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The Effect of Supplementation with Organic Acid and Oregano Oils in Drinking Water on Pekin Duck Growth and Welfare

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Simple Summary: The use of water supplements, such as phytogenics and organic acids, in poultry research, especially in Pekin ducks, has been very limited. Due to commercial Pekin ducks consuming a higher amount of water compared to chickens, it is hypothesized that water supplementation may prove more beneficial than standard feed supplementation. Oregano oils and organic acids have been popular feed additives in poultry diets due to their antimicrobial properties and gut health benefits. This study found significant benefits with both water additives mentioned for improving duck production, gut health, and reducing stress susceptibility. Therefore, water supplementation with either oregano oils or organic acid provides a possible alternative to antibiotic use in commercial Pekin ducks to improve production and animal well-being.

Abstract: This study evaluated duck growth, health, and welfare in response to water supplementation with organic acid (OA) and oregano oils (OOs) in Pekin duck. The treatments used in this study included a control (CON) treatment with no water additives given, an OA treatment (ProPhorce Exclusive NC®), and an OO treatment (Nubiotic 4X Concentrate®). The OA and OO improved the feed conversion ratio (FCR) and body weight (BW) ($p < 0.01$) compared to the control (CON). Both OA and OO showed differences ($p < 0.05$) in villus height and crypt depth compared to the CON. But only OA showed an increase ($p < 0.01$) in villus height and villus height/crypt depth ratio. On D 35, the total plasma corticosterone levels, heterophil-to-lymphocyte ratios, and asymmetry scores for OA and OO were decreased ($p < 0.05$) compared to CON, indicating lower stress susceptibility. The pH levels of OA ceca and jejunum were lower ($p < 0.05$) compared to CON. Tibia breaking strength was increased ($p = 0.02$) for OA compared to CON, while no differences were found with OO ($p > 0.05$). In conclusion, these experiments indicate that OA and OO can be used to improve duck growth, feed efficiency, stress susceptibility, and bird welfare.

Keywords: ducks; antibiotic alternatives; phytogenics; welfare; stress; growth production; water supplementation

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1. Introduction

The use of phytogenics and organic acids (OAs) as an antibiotic alternative (AA) additive has been widely researched throughout the past few decades in both the livestock and poultry industries. Phytogenics are typically derived from non-woody herbs and spices [1], also commonly referred to as essential oils (EOs) [2], that come from around the world. A few EOs have gained more popularity including thyme, oregano, anise, cinnamon, garlic, black pepper, and turmeric [3]. Due to the limited use of antibiotics for growth promoters, and therapeutic uses in the US, research has shown phytogenics and OAs to be viable AAs [2]. While it is known that maintaining homeostasis mechanisms throughout a bird's life span will promote efficient bird growth and health, various environmental stimuli often disrupt that homeostasis and increase chronic stress that may impact bird production and welfare [4]. Therefore, one typical approach to mitigate these negative effects is for feed additives to be implemented into the bird's diet in order to follow a therapeutic approach [1] for nutrition and bird growth and optimal economical production output.

One such group of feed additives are organic acids (OAs). Organic acids are organic carboxylic acids containing a chemical structure chain of R-COOH [5]. Organic acids include a large array of acids; however, commonly used OAs in animal feeds are short-chain fatty acids, including formic and propionic acids [6]. These OAs have been used as antibiotic alternative (AA) feed and water additives/acidifiers due to their growth-promoting attributes and pathogen control benefits [7]. Research has shown OAs to be an effective antimicrobial agent when compared to antibiotic use [7]. Due to having a pKa value between 3 and 5 [6], intestinal pH is reduced when OA is ingested in water or feed. This has aided in positive outcomes for lower FCR and FI in poultry production including chickens [7,8] and ducks [9,10]. Reduced FI may be due to a lack of palatability of OA when used in feed [5,7]. However, this trend of reduced FI remains in water additive studies as well [7,9]. Various studies have seen different outcomes with reduced FI, with some exhibiting a lower FCR with increased BW, while others show decreased BW and FI together in broilers [7].

