Effect of oxidized β-carotene-oxygen copolymer compounds on health and performance of pre- and post-weaned pigs

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Abstract

The discovery of naturally occurring, biologically active β-carotene-oxygen copolymer compounds in spontaneously oxidized β-carotene has stimulated interest in their potential health benefits. The copolymers, formed in Nature or synthetically by the air-oxidation of β-carotene, possess beneficial immune modulating activities that previously had been attributed to β-carotene itself. Support for these benefits is provided by previous studies showing that supplementation in feed with low parts-per-million levels of copolymer-rich, fully oxidized β-carotene (OxBC) helped reduce the negative impact of subclinical necrotic enteritis in broilers and improved growth in weaned piglets. To further assess these potential benefits, two trials were conducted in swine raised under commercial conditions in Vietnam. Trial 1, a 140-day full-grow, post-wean study with 500 28-day-old pigs, compared 2, 4 or 8 ppm OxBC against both an unsupplemented and an antibiotic control group. OxBC and antibiotics each improved growth rate, feed efficiency, and body weight compared to the control. Animals receiving 4 and 8 ppm OxBC performed better than did animals on antibiotics. In starter pigs, OxBC reduced the occurrence of diarrhea dose-dependently (4 and 8 ppm) and to a greater extent than did antibiotics. Trial 2, a 49-day study with 420 piglets, was conducted in two-stages. In Stage 1 (pre-wean), OxBC in the transition (creep) feed produced a dose-dependent trend toward increased body weight over 21 days, reaching significance at the highest inclusion level (16 ppm). In Stage 2 (post-wean), body weight gain showed a dose-dependent trend and was significant for both 8 ppm OxBC and the antibiotics at 28 days post-wean. Feed conversion was better at 8 ppm OxBC and for the antibiotic group. These findings support the concept that β-carotene-oxygen copolymers help optimize immune function, and

38 provide validation for the effectiveness of this strategy in enhancing animal performance in

39 the absence of in-feed antibiotics.

40 # Introduction

41 We have previously reported that the spontaneous non-enzymatic oxidation of β-
42 carotene produces predominantly an oxygen-rich family of copolymer compounds with
43 immunomodulatory properties [1, 2]. Fully oxidized β-carotene, termed OxBC and rich in
44 copolymer compounds, exerts its actions on the immune system through pathways that are
45 distinct from either vitamin A or intact β-carotene, which are both absent. We have proposed
46 that copolymer compounds are in fact the actual agents responsible for many of the
47 provitamin A-independent activities of β-carotene and other carotenoids [2].

48 OxBC exhibits dual immunological activities relating to: a) enhancing innate immune
49 detection and response to pathogens [2] and, b) an anti-inflammatory/pro-resolution action
50 that limits the extent of over-zealous immune responses and reduces the level of background
51 inflammation [3, 4].

52 The utility of OxBC as a feed additive and alternative to antibiotic growth promoters
53 has been demonstrated in studies with piglets [5], sows [4] and broiler chickens [6]. In piglets,
54 dietary supplementation with OxBC improved growth performance and prevented the
55 vaccine-induced growth-lag associated with the PRRS (porcine reproductive and respiratory
56 syndrome) vaccination [5]. In sows, supplementation with OxBC, begining at late gestation
57 and continuing through lactation, resulted in reduced proinflammatory cytokine levels in
58 colostriumn and milk concurrent with increased colostral and milk immunoglobulin levels
59 [4]. In broilers, dietary supplementation with OxBC reduced the level of pathogen
60 (*Clostridium perfringens)* recovered from the gut and protected against the reduction in
61 growth performance associated with induction of subclinical necrotic enteritis (NE) [6].

Several authors have proposed that the search for suitable alternatives to antibiotic growth promoters should focus on substances that achieve effects similar to the antibiotics that they are intended to replace, namely, reduction in both bacterial load and inflammation [5-8]. The results from trials with pigs and poultry highlight the utility of OxBC in achieving both of these outcomes. The benefits observed with piglets and gestating/lactating sows likely are consistent with the anti-inflammatory actions of OxBC, whereas, the reduction in *C. perfringens* in the poultry study supports the benefits of innate immune priming. Note that OxBC has no direct anti-microbial effect and that the reduction in *C. perfringens* in the poultry study reflects actions on the host's immune system, which is better able to detect and respond to the presence of pathogens.

In the current paper, we report the results of two dietary supplementation trials with OxBC in swine raised under commercial production conditions in Vietnam. The trials evaluate the utility of OxBC as a feed additive supporting the health and productivity of pigs. The first trial evaluated OxBC over an entire 140-day post-wean growth cycle. The second trial evaluated the effect of OxBC on the health and growth performance of pre-wean and post-wean pigs. These trials provide the first assessment of OxBC utility as a substitute for in-feed antibiotics in real-world, commercial, swine-production conditions.

Materials and Methods

All experimental protocols were approved by the animal care committee of the Institute of Animal Sciences for southern Vietnam.

Trial 1. Full Grow Study

The trial was carried out at a commercial farm, Thai My Pig farm, Thai My commune, in the Cu Chi district, Ho Chi Minh City, from 17 May to 30 November 2014.

85 Five hundred weaned barrows and gilts (28 days of age) were used in a 140-day
86 complete wean-to-finish feeding trial. The animals were reared on site and were the progeny
87 of Landrace x Yorkshire females and Duroc boars. The herd was asymptomatic for PRRS
88 (Porcine Reproductive and Respiratory Syndrome) and was vaccinated against hog cholera
89 (Coglapest™, Ceva-Phylaxia Veterinary Biologicals Co. Ltd, Hungary), foot-and-mouth
90 disease (Biotaftogen™, Biogénesis Bagó, Argentina) and PRRS (Tai Xanh™, Navetco,
91 Vietnam).

