Effects of grape seed extract on performance, immunity, antioxidant capacity, and meat quality in Pekin ducks

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ABSTRACT This study was conducted to evaluate the effects of grape seed extract (**GSE**) on growth performance, immunity, antioxidant capacity, relative organ weight, jejunum morphology, ileal microflora, and meat quality in Pekin ducks. A total of 1,500 female 1-day-old Pekin ducklings (52.0 ± 0.2 g) were blocked based on body weight (**BW**) and randomly allocated into 3 treatments with 10 replicates of 50 birds each. The experiment lasted for 6 wk, and dietary treatments included corn-soybean meal-based diet supplemented with 0, 0.01, and 0.02% GSE. The supplementation of GSE increased ($P < 0.05$) body weight gain (**BWG**) and final BW linearly but decreased ($P < 0.05$) feed-to-gain ratio (**F/G**) linearly during day (D) 22 to 42 and the entire experiment. The inclusion of GSE increased ($P < 0.05$) serum superoxide dismutase, glutathione peroxidase, total antioxidative capacity, catalase, complement4, immunoglobin G, interleukin-2, and interferon-γ linearly but decreased ($P < 0.05$) serum malondialdehyde linearly. The relative weight of carcass, breast meat, and spleen in GSE treatments was increased ($P < 0.05$) linearly, whereas the relative weight of abdominal fat was decreased linearly ($P < 0.05$). Birds fed GSE1 and GSE2 diets had lower ($P < 0.05$) cook loss, 2-thiobarbituric acid reactive substances, and drip loss on day 3 and 5 linearly but higher ($P < 0.05$) pH_{24h} and water-holding capacity. The addition of GSE decreased ($P < 0.05$) jejunum crypt depth and ileal *Escherichia coli* counts linearly but increased ($P < 0.05$) jejunum villus height: crypt depth ratio and ileal *Lactobacilli* linearly. Taken together, the inclusion of GSE increased final BW and BWG, decreased F/G during day 22 to 42 and day 1 to 42, partially improved antioxidant activities, immunity, meat quality, and gut health in Pekin ducks.

Key words: antioxidant capacity, ducks, grape seed extract, performance

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INTRODUCTION

It is well documented that oxidation damage could result in negative effect on growth performance, health status, meat quality, and hence economic losses in poultry, which may be considered as a major threat to poultry and poultry meat (Fellenberg and Speisky, 2006; Sihvo et al., 2013; Est'evez, 2015). Recent reviews and literatures have shown that oxidative stress could be attributed to the imbalance of pro-oxidants and the endogenous antioxidant mechanisms in living tissues (Kohen and Nyska, 2002), which may be initiated by reactive oxygen species (Cadenas and Davies, 2000). The oxidative reactions do not only cause adverse effect on growth performance and meat quality but also may damage food safety because of oxidized food for consumers (Bekhit et al., 2013; Est'evez, 2015). Moreover, the domestic poultry are particularly susceptible to oxidative reaction because of the modern genetic selection toward lean and large breast muscles and fast growth rates (Sihvo et al., 2013) as well as high unsaturation degree of the muscle lipids (Min et al., 2008).

Several strategies aimed to control oxidative reactions in poultry are applied to protect living tissues including dietary and technological strategies, which were reviewed by Est'evez (2015). Among them, there is a growing interest in the natural antioxidants (polyphenols) because of its natural, nontoxic, and residue-free properties (Surai, 2014; Diaz-Sanchez et al., 2015; Brenes et al., 2016). Polyphenols, known as polyhydroxyphenols, are mainly secondary plant metabolites containing natural bioactive compounds characterized by the presence of large multiples of phenol structural units (Zhang and Tsao, 2016), which can be divided into 3 main subclasses (flavonoids, phenolic acids, and stilbenoids). They are extensively

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found in fruits, vegetables, herbs, flowers, spices, and tea (Szliszka and Krol, 2011). In addition, studies show that polyphenols have a variety of biological activities and exert antioxidant, antiinflammatory, immunomodulatory, and antimutagenic effects (Cottart et al., 2014; Lipiński et al., 2017; Zhang et al., 2017) by regulating the activity of enzymes and cell receptors (D'Archivio et al., 2007).

