



Performance, Histology, and Meat Quality of Coccidiosis-Challenged Broilers Fed Essential Oils

Samantha K. Tauer¹, Jonathan P. Holt^{2,3}, Keith R. Underwood^{1*}, Crystal L. Levesque¹, and Robert C. Thaler¹

¹Department of Animal Science, South Dakota State University, Brookings, SD, 57007 USA

²Ralco Nutrition, Inc., Marshall, MN, 56258 USA

³Current: Department of Animal Science, North Carolina State University, Raleigh, NC, 27695 USA

*Corresponding author. Email: keith.underwood@sdstate.edu (K. R. Underwood)

Abstract: An experiment was conducted to determine the effects of Ralco's Microfused Essential Oils (MEO) on growth, jejunal histology, and meat quality of coccidiosis-challenged broilers. Day old broilers ($n = 768$; 64 pens; 12 broilers/pen) were randomly allotted to 1 of 5 dietary treatments fed in 4 phases: starter (d0–16), grower (d17–27), finisher (d28–34), and withdrawal (d35–41). Diets were a corn-soybean meal basal diet (CON), CON+BMD50/Coban90 at 55/121, 250/550, 250/495 and 0 mg/kg (ANTI), CON+MEO at 375, 250, 125, and 100 mg/kg (MEOD), CON+MEO at constant 500 mg/kg (MEO500), and CON+MEO at constant 250 mg/kg (MEO250). Broilers were offered a 10X dose of a coccidiosis vaccine on d 3, 15, 22, and 29. On d 14, jejunal histology was measured. Two broilers per pen were harvested on d 41 and breast pH was measured. Rancidity compounds (TBARS) were measured on whole breasts at d 7 postmortem and ground thighs on d 0, 3, 5, and 7 postmortem. Overall, MEO250-fed broilers had a tendency for greater gain over CON-fed (63.6 vs. 61.3 ± 0.87 g; $P < 0.08$). MEOD-fed broilers tended to have greater villus height compared to MEO500-fed (806.6 vs. 716.3 ± 27.7 μm ; $P = 0.09$). No differences were observed for pH or TBARS of breasts ($P > 0.05$). There were differences in L*, a*, and b* color values of breasts and there was an interaction for color values of thighs. ANTI, MEO500, and MEO250 had decreased TBARS values for thighs compared to CON at d 7 postmortem ($P \leq 0.05$). Overall, growth performance of MEO250 was not different than ANTI during peak challenge ($P > 0.05$). MEO250 could improve growth performance and meat quality when broilers are subjected to a coccidiosis disease challenge.

Key words: essential oils, broiler, growth, histology, meat quality

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Introduction

Consumer pressure for antibiotic-free meat products has led to increased research in the area of antibiotic alternatives, including essential oils. Essential oils have a wide variety of effects including antimicrobial, antioxidant, and digestive stimulant activities (Mathlouthi et al., 2012; Botsoglou et al., 2002; Platel and Srinivasan, 2004). Essential oils have been demonstrated to positively impact growth performance, gut health, and meat quality, but the

responses are inconsistent. The inconsistencies have been related to the species/subspecies of the plant, geographical location, harvest time, and plant part used that can affect the chemical composition of the oils (Brenes and Roura, 2010).

Microfused Essential Oils is a blend of oils, but is comprised mainly of oregano. The oils undergo a patented microfusion process that creates a surface area of oil droplets that is 20 times greater than other commercially available oils, increasing the stability and effectiveness of the oils. Due to its oregano content, MEO has a high antioxidant activity, which is attributed to its 2 main phenols, carvacrol and thymol (Economou et al., 1991; Yanishlieva and Marinova, 1995). Lipid oxidation affects meat quality by negatively impacting color, odor, flavor, and shelf-life (Maraschiello et al., 1998). Poultry

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meat is especially sensitive to lipid oxidation due to the high content of polyunsaturated fatty acids (Al-Hijazeen et al., 2016). Additionally, processes such as mincing and cooking can increase the oxidation potential of the meat (Tichivangana and Morrissey, 1985).

The objective of this experiment was to determine the effects of Ralco's Microfused Essential Oils (MEO) on growth performance, jejunal histology, and meat quality of coccidiosis-challenged broilers.

Materials and Methods

Experimental design, diets, and animal housing

Day old broiler cockerels ($n = 768 + 20$ for replacements) were transported from Cobb-Vantress Inc. in Siloam Springs, AR to Ralco's Poultry Research Facility in Lynd, MN. Chicks were left in shipping crates in the facility overnight with barn temperature maintained at 33°C. The following morning, chicks were wing-banded, weighed, and randomly allotted to treatment. Treatments consisted of 5 diets that were fed as follows: a corn-soybean meal basal diet (CON), the CON diet with BMD50/Coban90 added at 500/605, 250/550, 250/495 and 0 mg/kg in the starter, grower, finisher, and withdrawal phase, respectively (ANTI), CON+MEO at 375, 250, 125, and 100 mg/kg added in the starter grower, finisher, and withdrawal phase, respectively (MEOD), CON+MEO added at 500 mg/kg in all phases (MEO500), and CON+MEO added at 250 mg/kg in all phases (MEO250). The MEO was supplied by Ralco Nutrition, Inc. in Marshall, MN. All diets were fed in mash form in 4 phases: starter (d0 to 16), grower (d17 to 27), finisher (d28 to 34), and withdrawal (d35 to 41; Table 1). Samples of all experimental diets and the vitamin trace mineral premix were sent to Dairyland Laboratories, Inc. in Arcadia, WI for nutrient analysis.

The broilers ($n = 12$ per pen) were housed in 2 side-by-side battery brooders with four stacked rows and 32 pens ($0.91 \times 0.61 \times 0.61$ m) per brooder. There were 12 replications for the CON treatment and 13 replications for the remaining treatments. Any mortality that occurred within the first 5 d was replaced with extra chicks that had been fed the CON diet. Prior to the removal of one broiler per pen for histological assessment on d 14, feeder space was 7.6 cm per broiler and stocking density was 0.028 m² per broiler. After removal of one broiler, feeder space was 8.3 cm per broiler and stocking density was 0.031 m² per broiler. Each pen was equipped with 3 nipple waterers.

