Effect of oxidized β-carotene on the growth and feed efficiency of broilers

W. W. Riley,[*] J. G. Nickerson,[†] and G. W. Burton[†,1]

[*]*International School, Jinan University, Guangzhou, China; and* [†]*Avivagen, Inc., Ottawa, Ontario, Canada*

ABSTRACT Fully oxidized β-carotene (**OxBC**) containing β-carotene-oxygen copolymers is proposed as an alternative to antimicrobial growth promoters. Two trials were conducted to determine the efficacy of OxBC in enhancing growth and feed intake (**FI**) in male and female Ross × Ross 308 broilers in Ontario, Canada, and in Ross 308 male broilers in the United Kingdom. In the first trial, 0, 1, 2, or 5 ppm OxBC were added to diets in a 20% cornstarch premix, whereas in the second trial, 0, 2, or 5 ppm OxBC were added in a 1% cornstarch or 1% corncob grits premix. In trial 1, 2, and 5 ppm OxBC improved bird final body weights (**BW**) compared with the unsupplemented, nonmedicated (no bacitracin methylene disalicylate included), negative control birds after 39 d of feeding under commercial conditions ($P < 0.05$). All levels of OxBC improved feed conversion (**FCR**) during the finisher period ($P < 0.05$), whereas 2 and 5 ppm OxBC enhanced FCR relative to the negative control group during the full production cycle ($P < 0.05$). Average daily FI was not affected by OxBC, whereas 2 and 5 ppm OxBC increased broiler average daily gain (**ADG**) ($P < 0.05$). Oxidized β-carotene did not affect bird mortality. The optimal OxBC dose was 2 ppm under the conditions used. In trial 2, 2 or 5 ppm OxBC on cornstarch and 5 ppm OxBC on corncob grits improved ADG, BW, and FI when fed for 35 d, as compared with the negative, nonmedicated control ($P < 0.05$). Feed conversion was not improved in the OxBC groups compared with the control group ($P > 0.05$). There were no differences among the 3 OxBC groups ($P > 0.05$). When birds were fed 2 ppm OxBC on corncob grits, the overall ADG, BW, and FI were lower than the respective control values ($P < 0.001$). Overall bird mortality was higher than expected for all groups, including the control group, but no dose effect was evident. Cornstarch was the preferred carrier for OxBC, and 2 ppm OxBC was the optimal dose under the conditions used.

Key words: OxBC, oxidized β-carotene, β-carotene-oxygen copolymer, antimicrobial growth promoter alternative

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INTRODUCTION

Plant carotenoids represent the main dietary source of provitamins A, and β-carotene is the best-known example of these. However, more than 1,100 carotenoids have been identified in nature (Yabuzaki, 2017), and many of these have nonprovitamin A activity, including the xanthophylls, ß-cryptoxanthin, astaxanthin, canthaxanthin, zeaxanthin, and lutein, which all contain oxygen atoms, and lycopene, which is a pure hydrocarbon (Marounek and Pebriansyah, 2018). Carotenoid compounds, apart from β-carotene, most commonly found in poultry rations are zeaxanthin from corn and the xanthophylls from alfalfa. Both synthetic and natural carotenoids are used in broiler and layer feeds to increase skin yellowing and the yellow or orange color of egg yolks. The degree of pigmentation desired depends on the poultry breed (i.e., duck, goose, quail, chicken, turkey) and the cultural habits of domestic consumers. In general, carotenoids with high provitamin A activity have low pigmenting properties (Hencken, 1992).

In addition to serving as vitamin A precursors and a source of pigmentation for birds, carotenoids have also been implicated in the modulation of the avian innate immune system (Blount et al., 2003; McGraw and Ardia, 2003; Chew and Park, 2004; LeClaire et al., 2015; Lopez-Rull et al., 2015). Koutsos et al. (2003) used lipopolysaccharide or interleukin-1 to induce an acute phase response in chicks and concluded that this response was implicated in reducing tissue carotenoid levels during infectious disease. In a subsequent study, this same group demonstrated that chicks fed 0 mg lutein had greater body weight losses and higher plasma

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[1]Corresponding author: g.burton@avivagen.com

haptoglobin and relative thymus, bursa, and spleen weights post-lipopolysaccharide challenge compared with chicks fed 40 mg lutein/kg diet (Koutsos et al., 2006). The conclusion was that a lack of carotenoid exposure, either *in ovo* or after hatch, increased systemic inflammation, and that the innate immune response is critical as the first line of defense against invading pathogens. The components of this innate immune response possess the ability to regulate the transcription and translation of genes for additional mediators of the inflammatory response (i.e., cytokines and acute-phase proteins). For example, plasma haptoglobin and serum amyloid A protein are acute-phase proteins that respond positively to inflammatory stimulation in various species, including wild birds (Pepys et al., 1989; Cerón et al., 2005; Caliendo et al., 2013).

Burton et al. (2014) expanded on the potential role of dietary carotenoids in the modulation of the innate immune system in various animal species by describing the means by which naturally occurring carotenoids undergo spontaneous, autocatalytic oxidation to produce a large number of complex oxygen copolymers. They suggested that these copolymers are the source of immune-enhancing activity in several species, including poultry, that has been attributed to the carotenoid family of compounds. Fully oxidized β-carotene (**OxBC**), obtained when β-carotene reacts to the full extent with oxygen in air, is especially rich in β-carotene–oxygen copolymers (Burton et al., 2014). The copolymer-rich OxBC has been found to exert its effect by priming the innate immune system and by limiting the propagation of excessive inflammation (Duquette et al., 2014; Johnston et al., 2014). These immunological effects are achieved via mechanisms that are distinct from the provitamin A or antioxidant pathways commonly proposed as explanations for carotenoid actions.