Grape (*Vitis vinifera*) is one type of the world's largest fruit crops (FAO-STAT, 2010). Grape seed, a natural agricultural by-product of grapes, is a better source of antioxidative constituents than grape juice by-products because of its high content of vitamin E, flavonoids, and proanthocyanidins (Abu Hafsa and Ibrahim, 2018). Grape seed polyphenols have been widely used as human food supplement for health. Grape seed extract (**GSE**) is a heterogeneous mixture of polyphenols (anthocyanidins, catechins, and their derivatives) obtained from solvent extraction (Viveros et al., 2011). Previous studies have indicated that GSE has strong antioxidant capacity, whose activity is approximately 20 times stronger than that of vitamin E and 50 times greater than that of vitamin C (Carpenter et al., 2007). Several studies have been conducted to evaluate its effects on growth performance, antioxidant activities, gut health, immune function, and nutrient digestibility in broilers (Viveros et al., 2011; Iqbal et al., 2015; Yang et al., 2016; Abu Hafsa and Ibrahim, 2018), weaning pigs (Hao et al., 2015; Chedea et al., 2018), growing pigs (Fiesel et al., 2014), and sows (Wang et al., 2019), which indicated positive effects, especially on antioxidant function. Furthermore, numerous studies have demonstrated that GSE could improve meat quality in broilers (Smet et al., 2008), pigs (Zhang et al., 2015), cattle (Ahn et al., 2002), and lamb (Zhao et al., 2018). However, little information about the effect of GSE on ducks was available. Thus, we hypothesize that GSE may exert antioxidant capacity, stimulate immune system, and hence improve growth performance and meat quality in Pekin ducks. Therefore, the aim of this study was to evaluate the influence of GSE on growth performance, immune function, antioxidant capacity, relative organ weight, jejunum morphology, ileal microflora, and meat quality in Pekin ducks.

MATERIALS AND METHODS

Experimental Design and Duck Husbandry

All the experimental procedures have been approved by the Animal Welfare Committee of Dankook University (Cheonan, Choongnam, South Korea). The GSE was extracted from grape seed with the ethanol method, and the content of total polyphenols was 51.2%, which was analyzed by high-performance liquid chromatography (Agilent 1,100 series, Palo Alto, CA).

A total of 1,500 female Pekin ducklings (No. 4 strain) at 1 D of age with an average initial body weight (**BW**) of 52.0 ± 0.2 g were blocked based on BW in this 42-day experiment and placed in stainless steel battery brooders. The cages were equipped with feeder, nipple drinker, and raised plastic floors. All ducks were housed in an environmentally controlled facility. The dietary treatments were (1) CON, basal diet; (2) GSE1, CON + 0.01% GSE; (3) GES2, CON + 0.02% GSE. Grape seed extract was included at the expense of corn. There were 10 replications (cages) per treatment and 50 ducks per cage in a randomized complete block design. All diets were formulated to meet or exceed the NRC (1994) requirements of ducks for the starter period from 1 to 21 D and grower period from 21 to 42 D of age (Table 1). Diets were fed in pellet form, and ducks were provided with water and feed *ad libitum* throughout the experiment. The environmental temperature and humidity were kept at 29°C and 60%, respectively, during 1 to 14 D. Afterward, the temperature was kept at 24°C.

Feed samples were analyzed for dry matter (Method 934.01), crude protein (Method 990.03), total ash (Method 942.05), calcium, and phosphorus (Method 985.01) according to the standard procedures of the AOAC (2002). The amino acids of all diets were determined, following acid hydrolysis with 6 N HCl at 110°C for 24 h, using an amino acid analyzer (Biochrom 20, Pharmacia Biotech, Cambridge, England). Before acid hydrolysis, methionine and cystine were oxidized with formic acid (Liu et al., 2019).

Sampling and Measurements

BW and feed intake (**FI**) were recorded on day 1, 21, and 42 of the experiment on a per replicate basis. Body

Table 1. Diet composition (as-fed basis).