92 Animals were randomized by weight with each pen containing 20 pigs with the same
93 ratio of barrows to gilts. The trial was comprised of five dietary treatment groups with five
94 replicate pens per treatment as follows: Control (basal diet with no antibiotics or OxBC), AB
95 (basal diet with antibiotics; no OxBC) and OxBC (basal diet supplemented with 2, 4 or 8 ppm
96 (mg/kg) OxBC; no antibiotics). The compositions of the basal diets for the Starter, Grower
97 and Finisher phases of the study are shown in Table 1. No in-feed or water-administered
98 medications or feed additives were employed with the exception of the antibiotics used in the
99 AB group and OxBC used in the three OxBC groups. OxBC was provided in-feed as the OxC-
100 beta™ Livestock 10% commercial premix product (Avivagen Inc. Ottawa, Canada).
101 Chlortetracycline and colistin sulfate were purchased from Zhumadian Huazhong Chia Tai
102 Co. Ltd (China) and Lifecome Biochemistry Co. Ltd (China), respectively.

103 **Table 1**. **Composition of basal diets for Starter, Grower and Finisher phases of the Full**
104 **Grow Study.**

No.	Ingredient name	Post-Wean (g/kg)	Grower (g/kg)	Finisher (g/kg)
1	Corn	481.0	700.00	513.32
2	Rice bran	-	58.08	123.07

3	Cassava root	-	10.00	157.86
4	Soybean meal 47.5% CP	237.0	136.92	100.00
6	Whey powder 11% CP	100	-	-
7	Prelac 38% CP	100	-	-
8	Fish meal 50% CP	-	75.81	74.00
9	Fish meal 60% CP	20.0	-	-
10	Soybean oil	27.1	11.83	25.72
11	Premix Min-Vit	3.0	2.50	2.50
12	DCP	18.0	-	-
13	Seashell powder	3.4	-	-
14	Enzyme	1.0	-	-
15	Salt	2.9	2.45	2.51
16	Sweetener	0.3	-	-
17	L-Lysine	3.2	1.57	0.63
18	DL-Methionine	1.6	0.26	0.13
19	L-Threonine	1.3	0.48	0.26
20	DL-Tryptophan	0.2	0.11	0.0
	Total	1,000.0	1,000.0	1,000.0
Nutrient composition				
1	Dry Matter (%)	88.15	87.85	88.04
2	ME (Kcal/kg)	3200	3,265	3,265
3	Protein (%)	22.0	18.0	15.5

4	Fat (%)	6.23	5.84	7.03
5	Fiber (%)	2.34	2.41	2.77
6	Calcium (%)	0.90	0.60	0.60
7	Total Phosphorus (%)	0.70	0.57	0.57
8	Avail. P (%)	0.52	0.48	0.48
9	Lysine (%)	1.42	1.07	0,92
10	Methionine (%)	0.82	0.39	0,32
11	Meth + Cystine (%)	0.47	0.69	0,61
12	Threonine (%)	0.91	0.81	0,70
13	Tryptophan (%)	0.27	0.21	0,19

For logistical and space purposes, the trial was conducted in a time-replicated fashion with replicates one and two for all five treatment groups beginning on Study Day 1; replicates three and four for all five treatment groups began the trial 28 days later, and replicate five for all five treatment groups began the trial 56 days after the first cohort.

Each pen was equipped with a dry hopper feeder with six feed access holes, each measuring 13 cm x 13 cm, aligned parallel to the pen front. Feeders were located at the front of the pen, and a water drinker was located at the back of each pen. The pigs had *ad libitum* access to feed and water throughout the trial.

Pigs were individually weighed at the start of the study (Day 1) and at Days 28 (~20 kg), 84 (~50 kg) and 140 (~100 kg). All feed given was weighed daily, the feeders were emptied weekly and the remaining feed weighed.

117 Growth performance parameters were calculated for each stage of the production
118 cycle (Starter: Days 1-28, Grower: Days 29-84, and Finisher: Days 85-140) as well as for the
119 overall study period (Days 1-140).

120 Statistical analyses were performed using the Statistical Analysis System for Personal
121 Computers (SAS) Version 9.1 (SAS Institute, Cary, NC). Treatment effects were tested with
122 a combination of analysis of variance for continuous variables using the MIXED procedure
123 and Chi-square analysis, including the Cochrane-Armitage trend test. Dichotomous variables
124 (pigs clinically treated or removed) were created using the post-mortem and clinical results
125 from the data provided to analyze the number of pigs removed from or treated during the
126 study. The data were analyzed by logistic regression using the GLIMMIX procedure.

127 Trial 2. Pre-and Post-Wean Piglet Study

128 The trial was carried out at a commercial farm, Thai My Pig farm, Thai My commune,
129 in the Cu Chi district, Ho Chi Minh City from March 15 to May 30, 2016.

130 Forty-two (42) sows and their offspring were used in the two-stage, 49-day feeding
131 trial. Stage 1 evaluated the benefits of supplementation with OxBC in creep feed in pre-
132 weaned piglets. Stage 2 evaluated the benefits of continuing OxBC supplementation through
133 the post-wean Starter phase.

134 All piglets were the progeny of Landrace x Yorkshire females and Duroc boars and
135 were reared on site. The piglets were vaccinated against PRRS (Tai Xanh™, Navetco
136 Vietnam) at 14 days old.

137 After one day post-farrowing, sows with minimum litter sizes of ten piglets and
138 similar weights were selected. Litter sizes were also standardized by cross-fostering between

139 sows within treatment groups within three days post-farrowing. As most sows had litter sizes
140 greater than ten, surplus piglets were cross-fostered to sows that were not part of the trial.