Table 1. Composition and formulated nutrients of basal broiler diets for starter, grower, finisher, and withdrawal phases¹

Ingredient/Nutrient %	Starter	Grower	Finisher	Withdrawal
Corn	59.0	65.1	68.8	70.2
Soybean Meal, 46%	36.6	30.3	25.4	24.6
Limestone	1.25	1.28	1.30	1.30
Soy Oil	0.50	1.00	1.25	1.75
Monocal. Phosphate	1.35	1.18	1.10	1.13
Sodium Bicarbonate	0.30	0.30	0.29	0.29
DL-Methionine	0.28	0.26	0.27	0.21
Salt	0.24	0.23	0.24	0.24
L-Lysine	0.17	0.17	0.16	0.14
Choline 60 (dry)	0.08	0.07	0.06	0.06
L-Threonine	0.04	0.03	0.05	0.05
VTM Premix ²	0.20	0.17	0.14	0.14
Phytase	0.01	0.01	0.01	0.01
Total	100	100	100	100
Crude Protein	21.3	18.8	16.9	16.5
Net Energy (Kcal/kg)	2222	2317	2383	2410
Lysine	1.352	1.182	1.044	1.004
Calcium	0.90	0.85	0.83	0.83
Total Phosphorus	0.69	0.63	0.59	0.59

¹Broilers were fed starter from d 0 to 16, grower from d 17 to 27, finisher from d 28 to 34, and withdrawal from d 35 to 41.

²Vitamin/trace mineral premix was formulated to contain the following: 42,047 KIU/kg vitamin A; 8,190 KIU/kg vitamin D; 275, 579 IU/kg vitamin E; 24, 683 mg/kg vitamin K; 10,441 mg/kg biotin; 7,976 mg/kg folic acid; 195,778 mg/kg niacin; 130,827 mg/kg D-pantothenic acid; 40,281 mg/kg riboflavin; 6,614 mg/kg thiamine; 6,612 mg/kg vitamin B₆; 198.4 mg/kg vitamin B₁₂; 1,667,000 mg/kg manganese; and 9,900 mg/kg selenium.

Coccidiosis challenge

A coccidiosis challenge was induced to stimulate the conditions of a disease stress. Broilers, including replacements on d 3, were offered a commercial coccidiosis vaccine containing viable oocysts of *E. acervulina*, *E. maxima*, and *E. tenella* at 10 times the dose on top of the feed in a gel carrier dyed green on d 3, 15, 22, and 29 to ensure a challenge was maintained. To determine when peak infection occurred, fecal samples were collected on d 6, 8, 13, 15, 20, 22, 28, 34, and 36 from each pen and pooled by treatment. Samples were immediately placed on ice and sent to Best Veterinary Solutions, Inc. in Willmar, MN for analysis of oocyst counts.

Growth performance

Individual bird weights were taken on d 0, 15, 26, 35, and 41 and a pen mean was calculated for statistical analysis. Pen feed disappearance was measured on d 15, 26, 35, and 42 at the end of each feeding phase. All feeders were emptied on the previously mentioned

days and the new diet phase was added. Pens were checked daily for mortality or those needing to be euthanized due to leg or health problems. Any broilers that showed signs of disease and did not maintain or improve health over time were euthanized.

Jejunal histology

On d 14, a randomly selected broiler from each pen was removed and euthanized using a CO₂ chamber. A 5 cm section of the jejunum, beginning 5 cm proximal to Meckel's diverticulum, was collected and placed into 10% formalin for histological assessment. Slides were prepared and stained with hematoxylin and eosin at a commercial pathology diagnostics lab (Animal Disease Research and Diagnostic Laboratory, Brookings, SD). Villus height and crypt depth was measured using a Nikon microscope (Tokyo, Japan) equipped with a DS2MV Nikon camera (Tokyo, Japan) and NIS Elements software (Tokyo, Japan). Due to an unexpected amount of damaged villi, measurements were taken on all viable villus and the crypt associated with each villi. The villus height:crypt depth ratio was calculated.

Carcass fabrication and color

On d 41, two broilers were randomly selected from each pen, leg banded, and transported to a small harvest facility in Pipestone, MN for harvest the following morning. Seventeen hours after removal from pens, broilers were stunned, butchered, eviscerated, and chilled in water. Individually bagged whole chickens were transported to the meat laboratory at South Dakota State University, Brookings, SD in a refrigerated trailer. The carcasses were placed in a cooler (1 to 3°C) overnight and fabricated the following morning. Breasts were removed, deskinning, deboned, and placed on a foam tray and overwrapped. Thigh meat was deskinning, deboned, and ground twice with a 3.18 mm die. Two pens (four carcasses) were pooled and utilized as one replication for color analysis and shelf life of thigh meat. The grinder was rinsed between replications. Each replication was split into four 113.4 g patties, placed on a black polystyrene foam tray, overwrapped with oxygen permeable polyvinyl chloride (15,500 – 16,275 cm³/m²/24 h oxygen transmission rate), and labeled as d 1, 3, 5, or 7. Following tray overwrapping, both breasts and thighs were placed with one sample of each treatment per column on tables in an illuminated cooler using dual fluorescent lighting (2,950 lumens/bulb, at 4,100 K, and 40 inches from the

samples) at 4°C and rotated from front to back daily. Measurement of color using a Minolta colorimeter (CR-410; Minolta Corp., Ramsey, NJ; equipped with a 50 mm diameter measuring space, D65 illuminant, 0° viewing angle, and 2° observer) was taken on both breasts and thighs on d 0, 1, 2, 3, 4, 5, 6, and 7.

