dosage in a place without air flow. Malt extract and vinegar were gifted by Niroo Malt Khorasan Co. (Mashhad, Iran). All procedures used were approved by the Ferdowsi University of Mashhad (Mashhad, Iran) Animal Care and Use Committee.

Malt Extract and Vinegar Chemical Analysis

The chemical composition of malt extract was determined according to AOAC (International, 2000). The malt sample was analyzed for dry matter (DM, method 930.15), total ash (method 942.05), fat (method 954.02) and crude protein (CP, Kjeldahl N × 6.25, method 990.03) as well as acetic acid (method 925.34). The total phenol content of malt extract and vinegar was determined by Folin–Ciocalteu reagent (Qingming *et al*., 2010). About 1 mL of samples was mixed with 2 mL of Folin– Ciocalteu reagent followed by addition of 2 mL of 15% Na2CO3. After keeping for 1 h of reaction at room temperature, the absorbance was read at 760 nm (UV-2100, Unico Instruments Co., Shanghai, China). Total phenolic was expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight or per L. Vitamins content of malt extract were determined by the method described by Ekinci and Kadakal (2005).

One part of the malt extract (5 g) was added into four parts of deionized water (20 g). The mixture was homogenized by applying homogenizer (T25 Ultra-Turrax, IKA Labortechnik, Staufen, Germany) for 10 min at 14 × 10^3 g. The stationary phase was flushed with 10 mL methanol and 10 mL water was adjusted to pH 4.2 to activate the stationary phase. Liquid chromatography was performed utilizing a HPLC system (Camag Co., Muttenz, Switzerland). A reversed-phase discovery C18 (150 mm × 4.6 mm, 5 µm; #504955) HPLC column was used. The photodiode-array detector of 234 nm for thiamine, 204 nm for pantothenic acid,

266 nm for riboflavin, 282 nm for folic acid and 324 nm for pyridoxine were used. The mobile phase was 0.1 mol L-1 KH2PO4 (pH 7)–methanol, 90:10. The flow-rate was 0.7 mL min^{-1}.

Table 1. Composition of the basal control diets

Item	Starter (0-10 d)	Grower (11-24 d)	Finisher (25 d-end)
Ingredient, g/kg			
Corn grain	510.3	540.6	588.4
Soybean meal (CP=440 g/kg)	421.5	384.7	331.5
Soybean oil	23.8	35.0	43.8
Limestone	14.4	13.3	12.3
Dicalcium phosphate	15.2	13.2	11.3
Sodium chloride	3.0	3.0	3.0
Vitamin premix[1]	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5
DL-methionine	4.2	3.6	3.3
HCL-lysine	1.9	1.3	1.3
L-threonine	0.65	0.25	0.05
Phytase (10000)[3]	0.05	0.05	0.05
Calculated nutritional composition, per kg			
DM, g	883.2	882.9	881.7
ME, Kcal	3000	3100	3200
CP, g	230	215	195
Crude ash, g	57.7	53.1	47.8
Ca, g	9.6	8.7	7.8
Available phosphorus, g	4.8	4.3	3.9
Total lysine, g	14.4	12.9	11.6
Total methionine, g	7.7	7.0	6.4
Total methionine + cystine, g	10.8	9.9	9.1
Total threonine, g	9.7	8.8	7.8
Total Arginine, g	15.2	13.7	12.2

[1]Provided in kg of diet: vitamin A (retinyl acetate),13.5 mg; vitamin E (dl-α tocopherol acetate), 160 mg; vitamin D3 (cholecalciferol), 10 mg; vitamin B1 (thiamine mononitrate), 3.2 mg; vitamin B2 (riboflavin), 8.6 mg; vitamin B3 (niacinamide), 60 mg; vitamin B5 (calcium pantothenate), 17 mg; vitamin B6 (pyridoxine HCl), 5.4 mg; ; biotin, 0.30 mg; vitamin B9 (folic acid), 2.2 mg vitamin B12 (cyanocobalamin), 0.017 mg; vitamin K (menadione sodium bisulphate complex), 3.2 mg

[2]Provided (mg/kg of diet): manganese (manganese sulfate and manganous oxide), 120 mg; zinc (zinc oxide), 110 mg; iron (ferrous sulfate), 20 mg; copper (copper sulfate), 16 mg; iodine (calcium iodate), 1.25 mg; selenium (sodium selenite), 0.30 mg

[3] Phytafeed, Pintaluba, Tarragona, Spain.