141 **Stage 1. Pre-Wean (Study Days 0-21)**

142 In Stage 1, 42 lactating sows (one week post-farrow) and their one-week-old offspring
143 were randomly and evenly assigned to one of six treatments with seven replicate pens per
144 treatment. Each pen contained one sow and her litter of ten piglets. The mean piglet body
145 weight was 2.44 kg. As there were insufficient sows to carry out seven replicates
146 simultaneously, the trial was run in a time-replicated fashion, with two cohorts of sows. The
147 first cohort contained 18 sows with 180 piglets (six treatments and three replicates) and the
148 second cohort contained 24 sows with 240 piglets (six treatments and four replicates). The
149 second cohort began the trial one week after the first cohort.

150 The diets were introduced as soon as the piglets were ready to begin consuming creep
151 feed. Sows in all groups received a basal commercial lactation diet without OxBC
152 supplementation. Dietary treatments were delivered to piglets via supplementation of the
153 creep feed as follows: Control (basal diet with no antibiotic or OxBC), AB (basal diet with
154 antibiotics) and OxBC (basal diet with 2, 4, 8 or 16 ppm OxBC; no antibiotic). The
155 composition of the basal diet is shown in Table 2.

156 **Table 2. Composition of the basal diets for the Creep and Starter phases of the Pre- and**
157 **Post-Wean Study.**

Ingredient Name	**Pre-Wean** (g/kg)	**Post-Wean** (g/kg)
Corn	573.2	636.5
DDGS	-	30.0

Soybean meal 47%CP	120.0	200.0
Soya oil	30.0	31.5
Skimmed milk replacer	93.7	-
Whey powder	50.0	-
Blood Plasma Protein	43.0	39.3
Fish meal	70.0	33.5
Dicalcium phosphate	5.6	09.9
Calcium carbonate	02.5	06.8
Salt	-	02.5
Vitamin-mineral premix	5.0	03.0
L-Lysine HCl	2.7	03.6
Threamine	1.0	1.0
Methionine	1.8	1.6
Trypthophan	1.2	0.8
Sweetener	0.3	-
Total	**1000**	**1000**
Chemical composition		
Crude protein, %	22.21	21.29
Crude fiber, %	2.01	2.85
Crude fat, %	6.32	6.42
NE swine, kcal/kg	2557	2486
ME swine, kcal/kg	3275	3271
Calcium, %	0.85	0.75

Phosphorus, %	0.70	0.65
Available P, %	0.47	0.40
Sodium, %	0.29	0.28
Lysine, %	1.42	1.32
Methionine, %	0.54	0.45
Cystine, %	0.30	0.32
Met+Cys, %	0.85	0.79
Threonine, %	0.89	0.83
Tryptophan, %	0.31	0.29
Arginine, %	1.10	1.18
Isoleucine, %	0.79	0.73
Leucine, %	1.74	1.68
Valine, %	0.97	0.90
Histidine, %	0.52	0.52
Phenylalanine, %	0.92	0.92

Feed and water were provided *ad libitum*. Piglets were individually weighed at the start of the study (Day 1) and at weaning (Day 21). Feed given was weighed daily and unused feed was weighed at the end of each week.

Stage 2. Post-Wean (Days 22-49)

Stage 2 began at Study Day 22, one day post-wean, when the piglets were 29 days old. Three hundred and sixty (360) piglets were used in a random block design of six treatments with six replicate pens of ten piglets each. Piglets were randomized within their prior

166 treatment groups before being equally assigned into pens. Treatments were as follows:
167 Control (basal diet with no antibiotics or OxBC), AB (basal diet with antibiotics, no OxBC)
168 and OxBC (basal diet with 2, 4, 8 or 4 (16 in Stage 1) ppm OxBC; no antibiotics). The
169 composition of the basal diet is shown in Table 2. The piglets were provided feed and water
170 *ad libitum*. Feed given was weighed daily and any unused feed was weighed at the end of
171 each week.

172 The following measurements were performed: mean body weight (BW) at Days 1, 21
173 and 49 for each animal; average daily feed intake (ADFI): total feed intake per pen divided
174 by the product of the number of live animals and days on diet; ADG: total BW gained per pen
175 divided by the product of the number of live animals and days on diet; FCR: total feed
176 consumption divided by BW increase, defined as: (sum of final body weights of surviving
177 animals plus weight of mortalities and removals) – (sum of initial body weights including
178 initial bodyweight of animals dead or removed during the specified period); mortality and
179 diarrhea incidence, for each treatment group for both Stage 1 and Stage 2 of the study.
180 Diarrhea rate was determined as the percentage of animals presenting with loose or
181 watery stools.

182 Data were analyzed by ANOVA using Minitab Statistical Software (Minitab, State
183 College, PA, USA). Differences between means were tested using Fisher's multiple range
184 test when the F value was significant at $P< 0.05$. Data were considered significantly different
185 at $P<0.05$.

186 # Results

187 ## Trial 1. Full Grow Study

188 The effect of dietary supplementation with OxBC on the growth performance of pigs
189 is shown in Table 3. On Day 1, there were no significant differences in BWs among any of
190 the groups (P>0.05). Dietary supplementation with all three levels of OxBC during the Starter
191 period (Days 1 to 28) resulted in significant improvements in body weight (BW), average
192 daily weight gain (ADG), average daily feed intake (ADFI) and feed/gain ratio (F/G)
193 compared to the control group (P<0.001). Animals in the OxBC groups also performed better
194 than or equivalent to those receiving antibiotics during the starter period.