Lipid oxidation

Rancidity compounds were measured using 2-thiobarbituric reactive substances (TBARS) on 2 broilers per pen on whole breasts at d 7 postmortem, and on ground thigh meat on d 0, 3, 5, and 7 postmortem. Thighs were removed from the refrigerator, vacuum sealed, and frozen (–80°C) as labeled on d 1, 3, 5, or 7. Breasts were removed, vacuum sealed, and frozen (–80°C) on d 7. To create a homogenous sample, breasts and thighs were powdered by placing small cubes of meat into liquid nitrogen. Once frozen, the cubes were placed into a chilled Waring blender and blended until it was powdered. The powder was then placed into a Whirl-Pak bag, vacuum sealed, and stored at –80°C. All samples for TBARS were run in duplicate and one spiked sample using TEP (97% 1,1,3,3, tetraethoxypropane, Sigma-Aldrich T9889) was used per plate to determine percent recovery. For each sample, 1 mL of 0.2 mg/mL butylated hydroxyl toluene (BHT; MP Biomedicals, LLC, cat# 101162) and 45.5 mL 10% trichloroacetic acid (TCA) were added to 5 g of sample. TCA was prepared using 96.26 g of o-phosphoric acid (Acros Organics, 389025000) and 400 g of trichloroacetic acid (Fischer Scientific, A322–500) into a total volume of 4,000 mL with double distilled water. The spiked sample was prepared by weighing 5 g of sample, adding 1 mL BHT, 35.5 mL TCA, and 10 mL of 10 µM TEP. Each sample was homogenized using an IKA T25 Digital Ultra-Turrax (IKA Works, Inc., Wilmington, NC) for 1 min and filtered through Whatman No1 filter paper into a 100 mL glass sample bottle. A clean culture tube was then used to mix 5 mL of filtrate with 5 mL of 0.02 M thiobarbituric acid (TBA; 2-thiobarbituric acid, Sigma Aldrich T5500). A standard curve was also prepared using 25 µM TEP, TCA, and TBA. The tubes were inverted 5 times and placed in a shaker at room temperature for 15 to 20 h and then plated (250 µL) in duplicate and read using a plate reader (Molecular Devices SpectraMax 190, Molecular Devices, Sunnyvale, CA) at 530 nm (Witte et al., 1970). The procedure used by Tarladgis et al. (1960) was used for calculation of malondialdehyde (MDA) content using the percent recovery and absorbance values obtained.

Table 2. Broiler performance during the starter, grower, finisher, and withdrawal phases¹

Item	CON	ANTI	MEOD	MEO500	MEO250	Pooled SEM	<i>P</i> -value
Weight (g)							
Day 0	38.4	38.3	38.3	38.2	38.2	0.30	0.99
Day 17	494 ^b	543 ^a	525 ^{a,b}	521 ^{a,b}	545 ^a	8.40	0.0003
Day 28	1400 ^b	1486 ^a	1441 ^{a,b}	1440 ^{a,b}	1476 ^a	18.74	0.001
Day 35	1966 ^b	2071 ^a	2023 ^{a,b}	2011 ^{a,b}	2055 ^a	19.75	0.003
Day 41	2552 ^y	2617 ^{x,y}	2591 ^{x,y}	2572 ^{x,y}	2646 ^x	26.05	0.08
Starter							
ADG ² (g)	26.8 ^b	29.7 ^a	28.6 ^{a,b}	28.4 ^{a,b}	29.8 ^a	0.50	0.0003
ADFI ³ (g)	38.3	38.7	39.3	38.2	39.5	0.69	0.34
G:F ⁴	0.71 ^c	0.77 ^a	0.73 ^{b,c}	0.75 ^{a,b}	0.77 ^{a,b}	0.01	0.0002
Grower							
ADG (g)	82.0 ^b	85.4 ^a	83.0 ^{a,b}	83.2 ^{a,b}	84.3 ^{a,b}	0.99	0.04
ADFI (g)	120 ^y	124 ^{x,y}	123 ^{x,y}	123 ^{x,y}	125 ^x	1.65	0.09
G:F	0.68 ^{a,b}	0.69 ^a	0.67 ^{a,b}	0.67 ^{a,b}	0.67 ^b	0.005	0.01
Finisher							
ADG (g)	82.0	84.8	84.3	82.8	83.9	1.81	0.66
ADFI (g)	165	170	174	169	178	3.86	0.14
G:F	0.50	0.50	0.49	0.49	0.47	0.01	0.33
Withdrawal							
ADG (g)	96.7	90.4	94.9	93.4	97.3	2.41	0.20
ADFI (g)	179	180	179	183	183	2.87	0.74
G:F	0.54	0.51	0.54	0.51	0.54	0.009	0.02

^{a,b}Values with different superscripts indicate a significant difference within rows ($P \leq 0.05$).

^{x,y}Values with different superscripts indicate a tendency to be significantly different within rows ($P \leq 0.1$).

¹Experimental diets were fed in the starter phase as follows: corn-soybean meal basal diet (CON), CON diet with BMD50/Coban90 added at 55/121 mg/kg (ANTI), CON+MEO added at 375 mg/kg (MEOD), CON+MEO added at 500 mg/kg (MEO500), and CON+MEO added at 250 mg/kg (MEO250). MEO = Microfused Essential Oils (Ralco Nutrition, Inc, Marshall, MN). Broilers were fed starter from d 0 to 16, grower from d 17 to 27, finisher from d 28 to 34, and withdrawal from d 35 to 41.

²ADG = Average daily gain.

³ADFI = Average daily feed intake.

⁴G:F = Gain to feed.

pH

Two frozen, powdered, homogenous breast samples from each pen were used to determine pH. A volume of 90 mL of distilled water was added to 10 g of sample. The solution was then homogenized and read for pH, where pH 7 solution (Fischer Scientific, SB107–500) was used for a standard. The pH values were obtained using a Thermo Scientific Orion 370 Advanced PerpHecT LogR Meter (Chelmsford, MA). Samples were averaged for each pen and pen was considered the experimental unit.