Nutrient digestibility has also been linked to the relationship of lower pH in the GI. This improved digestibility leads to an improvement in overall bird performance, which encourages the use of the OAs for growth, disease control, and economical advantages [9,11]. Lower pHs, when further investigated, have shown that harmful microbials cannot thrive in this environment in chickens [5,7,12] and meat ducks [9] due to microbiome changes reducing epithelial permeability [7]. Villus height and crypt depth measurements of the small intestine in various studies have also shown significant trends for improved nutrient digestion, resulting in improved mineral absorption and microflora populations [6,7,10]. Furthermore, improvements in nutrient digestibility result in improvements in mineral and total ME absorption, including nitrogen [7,8]. Improved nitrogen absorption leads to reduced ammonia levels and results in an improved environment and bird welfare. Due to the higher absorption rate of phosphorus with OA, tibia ash content has also shown a significant increase, which can lead to stronger leg bones, leading to a reduction in lameness and bird mortality [5].

Another group of feed additives used as AAs are essential oils. One such essential oil is oregano oil (OO), which derives from the *Lamiaceae* family (*Origanum vulgare*) and involves extracting oils from the leaves, flowers, and roots [1]. Oregano oils have been shown to be an alternative for therapeutic [13] and antibiotic growth promoters (AGPs) due to the increased palatability and natural antioxidant and antimicrobial properties in poultry [14–17]. They have also shown positive improvements in meat quality in broilers and other poultry meat products [16]. Oregano oils contain phenolic compounds such as carvacrol, β-fenchyl alcohol, thymol, and γ-terpinene. Many other ingredients can be found in OO that derive from different parts of the plant including P-cymene, caryophyllene, 3-carene, palmitic acid, linoleic acid, and oleic acid [3,6,17]. Carvacrol and thymol, the two main phenolic compounds found in OOs, have proven antimicrobial and antioxidant effects in poultry [15]. The bioactive ingredients of OO mentioned above have also shown positive effects for intestinal microbiota anaerobic and aerobic digestive mechanisms which promote antimicrobial efficacy [13,15,17,18]. Oregano oil compounds show antifungal, antimicrobial, antiparasitic, and antioxidant properties which may provide therapeutic and disease treatment options for poultry [13,16]. With similar results to OA, OO has been shown to promote a significant increase in bird BW and FCR. The two main active ingredients (thymol and carvacrol) in OO provide a large portion of increased viability for bird growth and gut health. Improved GI health has also been linked to OO use due to the increase in mucous secretions that protect the epithelial cells in the intestines. Coating these cell walls leads to a decrease in disease volatility and further provides a stable microbiome for improved nutrition efficiency. Oregano oil increases villus height within the intestinal tract, allowing for nutrient absorption. The main OO components, thymol and carvacrol, are also membrane-soluble. This allows thymol and carvacrol to break down

Gram-negative bacteria through altering the permeability of their membrane for cell lysis and apoptosis [19]. The immune response increases through the use of OO, which aids in disease control and prevention in bird flocks for bacteria, Eimeria, and possibly viruses. In one study, the inflammatory immune response was reduced and showed antioxidant characteristics, improving overall meat quality and product shelf-life [20].

Within poultry research, the effects of EO and OA use have shown vast improvements within nutrient absorption and feed efficiency to enable ameliorated growth production [21], provide antioxidant mechanisms for lowered inflammation and improve meat quality [22], reduce microbial loads in the GI tract to promote pathogen defence, as well as enhance immune response [22]. Though the use of OA and OO has shown great potential, research lacks the understanding of its implementation in duck production. Therefore, this study was conducted to evaluate the effect of water supplementation with OA and OO on Pekin duck productivity and welfare.

2. Materials and Methods

2.1. General Husbandry

The study was conducted using 378 straight-run day-of-hatch Pekin ducklings. Pekin day-old ducklings were assigned using a complete block design to pens; each pen housed 14 birds per pen. Three treatments were used in this study with 9 replicate pens within each treatment, for a total of 27 pens utilized. Treatments used in this study included the following: a control (CON) treatment with no water additives given, an OA treatment (ProPhorce Exclusive NC®, Perstorp, Malmo, Sweden) at an application ratio of 1 milliliter (mL) of OA to 128 mL of water, and an OO treatment (Nubiotic 4X Concentrate®, AgSupplies Direct, Dallas, TX, USA) at an application ratio of 1 mL of OA solution to 128 mL of water. A Dosetron® 7 GPM: D128R Medicator (Dosetron International, Clearwater, FL, USA) for each water treatment was used for proper dosage and administration throughout the water lines accessible to the ducks' pens. Both organic acid and OO treatments were premixed to fit the capacity of a 18.9 L bucket. The OA mixture was dosed according to ProPhorce™ application guidelines with ~1 kg per 1000 L of water with an approximate water pH of 3.8–4.0. For the OO treatment, approximately 118 mL of oregano oil to 18.9 L of water was mixed. Each treatment had a separate Dosetron connected to blocked pen treatments with a 1:128 mL application rate. All treatments were given from D 1 to 35. Product composition is presented in Table 1.