195 During the Grower period (Days 29-84), supplementation with all levels of OxBC
196 significantly increased ADG relative to the Control (P<0.001). The ADG of the AB group
197 was also higher than the Control (P<0.001), but there were no differences among the OxBC
198 and the AB groups. There was an apparent dose dependent decrease in feed consumption
199 across the three OxBC groups, with the ADFI of the 8 ppm OxBC group being significantly
200 lower than the Control (P<0.05). The F/G ratios for all OxBC groups and the AB group were
201 significantly improved compared to the Control (P<0.001). The 8 ppm OxBC group had the
202 lowest F/G ratio, which was significantly lower than the ratios for the Control, AB and 2 ppm
203 OxBC groups (P<0.001).

204 **Table 3. Effect of dietary OxBC on ADG, ADFI, F/G and BW of pigs in the Starter, Grower, Finisher and Overall periods.[1,2]**

Treatment:	Control	AB[3]	OxBC 2 ppm	OxBC 4 ppm	OxBC 8 ppm	Pooled SEM	P-value
Starter Period: Days 1-28							
BW Day 1	7.7±0.1	7.8±0.1	7.6±0.1	7.6±0.1	7.8±0.1	0.11	0.152
BW Day 28	17.7±0.3[b]	18.4±0.3[b]	19.8±0.5[a]	20.0±0.5[a]	20.2±0.5[a]	0.41	<0.001
ADG	358±8[b]	381±12[b]	435±20[a]	440±19[a]	442±15[a]	15	<0.001
(% Difference)[4]	-	6	22	23	23	-	-
ADFI	601±10[b]	607±20[b]	689±30[a]	676±27[a]	684±21[a]	23	<0.001
(% Difference)[4]	-	1	15	13	14	-	-
Feed/Gain	1.68±0.03[a]	1.59±0.03[b]	1.59±0.01[bc]	1.54±0.02[d]	1.55±0.01[cd]	0.02	<0.001
(% Difference)[4]	-	-5.4	-5.4	-8.3	-7.7	-	-

Grower Period: Days 29-84							
BW Day 84	49.6±0.3[c]	51.9±0.8[b]	53.2±0.4[a]	53.6±0.5[a]	53.8±0.5[a]	0.51	<0.001
ADG	569±2[b]	598±17[a]	596±5[a]	601±14[a]	600±11[a]	11	0.001
(% Difference)[4]	-	5.1	4.7	5.6	5.4	-	-
ADFI	1645±78[a]	1581±35[ab]	1625±33[ab]	1590±34[ab]	1542±29[b]	46	0.018
(% Difference)[4]	-	-3.9	-1.2	-3.3	-6.3	-	-
Feed/Gain	2.89±0.14[a]	2.73±0.03[b]	2.73±0.05[b]	2.64±0.04[bc]	2.57±0.08[c]	0.08	<0.001
(% Difference)[4]	-	-5.5	-5.5	-8.7	-11.1	-	-
Finisher Period: Days 85-140							
BW Day 140	99.5±0.5[c]	101.7±0.5[b]	103.6±0.5[a]	104.2±0.3[a]	104.4±0.5[a]	0.44	<0.001
(% Difference)[4]	-	2.2	4.1	4.7	4.9	-	-
ADG	892±13	889±8	901±9	902±7	904±9	9.3	0.056

(% Difference)[4]	-	0.3	1.0	1.1	1.3	-	-
ADFI	3085±114[a]	3031±102[ab]	2959±87[ab]	2897±47[b]	2876±49[b]	84	0.004
(% Difference)[4]	-	-1.8	-4.1	-6.1	-6.8	-	-
Feed/Gain	3.46±0.14[a]	3.41±0.13[a]	3.28±0.08[ab]	3.21±0.05[b]	3.18±0.03[b]	0.09	<0.001
(% Difference)[4]	-	-1.4	-3.8	-2.1	-0.9	-	-
Overall: Days 1-140							
ADG	656±4[c]	671±3[b]	686±4[a]	690±1[a]	690±3[a]	3.2	<0.001
(% Difference)[4]	-	2.3	4.6	5.2	5.2	-	-
ADFI	2012±77[a]	1966±50[ab]	1971±41[ab]	1930±25[ab]	1904±30[b]	48	0.021
(% Difference)[4]	-	-2.3	-2.0	-4.1	-5.4	-	-
Feed/Gain	3.07±0.12[a]	2.93±0.06[b]	2.87±0.05[bc]	2.80±0.04[c]	2.76±0.04[c]	0.07	<0.001
(% Difference)[4]	-	-4.6	-6.5	-8.8	-10.1	-	-

206 [1] Values represent means ± SD. Values in the same row with different superscripts, a,b,c, are significantly different ($P<0.05$).

207 [2] Units: BW: kg/head; ADG, ADFI: g/head/d.

208 [3] Antibiotics were included in the feed as follows: Pre-wean (nursery): 100 ppm colistin sulfate; Starter (days 0-28): 150 ppm

209 chlortetracycline and 100 ppm colistin sulfate; Grower (days 29-84): 150 ppm chlortetracycline; Finisher (days 85-140): no antibiotics.

210 [4] Percent difference compared to Control.