In a study by Kang et al. (2018), dietary OxBC supplements exerted notable positive effects in a necrotic enteritis (**NE**) broiler chicken model by decreasing the level of *Clostridium perfringens* bacteria as well as pathogen-induced lesions in the small intestines of these birds, thus further indicating the immune-modulating role of these copolymers. The authors concluded that supplementation of broiler diets with 2 to 6 mg/kg of OxBC had a positive effect in improving productivity in outbreaks of NE and could contribute to the prevention of NE on commercial broiler farms.

The potential value of oxidized β-carotene copolymers as alternatives to antimicrobial growth promoters (**AGP**) has been of on-going interest, and earlier studies were conducted specifically to determine the efficacy of these copolymers in positively impacting feed intake (**FI**) and growth performance when used *in lieu* of AGP. The 2 studies described herein were conducted to refine the effective dose of oxidized carotenoids (**OxBC**) for improving growth and feed efficiency in Ross 308 broiler chickens and to determine a suitable carrier to be used with OxBC for further experimental and commercial purposes.

MATERIALS AND METHODS

Experimental Birds and Trial Design

In the first trial (trial 1), the Ross x Ross 308 pullets and cockerels were obtained from a commercial hatchery. Extra chicks were placed on the 0 ppm control diet in a separate pen for early mortality replacement, and all chicks were vaccinated in the hatchery against Marek's disease (1/4 dose per chick) and infectious bronchitis, but not against coccidiosis. The trial was conducted by a private research facility in the Province of Ontario, Canada. A total of 2,500 chicks were assigned to treatments on arrival. Birds within a block commenced the trial on the same day. There were 5 blocks in the study, and each comprised 10 pens with an equal number of male and female birds. The respective male and female pens in each block were randomly and equally assigned to treatments (A, B, C, D, or E), which represented the negative (nonmedicated; i.e., no bacitracin methylene disalicylate (**BMD**) added), positive (medicated; 55 ppm BMD added), 1 ppm OxBC, 2 ppm OxBC, and 5 ppm OxBC groups, respectively. There were 50 birds per pen on day 0, and the birds were randomly assigned so that each hatchery box contributed approximately an equal number of birds to each pen within a block.

For the second trial (trial 2), 4,655-day-old Ross 308 commercial hybrid male broiler chicks were used. Chicks were obtained from the Moy Park Hatchery, Donaghmore, Northern Ireland, and randomly allocated to 5 experimental treatments. The study was designed as a randomized block, with 19 blocks of 5 pens with 49 chicks per pen at the start of the study, and each dietary treatment being replicated 19 times. The pens in each block were randomly and equally assigned to treatments (A, B, C, D, or E), which represented the control (basal ration), 2 ppm OxBC on starch, 5 ppm OxBC on starch, 2 ppm OxBC on corncob grits, and 5 ppm OxBC on corncob grits groups, respectively. All chicks were vaccinated at the hatchery for infectious bronchitis, but not against coccidiosis. The trial was conducted by a contract research organization in the United Kingdom.

Bird Management

Trial 1 was conducted at a contract agricultural research facility in southern Ontario, Canada, under conditions typical of those used in commercial practice in the local geographical area. Birds which had obvious health problems were excluded from the study. Early mortalities (0–5 d of age) were replaced with extra chicks that had been maintained on the basal diet. Water and feed were provided ad libitum to birds throughout the trial. The trial was conducted in accordance with the Canadian Council on Animal Care guidelines for the care and use of farm animals in research, teaching, and testing. The trial protocol was approved by the Animal Care and Use Committee of the contract research organization.

Trial 2 was conducted at a contract agricultural research facility in Scotland, U.K. Birds were housed in floor pens with a floor area of 2.98 × 1.15 m, providing 699 cm^2 per bird. Fresh wood shavings were provided to a depth of ∼10 cm in each pen. A negative pressure ventilation system was set to operate so that it met commercial recommendations. Feed and water were made available to the birds on an ad libitum basis throughout the experimental period. The birds were housed in floor pens in an environmentally controlled room for the entire 35-day trial. The temperature was maintained as per the values recommended by the breeder (Aviagen, 2018). The study design was a randomized block, and on day 0, all birds were allocated randomly by pen replicate number. Only day-old birds, which appeared healthy, were included for selection. The trial was conducted in accordance with the applicable Codes of Recommendations for the Welfare of Livestock in the UK for Turkeys/Layers/Broilers, adhering in particular to the husbandry of the birds. The study protocol was approved by the Animal Experiments Committee of the contract research organization.

Test Product, Feed Preparation, and Analysis

Trial 1 The oxidized β-carotene copolymer product, OxBC, was supplied by the sponsor of the study as a 20% premix (starch diluent). The amount of test product used in the treatment diets was 0, 1, 2, or 5 ppm OxBC, and the inclusion of 55 ppm BMD in the basal diet (Table 1) served as the positive control. The treatment diets were introduced on day 0 and fed continuously until study termination on day 39. To manufacture final feeds, the 20% OxBC premix was diluted with corn starch, a 0.5% (w/w) free flow agent (Sipernat; silicon dioxide), and 1% mineral oil to produce a 2% (w/w) OxBC premix. The required amount of active ingredient was delivered by varying the amount of 2% OxBC premix per tonne of complete feed in corn, soybean-meal, wheat diets, formulated for 3 growth stages of the bird (starter-grower-finisher). All of these diets met or exceeded the requirements for broilers, as outlined by the National Research Council (1994) (Table 1). The form of the 3 growth stage feeds was medium crumb (starter), course crumb (grower), and short pellet (finisher) and they were only used upon confirmation that nutrient analyses met expectations. To minimize cross-contamination risk, feeds were manufactured in order of treatment code (A, B, C, D, E). A representative composite sample of feed (treatments A, B, C, D, and E) was taken from each batch, and a primary sample was divided into 2 0.5 kg subsamples for nutrient analysis and as a retainer sample. The nutrient analysis samples, representing the starter, grower, and finisher pelleted feeds, were analyzed for dry matter, crude protein, calcium, phosphorus, and sodium.