Statistical analysis

All statistical analysis was performed using the PROC MIXED procedure of SAS (Version 9.3, SAS Inst. Inc., Cary, NC). A completely randomized design was used with pen as the experimental unit for the performance and meat quality and bird was used as the

experimental unit for histology. The control treatment had 12 replications for all results, except $n = 5$ for thigh TBARS and color. All remaining treatments had 13 replications for all results, except $n = 6$ for thigh TBARS and color. Repeated measures analysis was used for color and thigh TBARS and Tukey's adjustment for means separation was used where main effect of treatment was significant. Gain to feed (G:F) was calculated by dividing average daily gain (ADG) per pen by the average daily feed intake (ADFI) per pen. Differences were considered significant when the P -value ≤ 0.05 and a tendency for significance when the P -value ≤ 0.10 .

Results

In-feed antimicrobials were intended to be included at manufacturers recommended levels; however, due to a mixing error, the levels of both antimicrobi-

Table 3. Broiler growth performance for the entire experimental feeding period (d 0 to 41)¹

Item	CON	ANTI	MEOD	MEO500	MEO250	Pooled SEM	P-value
ADG ² (g)	61.3 ^y	62.9 ^{x,y}	62.2 ^{x,y}	61.8 ^{x,y}	63.6 ^x	0.63	0.08
ADFI ³ (g)	98.9	102	102	101	103	1.33	0.13
G:F ⁴	0.62	0.62	0.61	0.61	0.61	0.007	0.67

^{x,y}Values with different superscripts indicate a tendency to be significantly different within rows ($P \leq 0.1$).

¹Experimental diets were fed in the starter phase as follows: corn-soybean meal basal diet (CON), CON diet with BMD50/Coban90 added at 55/121 mg/kg (ANTI), CON+MEO added at 375 mg/kg (MEOD), CON+MEO added at 500 mg/kg (MEO500), and CON+MEO added at 250 mg/kg (MEO250). MEO = Microfused Essential Oils (Ralco Nutrition, Inc, Marshall, MN). Broilers were fed starter from d 0 to 16, grower from d 17 to 27, finisher from d 28 to 34, and withdrawal from d 35 to 41.

²ADG = Average daily gain.

³ADFI = Average daily feed intake.

⁴G:F = Gain to feed.

als in the ANTI diet in the starter phase were approximately 10% of the levels indicated in the materials and methods. During transport to the harvest facility, one broiler fed the CON diet died, so no meat quality data was collected from that broiler.

Growth performance

Growth performance results were divided by phases as follows: starter, grower, finisher, withdrawal, and overall. The ANTI and MEO250 supplemented broilers were heavier than CON at d 17, 28, and 35, respectively ($P < 0.02$; Table 2). On d 41, MEO250 broilers had a tendency for increased weight over the CON ($P < 0.08$; Table 2). The ANTI and MEO250 broilers had increased daily gain compared to CON in the starter phase ($P < 0.01$; Table 2) and ANTI had significantly greater daily gain compared to CON ($P < 0.03$) in the grower phase (Table 2). Overall, ME250 broilers had a tendency for greater daily gain compared to CON ($P < 0.08$; Table 3). There were no differences in ADFI, except in the grower phase, where MEO250 broilers had a tendency for increased intake over CON ($P < 0.06$; Table 2). There were differences among treatments in the feed conversion ratio (FCR) in both the starter and

grower phase (Table 2). In the starter phase, ANTI, MEO500 and MEO250 broilers had increased G:F over CON ($P < 0.04$; Table 2), while ANTI-fed broilers had increased G:F over MEO250 in the grower phase ($P < 0.02$; Table 2). For performance parameters where treatments were different ($P \geq 0.05$) MEOD and MEO500 performed intermediate to MEO250, ANTI, and CON.

Jejunal histology

Jejunal collections were performed on Day 14 and peak infection appeared to have occurred on d 15 according to both the pooled oocyst counts and visual signs of lethargy, fever, and weakness. Pooled fecal oocyst counts were 0; 14,053; 13,467; 1052,533; 5227; 1734; 133; 0 and 0 on d 6, 8, 13, 15, 20, 22, 28, 34, and 36, respectively. The MEOD-fed broilers had a tendency for higher villus height than MEO500 ($P = 0.09$) and there were no differences in crypt depth or villus height to crypt depth (VH:CD) ratio among treatments (Table 4).

Meat quality

To determine meat quality from broilers, pH, color, and lipid oxidation were measured. There were no dif-

Table 4. Measurements of villus height, crypt depth, and VH:CD¹ ratio of the jejunum from broilers collected on d 142

Item	CON	ANTI	MEOD	MEO500	MEO250	Pooled SEM	P-value
Villus Height (μm)	774.4 ^{x,y}	752.1 ^{x,y}	806.6 ^x	716.4 ^y	800.6 ^{x,y}	27.7	0.09
Crypt Depth (μm)	103.0	100.7	111.8	108.7	103.4	4.9	0.42
VH:CD ²	8.01	7.93	7.95	7.07	8.27	0.36	0.12

^{x,y}Values with different superscripts indicate a tendency to be significantly different within rows ($P \leq 0.1$).

¹Experimental diets were fed in the starter phase as follows: corn-soybean meal basal diet (CON), CON diet with BMD50/Coban90 added at 55/121 mg/kg (ANTI), CON+MEO added at 375 mg/kg (MEOD), CON+MEO added at 500 mg/kg (MEO500), and CON+MEO added at 250 mg/kg (MEO250). MEO = Microfused Essential Oils (Ralco Nutrition, Inc, Marshall, MN).

²VH = villus height; CD = crypt depth.