211 During the Finisher stage (Days 85-140), the ADGs of animals in the OxBC groups
212 were greater than those of either the Control or AB groups, although the differences did not
213 reach statistical significance (P>0.05). Supplementation with 4 or 8 ppm OxBC resulted in
214 significant improvements in the F/G ratio compared to the Control and AB groups (P<0.001).
215 As was observed in the grower period, animals receiving OxBC had lower feed intake during
216 the finisher phase when compared to the Control. The feed intake was significantly lower
217 (P<0.005) in the 4 ppm OxBC and 8 ppm OxBC groups compared to the Control group. Note
218 that during the finisher stage, animals in the AB group did not receive antibiotics, which is a
219 common practice and legal requirement in many jurisdictions, including Vietnam.
220 Overall (Study Days 1-140), dietary supplementation with OxBC at 2, 4 or 8 ppm led
221 to significant increases in final BW and ADG, relative to both the Control and the AB groups
222 (P<0.001). OxBC also reduced the overall F/G (P<0.001) and ADFI (P<0.05) compared to
223 the Control with the 4 and 8 ppm groups also outperforming the AB group on F/G (P<0.001).
224 In terms of clinical health, the results presented in Table 4 show that the highest
225 incidences of both diarrhea and mortality were observed in the Control group for all study
226 periods, with the lowest being observed in the OxBC groups. The highest incidence of
227 diarrhea was during the Starter period, and OxBC significantly and dose-dependently reduced
228 the diarrhea rate (P<0.001). Diarrhea was the lowest (less than half that of the Control group)
229 in the 8 ppm OxBC group, and both the 4 and 8 ppm OxBC groups had significantly reduced
230 incidences relative to the AB group (P<0.001).

231 **Table 4**. **Effect of dietary OxBC on diarrhea incidence and overall mortality of pigs in the Starter, Grower, Finisher and Overall**

232 **periods.[1]**

Treatment:	**Control**	**AB[2]**	**OxBC 2 ppm**	**OxBC 4 ppm**	**OxBC 8 ppm**	**Pooled SEM**	**P-value**
Starter Period: Days 1-28							
Diarrhea (%)	7.9±0.6[a]	6.6±0.5[b]	6.0±0.2[b]	4.9±0.4[c]	3.7±0.6[d]	0.47	<0.001
(% Difference)[3]	-	-17	-25	-39	-54	-	-
Grower Period: Days 29-84							
Diarrhea (%)	2.7±0.2[a]	2.1±0.1[b]	2.0±0.2[b]	1.6±0.1[c]	1.4±0.1[c]	0.16	<0.001
(% Difference)[3]	-	-21	-26	-41	-48	-	-
Finisher Period: Days 85-140							
Diarrhea (%)	0.87±0.06[a]	0.70±0.06[b]	0.64±0.07[bc]	0.67±0.06[b]	0.55±0.08[c]	0.06	<0.001
(% Difference)[3]	-	-20	-26	-23	-37	-	-

Overall: Days 1-140

Diarrhea (%)	3.1±0.1[a]	2.5±0.1[b]	2.3±0.1[c]	1.9±0.1[d]	1.5±0.1[e]	0.10	<0.001
(% Difference)[3]	-	-19	-27	-39	-50	-	-
Mortality (%)	5.0±3.5	3.0±2.7	2.0±2.7	2.0±2.7	2.0±2.7	2.9	0.431

[1] Values represent means ± SD. Values in the same row with different superscripts, a,b,c, etc., are significantly different ($P<0.05$).

[2] Antibiotics were included in the feed as follows: Pre-wean (nursery): 100 ppm colistin sulfate; Starter (Days 1-28): 150 ppm chlortetracycline and 100 ppm colistin sulfate; Grower (Days 29-84): 150 ppm chlortetracycline; Finisher (Days 85-140): no antibiotics.

[3] Percent difference compared to Control.

240 The occurrence of diarrhea during the Grower and Finisher periods was markedly less
241 compared to the Starter period. Despite the lower background incidence of diarrhea in these
242 later growth phases, OxBC still continued to provide significant and dose-dependent
243 protection from the condition.

244 Overall, animals in the Control group had the highest incidence, whereas animals in
245 the 8 ppm OxBC group had the lowest incidence. OxBC significantly and dose-dependently
246 reduced the incidence relative to both the Control and AB groups ($P<0.001$).

247 Mortality was relatively low, overall, with few pigs dying during the study. The lowest
248 number of deaths occurred in the OxBC groups, but differences were not significant between
249 any of the groups.

250 Trial 2: Pre-and Post-Wean Piglet Study

251 Stage 1 Pre-Weaning (Days 1-21)

252 The effects of OxBC and antibiotics on the growth performance of pre-weaning
253 piglets are provided in Table 5.

254 Inclusion of OxBC in the creep feed resulted in a dose-dependent trend towards
255 increased ADG and BW that reached significance for 16 ppm OxBC compared to the Control
256 over the 21 days of Stage 1. The ADG and BW of the AB group were also higher than the
257 Control. Antibiotics and 16 ppm OxBC improved the F/G ratio compared to the Control,
258 while the ADFI was not significantly different across the 6 treatment groups ($P>0.05$).

259 The very low F/G ratio observed for all groups is attributed to the small amounts of
260 feed ingested by the piglets, which were still mainly consuming milk from the sows.

261

262 **Table 5. BW, ADG, ADFI and F/G of pre-weaning piglets in Stage 1 of Trial 2 (Days 1-21)[1,2]**

Treatment:	**Control**	**AB[3]**	**OxBC 2 ppm**	**OxBC 4 ppm**	**OxBC 8 ppm**	**OxBC 16 ppm**	**SEM**	**P-Value**
BW Day 0	2.45±0.07	2.44±0.09	2.43±0.08	2.45±0.10	2.46±0.05	2.45±0.07	0.078	0.976
(% Difference)[4]	-	-0.4	-0.8	0.0	0.8	0.4		
BW Day 21	6.88±0.07[c]	7.21±0.07[a]	6.92±0.17[bc]	6.94±0.12[bc]	6.98±0.08[bc]	7.10±0.13[ab]	0.11	0.001
(% Difference)[4]	-	4.8	0.6	0.9	1.3	3.2		
ADG	211±2.3[c]	227±2.8[a]	214±7.5[bc]	214±2.9[bc]	215±4.2[bc]	222±7.8[ab]	5.1	0.001
(% Difference)[4]	-	8.1	1.9	1.9	2.4	5.2		
ADFI	79.9±0.9	80.1±0.9	79.9±0.9	79.7±1.2	80.5±1.1	79.5±1.5	1.1	0.592
(% Difference)[4]	-	0.9	0.1	-0.3	0.9	-0.4		
Feed/Gain	0.38±0.01[a]	0.35±0.01[c]	0.37±0.01[ab]	0.37±0.01[ab]	0.37±0.01[ab]	0.36±0.02[bc]	0.01	0.001
(% Difference)[4]	-	-7.9	-2.6	-2.6	-2.6	-5.3		
Intake of Active[5]	0	8.05	0.16	0.32	0.64	1.27	-	-

263

264 [1] Values represent means ± SD. Values in the same row with different superscripts, a,b,c, are significantly different (P<0.05).