Table 1. Feed formulations: calculated and analyzed nutrient values (trial 1).

Ingredient (%)	Starter (0–18 d)	Grower (19–31 d)	Finisher (32–38 d)
Corn	55.04	59.72	62.85
Soybean meal (48% CP)	19.42	18.32	15.89
Wheat	10.00	10.00	10.00
Pork meal	7.50	7.50	6.62
Canola meal	3.00	–	–
Poultry fat	–	0.66	2.05
Feather meal	2.20	1.15	–
Calcium carbonate	0.74	0.65	0.75
Lignosol[1]	0.63	0.63	0.63
Dicalcium phosphate	0.41	0.23	0.16
Mineral/vitamin premix[2]	0.25	0.25	0.25
Salt	0.21	0.21	0.25
Methionine (liquid)[3]	0.15	0.19	0.17
Sodium bicarbonate	0.15	0.16	0.12
Lysine	0.11	0.14	0.07
Myco CURB[4]	0.10	0.10	0.10
Choline (liquid)	0.08	0.06	0.04
Threonine	–	0.04	0.04
Quinguard[5]	0.02	0.02	0.02
Calculated nutrients (as fed)			
Crude protein	21.0	21.0	18.5
Calcium	1.02	0.90	0.85
Total phosphorus	0.67	0.61	0.55
Sodium	0.18	0.18	0.18
Analyzed nutrients (as fed)			
Moisture	12.46	12.44	12.51
Crude protein	21.6	21.5	17.9
Calcium	1.00	0.81	1.01
Total phosphorus	0.71	0.72	0.68
Sodium	0.17	0.18	0.18

[1]Lignin sulfonate.
[2]Added as a commercial premix, which met all NRC requirements (1994).
[3]Methionine hydroxyl analog (MHA).
[4]Mold inhibitor.
[5]Ethoxyquin (antioxidant).

Trial 2 The birds were fed a standard commercial basal ration from 0 to 10 d of age, a grower ration from 10 to 21 d of age, and a finisher ration from 21 to 35 d of age. The basal diet did not contain any coccidiostat or AGP, prophylactic or similar additives. The control birds were fed the basal rations without added OxBC, and the supplemented groups were fed the same basal rations including either 2 or 5 ppm OxBC on a cornstarch or a corncob grit premix for a period of 35 d. The composition and the calculated analysis of the control diet are presented in Table 2. All of the trial 2 diets met or exceeded the requirements for broilers, as outlined by the National Research Council (1994). The test product was supplied by the sponsor as a 1% premix on cornstarch or corncob grits carrier. These were mixed with wheat to be included in the diet at rates of 200 g per kg and 500 g per kg of wheat. Each ration was made from a single batch of the basal feed. The interim premixes of treated wheat were used to prepare the final test diets to give final OxBC inclusion levels of 0, 2, and 5 ppm. The final feeds were pelleted, and pelleting temperatures were recorded as being between 61°C and 70°C for the starter diet and between 64°C and 70°C for the grower and finisher diets. Samples were taken before pelleting, and they were collected into a composite sample for each diet (starter, grower, finisher) in an appropriately labeled bag (8–10 kg in total). The scoop used for sampling was reused only for diets of a higher OxBC concentration with the same premix (cornstarch or corncob grits), and it was changed when the premix was changed or when there was a lower OxBC inclusion level in the diet.

Growth Data (Trials 1 and 2) and Adverse Events (Trial 2)

Trial 1 The following variables were compiled for each growth period (starter, day 0–18; grower, day 19–31; finisher, day 32–38): cumulative average daily feed intake, calculated as total FI per pen divided by the number of live bird days in the specified period; cumulative average daily body weight gain, calculated as total body weight gain per pen divided by the number of live bird days in the specified period; and cumulative feed efficiency, calculated as total feed consumption divided by body weight increase. Body weight increase was defined as follows: (sum of final body weights of surviving birds + weight of mortalities and removals)–(sum of initial body weights, including initial body weights of birds dead or removed during the specified period). Average body weight was calculated on a pen basis on day 0, 18, 31, and at market (day 39).

Pen live weights were recorded on day 0, 18, 31, and 39. Pen feed consumption was recorded for day 0–18, 19–31, and 32–39. Morbidity and mortalities were recorded, including the apparent causes of death or the reasons for culling. The minimum and maximum barn temperature was recorded on a daily basis. Daily observations of feed texture, litter quality, and bird activity were also recorded.

Table 2. Feed formulations: calculated and analyzed nutrient values (trial 2).