Items	Starter[1]	Grower[1]
Ingredients, %		
Corn	59.20	64.22
Soybean meal (CP 46%)	31.36	24.69
Wheat bran	0.50	0.40
Soybean oil	2.03	2.83
Corn gluten meal	2.00	4.00
Dicalcium phosphate	1.39	1.27
Limestone	1.10	0.97
Bentonite	0.90	-
Sodium chloride	0.20	0.25
Choline chloride (60%)	0.10	0.10
DL-Methionine (99%)	0.15	0.11
L-Lys·HCl (78%)	0.07	0.16
Vitamin premix[2]	0.70	0.70
Trace mineral premix[3]	0.30	0.30
Total	100.00	100.00
Analyzed composition		
ME, kcal/kg[4]	3,000	3,200
Crude protein, %	22.27	18.30
Lysine, %	1.00	0.80
Methionine, %	0.50	0.45
Methionine + Cystine, %	0.82	0.75
Threonine, %	0.98	0.82
Calcium, %	0.70	0.60
Available phosphorus, %	0.40	0.35

[1]Starter diets, provided during day 1 to 21; grower diets, provided during day 22 to 42.

[2]Provided per kg of diet: choline chloride, 1,000 mg; vitamin A, 10,000 IU; vitamin D_3, 3,000 IU; vitamin E, 20 IU; vitamin K_3, 2 mg; thiamin, 2 mg; riboflavin, 8 mg; pyridoxine hydrochloride, 4 mg; cyanocobalamin, 0.02 mg; calcium-D-pantothenate, 20 mg; nicotinic acid, 50 mg; folic acid, 1 mg; biotin, 0.2 mg.

[3]Provided per kg of diet: Cu, 10 mg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg; Se, 0.3 mg; I, 0.2 mg; Cr, 0.15 mg.

[4]Calculated values.

weight gain (**BWG**), FI, and feed-to-gain ratio (**F/G**) were calculated accordingly. Mortality was recorded as it occurred, and the weights of dead birds were used to adjust F/G.

At the end of the experiment, 8 birds from each replicate were randomly selected from each cage, and blood samples were collected from the jugular vein into a sterile syringe and stored at 4°C. Blood samples were then centrifuged at 3,000 × *g* for 15 min, and serum was separated. The levels of superoxide dismutase (**SOD**), catalase (**CAT**), glutathione peroxidase (**GSH-PX**), total antioxidative capacity (**T-AOC**), malondialdehyde (**MDA**), immunoglobin A, immunoglobin M, immunoglobin G (**IgG**), complement3, complement4 (**C4**), interleukin-2 (**IL-2**), interleukin-6, tumor necrosis factor-α (**TNF-α**), and interferon-γ (**IFN-γ**) in the serum were measured using ELISA method (Jiancheng Biotechnology Institute, Nanjing, China) following the kit instructions (Ao and Kim, 2019; Liu et al., 2020a).

After blood collection, the same birds were weighed individually and then sacrificed by cervical dislocation and exsanguinated (Liu et al., 2020b). The carcass weight (without neck and feet), breast meat, liver, gizzard, pancreas, thymus, bursa of fabricius, spleen, and abdominal fat were removed by trained personnel and weighed after flushing with saline. Organ size was expressed as a percentage of BW. The pH of the breast meat was measured by a calibrated, glass-electrode pH meter (WTW pH 340-A; WTH Measurement Systems Inc., Ft. Myers, FL). The breast meat lightness (L*), redness (a*), and yellowness (b*) values were determined (Minolta CR410 Chromameter; Konica Minolta Sensing Inc., Osaka, Japan). The water-holding capacity (**WHC**) was measured in accordance with the methods described by Kauffman et al. (1986). Drip loss was measured with approximately 2 g of heat sample according to the plastic bag method described by Honikel (1998). Cook loss was determined as described previously by Sullivan et al. (2007). The 2-thiobarbituric acid reactive substances (**TBARS**) were measured by the method described by Witte et al. (1970). The TBARS values were expressed in terms of milligrams of MDA per kilogram of muscle. Trichloroacetic acid solution (20% wt/vol) was utilized for the extraction. The chromium concentration was determined by spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan).