Table 1. Composition of ingredients for experimental Pekin duck water supplementation treatments.

Organic Acid Ingredients	OA, (%) [1]	Oregano Oil Active Ingredients (Proprietary Mixture)
Formic acid	25–35	Oregano oil
Propionic acid	5–15	Cinnamon oil
1,2,3-propanetriol, glycerol	5–10	Citric acid
Castor oil, ethoxylated	5–10	Sodium chloride
Cinnamaldehyde	1–5	

[1] remainder of composition: water.

Each pen was 0.914 m by 1.829 m, with each pen containing three nipple waterline drinkers, one tube feeder, and fresh pine shavings as litter. Both the tube feeder and waterline were adjusted throughout the duration of the trial to accommodate bird growth. Feed and water were provided ad libitum during the duration of the trial. The lighting program was 24 h of daylight from D 0–3 and 16 h of daylight at D 4–35. The barn temperature was recorded twice daily and adjusted in response to bird comfort. A basal diet was formulated and composed of a corn and soybean meal-based diet with vegetable oil as a liquid fat source. A 2-phase feeding program was used in this study (starter D 0–14 and grower D 15–35). The starter feed was offered as crumbles and the grower feed as a pellet. During the starter phase (D 0–14), the diet contained 5% BMD 50 (bacitracin methylene disalicylate, Zoetis, Parsipanny, NJ, USA) per ton. No BMD was used in the grower phase. All feed was manufactured by the Texas A&M feed mill.

All animal husbandry procedures are conducted in accordance with an approved animal use protocol (IACUC #2021-0314) and methods described below similar to those presented in previously [23,24].

2.2. Growth Parameters

Growth and production parameters were measured on D 14 and D 35 of the study. Feed was weighed before being added to feeders in each pen, and residual feed was weighed back on D 14 at the end of the starter phase and again on D 35 upon conclusion of the study to allow FI calculations. The feed conversion ratio was calculated by dividing the total FI per pen by the total BWG per pen and was corrected for mortality. Mortalities and culls found before D 5 were replaced with a duck of the same weight being given the same treatment in an extra pen.

2.3. Gut Health Parameters

2.3.1. Intestinal pH levels

The pH of 4 key areas of the intestinal tract including the proventriculus, jejunum, ileum, and ceca were measured and recorded on D 35. Three birds per pen were randomly selected from the control and OA treatment (N = 54). None were sampled from the OO treatment. Each bird was sampled from the medial cross section of the proventriculus, jejunum, ileum, and ceca. A pH meter (Hanna Instruments Inc., Smithfield, RI, USA) was calibrated and inserted into the medial section of each sampled section listed above until the reading was shown and recorded. Once the pH was recorded, the probe was sanitized with distilled water to ensure no contamination affected the reading between each pH sampling.

2.3.2. Ileum Sampling and Histomorphology

On D 35, 20 birds were randomly selected from each treatment (N = 60). A 1–3 cm long section of ileum was collected from the medial point between the ileocecal junction and Meckel's diverticulum. Samples were rinsed with phosphate-buffered saline and stored in 15 mL centrifuge tubes (VWR International, LLC, Radnor, PA, USA) of 10% neutral buffered formalin at room temperature. The samples were sent to StageBio Laboratories (Mount Jackson, VA, USA) to be processed and stained with Periodic Acid–Schiff (PAS) in combination with Alcian Blue (AB). The mounted and stained ileum sections were photographed at 4× magnification using a Nikon Eclipse Ci-L microscope (Nikon Corporation, Tokyo, Japan). The accompanying Elements software (Version D) program was used to obtain the villus length, crypt depth, and V/C ratio from 6 intact villus samples per bird. The villus height was measured from the top of the villus to the villus–crypt junction. The crypt depth was measured from the invagination base of the villi to the muscularis mucosae. Both measurements were used to estimate villi height over crypt depth ratio.