Ingredient (g/kg)	Starter (0-10 d)	Grower (11-21 d)	Finisher (22–35 d)
Wheat	610.4	616.0	647.8
Rapeseed meal	41.0	41.0	41.0
Soybean meal (48% CP)	230.0	210.0	141.9
Soybean meal (full fat)	44.6	44.6	82.0
Vegetable oil	30.0	46.0	47.0
Salt	2.7	2.7	2.7
$NaHCO_3$	1.5	1.5	1.5
Lysine	2.0	2.0	2.0
DL-Methionine	3.3	3.2	2.6
Limestone	13.0	12.0	12.0
Dicalcium phosphate	15.0	14.5	13.0
Vitamin premix[1]	6.5	6.5	6.5
Calculated nutrients (as fed)			
Crude protein, g/kg	229.9	220.5	203.3
AME, MJ/kg	12.68	13.20	13.51
Calcium, g/kg	9.4	8.9	8.5
Phosphorus, g/kg	8.2	8.0	7.6
Available phosphorus, g/kg	4.9	4.7	4.3
Fat, g/kg	51.5	67.4	75.2
Fiber, g/kg	37.1	36.4	36.8
Lysine, g/kg	13.6	13.0	11.9
Methionine, g/kg	6.0	5.7	5.0
Methionine + cysteine, g/kg	9.7	9.4	8.5
Analyzed nutrients (as fed) g/kg			
Ash	69.6	59.9	57.3
Dry matter	869.0	877.0	871.0
Crude protein	185.0	201.0	185.0
Lysine	11.3	11.2	10.0
Methionine	5.7	6.0	5.2
Starch	443.0	431.0	453.0
Neutral detergent fiber	89.5	91.2	88.8
Acid hydrolyzed ether extract	66.7	77.8	83.5

[1]Added as a commercial premix, which met all NRC requirements (1994).

Trial 2 Body weight and FI data were recorded for each period (starter, day 0–10; grower, day 11–21; finisher, day 22–35) to calculate weight gain, feed efficiency, and feed conversion ratios (**FCR**). Pen bulk weights of birds were obtained on day 10, 21, and 35, and the weight of birds that were removed (dead or culled) was recorded and body weights adjusted accordingly. Feed weigh-backs were conducted on day 10, 21, and 35. Birds were inspected daily for any adverse or serious adverse events, and all inspections were recorded on the appropriate capture form. An adverse event, for the sake of this trial, was considered to be any observation in the birds, whether or not product-related, that was unfavorable or unintended. Included were events related to a suspected lack of efficacy or noxious reaction after exposure. A serious adverse event was considered to be any adverse event which resulted in death, was life-threatening, or resulted in persistent or significant disability/incapacity, or congenital anomaly or birth defect. In this trial, a serious adverse event was mortality higher than 1.5% in any week in a treatment group, with the sponsor being notified within one working day. All deaths were recorded, and where the cause of the mortality was not obvious, the carcass was sent for postmortem examination. All postmortem reports were subsequently sent to the sponsor.

Statistical Analysis

Trial 1 Analysis of variance was used for the randomized complete block design, and pairwise comparisons of treatment means using least-significant differences were used to separate means when statistically significant ($P < 0.05$) treatment effects were observed (Minitab, 2000; Statistix 7, 2000). Mortality data in percentages were arcsine transformed before statistical analyses.

Trial 2 Statistical analysis was carried out using GENSTAT 11th Edition for Windows. Data from the treated groups were compared with those of the control group using a linear mixed model (REML), with a level of probability of less than or equal to 0.05 indicating significance. Differences among diets were determined by least significant difference tests. Initial weights were used as a covariate. Average daily gain, average daily FI, and FCR were all reported. Body weights were reported as the initial weight and the final weight for each growth phase.

RESULTS

Growth Performance

Trial 1 The live weights of birds in the negative, nonmedicated control group (no BMD) and the 1 and 2 ppm OxBC groups were significantly higher than those of birds in the medicated group (55 ppm BMD) at day 18, whereas the birds in the 5 ppm OxBC group had live weights at day 18 equivalent to all other groups ($P < 0.05$) (Table 3). On day 39, birds fed 2 and 5 ppm OxBC had significantly higher live weights than did birds fed the negative control or the medicated diets, while the 2 ppm OxBC group also had a significantly higher live weight than did the 1 ppm OxBC group ($P < 0.05$) (Table 3).

The average daily gain (ADG) of birds fed 0, 1, and 2 ppm OxBC was significantly higher than of birds fed the medicated diet during the starter period (day 0–18; $P < 0.05$), whereas birds fed 5 ppm OxBC had ADG intermediate between the 0, 1, and 2 ppm OxBC groups and the medicated group (Table 3). Birds fed OxBC (1 ppm, 2 ppm, and 5 ppm) had significantly higher ADG compared with those fed either control or medicated diet during the finisher phase (day 32–39; $P < 0.05$) (Table 3). For the full duration of the trial, birds fed 2 ppm or 5 ppm OxBC had significantly higher ADG (4.1 and 3.8%, respectively) relative to birds fed either control or medicated diet (day 0–39; $P < 0.05$) (Table 3), with birds fed 1 ppm OxBC having an intermediate gain that was not significantly different from birds fed any of the other treatments.

Feed conversion ratios were not significantly affected during the starter period (day 0–18). During the grower period (day 19–31), the FCR were significantly higher in birds fed 1 ppm OxBC but not in those fed 2 or 5 ppm OxBC relative to birds fed the negative control diet (Table 4). By contrast, FCR were significantly lower in birds fed all levels of OxBC during the finisher period compared with birds fed either control or medicated diet (day 32–39; $P < 0.05$). For the entire duration of the trial, birds fed OxBC had lower FCR than the negative control group, whereas only birds fed 5 ppm OxBC had a significantly lower FCR than those fed the medicated diet (day 0–39; $P < 0.05$) (Table 4).