265 [2] Units: BW: kg/head; ADG, ADFI: g/head/d; Intake of Active: mg/d.

266 [3] The AB diet contained 100 ppm colistin sulfate.

267 [4] Percent difference compared to Control.

268 [5] Average daily intake (mg) of antibiotic or OxBC.

269 No evidence of diarrhea or mortality was observed in Stage 1.

270 **Trial 2: Stage 2 (Days 22-49)**

271 The effects of OxBC and antibiotics on BW, ADG, ADFI and F/G for weaned piglets
272 are shown in Table 6.

273 At the beginning of Stage 2, a subset of select piglets from each treatment group was
274 randomized within treatment groups to continue the trial. Piglets were selected such that the
275 average BW among the six treatment groups did not differ on Day 22 (the beginning of Stage
276 2). After 28 days (Day 49, the end of Stage 2), average BWs were highest for the AB group
277 followed by the four OxBC groups and the Control. Increases in average BW reached
278 statistical significance for both the AB and the 8 ppm OxBC groups ($P<0.001$).

279 The increases in BW were reflected in the ADG. The AB group had the highest ADG,
280 which was significantly higher than the Control ($P<0.001$). ADG showed a trend towards a
281 dose-dependent increase in the OxBC groups, reaching statistical significance for the 8 ppm
282 OxBC group compared to the Control. However, there was no statistical difference in ADG
283 across the four OxBC groups.

284 There were no significant differences in ADFI among the treatment groups. F/G ratios
285 were significantly improved for both the AB group and the 8 ppm OxBC group compared to
286 all other groups ($P<0.001$).

287 The effects on the incidence of diarrhea and mortality are given in Table 7.

288 **Table 6. BW, ADG, ADFI and F/G of weaned piglets in Stage 2 of Trial 2 (Days 22-49).[1,2]**

Treatment:	Control	AB[3]	OxBC 2 ppm	OxBC 4 ppm	OxBC 8 ppm	OxBC 4 ppm[4]	SEM	P-Value
BW Day 21	6.91±0.07	6.95±0.08	6.93±0.06	6.94±0.09	6.93±0.06	6.98±0.07	0.07	0.697
(% Difference)[5]	-	0.4	0.3	0.4	0.1	1.0		
BW Day 49	17.74±0.12[c]	18.80±0.25[a]	17.94±0.50[bc]	18.00±0.47[bc]	18.32±0.09[ab]	18.23±0.07[bc]	0.31	0.001
(% Difference)[5]	-	6.0	1.1	1.5	3.3	2.8		
ADG	387±5[c]	423±7[a]	393±16[bc]	395±16[bc]	407±3[ab]	402±2[bc]	10	0.001
(% Difference)[5]	-	9.6	1.8	2.3	5.4	3.9		
ADFI	639.7±7.4	652.4±7.5	640.2±15	639.5±3.7	637.5±7.1	643.7±5.7	2.2	0.087
(% Difference)[5]	-	2.0	0.1	0.0	-0.3	0.6		
Feed/Gain	1.65±0.04[a]	1.54±0.03[c]	1.63±0.08[ab]	1.62±0.06[ab]	1.57±0.01[bc]	1.60±0.01[ab]	0.035	0.001
(% Difference)[5]	-	-6.7	-1.2	-1.8	-5.5	-3.0		
Intake of Active[6]	0	59.2 CS 88.8 CTC	1.16	2.32	4.62	2.33	-	-

289

290 [1] Values represent means ± SD. Values in the same row with different superscripts, a,b,c, are significantly different ($P<0.05$).

291 [2] Units: BW: kg/head; ADG, ADFI: g/head/d; Intake of Active: mg/d.

292 [3] The AB diet contained 100 ppm colistin sulfate (CS) and 150 ppm chlortetracycline (CTC).

293 [4] The treatment provided 16 ppm OxBC during Stage 1 and 4 ppm OxBC during Stage 2. The supplementation was higher during Stage

294 1 to account for the low feed intake of nursing piglets.

295 [5] Percent difference compared to the Control.

296 [6] Average daily intake (mg) of antibiotic or OxBC.

297 **Table 7. Diarrhea incidence and mortality in weaned piglets during Stage 2 (Days 22-49)**

298

Treatment:	**Control**	**AB[1]**	**OxBC 2 ppm**	**OxBC 4 ppm**	**OxBC 8 ppm**	**OxBC 4 ppm[2]**
Diarrhea (%)	4.7±0.8	3.4±1.1	4.6±1.3	4.1±0.9	3.6±1.1	3.9±0.8
(% Difference)[3]	-	-28	-1	-12	-24	-18
Mortality (%)	5.0	0	3.4	3.4	0	0
% of Control	(100)	0	67	67	0	0

299

300 [1] The AB diet contained 100 ppm colistin sulfate and 150 ppm chlortetracycline.

301 [2] The treatment provided 16 ppm OxBC during Stage 1 and 4 ppm OxBC during Stage 2. The supplementation was higher during

302 Stage 1 to account for the low feed intake of nursing piglets.

303 [3] Percent difference compared to the Control.