Table 5. pH, L*, a*, and b* color values of raw whole chicken breasts during retail display for 7 d¹

Item	CON	ANTI	MEOD	MEO500	MEO250	Pooled SEM	P-value
pH	6.08	6.04	6.04	6.07	6.08	0.027	0.54
L*	60.17 ^c	61.97 ^a	59.96 ^c	61.32 ^b	61.72 ^{a,b}	0.12	< 0.0001
a*	9.97 ^{b,c}	9.19 ^d	10.44 ^a	10.09 ^c	9.67 ^b	0.079	< 0.0001
b*	12.60 ^a	11.82 ^{c,d}	12.23 ^b	12.10 ^{c,b}	11.74 ^d	0.094	< 0.0001

^{a-d}Values with different superscripts indicate a significant difference within rows ($P \leq 0.05$).

¹Experimental diets were fed in the starter phase as follows: corn-soybean meal basal diet (CON), CON diet with BMD50/Coban90 added at 55/121 mg/kg (ANTI), CON+MEO added at 375 mg/kg (MEOD), CON+MEO added at 500 mg/kg (MEO500), and CON+MEO added at 250 mg/kg (MEO250). MEO = Microfused Essential Oils (Ralco Nutrition, Inc, Marshall, MN). Broilers were fed starter from d 0 to 16, grower from d 17 to 27, finisher from d 28 to 34, and withdrawal from d 35 to 41.

ferences in pH among treatments for breasts at d 7 of retail display (Table 5). There were no treatment by day interactions for breast meat color. Therefore, main effects of treatment and day are reported in Table 5, Fig. 1, Fig. 2, and Fig. 3. For analysis of color, there were differences observed for L* (lightness), a* (redness), and b* (yellowness) of breasts. The CON and MEOD treatments exhibited a lower L* value (overall average across all 7 d) compared to ANTI, MEO500, and MEO250 for breasts, while the ANTI group had a higher L* value over CON, MEOD, and MEO500 ($P < 0.0008$; Table 5). The value for L* of breasts did not change from d 0 to 1, but decreased from d 1 to 7, with the exception of increases in L* value on d 4 and 6 ($P < 0.04$; Fig. 1). During the 7-d retail display period, the ANTI treatment exhibited a lower a* value compared to other treatments, while MEOD exhibited a higher a* value compared to CON, ANTI,

and MEO250 ($P < 0.02$; Table 5). Breast a* values increased from d 0 to 1, plateaued from d 1 to 4, decreased from d 4 to 5, and then plateaued from d 5 to 7 ($P < 0.03$; Fig. 2). As an average value over all 7 d, the ANTI and MEO250 treatments exhibited the lowest b* value, the CON treatment exhibited the highest b* value, and MEOD and MEO500 were intermediate ($P < 0.05$; Table 5). The b* value increased from d 0 to 4 and then plateaued from d 4 to 7 ($P < 0.0001$; Fig. 3). Overall, breast meat decreased in lightness, decreased in redness after d 3, and increased in yellowness.

For thigh color, there was an interaction of treatment*day for L*, a*, and b*. The 2 treatments that did not change in L* value over time were MEOD and MEO250. The CON and ANTI treatments performed similarly in their L* values over time. Both treatments did not change from d 0 to 1, increased from d 1 to 3, and plateaued from d 3 to 7 ($P \leq 0.007$;

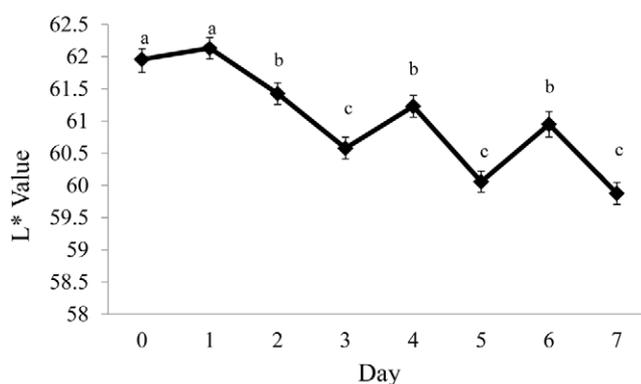


Figure 1. L* color value of raw whole chicken breast during a 7 d retail display for broilers fed corn-soybean meal basal diet (CON), CON diet with BMD50/Coban90 added at 55/121 mg/kg (ANTI), CON+MEO added at 375 mg/kg (MEOD), CON+MEO added at 500 mg/kg (MEO500), and CON+MEO added at 250 mg/kg (MEO250). MEO = Microfused Essential Oils (Ralco Nutrition, Inc, Marshall, MN). Broilers were fed starter from d 0 to 16, grower from d 17 to 27, finisher from d 28 to 34, and withdrawal from d 35 to 41. ^{a-c} Values with different superscripts indicate a significant difference ($P \leq 0.05$).

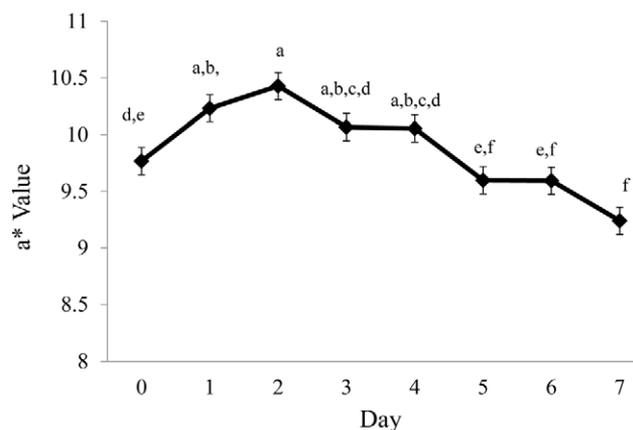


Figure 2. a* color values of raw whole chicken breasts during a 7 d retail display for broilers fed corn-soybean meal basal diet (CON), CON diet with BMD50/Coban90 added at 55/121 mg/kg (ANTI), CON+MEO added at 375 mg/kg (MEOD), CON+MEO added at 500 mg/kg (MEO500), and CON+MEO added at 250 mg/kg (MEO250). MEO = Microfused Essential Oils (Ralco Nutrition, Inc, Marshall, MN). Broilers were fed starter from d 0 to 16, grower from d 17 to 27, finisher from d 28 to 34, and withdrawal from d 35 to 41. ^{a-f} Values with different superscripts indicate a significant difference ($P \leq 0.05$).