Growth Performance

The mortality was recorded daily and used for correction of average daily gain (ADG) feed intake (FI), and feed conversion ratio (FCR) in each period and the entire experimental period. The European production efficiency factor (EPEF) was calculated according to the following formula: {[BW (kg) × livability (%)]/ [age (d) × FCR]} × 100.

Carcass Yield

At the end of the experiment, after 6- hours feed withdrawal, two male birds per pen were randomly selected and were euthanized for organ sampling; carcass, liver, pancreas, spleen, bursa and thymus were weighed by digital weighing (0.001-g digital balance, model GF 400, A&D Weighing, CA). Breast and thigh muscles were vacuum packed for further analysis.

Jejunal Histomorphology

Intestinal segment sample (approximately 0.5 cm in length) from mid-part of the jejunum (from the pancreatic loop to Meckel's diverticulum) was excised and digesta was eliminated by saline 0.9% flushing. Dehydrated samples were cleared and embedded in paraffin. Then, jejunal segments were sliced at a 6-µm thickness, and were placed on glass slides for examination by light microscopy (SMZ-168 series, Motic stereo microscope, Motic Co. Hong Kong). The evaluated variables for jejuna morphology were including villus height from apex to the junction of the villus, crypt depth from the base of the villi to the submucosa and crypt and villus width

from the junction to the basement membrane of the epithelial cell at the bottom of the crypt. A total of 10 sections were counted from one bird and their average was considered for one sample. The villus height to crypt depth ratio and apparent villus surface area were measured according to Maneewan and Yamauchi (2004).

Muscle pH, Drip Loss and Cooking Loss Measurements

pH of the breast and thigh muscles was measured using a pH meter (model 691 Laboratory pH Meter, Metrohm Co, Herisau, Switzerland) at a depth of 2.0 cm below the surface, in breast and thigh muscle at 24 h after slaughter, as described by Akbari Moghaddam Kakhki *et al*. (2017). The left breast and thigh muscles were deboned and striped (2 cm × 2 cm × 2 cm) then were weighed individually and stored at 4°C in a polyethylene bag. The muscle and thigh strips were removed from the bags after 24 h, were wiped, and reweighed to calculate drip loss as the method described by Akbari Moghaddam Kakhki *et al*. (2017) and expressed as a percent of initial muscle weight. For cooking loss measurement samples (2 cm × 2 cm × 2 cm) were weighed and wrapped in aluminum foil and cooked in an oven at 100°C until the external temperature reached 85°C and the interior temperature reached 72 ± 2°C. Cooked muscles were cooled to room temperature and weighed to determine the cooking loss. Cooking loss (%) was calculated as described by Akbari Moghaddam Kakhki *et al*. (2017).

Malondialdehyde concentration measurements

Malondialdehyde (MDA) concentration in the breast and thigh muscles at 24-h after slaughter was considered as the indicator of lipid oxidation. The MDA concentration was measured using a commercial MDA assay Kit procedure (Malondialdehyde Assay kit, ZellBio GmbH Co., Ulm, Germany). In summary, MDA is measured in acidic media and is heated (90-100°C) colorimetrically at 532 nm (UV-2100, Unico Instruments Co., Shanghai, China). The absorbance was determined at 532 nm against a blank containing 5 mL of distilled water and 5 mL of 0.02 M TBA solution. All meat quality and oxidation stability measurement were performed twice and the average of them was considered for statistical analysis.