After weighing, the samples of small intestine tissues (approximately 2 cm from jejunum and ileum, respectively) were collected for determination of mucosal morphology and microflora. The tissues from jejunum were cleaned with saline and then fixed in 10% neutral formalin. The fixed tissues were trimmed and embedded in paraffin for mucosal morphology and integrity. Thin sections (5 μm) were sliced and mounted on slide and then stained with hematoxylin and eosin (staining procedure) for histopathological examination by an optical microscope (Olympus, Tokyo, Japan). Jejunum morphological variables measured were villus height (**VH**) and crypt depth (**CD**). The villus height: crypt depth ratio (**VH/CD**) was calculated accordingly. These indexes were quantified according to the method described previously (Viveros et al., 2011). Mean values of VH, CD, and VH/CD within each segment (10 villi per bird) were calculated. Samples of fresh digesta (2 g) from the ileum were collected aseptically in preweighed 20-mL sterilized plastic tubes. One gram of the composite ileal digesta sample from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenized. Viable counts of bacteria in ileal digesta samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates and *lactobacilli spp.* medium III agar plates to isolate the *Escherichia coli* and *Lactobacilli*, respectively. The *lactobacilli* medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. MacConkey agar plates were incubated for 24 h at 37°C. *E. coli* and *lactobacilli* colonies were counted immediately after removal from the incubator (Ao and Kim, 2019).

Statistical Analysis

All data were analyzed using Mixed procedures of SAS (SAS Institute, 2003). Orthogonal polynomials were used to assess the linear and quadratic effects of increasing dietary concentrations of supplemental GSE. Variability in the data is expressed as the standard error of the means, and a probability level of $P < 0.05$ were considered to be statistically significant.

RESULTS

Growth Performance

During day 1 to 21, dietary treatments did not affect ($P > 0.05$) BWG, FI, or F/G (Table 2). During day 22 to 42, the supplementation of GSE increased ($P < 0.05$) BWG linearly but decreased ($P < 0.05$) F/G linearly without any effect on FI ($P > 0.05$). Overall, BWG and final BW increased ($P < 0.05$) linearly in GSE treatments compared with CON, whereas F/G was reduced ($P < 0.05$) linearly.

Antioxidant Activities and Immune Function

The administration of GSE improved ($P < 0.05$) serum SOD, GSH-PX, T-AOC, and CAT linearly, whereas reduced ($P < 0.05$) serum MDA linearly (Table 3).

The inclusion of GSE increased ($P < 0.05$) serum C4, IgG, IL-2, and INF-γ linearly (Table 4). There was no difference ($P > 0.05$) in serum complement3, immunoglobin A, immunoglobin M, interleukin-6, or TNF-α among dietary treatments.

Relative Organ Weight and Meat Quality

The GSE supplementation increased ($P < 0.05$) relative weight of carcass, breast meat, and spleen linearly (Table 5). The relative weight of abdominal fat

Table 2. Effects of grape seed extract on growth performance in ducks[1].

Item[2]	CON[3]	GSE1[3]	GSE2[3]	SEM[4]	*P*-value	
					Linear	Quadratic
Initial BW, g	52.0	52.0	52.0	0.11	0.731	0.872
Final BW, g	2,903	2,951	2,993	17	0.042	0.754
Day 1–21						
BWG, g	1,203	1,192	1,195	11	0.546	0.221
FI, g	2,496	2,486	2,479	13	0.143	0.720
F/G	2.07	2.09	2.07	0.02	0.635	0.156
Day 22–42						
BWG, g	1,648	1,707	1,746	16	0.033	0.821
FI, g	4,620	4,601	4,593	20	0.225	0.692
F/G	2.80	2.70	2.63	0.02	0.025	0.786
Day 1–42						
BWG, g	2,851	2,899	2,941	17	0.031	0.586
FI, g	7,116	7,087	7,072	19	0.305	0.822
F/G	2.50	2.44	2.40	0.02	0.042	0.745

[1]Means represent 10 replicates with 50 birds per cage (n = 10/group).
[2]BWG, body weight gain; FI, feed intake; F/G, feed-to-gain ratio; d, day.
[3]CON, basal diet; GSE1, basal diet containing 0.01% GSE; GSE2, basal diet containing 0.02% GSE.
[4]Standard error of the means.

decreased linearly ($P < 0.05$). No difference was observed ($P > 0.05$) in relative weight of liver, pancreas, gizzard, bursa of fabricius, or thymus among dietary treatments.