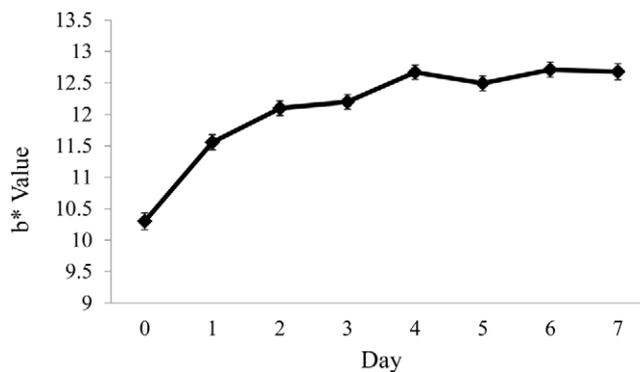


Figure 3. b^* color values of raw whole chicken breasts during a 7 d retail display for broilers fed corn-soybean meal basal diet (CON), CON diet with BMD50/Coban90 added at 55/121 mg/kg (ANTI), CON+MEO added at 375 mg/kg (MEOD), CON+MEO added at 500 mg/kg (MEO500), and CON+MEO added at 250 mg/kg (MEO250). MEO = Microfused Essential Oils (Ralco Nutrition, Inc, Marshall, MN). Broilers were fed starter from d 0 to 16, grower from d 17 to 27, finisher from d 28 to 34, and withdrawal from d 35 to 41. ^{a-f} Values with different superscripts indicate a significant difference ($P \leq 0.05$).

Fig. 4). MEO500 did not change in L^* value from d 0 to 1, increased from d 1 to 2, plateaued again from d 2 to 3, decreased from d 3 to 4, and then plateaued again from d 4 to 7 ($P \leq 0.05$). All treatments decreased in a^* value from d 0 to 1 and increased from d 1 to 3 ($P < 0.02$; Fig. 5). Following d 3, ANTI, MEO500, and MEO250 performed similarly, plateauing from d 3 to 7, while CON and MEOD a^* value plateaued from d 3 to 6 and then decreased from d 6 to 7 ($P < 0.03$). For b^* of thighs, all treatments increased from d 0 to 2 ($P \leq 0.0001$; Fig. 6). The CON, MEOD, and MEO500 treatments plateaued in b^* value from d 2 to 7, while the ANTI and MEO250 treatments plateaued from d 2 to 6 and then decreased from d 6 to 7 ($P < 0.0001$).

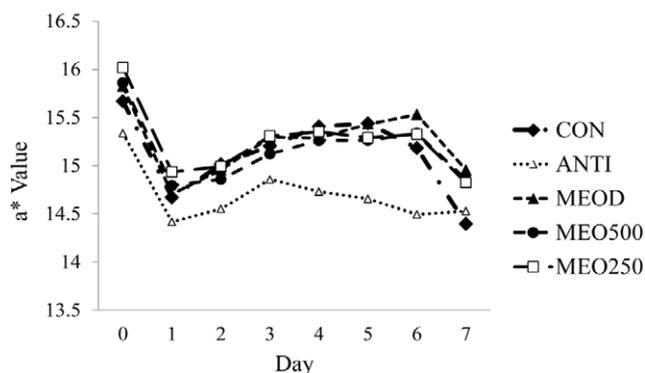


Figure 5. a^* color values of raw ground chicken thighs during a 7 d retail display for broilers fed corn-soybean meal basal diet (CON), CON diet with BMD50/Coban90 added at 55/121 mg/kg (ANTI), CON+MEO added at 375 mg/kg (MEOD), CON+MEO added at 500 mg/kg (MEO500), and CON+MEO added at 250 mg/kg (MEO250). MEO = Microfused Essential Oils (Ralco Nutrition, Inc, Marshall, MN). Broilers were fed starter from d 0 to 16, grower from d 17 to 27, finisher from d 28 to 34, and withdrawal from d 35 to 41. (SEM = 0.29; Treatment \times d $P = 0.002$).



Figure 4. L^* color values of raw ground chicken thighs during a 7 d retail display for broilers fed corn-soybean meal basal diet (CON), CON diet with BMD50/Coban90 added at 55/121 mg/kg (ANTI), CON+MEO added at 375 mg/kg (MEOD), CON+MEO added at 500 mg/kg (MEO500), and CON+MEO added at 250 mg/kg (MEO250). MEO = Microfused Essential Oils (Ralco Nutrition, Inc, Marshall, MN). Broilers were fed starter from d 0 to 16, grower from d 17 to 27, finisher from d 28 to 34, and withdrawal from d 35 to 41. (SEM = 0.52; Treatment \times d $P = 0.046$).

Differences among treatments were also noted for TBARS of thighs, but not breasts (Fig. 7). All treatments had decreased levels of MDA per kg of wet tissue for ground thighs compared to CON on d 5 ($P \leq 0.05$). The ANTI, MEO500, and MEO250 treatments had decreased levels of MDA per kg of wet tissue for ground thighs compared to CON on d 7 ($P \leq 0.05$) indicating decreased levels of lipid oxidation.