Ceca microflora

Ceca were moved under aseptic freeze condition to the microbial laboratory for bacteria counting of *E.Coli*, *Lactobacillus*, *Coliforms* and *Clostridiums* as well as the total bacteria count. For bacteria counting of Lactobacillus, *E. Coli*, and total counts, samples were cultured using LBS agar, MacConkey and nutrient agar, respectively, (Qingdao High-tech Industrial Park Haibo Biotechnology Co., Ltd., Shandong, China). For *Coliforms* enumeration squeezed fresh ceca content were collected in sterilized 25-mL tubes and three grams of them were diluted with 10 mL distilled water. Samples were serially diluted from 10^{-1} to 10^{-7}. One deciliter of each diluted sample was plated on the violet bile agar for the enumeration of coliforms bacterial populations as described in much detail by Zhao *et al*. (2013) and Zhang *et al*. (2014). Formed colonies were expressed as $\log^{10}$ CFU per gram of fresh digesta. All microbiological assays were measured in triplicate and the average values were used for statistical analysis.

Statistical analysis

All obtained data were subjected to SAS software and considered for normality with UNIVARIATE PLOT NORMAL procedure. Data were analyzed in a completely randomized design and significant effects ($P < 0.05$) were made by the treatments through GLM procedure with the help of Tukey test.

Results

Chemical Composition

Chemical composition showed that malt extract contains 28.7 g/100g of moisture, 15.22 MJ/kg Gross energy, 67 g/kg of crude protein, 8 g/kg of ether extract, 21 g/kg of ash, 1.5 mg/100g of B1, 5.21 mg/100g of B2; 14.32 mg/100g of B5, 4.99 mg/100g of B6, 72.2 µg/100g of B9, 3.5 mg GAE/g of total Phenolic Content. In addition, chemical analysis showed that malt vinegar has pH of 3.0 and contains 5.8% of acetic acid and 2010 mg GAE/L of total Phenolic Content.

Growth Performance

The ADFI, ADG and FCR were not affected by dietary treatments throughout starter and grower periods (Table 2). In the finisher phase, ME2MV4 had higher ADG than the other treatments. The ADFI was not influenced by dietary treatments throughout finisher phase, but FCR tended to alter in response to dietary treatments ($P = 0.065$). In addition, ADG, FCR and EPEF were

influenced by dietary treatments throughout the whole experimental period. Dietary supplementation of malt extract and vinegar did not alter ADFI and mortality rate in the whole experimental period. Supplementation of ME2MV4 led to a significant increment in overall ADG compared to birds were fed CON and ME2MV8 diets (P = 0.007).

Table 2. Effect of dietary treatments on growth performance of broilers during 1 to 43 d[1]

Treatment[2]	CON	ME2	ME2MV4	ME2MV8	*SEM*	*P*-values
ADFI[3] (g/b/d)						
Starter	26.3	26.2	26.4	26.8	0.768	0.931
Grower	79.6	76.0	76.9	80.5	2.118	0.421
Finisher	158.6	155.7	160.5	158.1	2.791	0.183
Overall	102.3	100.2	101.9	101.5	1.743	0.846
ADG[3] (g/b/d)						
Starter	23.0	22.7	22.9	23.7	0.520	0.608
Grower	53.4	52.9	53.5	51.8	0.800	0.462
Finisher	82.6[b]	87.0[ab]	90.2[a]	83.0[b]	1.524	0.008
Overall	59.2[b]	61.0[ab]	62.6[a]	59.1[b]	0.695	0.007
FCR[3]						
Starter	1.14	1.18	1.16	1.13	0.023	0.908
Grower	1.49	1.44	1.44	1.55	0.035	0.109
Finisher	1.92	1.79	1.78	1.91	0.050	0.065
Overall	1.72[a]	1.64[b]	1.63[b]	1.71[a]	0.038	0.047
Mortality (%)	5.0	7.2	4.0	8.0	1.861	0.422
EPEF[3]	329[b]	352[ab]	375[a]	322[b]	12.124	0.028