2.4. Stress Parameters

2.4.1. Heterophil-to-Lymphocyte Ratio and Plasma Corticosterone

At 35 days of age, 20 ducks per treatment (N = 60) were randomly selected for plasma corticosterone (CORT) and heterophil-to-lymphocyte ratio (H/L) analysis. Approximately 1–2 mL of blood was collected from the brachial wing vein of each duck. A small drop of blood from each bird sampled was smeared on a glass plate for heterophil-to-lymphocyte ratio (H/L) analysis. The remaining blood collected was injected into a plasma separation gel and lithium heparin vacutainer (BD 368056, BD, Franklin Lakes, NJ, USA) and temporarily stored in an ice bath. Once all blood samples had been collected, all vacutainers were spun down using a centrifuge (Eppendorf 5804, Eppendorf North America, Hauppauge, NY, USA) at 4000 RPM for 15 min to separate the plasma and blood cells. Each blood plasma sample was then poured into a 2 mL microcentrifuge tube and stored at −19 °C until further analysis was performed. A hematology staining kit (Cat# 25034, Polysciences Inc., Warrington, PA, USA) was used for staining blood smear slides used for the H/L ratio.

The plasma corticosterone concentration from each sample was analyzed using a commercially available ELISA kit (Enzo Life Sciences, ADI-901-097, Farmingdale, NY, USA). The inter- and intra-assay % CV were both under 5%.

To determine the heterophil-to-lymphocyte ratio of the collected samples, one layer of stained blood cells on glass slides was observed under 40× magnification using an oil immersion lens on a microscope (Omax DCE-2, Kent, WA, USA). The number of heterophils and lymphocytes observed in an area of the blood smear slide without overlapping cells was counted using a keystroke counter (SEOH B4001-5LC, Navasota, TX, USA) until a total of 100 cells had been recorded. Increased total plasma corticosterone levels and heterophil-to-lymphocyte ratios indicate higher stress susceptibility in poultry.

2.4.2. Footpad Lesion Parameters

Footpad lesions (FPLs) were measured at D 35 to evaluate duck welfare on all birds (N = 378). Each bird was selected from its respective pen and cradled with the holder's arm on its back. Each leg was supported by the holder's hands for further restraint. An observer evaluated both footpads, and each footpad was given an individual score. A 3-point paw scoring system was utilized for FPL determination. A paw score of 0 means the bird does not express lesions, injury, or redness to the footpad area. A paw score of 1 means the bird expresses 50% or less lesions, injury, or redness to the footpad area. A paw score of 2 means the bird expresses more than 50% profound lesions, injury, or redness to the footpad area. Once the bird was scored and recorded, each bird was sprayed with livestock paint and placed back in its respective pen. The left and right footpad lesion scores were averaged for one FPL score per bird.

2.4.3. Composite Asymmetry Score

Asymmetry was measured on D 35 to evaluate bird physiological growth stress by methods discussed by Archer [25]. Sixty birds per treatment were selected at random (N = 180). This study analyzed the physical asymmetry of 3 bilateral traits including the metatarsal length (ML), metatarsal width (MW), and middle toe length (MTL). These 3 bilateral traits were measured for the right and left legs of each duck using a calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL, USA). The sum of the absolute value of the right measurement was subtracted from the left measurement of each trait, then divided by the total number of traits in order to calculate the composite asymmetry score. The equation for ASYM is as follows:

$$(|\text{L-R}|\text{MTL} + |\text{LR}|\text{ML} + |\text{L-R}|\text{MW}) = \text{composite asymmetry score}$$

Asymmetry has been used as a tool for welfare indicators for environmental and developmental stress [22,25–29].

2.4.4. Bone Breaking Strength and Bone Ash Determination

On day 35, twenty birds per treatment (N = 60) were randomly selected and euthanized via a Zephyr-EXL Pneumatic Stunner (Schuyler, NE 68661); both the left and right tibia were removed. The muscle, connective tissue, and fibula were removed from each tibia, and the bones were dried using a Forced Air Oven (VWR 89511-410, Radnor, PA, USA) at 100 °C for 12 h. The left tibias were used to measure bone mineral content. The right tibias were used to measure bone breaking strength. The left tibias were defatted using diethyl ether for 6–8 h and air-dried under a chemical hood, allowing all remaining ether to evaporate. The defatted tibias were then dried again at 100 °C for 12 h and then ashed at 600 °C in ceramic crucibles for 24 h. To determine the percentage of tibia mineral ash content, all tibias and crucibles were weighed before and after ashing. To minimize moisture content, crucibles were kept at 100 °C for 12 h prior to ashing. The right tibia breaking strength (g) was determined by the 3-point bending test using a TA.XT plus100 Texture Analyser

(Stable Micro Systems, Surrey, UK) to break each tibia bone at the center point of the tibial shaft charged with a 100 kg load cell and a crosshead speed of 1.67 mm/s, with the tibia supported on a 3-point bend platform with a 4 cm constant span.