Discussion

The objective of this experiment was to determine the effects of MEO on growth performance, jejunal histology, and meat quality of coccidiosis-challenged broilers. Furthermore, it was of interest to determine if MEO could

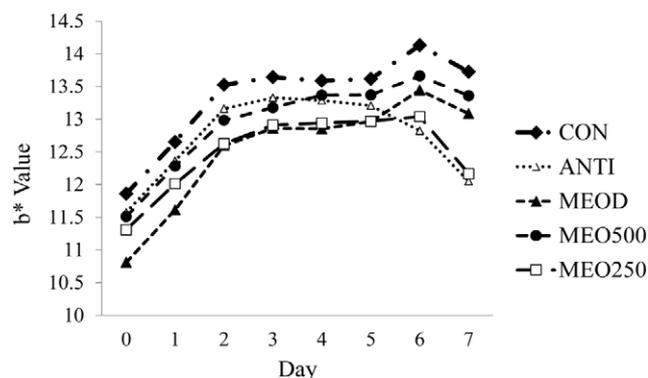


Figure 6. b^* color values of raw ground chicken thighs during a 7 d retail display for broilers fed corn-soybean meal basal diet (CON), CON diet with BMD50/Coban90 added at 55/121 mg/kg (ANTI), CON+MEO added at 375 mg/kg (MEOD), CON+MEO added at 500 mg/kg (MEO500), and CON+MEO added at 250 mg/kg (MEO250). MEO = Microfused Essential Oils (Ralco Nutrition, Inc, Marshall, MN). Broilers were fed starter from d 0 to 16, grower from d 17 to 27, finisher from d 28 to 34, and withdrawal from d 35 to 41. (SEM = 0.29; Treatment \times d $P = 0.0006$).

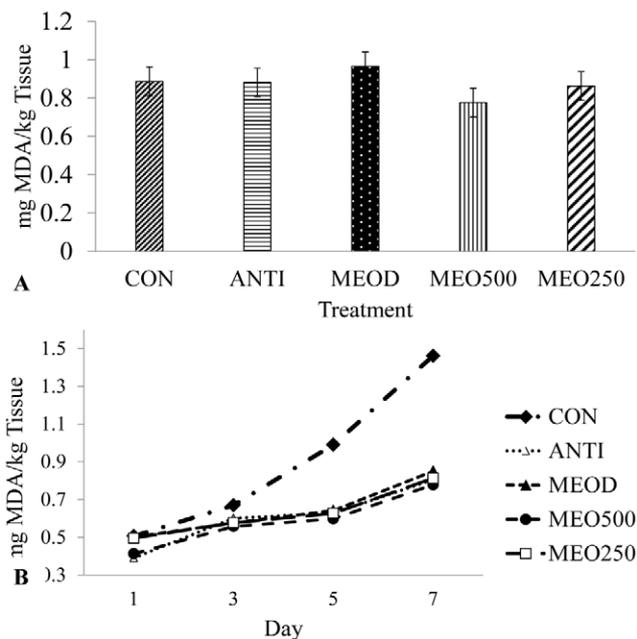


Figure 7. Malondialdehyde levels (MDA) per kg of wet tissue of raw chicken breasts (A; SEM = 0.08, $P > 0.05$) and raw ground chicken thighs (B; SEM = 0.06, Treatment*Day $P = 0.01$) during a 7 d retail display for broilers fed corn-soybean meal basal diet (CON), CON diet with BMD50/Coban90 added at 55/121 mg/kg (ANTI), CON+MEO added at 375 mg/kg (MEOD), CON+MEO added at 500 mg/kg (MEO500), and CON+MEO added at 250 mg/kg (MEO250). MEO = Microfused Essential Oils (Ralco Nutrition, Inc, Marshall, MN). Broilers were fed starter from d 0 to 16, grower from d 17 to 27, finisher from d 28 to 34, and withdrawal from d 35 to 41.

be a suitable replacement for antimicrobials in commercial poultry facilities. If the antimicrobials would have been included at the appropriate inclusion rate in the starter, it would have been expected for the ANTI-fed broilers to have better performance than what was observed.

Based on performance measures, it is evident that MEO fed at a constant 250 mg/kg was the most effective method of combatting the negative effects of the coccidiosis challenge. One reason for these results could be that MEO at 500 mg/kg is too high, especially in the starter phase. Once the normal microbiota composition is altered, pathogens may be able to proliferate in the small intestine (Li et al., 2017). Therefore, with the strong antimicrobial potential of MEO, it is possible that MEO at 500 mg/kg negatively impacts the normal microflora of the gut. However, it can be noted that neither level of MEO nor the ANTI treatment significantly impacted ADFI. The effects of MEO250 and ANTI treatments were most apparent during the starter and grower phases, and the differences started to diminish as the broilers started gaining immunity to the coccidiosis. However, the effects of these treatments on broiler weight were still present during the finisher and withdrawal phases. The ANTI-

and MEO250-fed broilers had higher weights over CON-fed broilers at the end of the starter, grower, and finisher, but only the MEO250-fed broilers had a tendency for increased weight over CON-fed broilers at the end of the withdrawal phase. More than likely, this is due to the fact that the antimicrobials were removed from the ANTI treatment in the withdrawal phase, so the increase in weight advantage was not maintained.

While there were positive effects on growth performance of MEO250-fed broilers, there was no effect of this treatment on jejunal histology. However, MEOD had a tendency for increased villus height over MEO500. Increased villus height can serve as an indicator of increased surface area for nutrient absorption (Heak et al., 2017). Although MEO was fed at diminishing levels for the MEOD treatment, the only level the broilers received prior to histological assessment was 375 mg/kg.