[a–d] Values with uncommon superscripts within each row are significantly different (P < 0.05)
[1] Growth performance data are means of 6 pens of broilers with 25 broilers per pen.
[2] CON = control; ME2 = dietary supplemented with 0.2% malt extract; ME2MV4 = dietary supplemented with 0.2% malt extract and 0.4% malt vinegar; ME2MV8 = dietary supplemented with 0.2% malt extract and 0.8% malt vinegar
[3] ADG = average daily gain, ADFI = average daily feed intake, FCR = feed conversion ratio, EPEF = European production efficiency factor

Carcass and Organs Yield

Effects of dietary treatments on carcass cut-yields and percentage of organs are shown in Table 3. Carcass yield in the birds fed by ME2MV4 treatment was greater than CON and ME2 groups (P = 0.031). Percentage of the liver, abdominal fat, pancreas, thymus, bursa of Fabricious and spleen was not influenced by dietary treatments (P > 0.05).

Table 3. Effect of dietary treatment on carcass and organs cut-yields at the 43 d of age (g/100 g of live body weight)

Treatment [1]	CON	ME2	ME2MV4	ME2MV8	*SEM*	*P*-values
Carcass [2]	71.68[b]	71.86[b]	72.06[a]	71.90[ab]	0.145	0.031
Liver	2.22	2.22	2.22	2.24	0.005	0.893
Abdominal fat	1.92	1.91	1.89	1.83	0.014	0.096
Pancreas	0.22	0.23	0.23	0.24	0.001	0.156
Thymus [3]	0.3	0.32	0.31	0.3	0.002	0.542
Bursa	0.14	0.15	0.15	0.14	0.003	0.653
Spleen	0.11	0.12	0.12	0.12	0.002	0.841

[a–b] Values with uncommon superscripts within each row are significantly different (P < 0.05)
[1] CON = control diets; ME2 = dietary supplemented with 0.2% malt extract; ME2MV4 = dietary supplemented with 0.2% malt extract and 0.4% malt vinegar; ME2MV8 = dietary supplemented with 0.2% malt extract and 0.8% malt vinegar
[2] Skin was removed and bone-in the part.
[3] All thymus lobes from both sides of the neck were weighed for each chick.

Meat Quality

Dietary treatments did not influence pH, drip loss and cook loss percentage of the breast and thigh muscles (Table 4) although drip loss of the breast and thigh muscles was tended to be lowered in ME2 group ($P < 0.10$). The MDA concentration was considered as a lipid oxidation indicator; supplementation of ME2, ME2MV4 and ME2MV8 led to the significant reduction of MDA concentration in the breast muscle ($P < 0.01$). In the thigh muscle, the ME2MV8 group had significant lower MDA concentration compared to CON and ME2 treatments ($P < 0.001$).

Jejunal histology

The villus height, crypt depth and their ratio were not altered by malt extract and vinegar supplementation (Table 4). Villus width was increased by the ME2 treatment compared to the ME2MV4 and ME2MV8 ($P = 0.045$) and lead to the greater apparent absorption surface area in ME2 treatment ($P = 0.024$).

Ceca microflora

Ceca microflora enumeration including: total count, *E. Coli*, *Coliforms*, *Lactobacillus*, and *Clostridium* was not affected by dietary treatments ($P > 0.05$; data are not presented). The average of $\log_{10}$ CFU of the total count, *E. Coli*, *Coliforms*, *Lactobacillus*, and *Clostridium* were 6.89, 5.18, 5.89, 4.91 and 4.11, respectively.

Table 4. Effect of dietary treatments on meat quality characteristics and jejunal morphology