2.5. Statistical Analysis

One-way ANOVA was used to investigate the treatment effects on mortality, FCR, BW (kg), total plasma corticosterone concentration, H/L ratio, gut health measures, bone quality measures, FPL, and asymmetry. The Kruskal–Wallis test was used to determine treatment effects for footpad lesions. General Linear Model (GLM) assumptions were tested using Levene's test for homogeneity of variance and the Shapiro–Wilk test for linear and quadratic response. Minitab 17.1.0. was used to perform the described analyses. All data are expressed as mean pooled SEMs, and $p < 0.05$ was used to determine all significant differences.

3. Results

3.1. Performance and Mortality

The performance results are presented in Table 2. Organic acid (0.82 ± 0.01 kg) and OO (0.80 kg ± 0.01) did not differ in BW at D 14 ($p > 0.05$) compared to CON (0.805 kg ± 0.01). At D 35, BW was higher in both OA ($p < 0.001$) and OO ($p < 0.03$) compared to CON.

Table 2. Body weights, average daily feed intake (ADFI), and feed conversion (FCR) measurements of Pekin ducks supplemented with organic acid or oregano oil in the water for 35 days.

Treatment	Avg. Bird Weight (kg)	14 D Avg. Bird Weight (kg)	35 D Avg. Bird Weight (kg)	ADFI 1–15 D (g/d)	ADFI 15–35 D (g/d)	ADFI 1–35 D (g/d)	FCR 1–15 D	FCR 15–35 D	FCR 1–35 D
Control (1)	0.06	0.81	3.21 [b]	66.33	283.90 [a]	174.13 [a]	1.24 [a]	1.66 [a]	1.56 [a]
Organic acid (2)	0.05	0.82	3.52 [a]	66.04	279.28	171.76	1.21	1.49 [b]	1.43 [b]
Oregano oils (3)	0.05	0.80	3.36 [a]	65.85	265.43 [b]	165.51 [b]	1.23 [b]	1.50 [b]	1.44 [b]
p-value 1 vs. 2	-	0.32	0.001	0.85	0.53	0.58	0.4	0.0001	0.0001
p value 1 vs. 3	-	0.9	0.03	0.72	0.0001	0.006	0.003	0.0001	0.0001
Pooled SEM	0.0005	0.004	0.04	0.52	2.83	1.54	0.01	0.02	0.02

[a,b] means within columns with different superscripts differ significantly at $p < 0.05$.

The average daily feed intake (ADFI) did not differ between the treatments during the starter phase ($p > 0.05$). During the grower phase, the OA and CON treatments did not differ ($p > 0.05$); however, the OO birds consumed less feed (265.43 g ± 1.10) than the CON ($p < 0.0001$). As a result, OO also had an overall lower cumulative ADFI (165.51 g ± 0.75) than the CON (174.13 g ± 2.61, $p = 0.006$).

The OA treatment did not differ from CON during the starter phase for FCR ($p > 0.05$). The OO treatment had a lower FCR (1.23 ± 0.01) than CON (1.24 ± 0.02) during the starter phase ($p < 0.003$). Both the OO (1.50 ± 0.03) and OA (1.49 ± 0.03) had lower FCRs during the grower phase compared to the CON (1.66 ± 0.01) ($p < 0.0001$). Cumulatively, the FCR for OA (1.43 ± 0.02) and OO (1.44 ± 0.02) also showed differences compared to CON (1.56 ± 0.01) ($p < 0.0001$).

The mortality results are presented in Table 3. No treatment differences were observed in mortality ($p > 0.05$). No differences were found in mortality with culls ($p > 0.05$).

Table 3. Mortality (%) and mortality w/culls (%) of Pekin ducks supplemented with organic acid or oregano oil in the water for 35 days.