304

305 Diarrhea and mortality incidence were generally low in all groups, with the highest
306 incidence of diarrhea and mortality being observed in the Control group. Treatment with 8
307 ppm OxBC or antibiotics decreased diarrhea incidence by 24% and 28%, respectively.
308 No mortalities were recorded in the AB group, the 8 ppm OxBC group or the group
309 fed 16 ppm OxBC in Stage 1 and 4 ppm OxBC in Stage 2. The differences in mortalities
310 between the groups were not significant.

311 Discussion

312 Results from the two trials reported here demonstrate that dietary supplementation
313 with low parts-per-million levels of OxBC improves the health and productivity of pigs reared
314 under commercial production conditions. In Trial 1, conducted over the entire 140-day post-
315 wean grow-out period, inclusion of OxBC in the feed led to improvements in growth
316 performance during each of the three stages of the production cycle and also significantly
317 reduced the incidence of diarrhea in post-wean starter piglets. Results from the second trial
318 confirm the benefits of dietary OxBC on growth performance of post-wean starter piglets and
319 indicate that further benefits on piglet performance are possible when OxBC is added to the
320 transition (creep) feed of nursing piglets prior to weaning.
321 In Trial 1, the observation that the benefits of OxBC were most apparent during the
322 starter period compared to the grower and finisher periods is not surprising, given that OxBC
323 plays a role in supporting immunity [1-3] and the fact that young piglets experience high
324 levels of stress associated with weaning and that they are immunologically immature. The
325 highest incidence rates of diarrhea occurred during the starter period, consistent with the well-
326 known susceptibility of young piglets to post-wean diarrhea [7]. The reduced incidence of
327 diarrhea observed in the OxBC supplemented groups relative to controls is consistent with

328 the immune priming actions of OxBC, which center upon an ability to increase the level of
329 pathogen pattern recognition receptors (PPRRs) and enhanced down-stream innate immune
330 response to receptor activation [2].

331 More specifically, OxBC has been shown to increase the level of toll-like receptor
332 subtypes 2 and 4 (TLR-2, TLR-4) and their coreceptor CD14 in both *in vitro* and *in vivo*
333 studies [2]. TLR-4 and CD14 are well-characterized co-receptors responsible for recognizing
334 the lipopolysaccharide (LPS) moiety present in gram negative bacteria, such as *E. coli,* while
335 TLR-2 recognizes the lipoteichoic acid (LTA) moiety present on gram positive bacteria, such
336 as *Clostridium perfringens* [8-10]. Binding of bacterial LPS or LTA to the TLR-4/CD14 or
337 TLR-2 receptor triggers an innate immune response aimed at clearing the bacteria [11, 12].

338 A study comparing *E. coli* resistant versus susceptible lineages of pigs found that the
339 genetic basis for the resistance to *E. coli* colonization of the gut was higher levels of CD14
340 expression [13]. The authors concluded that the TLR-4/CD14 pathway plays a significant role
341 in reducing *E. coli* colonization and the incidence of diarrhea in piglets. While we did not
342 measure intestinal receptor or *E. coli* levels in the current trials, an increase in intestinal CD14
343 and TLR-4 content (as we have observed in mice fed OxBC [2]) and the subsequent reduction
344 in *E. coli* colonization offers a plausible explanation for the reduced incidence of diarrhea
345 observed in the OxBC-supplemented piglets. Results from an infectious challenge study in
346 broilers, where dietary supplementation with OxBC reduced *Clostridium perfringens* levels
347 by 2 to 3 log units, were consistent with the concept that OxBC supports the intestinal immune
348 system and allows the host to resist pathogen colonization [6].

349 The improvement in clinical health (reduced incidence of diarrhea) likely explains, in
350 part, the improved growth performance of piglets in the OxBC-supplemented versus control
351 groups during the Starter period in Trial 1. As would be expected for older, more

352 immunocompetent pigs, there were fewer incidences of diarrhea in grower and finisher pigs
353 compared to starter pigs across all treatment groups in the first trial. While there tended to be
354 a lower incidence of diarrhea in the OxBC supplemented groups compared to the control
355 during the grower and finisher periods, it seems unlikely that these small reductions in disease
356 incidence would explain the improved growth performance observed in the OxBC groups
357 during the latter stages of the trial. Instead, the ability of OxBC to reduce inflammatory tone
358 represents the most plausible mechanism to explain the improved growth of older pigs in the
359 OxBC groups.

360 The intestine has been described by some authors as a tissue in a constant state of
361 controlled inflammation, and there is a need for organisms to tightly control the level of
362 intestinal inflammation [14, 15]. Commercial production practices are known to further
363 exacerbate the inflammatory state of the intestine, as stresses associated with weaning, *ad*
364 *libitum* feeding of high energy diets, and the presence of anti-nutritional components in the
365 diet all contribute to increased inflammation of the gut [16]. The contribution that reduced
366 inflammation plays in improving growth performance is further highlighted by the fact that
367 an anti-inflammatory action has been proposed as an underlying mechanism contributing to
368 the growth promoting effects of subtherapeutic antibiotic growth promoters [15].

369 Results from studies in an experimental model of bovine respiratory disease (BRD)
370 and in gestating/lactating sows demonstrate an anti-inflammatory action for OxBC. In the
371 experimental BRD trial, animals that received dietary supplementation with OxBC had a
372 significant increase in anti-inflammatory or pro-resolution activity in the lung [3] while
373 OxBC-supplementation in gestating/lactating sows led to reductions in the concentration of
374 pro-inflammatory cytokines, TNFα and IL-8 in colostrum and milk [4]. Reduced

375 inflammation may also be partly responsible for the observed reduction in intestinal lesion
376 severity in *C. perfringens*-challenged broilers [6].