No significant differences were noted in FI among treatments throughout the starter (day 0–18), grower (day 19–31), or finisher periods (day 32–39), or over the entire production cycle (day 0–39) ($P > 0.05$) (Table 4). However, FI in the 2 ppm OxBC group at day 31 was numerically higher than FI in both control and medicated groups and for the 1 ppm and 5 ppm OxBC groups, and the difference approached significance ($P = 0.07$).

Dietary OxBC supplementation did not affect broiler mortality over the duration of the experiment (Table 5). However, birds fed 1 ppm OxBC had a similar mortality level to the birds fed the medicated diet, and both of these were significantly lower than birds fed 2 ppm OxBC ($P < 0.05$; LSD comparison of means).

Trial 2 The average body weights (**ABW**) of the day-old birds in this trial were similar to those of the birds in trial 1, and they were consistent with Aviagen guidelines for Ross 308 male broiler chicks (Aviagen, 2018) (Table 3). The final body weights of the birds at 35 d were slightly higher than the final body weights of the birds at 39 d in trial 1, but that is not unexpected, given the fact that trial 1 birds were a mixture of male and female broilers, whereas only male broilers were used in trial 2.

There were no differences ($P > 0.05$) among the ABW of the birds at the beginning of the trial (day 0)

Table 3. Effect of dietary OxBC supplementation on average weights and average daily gain of male and female broiler chickens over 39 d (trial 1) and of male broiler chicks over 35 d (trial 2).

	Average body weight[1] (kg)					Average daily gain (g/day)		
Trial 1	Day 0	Day 18	Day 31	Day 39	0–18 d	19–31 d	32–39 d	0–39 d
0 ppm control	0.044	0.605^{a}	1.739	2.184^{c}	31.1^{a}	80.7	74.1^{b}	56.0^{b}
0 ppm + Med[2] control	0.044	0.588^{b}	1.724	2.182^{c}	30.1^{b}	81.1	75.8^{b}	55.9^{b}
1 ppm OxBC	0.044	0.604^{a}	1.710	2.216^{b,c}	30.9^{a}	79.1	84.0^{a}	57.1^{a,b}
2 ppm OxBC	0.044	0.605^{a}	1.751	2.282^{a}	30.9^{a}	81.7	87.2^{a}	58.3^{a}
5 ppm OxBC	0.044	0.594^{a,b}	1.727	2.262^{a,b}	30.4^{a,b}	80.6	89.0^{a}	58.1^{a}
Trial 2	Day 0	Day 10	Day 21	Day 35	0–10 d	11–21 d	22–35 d	0–35 d
0 ppm control	0.043	0.262^{b}	0.924^{b}	2.243^{b}	21.9^{b}	60.3^{b}	94.1^{a}	62.9^{b}
2 ppm OxBC starch	0.043	0.268^{a}	0.965^{a}	2.309^{a}	22.5^{a}	63.4^{a}	96.1^{a}	64.7^{a}
5 ppm OxBC starch	0.043	0.269^{a}	0.959^{a}	2.320^{a}	22.6^{a}	62.7^{a}	97.2^{a}	65.1^{a}
2 ppm OxBC CCG[3]	0.043	0.271^{a}	0.935^{b}	2.19^{c}	22.9^{a}	60.4^{b}	89.8^{b}	61.4^{c}
5 ppm OxBC CCG[3]	0.043	0.269^{a}	0.956^{a}	2.300^{a}	22.6^{a}	62.5^{a}	96.0^{a}	64.5^{a}

Abbreviation: OxBC, oxidized β-carotene.
[1a-c]Values within a column bearing a common letter are not significantly different ($P > 0.05$), based on least significant differences of means.
[2]55 ppm BMD.
[3]CCG: corncob grits.

(Table 3). Overall, compared with the OxBC-fed birds, the chicks fed the control diet had a lower ABW at day 10 ($P < 0.001$), day 21 ($P < 0.001$), and day 35 ($P < 0.05$). However, the body weight of the control-fed birds at day 35 was higher than the body weight of the birds fed the 2 ppm OxBC on the corncob grits carrier. At day 21 and 35, birds fed OxBC on the cornstarch carrier had higher body weights than the birds fed OxBC on corncob grits ($P < 0.001$). In terms of OxBC levels, there was a clear carrier by level interaction on day 21 and 35 ($P < 0.001$) and a probable interaction on day 10 ($P = 0.07$). This indicated a difference between the weights of the birds fed 2 and 5 ppm OxBC on corncob grits, with the 5 ppm group being significantly higher, but there was no difference between the 2 cornstarch carrier treatment groups.

The OxBC-treated birds had a higher ADG compared with the control birds for the starter ($P < 0.001$), grower ($P < 0.001$), and overall ($P < 0.005$) periods, but not for the finisher ($P > 0.05$) period (Table 3). The ADG of the control birds for the overall period was higher ($P < 0.05$) than the ADG for the 2 ppm OxBC on corncob grits birds. Birds fed OxBC on cornstarch had a higher ADG for the grower, finisher, and overall periods ($P < 0.001$) than did the OxBC on corncob grits birds. A carrier by level interaction was observed for ADG for the grower, finisher, and overall periods ($P < 0.001$), indicating that birds fed the 5 ppm OxBC on corncob grits feed had higher ADG than did the birds fed the 2 ppm OxBC on corncob grits ration. There was no difference between the 2 OxBC on cornstarch carrier groups.

The average feed intake (**AFI**) of the control birds for the first 10 d of the study was not significantly different from the OxBC fed birds (Table 4). On average, the control birds exhibited lower FI than the OxBC fed birds for the periods between day 10 to 21 ($P < 0.005$), day 21 to 35 ($P < 0.05$), and day 0 to 35 ($P < 0.01$). For the last 2 treatment periods, however, the AFI of the control fed

Table 4. Effect of dietary OxBC supplementation on average daily feed intake and the feed conversion ratio of male and female broiler chickens over 39 d (trial 1) and of male broiler chicks over 35 d (trial 2).