Treatment [1]	CON	ME2	ME2MV4	ME2MV8	*SEM*	*P*-values
Meat quality						
Breast pH[2]	5.83	5.84	5.84	5.82	0.004	0.724
Thigh pH[2]	5.54	5.56	5.54	5.53	0.003	0.635
Breast drip loss (%)[2]	1.56	1.54	1.56	1.55	0.042	0.086
Thigh drip loss (%)[2]	0.68	0.63	0.68	0.67	0.051	0.072
Breast cook loss (%)	33.02	33.00	33.01	33.01	0.011	0.217
Thigh cook loss (%)	31.42	31.4	31.41	31.41	0.022	0.237
Breast MDA (mg/g)	0.451[a]	0.367[b]	0.358[b]	0.352[b]	0.011	<0.001
Thigh MDA (mg/g)	0.547[a]	0.457[b]	0.445[bc]	0.418[c]	0.01	<0.001
Jejunal Morphology						
Villus height (µm)	1188	1285	1240	1167	81.3	0.822
Villus width (µm)	165[ab]	218[a]	148[b]	130[b]	21.71	0.045
Crypt depth (µm)	230	290	273	251	25.15	0.375
Villus height / Crypt depth	5.17	4.43	4.54	4.65	0.356	0.455
Absorption surface area (mm^2)	0.20[b]	0.28[a]	0.19[b]	0.16[b]	0.026	0.024

[a–b] Values with uncommon superscripts within each row are significantly different ($P < 0.05$)

[1] CON = control diets; ME2 = dietary supplemented with 0.2% malt extract; ME2MV4 = dietary supplemented with 0.2% malt extract and 0.4% malt vinegar; ME2MV8 = dietary supplemented with 0.2% malt extract and 0.8% malt vinegar

[2] Measurements were performed at 24-h post mortem.

Discussion

There is no more evidence regarding the evaluation of malt extract and vinegar supplements in poultry nutrition or other animals, so we focused on primary components of malt extract and vinegar and also discussed the results of other studies that have been used ingredients and supplements with the same components.

Based on the results obtained from this study, dietary inclusion of malt extract and malt vinegar may have the beneficial effects on broiler performance. Feed conversion ratio was significantly affected (P=0.047) by supplementation of malt extract and vinegar, in which the birds fed ME2 and ME2MV4 diets had 4.65 and 5.23% lower FCR compared to the CON group, respectively. European production efficiency factor significantly differed among the treatments; ME2MV4 group had higher EPEF than CON and ME2MV8 treatments (P=0.011). Higher EPEF in birds fed ME2MV4 diet was resulted from heavier body weight, lower FCR and mortality rate compared to the other treatments.

Hosseini *et al*. (2010) conducted a study to evaluate the effects of feeding broilers with brewers byproducts and reported that inclusion of 7.5 and 15% brewers spent grain reduced ADG

accompanied by an increase in FCR and viscosity of intestinal content. Carías and Millán (1996) evaluated the substitution of soybean isolated protein by solid fraction of brewery liquid waste. The 20% replacement of brewery waste protein had no effect on ADG and ADFI of chickens compared to birds were fed soybean protein. In addition, the protein efficiency and net protein ratios of the diets did not show any difference.

Numerous studies were examined the effects of organic acids such as propionic, butyric, fumaric and formic acids on growth performance of broilers (Ricke, 2003). Some weak organic acids like acetic acid, citric acid and fumaric acid have been used as an organic acid supplement in poultry diets and as a natural preservative substance (Dibner and Buttin, 2002). The results of some published studies illustrated that dietary addition of citric acid in broiler diets resulted in an increment in ADG and FCR, but it diminished feed consumption (Dibner and Buttin, 2002). The reasons described to clarify improvement of broilers performance are associated with acidification characteristics of organic acids, including improvement of digestive enzymes, pancreatic secretions, affecting microbial populations of the gut and better nutrients digestibility (Menconi *et al*., 2014). Direct stimulation of gastrointestinal cell proliferation seems to be another mechanism for the beneficial effect of organic acids on broiler performance.

Carcass yield in birds fed diet ME2MV4 was greater than CON and ME2 groups. This might be resulted from greater body weight in the birds fed ME2MV4 diet. As described before, acidifier characteristic of malt vinegar (that could positively affect microbial population and improve digestive enzyme activity, pancreatic secretion and nutrient digestibility) accompanied by a desirable modification in intestinal histomorphology, resulted in greater body weight in the ME2MV4 treatment. Seemingly, supplementation of malt extract without using malt vinegar reduced drip loss more effectively than other treatments. This can be explained by the existence of phenolic compounds, which have an antioxidant function in malt extract (Qingming *et al*., 2010). Antioxidants reduce leakage of sarcoplasmic components from muscle cells by maintaining the integrity of cellular membrane and thereby reduce drip loss (Akbari Moghaddam Kakhki *et al*., 2017).