Dietary treatments did not affect ($P > 0.05$) pH_{45min}, lightness (L*), redness (a*), yellowness (b*), or drip loss on day 1 (Table 6). The inclusion of GSE decreased ($P < 0.05$) cook loss, TBARS, drip loss on day 3 and 5 linearly but increased ($P < 0.05$) pH_{24h} and WHC linearly.

Jejunum Morphology and Ileal Microflora

Jejunum CD in GSE treatments decreased ($P < 0.05$) linearly, whereas VH/CD increased ($P < 0.05$) linearly (Table 7). Dietary treatments did not influence ($P > 0.05$) jejunum VH.

The supplementation of GSE increased ($P < 0.05$) ileal microbial shedding of *Lactobacilli* linearly but decreased ($P < 0.05$) *E. coli* linearly (Table 8).

DISCUSSIONS

Growth Performance

This is the first study about the effect of GSE on ducks. In the present study, the supplementation of GSE (0.01-0.02%; 0.005-0.015 g polyphenols/kg diet) increased final BW and BWG linearly but decreased F/G linearly over the grower and overall periods. In agreement with our results, Abu Hafsa and Ibrahim (2018) indicated that 2% grape seed (11.14 g polyphenols/kg diet) increased final BW and BWG, whereas reduced F/G in broilers. They observed that higher grape seed (4%) decreased growth performance in broilers. Similar results were observed by Chamorro et al. (2013) in broilers. The phenolic compound gallic acid (0.0075–0.01%) from grape seed decreased F/G during day 22 to 42 and during day 1 to 42 in broilers (Starčevi'c et al., 2015; Samuel et al., 2017). On the contrary, Viveros et al. (2011) reported that GSE (0.72%; 0.019 g polyphenols/kg diet) decreased BWG in broilers. Feeding 0.2-1% GSE did not influence growth performance, but 3% GSE led to a growth depression in broilers (Hughes et al., 2005), which may be because of the higher total polyphenols expressed as gallic acid (27 g polyphenols/kg diet). The above results showed both dosage-dependent and time-dependent effects of GSE in poultry. The high inclusion of grape polyphenols might result in adverse effect on growth performance in broilers (Hughes et al., 2005; Viveros et al., 2011; Chamorro et al., 2013; Yang et al., 2016; Abu Hafsa and Ibrahim, 2018), which was supported by the previous studies (Nyachotti et al., 1997). Similarly, Yang et al.

Table 3. Effects of grape seed extract on antioxidant activities in ducks[1].

Item[2]	CON[3]	GSE1[3]	GSE2[3]	SEM[4]	*P*-value	
					Linear	Quadratic
SOD, U/mL	132	159	163	5.9	0.022	0.734
GSH-PX, U/mL	268	304	311	5.4	0.041	0.910
MDA, nmol/mL	4.28	3.19	3.05	0.2	0.024	0.652
T-AOC, U/mL	15.7	18.3	19.6	1.1	0.042	0.632
CAT, U/ml	127	151	153	5.2	0.030	0.701

[1]Means represent 10 replicates with 8 birds per cage (n = 80/group).
[2]SOD, superoxide dismutase; GSH-PX, glutathione peroxidase; MDA, malondialdehyde; T-AOC, total antioxidative capacity; CAT, catalase.
[3]CON, basal diet; GSE1, basal diet containing 0.01% GSE; GSE2, basal diet containing 0.02% GSE.
[4]Standard error of the means.

Table 4. Effects of grape seed extract on immune function in ducks[1].

Item[2]	CON[3]	GSE1[3]	GSE2[3]	SEM[4]	*P*-value	
					Linear	Quadratic
C3, g/L	0.16	0.17	0.15	0.01	0.372	0.182
C4, g/L	0.05	0.08	0.08	0.01	0.041	0.217
IgA, μg/mL	24.8	23.8	23.9	1.6	0.334	0.185
IgM, μg/mL	45.6	47.3	48.2	2.2	0.215	0.810
IgG, μg/mL	84.9	91.4	93.2	2.6	0.043	0.722
IL-2, ng/mL	121	132	134	2.9	0.032	0.553
IL-6, ng/mL	21.3	21.6	21.8	0.4	0.364	0.615
IFN-γ, ng/mL	19.0	21.9	22.2	0.4	0.031	0.844
TNF-α, pg/mL	19.9	20.6	21.3	0.5	0.090	0.693

[1]Means represent 10 replicates with 8 birds per cage (n = 80/group).
[2]C3, complement3; C4, complement4; IgA, immunoglobin A; IgM, immunoglobin M; IgG, immunoglobin G; IL-2, interleukin 2; IL-6, interleukin 6; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α.
[3]CON, basal diet; GSE1, basal diet containing 0.01% GSE; GSE2, basal diet containing 0.02% GSE.
[4]Standard error of the means.