	Average daily feed intake (g/day)				Feed conversion ratio[1] (kg/kg gain)			
Trial 1	0–18 d	19–31 d	32–39 d	0–39 d	0–18 d	19–31 d	32–39 d	0–39 d
0 ppm control	45.5	134.2	208.5	103.2	1.469	1.665^{b,c}	2.872^{a}	1.851^{a}
0 ppm + Med[2] control	44.7	133.4	204.3	102.4	1.484	1.650^{c}	2.781^{a}	1.831^{a,b}
1 ppm OxBC	45.3	134.2	203.4	102.6	1.462	1.701^{a}	2.429^{b}	1.806^{b,c}
2 ppm OxBC	45.3	137.1	206.5	104.1	1.466	1.684^{a,b}	2.389^{b}	1.789^{b,c}
5 ppm OxBC	46.2	134.3	206.3	103.6	1.520	1.670^{a,b,c}	2.328^{b}	1.788^{c}
Trial 2	0–10 d	11–21 d	22–35 d	0–35 d	0–10 d	11–21 d	22–35 d	0–35 d
0 ppm control	28.2	88.8^{b}	161.9^{b}	100.7^{b}	1.286^{a}	1.475^{a}	1.720	1.603
2 ppm OxBC starch	28.4	90.8^{a}	166.1^{a}	103.1^{a}	1.265^{b}	1.435^{d}	1.731	1.593
5 ppm OxBC starch	28.8	90.8^{a}	166.8^{a}	103.5^{a}	1.275^{b}	1.448^{c}	1.717	1.591
2 ppm OxBC CCG[3]	28.7	88.5^{b}	156.8^{b}	98.7^{c}	1.254^{c}	1.469^{b}	1.748	1.609
5 ppm OxBC CCG[3]	28.2	90.6^{a}	167.1^{a}	103.4^{a}	1.250^{c}	1.449^{c}	1.743	1.604

Abbreviation: OxBC, oxidized β-carotene.
[1a-c]Values within a column bearing a common letter are not significantly different ($P > 0.05$), based on least significant differences of means.
[2]55 ppm BMD.
[3]CCG: corncob grits.

Table 5. Effect of dietary OxBC supplementation on broiler mortality over 39 d in trial 1 and over 35 d in trial 2.

Trial 1	Mortality (%)[1]
0 ppm control	3.4[a,b]
0 ppm + Med. control	2.0[b]
1 ppm OxBC	1.8[b]
2 ppm OxBC	3.8[a]
5 ppm OxBC	2.8[a,b]
Trial 2	
0 ppm control	6.1
2 ppm OxBC cornstarch	6.2
5 ppm OxBC cornstarch	4.6
2 ppm OxBC CCG[2]	10.9
5 ppm OxBC CCG[2]	6.1
Total	6.9

Abbreviation: OxBC, oxidized β-carotene.
[1a,b]Values bearing a common letter are not significantly different ($P > 0.05$), based on least squares differences of means.
[2]CCG: corncob grits.

birds was higher ($P < 0.05$) than the AFI of the birds fed the 2 ppm OxBC on corncob grits. Birds fed OxBC on the cornstarch carrier (2 and 5 ppm) had a higher overall FI for the grower ($P < 0.005$), finisher ($P < 0.001$), and overall ($P < 0.001$) periods than did the birds fed OxBC (2 and 5 ppm) on corncob grits. In terms of OxBC levels, there was a carrier by level interaction during all study periods, which confirmed a difference between the FI of the 2 and 5 ppm OxBC on corncob grits groups (higher FI in the 5 ppm group). There was, however, no difference between the 2 OxBC on cornstarch carrier treatment groups.

On average, the OxBC-treated birds had a lower FCR than did the control birds for the starter ($P < 0.005$) and the grower ($P < 0.01$) periods, but not for the finisher or overall periods (Table 4). For the starter period, the OxBC on corncob grits fed birds had a significantly lower ($P < 0.05$) FCR than the OxBC on cornstarch fed birds, but this effect was reversed for the subsequent periods and for the overall study period. There was no difference between 2 and 5 ppm OxBC ($P > 0.05$). There was a carrier by level interaction for FCR ($P < 0.05$) during the grower period, which meant that the FCR for the 5 ppm OxBC on corncob grits was lower than the FCR for the 2 ppm OxBC on corncob grits birds. The opposite was true for the OxBC on cornstarch groups during the grower period, with the 2 ppm OxBC FCR being lower than the FCR for the 5 ppm OxBC group.

The mortality data for trial 2 are presented in Table 5. Mortality was relatively high during the trial, but there were no differences among the treatment groups. The overall mortality during the first and the last weeks of the trial was >1.5% and was recognized as an adverse event and reported to the sponsor (data not shown). The higher than expected mortality did not appear to be treatment related, as the control birds were also affected, and there was no trend for a higher mortality in the 5 ppm OxBC groups compared to the 2 ppm groups. Because of the relatively high mortality, a representative number of dead birds were examined postmortem by the research site veterinarian. The final diagnosis was that most of the deaths during the first week of the study were due to yolk sac infection, often with evidence of a thickened umbilicus and sometimes with secondary septicemia (i.e., not related to treatment but characteristic of a hatchery-related condition). The diagnosis for the deaths that occurred in the final week of the study was that this was due to a Staphylococcal infection of the respiratory tract. However, no medical intervention was recommended.