In the biological systems, MDA is considered to be the most important derivative of aldehyde products generated by lipid peroxidation. The CON treatment resulted in higher MDA concentration in the breast muscle than the other treatments supplemented either by malt extract or malt extract along with malt vinegar. Moreover, in the thigh muscle, the ME2MV8 treatment had a lower concentration of MDA than CON and ME2. Cell membrane perturbation and cell damage are mainly caused by lipid peroxidation. Superoxide and hydroxyl radicals are initiators for peroxidation of lipid and lead to peroxy radicals formation. Thus, scavenging peroxy radicals by antioxidants can prohibit lipid peroxidation. Various endogenous phenolic compounds of barley and Maillard reaction products which engendered during malting process can play important roles through their antioxidative properties (Maillard *et al*., 1996). Qingming *et al*. (2010) reported that enzymatic extrication of bound phenolic compounds could increase total phenolic content in barley from 3.11 mg gallic acid (GAE) to 3.19 mg per g of dry weight during malting or kilning. Furthermore, the phenolic hydroxyl group of ferulic acid, which is phenolic compound in malt extract and produced during Maillard reaction, capable of accepting electrons. This property can be combined with free radical competitively to decrease hydroxyl radical. Qingming *et al*. (2010) figured out that malt extract and Trolox (a water-soluble analog of vitamin E) have 47.1% and 42.1% scavenging effect, respectively. So, the reduction in MDA concentration among the treatments can be explained by antioxidant characteristic of malt extract. Furthermore, lower MDA concentration in the thigh muscle of birds in ME2MV8 treatment may result from the presence of gallic acid, catechin and vanillic acid in the malt vinegar and its synergistic effect with malt extract; which both of them consist of phenolic compounds and have antioxidant functionality (Zheng and Wang, 2001).

Generally, increasing in crypt depth represents higher tissue turnover and cell production. In addition, shortening of the villi height reduces the surface area for nutrient absorption (De Verdal Mignon-Grasteau *et al.* 2010). Our results indicated that ME2 group had greater villus width and subsequently, widened apparent absorption surface area than other treatments. The intestinal morphology is associated with chickens growth and intestinal functions. The epithelium of the small intestine is regenerated by proliferating crypt cells. There is

contradictory observation regarding the effect of organic acid on intestinal morphology. Some reports have shown that formic acid had no impact on intestinal morphology (Smulikowska *et al*., 2010; Hernández *et al*., 2006), while the results of Leeson *et al*. (2005) studies illustrated that butyric acid seemed to be the stimulant of villi growth and consequently, increased absorption area. Samanya and Yamauchi (2001) observed an improvement in jejunal villus height by the inclusion of 1% wood vinegar; however, the inclusion of 2% wood vinegar reduced villus height. It was demonstrated by Yoshimura and Hayakawa (1993) that the effect of wood vinegar was induced by its components such as propionic acid, acetic acid, dimethylphenol, butanoic acid and methoxyphenol. Although ME2MV4 had smaller absorption surface area than ME2, it had better growth performance. This phenomenon can be explained by adequate absorption surface area existed in the birds fed ME2MV4 diet that supports their growth requirements.

The demographics and digestive activity of bacterial population in the gastrointestinal tract can be influenced by strain, sex, age, rearing environment, accessible nutrients, passage rate and presence, amount and actualize ability of antimicrobial substances in a diet (Smulikowska *et al*., 2010). Antibacterial effects of organic acids depend on their pKa value, chemical form of the acid, animal species, type of microorganism, location in the gastrointestinal tract, sanitation of the environment and buffering capacity of the feed (Smulikowska *et al*., 2010).

In conclusion, the results of this study revealed that dietary supplementation of 0.2% malt extract along with 0.4% malt vinegar could improve growth performance and carcass yield in concomitant with improvement in oxidation stability of breast and thigh muscles.

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