Treatment	Mortality 15–35 D	Mortality w/Culls 15–35 D	Mortality 1–35 D	Mortality w/Culls 1–35 D
Control (1)	2.38	2.38	2.38	2.38
Organic acid (2)	0.79	1.59	0.79	1.59
Oregano oils (3)	0.00	0.79	0.00	0.79
p-value 1 vs. 2	0.28	0.62	0.28	0.62
p-value 1 vs. 3	0.06	0.28	0.06	0.28
Pooled SEM	0.50	0.58	0.50	0.58

3.2. Intestinal Health and Welfare Parameters

The villus height, crypt depth, and V/C ratio measurements are presented in Table 4. The villus height was greater ($p < 0.016$) in the OA treatment (377.42 ± 9.61 µm) compared to the control (343.99 ± 9.89 µm). The OO treatment (300.19 ± 7.65 µm) had shorter villi ($p < 0.001$) compared to CON (343.99 ± 9.89 µm). The crypt depth showed a significant decrease for both OA (122.20 ± 3.41 µm, $p < 0.04$) and OO (119.33 ± 4.22 µm, $p < 0.02$) compared to CON (137.23 ± 6.44 µm). The V/C ratio was increased in OA (1.36 ± 0.07, $p < 0.01$) compared to CON (1.13 ± 0.05), while OO (1.14 ± 0.06) did not differ ($p > 0.05$) compared to CON (1.13 ± 0.05).

Table 4. Villus height, crypt depth and villi/crypt ratio measurements of Pekin ducks supplemented with organic acid or oregano oil in the water for 35 days.

Treatment	Villus Height (µm)	Crypt Depth (µm)	V/C Ratio
Control (1)	343.99 ^{b}	137.23 ^{a}	1.13 ^{b}
Organic acid (2)	377.42 ^{a}	122.20 ^{b}	1.36 ^{a}
Oregano oil (3)	300.19 ^{a}	119.33 ^{b}	1.14 ^{b}
p-value 1 vs. 2	0.016	0.036	0.014
p-value 1 vs. 3	0.001	0.019	0.931
Pooled SEM	5.52	2.78	0.04

^{a,b} means within columns with different superscripts differ significantly at $p < 0.05$.

The pH levels for the proventriculus, jejunum, ileum, and ceca are presented in Table 5. The OO was not measured due to there being no expected effect on pH. Organic acid did not affect ($p > 0.05$) the pH levels in the proventriculus and ileum sections compared to CON. The pHs in the jejunum (6.14 ± 0.10, $p < 0.04$) and ceca (5.69 ± 0.08, $p < 0.02$) were both lower compared to CON (6.31 ± 0.11 and 6.05 ± 0.09, respectively).

Table 5. pH measurements of selected GI tract sections of Pekin ducks supplemented with organic acid in the Water for 35 days.

Treatment	Proventriculus	Jejunum	Ileum	Ceca
Control	4.70	6.31 ^{a}	6.74	6.05 ^{a}
Organic acid	4.80	6.14 ^{b}	6.82	5.69 ^{b}
p-value	0.630	0.040	0.32	0.017
Pooled SEM	0.10	0.04	0.04	0.08

^{a,b} means within columns with different superscripts differ significantly at $p < 0.05$.

The total plasma corticosterone concentration and H/L ratios on D 35 are shown in Table 6. The total plasma corticosterone concentrations were lower ($p < 0.01$) in OA (22,423 ± 3971.49 pg/mL) and OO (21,269 ± 3454.76 pg/mL) compared to CON (57,787 ± 12,887.57 pg/mL). The H/L ratio was also smaller ($p < 0.03$) in OA (0.34 ± 0.03) and OO (0.33 ± 0.04) compared to CON (0.47 ± 0.05). The asymmetry scores on D 35 were lower ($p < 0.01$) for both the OA (1.99 ± 0.11 mm) and OO (1.98 ± 0.11 mm) treatments compared to CON (2.49 ± 0.13 mm).

Table 6. Total plasma corticosterone measurements (pg/mL), heterophil/lymphocyte ratios, and asymmetry scores of Pekin ducks supplemented with organic acid or oregano oil in the water for 35 days.