DISCUSSION

Previous trials to evaluate the effects of OxBC in livestock have been conducted in piglets, dairy calves, and broiler chickens. Dietary supplementation with OxBC improved the growth performance of vaccinated (PRRS) and nonvaccinated piglets, and it resulted in significant anti-inflammatory activity in the lungs of *Mannheimia haemolytica*–challenged dairy calves (Hurnik et al., 2011; Duquette et al., 2014). Most recently, Kang et al. (2018) found that dietary supplementation with OxBC reduced the impact of subclinical necrotic enteritis challenge and enhanced the body weight of broilers relative to unsupplemented, nonantibiotic treated birds. These results as well as the presence and natural occurrence of β-carotene copolymers have led to the suggestion that these compounds may be the source of β-carotene's provitamin-A–independent effects (Burton et al., 2014, 2016; Duquette et al., 2014; Johnston et al., 2014; Schaub et al., 2017; Kang et al., 2018).

Kang et al. (2018), in their study on the ability of OxBC to mitigate the effects of a *C. perfringens* challenge in Ross broiler chickens, treated birds with 0, 2, 4, or 6 ppm OxBC while employing 2 positive control groups, one of which was similar to the positive control in the present study (55 ppm BMD). They found 2 ppm OxBC to be equally effective to 4 and 6 ppm OxBC in reducing challenge-induced lesions as well as intestinal pathogen level relative to a negative control group (no antibiotic or OxBC). With regard to lesion severity, 2, 4, and 6 ppm OxBC were equally effective as the antibiotics (55 ppm BMD and 2 ppm virginiamycin) in the 2 positive control groups. At the end of the 28-day challenge trial, the average body weight of birds treated with 2, 4, or 6 ppm OxBC was higher than the mean body weight of birds in the negative control group, showing that OxBC improved bird growth and mitigated the effects of *C. perfringens* on the intestinal lining. There was no additional benefit to higher dosages beyond 2 ppm, and no level of OxBC was capable of achieving the same growth recovery as were the 2 positive control groups.

In trial 1, the birds in the medicated group did not exhibit a greater live weight at the conclusion of the trial nor did they have a higher average daily weight gain than did the negative, nonmedicated control birds. The AGP used in this group, BMD, targets primarily gram-positive bacteria, including *C. perfringens,* by interfering with protein synthesis and cell wall production

in these microorganisms (Butaye et al., 2003; Proctor and Phillips, 2019). The lack of a positive growth response to BMD may have indicated a relatively low level of pathogenic stress on the birds during this 39 d trial. However, both average body weight and average daily gain were improved relative to the control and medicated groups by inclusion of OxBC in the diet of the Ross 308 pullets and cockerels. This effect was most consistent in the 2 ppm OxBC group relative to the control and medicated groups. Feeding 2 ppm OxBC was superior to feeding 1 ppm OxBC in enhancing average body weight, but 5 ppm OxBC showed no clear advantage over 2 ppm OxBC. The trial 1 data, therefore, suggest that 2 ppm OxBC would be sufficient to elicit a significant improvement in growth under commercial production conditions.

The presumed mechanism of action of OxBC is to prime the innate immune system. This includes both pathogen recognition responses as well as the activation of anti-inflammatory mediators (Burton et al., 2014; Johnston et al., 2014). In this first trial, it is presumed that the anti-inflammatory mode of action was responsible for the positive effects on growth and performance evident in birds treated with OxBC, given the lack of efficacy of the AGP used.

The trial 2 results confirmed the efficacy of 2 ppm OxBC in improving the average daily weight gain and the average body weight of male broilers fed for 35 d, with no additional benefit afforded by 5 ppm OxBC, when the carrier used was cornstarch. When corncob grits were used as the carrier for OxBC, there was a significant negative effect on the growth performance of birds at 2 ppm OxBC, although this effect was not evident at 5 ppm OxBC. Given the consistent positive response seen with the cornstarch carrier in both trials 1 and 2, and the negative result in trial 2 with corncob grits, it is preferable that cornstarch be used as the carrier for OxBC in future trials and for commercial purposes.

The absence of any effect of the treatments on FI in trial 1 during this study suggests that the improved average weight of birds in this first trial was due to other factors. Improved nutrient utilization, diminished stress and more optimal health of the animals are possible considerations, and these would be consistent with the suggested role of the OxBC as a modulator of the innate immune system in monogastric and ruminant species (Duquette et al., 2014; Johnston et al., 2014; Kang et al., 2018). However, in trial 2, there was a significant effect of OxBC on the FI of the male broilers. This effect was evident during all growth stages and for the entire 35-day period when 2 ppm or 5 ppm OxBC was fed using cornstarch as the carrier. The highly significant effect on FI in trial 2 ($P < 0.001$ for the grower, finisher, and entire 35-day period) was in contrast to the absence of a treatment effect of OxBC in trial 1. In the first trial, the sexes were mixed, and there were fewer total birds tested compared with trial 2 (2,500 vs 4,655). If male broilers are more sensitive to FI in the presence of OxBC than female broilers, then trial 2 had a far greater chance to show an effect of OxBC on FI than did trial 1. One cannot discount the possibility that the FI differences were partially or in total a result of management differences between Canada and the United Kingdom or a response to the different diets used in the 2 trials. The broilers in trial 1 were fed a corn, soybean-meal, wheat diet that is common to broiler feeds used in eastern Canada, whereas the birds in trial 2 were fed a wheat, soybean-meal, rapeseed-meal diet that is more typical of northern Europe.