377 The performance benefits of OxBC in weaned piglets observed in Trial 1 are
378 supported by the results from Trial 2 in which inclusion of OxBC in the Starter feed led to an
379 apparent dose-dependent trend for improved growth performance and feed efficiency, with
380 the highest inclusion level of OxBC (8 ppm) reaching significance. In terms of clinical health,
381 the results from Trial 2 indicate that, while the incidences of diarrhea were numerically lower
382 in the antibiotic and OxBC supplemented groups compared to the control, there was no
383 significant treatment effect (Table 7). There were considerably fewer cases of diarrhea
384 observed in the control group in Trial 2 (4.7%) compared to Trial 1 (7.9%). This difference
385 in the background incidence of diarrhea in the two trials likely reflects a lower level of
386 endemic pathogen pressure during Tial 2 relative to Trial 1. With a relatively low incidence
387 of diarrhea observed in the control group in Trial 2, further treatment-induced reductions may
388 not have been possible. Thus, in Trial 2 the benefits of OxBC on growth performance of post-
389 wean piglets is more plausibly due to anti-inflammatory actions rather than a reduction in the
390 incidence of diarrhea. It is interesting to note that the magnitude of the performance benefits
391 in piglets was much larger in Trial 1 compared to Trial 2. The difference in the magnitude of
392 the benefits may have been due to a combined benefit of reducing diarrhea and background
393 inflammation in the gut in Trial 1, while in the second trial, where there was lower endemic
394 pathogen pressure, only the anti-inflammatory activity may have factored in the benefit.

395 The results from the pre-wean period (Stage 1) of Trial 2 indicate that inclusion of
396 OxBC in transition or creep feed can also improve weight gain and body weight of nursing
397 piglets. OxBC appeared to dose-dependently increase ADG and body weight at weaning with
398 the highest inclusion rate of 16 ppm OxBC reaching statistical significance (Table 6). The

399 lack of a statistically detectable effect at the lower OxBC inclusion rates likely reflects the
400 very low amounts of OxBC received by nursing piglets that consumed relatively little feed.
401 Given the apparent dose-dependent improvements in growth performance, it would be useful
402 to investigate higher inclusion rates of OxBC in transition feed or OxBC-supplementation of
403 milk replacer. There were no mortalities or incidences of diarrhea observed during the pre-
404 wean stage of Trial 2, consistent with the presence of a relatively low level of endemic
405 pathogen pressure.

406 Overall, the results of the two trials reported here demonstrate the benefits of dietary
407 supplementation with OxBC on growth performance, feed efficiency, and the health of pre-
408 and post-weaned piglets. These findings have significance for both the commercial feed
409 industry and the field of nutrition. For the feed industry, the findings support the application
410 of OxBC as an effective alternative to antibiotic growth promoters. The performance and
411 health benefits obtained with OxBC were comparable to those of the antibiotic growth
412 promoters in both trials. For the field of nutrition, the results lend further support to the
413 identification of the copolymers present in OxBC as a source of the benefits of dietary β-
414 carotene. The concept that β-carotene is a source of beneficial compounds beyond vitamin A
415 is not new; however, there is a dearth of reproducible evidence as to the identity and biological
416 actions of these compounds. A scientific panel of the European Food Safety Association
417 (EFSA) recently concluded there was insufficient scientific evidence to support a proposed
418 benefit of β-carotene, as a feed additive, on host immunity [17]. The EFSA panel cited an
419 inconsistency of results with β-carotene as the major problem. The inconsistent results cited
420 by EFSA likely stem from the fact that the proposed immunological benefits arise not from
421 vitamin A or a direct action of β-carotene itself but rather from the presence of copolymers,
422 which were unknown at the time. Dietary supplementation with β-carotene potentially

423 provides variable levels of copolymers, depending upon the extent of adventitious oxidation.
424 β-Carotene's susceptibility to oxidation is recognized by manufacturers who nowadays
425 formulate the product to help prevent its oxidation.

426 In the trials reported here, the source of the β-carotene copolymers is a commercial
427 product manufactured by a highly reproducible process under stringent quality control
428 standards. However, the copolymers have also been shown to exist naturally at varying levels
429 in various food and feedstuffs [18, 19]. The presence of naturally occurring copolymers may
430 provide an explanation, at least in part, for the health and productivity benefits associated
431 with the use of certain traditional forages. For example, alfalfa, a forage rich in β-carotene,
432 has been proposed to contain unidentified growth factors that benefit the growth and
433 reproductive performance of various species [20-22]. The mechanisms of action of the so-
434 called unidentified growth factors remains largely unknown; however the presence of
435 naturally occurring copolymers arising from oxidation of β-carotene when alfalfa is dried [18]
436 represents one possible growth factor.

437 We propose that β-carotene is the source of two classes of compounds that are
438 biologically active and which both benefit health, the first being vitamin A and the second
439 being β-carotene oxygen copolymers. Driven by the desire to reduce costs, the modern feed
440 industry has largely replaced its use of β-carotene, whether synthetic or natural, with synthetic
441 vitamin A. Removal of β-carotene, particularly natural sources, such as alfalfa, from diets has
442 resulted in the simultaneous, albeit unintended, removal of the naturally present β-carotene
443 copolymers and their associated benefits. The positive effects on growth performance and
444 health observed with OxBC in these trials coupled with similar findings in previous trials in
445 swine [4, 5], poultry [6] and dairy calves [3] demonstrate that re-introduction of very low

446 amounts of β-carotene oxygen copolymer into feeds can beneficially support the health and
447 productivity of livestock and substantially reduce the need for in-feed antibiotics.

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