The beneficial effects of MEO fed at 250 mg/kg were also shown in the meat quality data. There were no differences among treatments for pH of breasts, which suggests that no treatment has an impact on this meat quality measure. This is consistent with research performed by Simitzis et al. (2010) and Young et al. (2003), which found no impact on the pH of meat from finishing pigs fed oregano essential oil. In regards to color, the L^* value of breasts decreased over time. Al-Hijazeen et al. (2016) also found that broilers fed oregano essential oil had decreased L^* values for breast meat over time. The a^* value of breasts peaked on d 2 and then decreased until d 7 and the b^* value increased from d 0 to 7. However, Al-Hijazeen et al. (2016) found that both the a^* and b^* value decreased from d 0 to 7. Moreover, the potentially lower fat content of the breasts as compared to the thighs could be the reason there were no differences in the level of lipid oxidation among treatments for breasts, but there were differences in lipid oxidation of ground thighs. Not only is breast meat lower in fat content, but the breasts were analyzed whole and the thighs were ground (Kirkpinar et al., 2014). Processing such as mincing, grinding, and cooking of the meat will increase the lipid oxidation potential of the meat (Tichivangana and Morrissey, 1985). Furthermore, it was not expected for the antimicrobial treatment to decrease the level of lipid oxidation in the meat to the same extent as the essential oil treatments. However, research performed by Knarreborg et al. (2004) found that supplementation of antibiotics (salinomycin, 40 mg/kg feed and avilamycin, 10 mg/kg feed) in broiler diets results in significantly increased plasma concentration of α -tocopherol, which is an antioxidant.

The objectives of the experiment were met and effects of MEO were quantified. Overall, MEO- and MEO500-fed broilers performed intermediate to CON- and ANTI-fed broilers and MEO250-fed broilers performed similarly to ANTI-fed broilers. Based on this study, it appears that MEO250 is the optimal level to feed MEO. Microfused Essential Oils fed at 250 mg/kg has the potential to improve growth performance when broilers are experiencing a coccidiosis disease challenge and decrease lipid oxidation of ground thigh meat during illuminated storage.

Literature Cited

- Al-Hijazeen, M., E. Lee, A. Mendonca, and D. Ahn. 2016. Effect of oregano essential oil (*Origanum vulgare* subsp. *hirtum*) on the storage stability and quality parameters of ground chicken breast meat. *Antioxidants* 5:18. doi:10.3390/antiox5020018
- Botsoglou, N., E. Christaki, D. Fletouris, P. Florou-Paneri, and A. Spais. 2002. The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. *Meat Sci.* 62:259–265. doi:10.1016/S0309-1740(01)00256-X
- Brenes, A., and E. Roura. 2010. Essential oils in poultry nutrition: Main effects and modes of action. *Anim. Feed Sci. Technol.* 158:1–14. doi:10.1016/j.anifeedsci.2010.03.007
- Economou, K. D., V. Oreopoulou, and C. D. Thomopoulos. 1991. Antioxidant properties of some plant extracts of the Labiatae family. *J. Am. Oil Chem. Soc.* 68:109–113. doi:10.1007/BF02662329
- Heak, C., P. Sukon, S. Kongpechr, B. Tengjaroenkul, and K. Chuachan. 2017. Effect of direct-fed microbials on intestinal villus height in broiler chickens: A systematic review and meta-analysis of controlled trials. *Int. J. Poult. Sci.* 16:403–414. doi:10.3923/ijps.2017.403.414
- Kirkpinar, F., H. Ünlü, M. Serdaroglu, and G. Turp. 2014. Effects of dietary oregano and garlic essential oils on carcass characteristics, meat composition, colour, pH and sensory quality of broiler meat. *Br. Poult. Sci.* 55:157–166. doi:10.1080/00071668.2013.879980
- Knarreborg, A., C. Lauridsen, R. M. Engberg, and S. K. Jensen. 2004. Dietary antibiotic growth promoters enhance the bioavailability of α -tocopheryl acetate in broilers by altering lipid absorption. *J. Nutr.* 134:1487–1492. doi:10.1093/jn/134.6.1487
- Li, J., H. Hao, G. Chang, C. Liu, S. Ahmed, M. A. B. Shabbir, H. I. Hussain, M. Dai, and Z. Yuan. 2017. Microbial shifts in the intestinal microbiota of salmonella infected chickens in response to enrofloxacin. *Front. Microbiol.* 8:1711. doi:10.3389/fmicb.2017.01711
- Mathlouthi, N., T. Bouzaienne, I. Oueslati, F. Recoquillay, M. Hamdi, M. Urdaci, and R. Bergaoui. 2012. Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: In vitro antimicrobial activities and effects on growth performance. *J. Anim. Sci.* 90:813–823. doi:10.2527/jas.2010-3646
- Maraschiello, C., E. Esteve, and J. A. Garcia-Regueiro. 1998. Cholesterol oxidation in meat from chicken fed α -tocopherol and beta-carotene-supplemented diets with different unsaturation grades. *Lipids* 33:705–713. doi:10.1007/s11745-998-0260-4
- Platel, K., and K. Srinivasan. 2004. Digestive stimulant action of spices: A myth or reality? *Indian J. Med. Res.* 119:167–179.
- Simitzis, P., G. Symeon, M. Charismiadou, J. Bizelis, and S. Deligeorgis. 2010. The effects of dietary oregano oil supplementation on pig meat characteristics. *Meat Sci.* 84:670–676. doi:10.1016/j.meatsci.2009.11.001
- Tarladgis, B. G., B. M. Watts, M. T. Younathan, and L. R. Dugan, Jr. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid flavor. *J. Am. Oil Chem. Soc.* 37:44–48. doi:10.1007/BF02630824
- Tichivangana, J. Z., and P. A. Morrissey. 1985. Metmyoglobin and inorganic metals as prooxidants in raw and cooked muscle systems. *Meat Sci.* 15:107–116. doi:10.1016/0309-1740(85)90051-8
- Witte, V. C., G. F. Krause, and M. E. Bailey. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. *J. Food Sci.* 35:582–585. doi:10.1111/j.1365-2621.1970.tb04815.x
- Yanishlieva, V., and M. Marinova. 1995. Antioxidant activity of selected species of the family *Lamiaceae* grown in Bulgaria. *Nahrung* 39:458–464. doi:10.1002/food.19950390510
- Young, J., J. Stagsted, S. Jensen, A. Karlsson, and P. Henckel. 2003. Ascorbic acid, α -tocopherol, and oregano supplements reduce stress-induced deterioration of chicken meat quality. *Poult. Sci.* 82(8):1343–1351. doi:10.1093/ps/82.8.1343