(2016) demonstrated that the dosage should attract careful attention because higher GSE supplementation may lead to detrimental effect on growth performance. The improvement in F/G in the current study was presented in the grower phase, which was consistent with previous studies (Starčevi'c et al., 2015; Samuel et al., 2017). Brenes and Roura (2010) indicated that the growth performance was not affected by the supplementation of GSE (0.06-0.36%) in broilers. Similar results were observed in broilers fed diets with 0.5-6% grape by-products (Goñi et al., 2007; Brenes et al., 2008). The inconsistent results may be attributed to the different supplementation dosages, sources, diet composition, and age. Previous studies indicated that GSE could exert better effects on growth performance in broilers in wheat-type diets compared with corn-type diets (Viveros et al., 2011; Yang et al., 2016), which may be because of the serum glucose balance (Andersen et al., 2008). Notwithstanding, the positive effect of GSE on growth performance in ducks fed corn-type diet were observed in our study, which may be species-dependent. However, more studies are needed to determine the effects of GSE on growth performance in ducks to verify this hypothesis.

Antioxidant Activities and Immune Function

Many studies have indicated that grape seed byproducts might improve antioxidant capacity in broilers (Iqbal et al., 2015; Yang et al., 2016; Samuel et al., 2017; Abu Hafsa and Ibrahim, 2018), weaning pigs (Hao et al., 2015), and sows (Wang et al., 2019). Our study also confirmed that GSE could exert antioxidative activities by improving serum SOD, GSH-PX, T-AOC, and CAT and decreasing serum MDA. This means that the GSE may be an effective antioxidant, which could decrease reactive free radicals and oxidative stress by activating the antioxidant enzyme system (Lipiński et al., 2017).

The immunity may be influenced by oxidative stress, and the improved antioxidant function may enhance their immune function in poultry (Iqbal et al., 2015; Kamboh et al., 2015). Polyphenols might boost immune function by suppressing the inflammatory process via nuclear factor-kappaB and nuclear factor-2–dominated pathways in the small intestine (Chiva-Blanch and Visoli, 2012; Paszkiewicz et al., 2012), which has been verified in pigs (Gessner et al., 2013).

Table 5. Effects of grape seed extract on relative organ weight in ducks[1].

Item	CON[2]	GSE1[2]	GSE2[2]	SEM[3]	*P*-value	
					Linear	Quadratic
Carcass weight, %	70.1	71.4	71.5	0.31	0.040	0.210
Breast meat, %	18.2	19.3	19.6	0.19	0.033	0.624
Abdominal fat, %	2.61	2.13	2.12	0.06	0.012	0.782
Liver, %	2.78	2.73	2.77	0.05	0.381	0.223
Gizzard, %	2.08	2.13	2.16	0.04	0.095	0.455
Pancreas, %	0.35	0.39	0.41	0.02	0.153	0.613
Thymus, %	3.42	3.54	3.56	0.06	0.114	0.785
Bursa of fabricius, %	0.13	0.16	0.18	0.02	0.083	0.594
Spleen, %	0.12	0.16	0.19	0.01	0.022	0.801

[1]Means represent 10 replicates with 8 birds per cage (n = 80/group).
[2]CON, basal diet; GSE1, basal diet containing 0.01% GSE; GSE2, basal diet containing 0.02% GSE.
[3]Standard error of the means.

Table 6. Effects of grape seed extract on meat quality in ducks[1].