The FCR in trial 1 showed an overall positive effect of OxBC on broiler performance during the finisher and overall trial periods. The FCR for birds in the 2 ppm and 5 ppm OxBC groups were superior to that of birds in the 1 ppm group and equal to the FCR of the negative control birds during the grower period. However, the medicated group birds had the lowest FCR during the grower period, and this was also significantly lower than the FCR of the 2 ppm OxBC birds. By contrast, FCR were significantly lower in birds fed 1, 2, or 5 ppm OxBC than in birds fed both the negative control and medicated diets during the finisher period. For the entire duration of trial 1, the birds fed the 5 ppm OxBC diet had the lowest FCR, and this was significantly lower than the FCR of the negative control and medicated groups. Still, all 3 OxBC groups had significantly lower FCR than did the negative control group, suggesting that this feed additive can replace BMD and provide similar performance benefits under conditions present in this study.

The FCR results from trial 2 were inconsistent with the trial 1 results. The OxBC-treated birds only had a lower FCR than did the control birds in the starter and the grower periods but not for the finisher or overall periods. Thus, it must be concluded that the FCR was not significantly improved in the OxBC supplemented groups when the full trial period (35 d) is used as the benchmark. Although OxBC improved weight gain in broilers in trial 2, it also increased FI. The combined improvement in both parameters would indicate that the gain in weight in trial 2 was either a direct effect of the increased FI or a combination of increased FI and improved nutrient utilization that was more difficult to ascertain through FCR due to the simultaneous enhancement in FI.

Mortality during trial 1 was low (<5%), and differences among treatments were insignificant. The low morbidity and mortality experienced during the course of this study suggest that the environmental stress on the birds, including that derived from disease, was not excessive. It is noteworthy that OxBC still evinced a growth performance benefit to the Ross 308 broilers compared with the control groups despite the relatively low level of stress, and that this benefit occurred at a 2 ppm OxBC dietary inclusion level.

By contrast, trial 2 mortality was abnormally high, but given the large number of birds used in the trial, the losses were acceptable, especially given that postmortem analysis demonstrated that mortalities were not treatment related. In addition, there were no

differences in the mortality rates between the treatment groups. The dissimilarities in trial mortalities were likely the result of geographical, seasonal, or management differences.

The development of natural immune modulators as replacements for antimicrobial drugs has been advocated as a means to boost the immunity of poultry and protect them from the myriad of pathogens that they face in their environment (Kogut, 2009). This challenge has taken on even more importance with the appearance of antibiotic resistance to commonly used antibiotics in the poultry and other livestock industries, and the demands by consumers and poultry processors to ban the use of these antibiotic growth promoting compounds except for therapeutic purposes (Diaz-Sanchez et al., 2015). OxBC may also have immune-modulating effects in poultry, as described by the work of Kang et al. (2018), in addition to its growth promoting actions evident in the work described herein. Importantly, OxBC can be used at relatively low levels in the ration of commercially important avian species, and which has also been shown to be an effective means of protection against a *C. perfringens* challenge in broilers (Kang et al., 2018).

SUMMARY

Trial 1

Two (2) and 5 ppm dietary OxBC supplementation significantly improved average final body weights of birds by 4.5 and 3.6%, respectively, compared with unsupplemented negative controls after 39 d of growth under commercial rearing conditions ($P < 0.05$). All tested levels of dietary OxBC supplementation significantly improved FCR during the finisher period (day 32–39; $P < 0.05$), whereas 2 and 5 ppm dietary OxBC supplementation significantly improved FCR relative to the negative, nonmedicated control group over the entire production cycle by 3.3% (day 0–39; $P < 0.05$). Average daily FI were not affected by dietary OxBC, whereas average daily gains were significantly improved with 2 ppm and 5 ppm dietary OxBC ($P < 0.05$). Dietary OxBC supplementation did not affect broiler mortality. The optimal dose of OxBC, as determined from the present investigation, is 2 ppm, under the conditions used.

Trial 2

In comparison with a negative control group, diets supplemented with 2 or 5 ppm OxBC on cornstarch carrier or 5 ppm OxBC on corncob grits carrier significantly ($P < 0.05$) improved average daily gain, body weight, and FI of male broilers fed for 35 d. The FCR was not significantly improved in the OxBC-supplemented groups compared with the control group. There were no significant differences among the 3 OxBC groups. When birds were fed 2 ppm OxBC on corncob grits carrier, the overall daily gain, body weight, and FI were significantly lower than the respective values for the control group. Overall bird mortality was higher than expected for all treatment groups, including the control, but no dose effect was evident. It is concluded that a cornstarch carrier is preferable to a corncob girts and that, consistent with trial 1, 2 ppm OxBC is the optimal dose in Ross 308 broilers, as determined under the conditions used in this study.

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Avivagen Inc. was the study sponsor for the Canadian and Scottish trials. In the study design, Avivagen stipulated that birds would be reared and managed under conditions typical of commercial production conditions in each region and specified the dietary inclusion levels of oxidized β-carotene (OxBC) used in each trial. Details such as number of replicate pens and number of birds per pen were largely determined by the infrastructure available at each research facility. Avivagen was not involved in collection of data or statistical analysis. The study report provided by the research organizations included a detailed description of the methods, statistical model, results, and raw data. Avivagen, having background knowledge of OxBC's mode of action and results of previous research trials, was responsible for interpretation of findings at the biological and practical commercial levels. The decision to prepare and submit a manuscript was made by Avivagen.

DISCLOSURES

G.W. Burton and J.G. Nickerson are employees of Avivagen Inc. W.W. Riley serves as a technical consultant to Avivagen Inc.

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