Treatment	D 35 Total Plasma Corticosterone (pg/mL)	D 35 H/L Ratios	D 35 Asymmetry
Control (1)	57,787 ^{a}	0.47 ^{a}	2.49 ^{a}
Organic acid (2)	22,423 ^{b}	0.34 ^{b}	1.99 ^{b}
Oregano oil (3)	21,269 ^{b}	0.33 ^{b}	1.98 ^{b}
p-value 1 vs. 2	0.01	0.02	0.005
p-value 1 vs. 3	0.01	0.03	0.004
Pooled SEM	5100.42	0.02	0.07

^{a,b} means within columns with different superscripts differ significantly at $p < 0.05$.

The footpad lesions, tibia breaking strength, and bone ash results are shown in Table 7. On D 35, the FPLs did not show differences ($p > 0.05$) between OA (0.11 ± 0.02), OO (0.08 ± 0.03), or CON (0.12 ± 0.03). The bone breaking strength differed ($p < 0.02$) between OA (27.47 ± 0.68 g) and CON (24.74 ± 0.85). Oregano oil (24.27 ± 0.79 g) did not differ ($p > 0.05$) from the control in the tibia breaking strength. The tibia ash content did not show differences ($p > 0.05$) between OA (57.51 ± 0.72%), OO (57.14 ± 0.60%), or CON (57.45 ± 0.64%).

Table 7. Footpad lesion scores, tibia breaking strength (kg), and tibia ash content (%) of Pekin duck supplemented with organic acid or oregano oil in the water for 35 days.

Treatment	D 35 Footpad Lesion Score	D 35 Tibia Breaking Strength (kg)	D 35 Tibia Ash Content (%)
Control (1)	0.12	24.74 ^{b}	57.46
Organic acid (2)	0.11	27.47 ^{a}	57.51
Oregano oil (3)	0.08	24.270	57.14
p-value 1 vs. 2	0.374	0.017	0.945
p-value 1 vs. 3	0.165	0.705	0.723
Pooled SEM	0.02	0.49	0.37

^{a,b} means within columns with different superscripts differ significantly at $p < 0.05$.

4. Discussion

There is limited research on the utilization of AAs in commercial Pekin duck production and their effects on duck health and welfare. The use of phytogenics and OAs as feed and water supplements has increased in poultry research in the last twenty years. Diet supplementation with phytogenic and OAs has shown similar benefits to using antibiotics, making them a possible alternative to growth promoters [2,3,7,8,15,16,18,21,30] and therapeutic treatments [5,6,13] in other poultry species. However, utilizing them as water supplements instead of in the feed of poultry remains limited, with even fewer studies investigating their use in Pekin duck production and welfare practices.

Organic acids and OOs have viable potential as AAs for poultry production and overall bird growth [21]. In the current study, the OA and OO ducks showed significant BW improvement compared to the CON ducks. Previous research in microencapsulated blends of OAs and EOs (thymol and vanillin) demonstrated increased cumulative BW in meat ducks [31]. Moreover, other studies have shown cumulative improvements in BW in other poultry when utilizing OAs [5,7,21] and OOs [16,30,32]. Organic acid and OO did not show ADFI differences compared to CON in the current study. Similarly, a study of OO supplements in ducks did not show differences in FI [18]. However, other studies have shown some reduction in FI for OO compared to CON for broilers [16] and ducks [18,31]. Therefore, FI may not be a consistently affected parameter of OO supplementation. It has been documented that the FCR significantly improves when diets are supplemented with either OOs or OAs in laying hens, broilers [2,7,8], and ducks [32]. The current study supports these findings as the overall FCR was improved in both the OO and OA treatments compared to CON. Previous research using OO showed a reduction in mortality rate with induced disease challenges [16] and overall mortality in diet supplementation studies [13]; however, the current study did not observe any difference in mortality between the treatments. The current study, along with previous research, demonstrates that OA and OO may both be used to improve performance and feed efficiency.

Overall gut health and nutrient absorption are thought to be related to the villus height and crypt depth of the intestinal lining. The villus height has been shown to increase in the ileum, duodenum, and jejunum following OA supplementation [7,11]. A few studies conducted in broilers [13,15] and Pekin ducks [30,32] have demonstrated that OO supplementation can improve villus height and crypt depth. In this current study, the villus height and V/C ratio were greater in the OA treatment ducks, while the OO treatment ducks had a lower villus height compared to those of the CON. The crypt depth showed a significant decrease for both OA and OO ducks compared to those of the CON. These results indicate that OA supplementation in ducks can improve gut health and likely nutrient absorption, though further research is needed to confirm this. The OO supplementation did not increase villi height but in fact lowered villus height compared to the CON; however, OO did reduce crypt depth, which is also thought to be related to gut health and, therefore, may still be a viable tool to improve gut health in ducks as well, but this definitely merits further investigation.