Item[2]	CON[3]	GSE1[3]	GSE2[3]	SEM[4]	*P*-value	
					Linear	Quadratic
pH_{45min}	6.62	6.61	6.63	0.03	0.524	0.235
pH_{24h}	5.81	5.95	6.08	0.02	0.042	0.783
WHC, %	47.3	51.1	51.9	1.21	0.021	0.694
Cook loss, %	29.9	26.3	26.0	0.53	0.044	0.265
TBARS, mg MDA/kg	1.69	1.27	1.26	0.06	0.033	0.800
Meat color						
Lightness (L*)	55.1	56.2	55.8	0.65	0.584	0.864
Redness (a*)	15.5	15.8	16.1	0.32	0.235	0.613
Yellowness (b*)	14.6	14.9	15.1	0.25	0.176	0.712
Drip loss, %						
Day 1	1.58	1.56	1.53	0.10	0.192	0.771
Day 3	3.59	3.28	3.27	0.12	0.043	0.424
Day 5	6.17	5.92	5.93	0.09	0.034	0.255

[1]Means represent 10 replicates with 8 birds per cage (n = 80/group).
[2]WHC, water holding capacity; TBARS, 2-thiobarbituric acid reactive substances; d, day.
[3]CON, basal diet; GSE1, basal diet containing 0.01% GSE; GSE2, basal diet containing 0.02% GSE.
[4]Standard error of the means.

The inclusion of GSE increased serum IgG in the present study. Similar results were observed in weaning pigs fed diets containing 0.01 to 0.015% grape seed procyanidins (Hao et al., 2015) and sows fed diets containing 0.02 to 0.03% GSE (Wang et al., 2019). IFN-γ serves as an important regulator in the activation of lymphocytes and monocytes and serum IL-2 promotes the proliferation of activated natural killer cells, B lymphocytes, T lymphocytes, and antibody production (Ao and Kim, 2019). Complement4 plays an important role in immune response and is an important part of the body's immune defense system. The present results showed that the supplementation of GSE improved serum C4, IL-2, and INF-γ, indicating GSE could enhance immune response by regulating antibodies, complements, and cytokines (Lipiński et al., 2017).

Relative Organ Weight and Meat Quality

Previous studies indicated that dietary GSE supplementation did not influence the relative weight of carcass and breast meat in broilers (Hajati et al., 2015; Lipiński et al. 2017). In contrast, we observed increased relative weight of carcass and breast meat in the present study. In accordance with our results, Abu Hafsa and Ibrahim (2018) observed that the addition of 1 to 2% grape seed increased relative weigh of carcass and dressing weight in broilers. Breast muscle yield was increased by the supplementation of 0.0075-0.01% phenolic compound gallic acid from grape seed in broilers (Samuel et al., 2017). The GSE supplementation decreased relative weight of abdominal fat in our study, which was similar to the findings of Abu Hafsa and Ibrahim (2018). They found that feeding 1 to 4% grape seed decreased the relative weight of abdominal fat. However, Brenes et al. (2008) reported increased abdominal fat percentage in broilers fed 1.5% grape pomace concentrate. Moreover, several studies observed no effect of GSE or grape proanthocyanidins on the relative weight of abdominal fat in broilers (Hajati et al., 2015; Yang et al., 2016). The relative weight of spleen was increased by GSE supplementation in the present study, which was not consistent with Brenes et al. (2008) and Abu Hafsa and Ibrahim (2018). Dietary treatments did not affect relative weight of liver, pancreas, gizzard, bursa of fabricius, or thymus in our study. Hajati et al. (2015) also demonstrated that dietary supplementation of GSE did not influence the percentage of liver or gizzard in broilers.

It is proposed that GSE may exert antioxidative activities to decrease water loss, which may improve meat quality. As expected, the supplementation of GSE reduced cook loss, TBARS, drip loss on day 3 and 5 linearly, but increased pH_{24h} and WHC in our study. Similarly, Wu et al. (2018) observed that dietary supplementation of grape proanthocyanidins (0.005-0.01%) decreased drip loss on day 1 and 2 as well as water loss rate in broilers. Others also observed decreased TBARS in broilers (Selani et al., 2011) and pigs fed GSE diets (Yan and Kim, 2011). On the contrary, Abu Hafsa and Ibrahim (2018) failed to observe positive effect of grape seed on pH, meat color, or WHC in broilers. Further studies are needed to determine the effects of GSE on meat quality in ducks.