Recent studies have noted OAs lower pH when added for water supplementation in order to promote antimicrobial effects and intestinal benefits [5,11,21]. However, intestinal pH levels are rarely measured with the addition of OA supplementation as alterations to the lower digestive tract are not thought as probable. Therefore, the crop, proventriculus, gizzard, and ceca pH levels have been the main focal point in many studies, with no additional emphasis on the small intestine in poultry [5,7,21]. Analysis of pH levels in the GI tract has been performed in swine [33] and geese [34], though other poultry including turkeys and ducks have not undergone research. One study recorded OA water supplementation's effects in broiler chickens and included pH levels, though none were significant [35]. Therefore, the current study analyzed intestinal pH levels for sections of the proventriculus, jejunum, ileum, and ceca at D 35. Organic acid supplementation did not result in pH changes compared to the control in the proventriculus or ileum. However, the jejunum and ceca showed a reduction in pH levels compared to CON after OA supplementation. It is possible that the alteration of the lower gastrointestinal tract in the current study is related to the increased water consumption by ducks compared to chickens, which have mainly been investigated with OA supplementation. Antimicrobial and microbiota population effects in relation to pH reduction in the ceca have also been noted in broiler studies [7,12]. This may, therefore, make OA a very useful tool in the mitigation of harmful bacteria and further lead to improved overall health in commercial ducks.

Stress measures are typically measured through hormones or heterophil and lymphocyte immune cell analysis, as well as acute stress plasma corticosterone measurements [36,37]. Understanding bird stress aids animal welfare and poultry production to alleviate detrimental health problems or growth development [37]. For the current study, total plasma corticosterone measurements and H/L ratios on D 35 showed a significant decrease for both treatments compared to CON. A study conducted for OO supplementation on laying hens showed a similar reduction in total serum corticosterone levels [17]. Broilers fed OOs showed comparable results with reduced H/L ratios [16]. OA supplementation in broilers has also demonstrated a reduction in corticosterone concentrations in some studies [38], while in others, it has shown increased H/L ratios [39]. Therefore, it is inconclusive whether OA reduces stress susceptibility consistently; therefore, further research is needed. Asymmetry scores showed a significant reduction for both OO and OA compared to CON. Asymmetry is often used to demonstrate more long-term stress susceptibility. It is important to minimize chronic and acute Pekin duck stress, and nutritional supplementation may provide a method to illicit positive effects for flock health and animal well-being. Accordingly, more research is needed to conclude that it has positive effects on Pekin duck welfare. In both cases, OO and OA likely aid in stress susceptibility improvements via the improved gut health and immune function they often provide.

The current study observed no differences in FPLs. Thymol, the main ingredient of OO, has shown potential to have antimicrobial properties for litter, as well as reducing ammonia levels [40]. However, the results of this current study are similar to those found in others [40]. While some studies have seen improvements in FPLs with OA supplementation [41], others have observed similar results to this study [42]. Therefore, while in theory, OO and OA may help improve footpad health, this was not observed in this study.

Both OO and OA are thought to improve nutrient digestibility [43,44]. Bone health measurements provide further understanding of nutrient indicators for mineral absorption. Oregano oil supplementation in laying hens did not show differences with bone strength or ash content [17]. Similarly, the current study saw no improvement in bone quality with

OO supplementation. Bone quality characteristics with OA supplementation in another study utilizing broiler chickens showed no differences for bone breaking strength and bone ash mineral content [44,45]. In the current study, the results for bone breaking strength showed a significant increase for OA compared to the control, while the tibia ash content did not show differences between the control and either OA or OO. So, while both OO and OA improved gut health, it did not result in increased bone mineralization via improved nutrient absorption.

5. Conclusions

In this study, the use of two separate water supplementation products (OA and OO) was investigated to understand the usefulness in improving commercial Pekin duck production and animal well-being. This study found that both OA and OO improved Pekin duck production, growth, and gut health and reduced stress susceptibility. Both OA and OO improved Pekin duck performance by increasing BW and improving feed efficiency. Both OA and OO altered gut health, leading to possible improvements in nutrient absorption and pathogen resistance and thus providing an avenue to reinforce duck welfare and improve performance in commercial production without the use of antibiotics.

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