Table 7. Effects of grape seed extract on jejunum morphology in ducks[1].

Item[2]	CON[3]	GSE1[3]	GSE2[3]	SEM[4]	*P*-value	
					Linear	Quadratic
VH, μm	1,093	1,069	1,084	16.4	0.774	0.142
CD, μm	348	314	303	6.6	0.032	0.825
VH/CD	3.17	3.40	3.58	0.4	0.041	0.464

[1]Means represent 10 replicates with 8 birds per cage (n = 80/group).
[2]VH, villus height; CD, crypt depth; VH/CD, villus height: crypt depth ratio.
[3]CON, basal diet; GSE1, basal diet containing 0.01% GSE; GSE2, basal diet containing 0.02% GSE.
[4]Standard error of the means.

Table 8. Effects of grape seed extract on ileal microflora in ducks[1].

Item	CON[2]	GSE1[2]	GSE2[2]	SEM[3]	*P*-value	
					Linear	Quadratic
Escherichia coli, $\log^{10}$ cfu/g	4.37	3.92	3.38	0.08	0.023	0.483
Lactobacilli, $\log^{10}$ cfu/g	7.44	7.73	7.95	0.07	0.034	0.665

[1]Means represent 10 replicates with 8 birds per cage (n = 80/group).
[2]CON, basal diet; GSE1, basal diet containing 0.01% GSE; GSE2, basal diet containing 0.02% GSE.
[3]Standard error of the means.

Jejunum Morphology and Ileal Microflora

The jejunum is the main part for nutrient absorption in poultry intestine, whose morphology can reflect the feed efficiency indirectly (Varel et al., 1987; Leeson and Summers, 2001). The VH/CD might indicate the absorption function of villi comprehensively (Mahdavi et al., 2010). The present study showed that dietary GSE supplementation caused a positive effect on jejunum CD and VH/CD, which was in line with Viveros et al. (2011). They reported that GSE (0.72%) supplementation decreased jejunum CD but increased VH/CD in broilers, which may be beneficial to gut health and nutrient absorption. Similarly, dietary grape proanthocyanidins (0.00075-0.0015%) positively modulated jejunum morphology in broilers (Yang et al., 2016), which may be attributed to antioxidant and antibacterial activities (Oliveira et al., 2013; Surai, 2014). The phenolic compound gallic acid (0.0075-0.01%) from grape seed also decreased jejunum CD and improved VH/CD in broilers (Samuel et al., 2017). The improved jejunum morphology may partly mirror the increased F/G in the present study. Besides, Boka et al. (2014) observed that heavier broilers had lower CD but higher VH/CD than lighter counterparts.

Likewise, the stabilization of ileal microflora is critical to gut health and function (Song et al., 2014). Grape by-products might enhance the growth of specific beneficial bacteria strains in the intestinal tract while competitively excluding certain pathogenic bacteria (Brenes et al., 2016). In the present study, the GSE supplementation exerted a positive effect on ileal bacterial populations, which was consistent with Abu Hafsa and Ibrahim (2018). They reported increased ileal *Lactobacilli* counts but decreased *E. coli* counts in broilers fed diets containing 1 to 4% grape seed. Similarly, Viveros et al. (2011) showed that GSE could effectively increase the ileal populations of beneficial bacteria and reduce the counts of pathogenic bacteria in broilers. Previous studies also confirmed the antibacterial activity of the GSE against *E. coli* in vitro (Baydar et al., 2006; Rodríguez-Vaquero et al., 2007). It is proposed that the increased ileal *Lactobacilli* counts may be because of its capability to use and metabolize phenolic compounds as nutritional substrates (García-Ruíz et al., 2008; Viveros et al., 2011). Furthermore, the increased immunity may also mirror the positive effect of GSE on ileal microflora in our study because polyphenols could increase the shedding of microbial beneficial bacteria (*Lactobacillus*) to indirectly enhance immunity and gut health (Paszkiewicz et al., 2012).

CONCLUSIONS

The supplementation of GSE (0.01-0.02%) caused a positive effect on feed efficiency, antioxidant activities, immunity, meat quality, jejunum morphology, and ileal microflora in Pekin ducks over the grower and the whole experiment and thus improved final BW and BWG.

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