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Original XPC™ reduces *in vitro* growth of *Clostridium perfringens* & *septicum*

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Introduction

In a previous issue of *Poultry Advisor* (July 2013), results were presented from a recent study on the effects of feeding Original XPC™ (1.25 kg/MT or 2.5 lb/t) to broilers challenged with *Clostridium perfringens*. In that study, feeding Original XPC significantly 1) reduced intestinal lesions; 2) increased body weight gain; and 3) improved feed conversion in birds challenged with *C. perfringens* compared to challenged untreated controls. Interestingly, when Original XPC was included with either virginiamycin (Stafac) or bacitracin (BMD), these product combinations were statistically more effective than the inclusion of either product alone. Furthermore, feed conversion of Original XPC-Stafac fed birds was similar to that of the unchallenged control group.

To further investigate the effects of Original XPC on clostridia, the results reported here are from challenge trials using an *in vitro* model (IAMM: Intestinal Activity Modifier Model[1]).

Trial Design

Two separate trials were conducted to evaluate the effects of Original XPC using an *in vitro* intestinal model on terminal pH, volatile fatty acids (VFA) and subsequent growth of either *C. perfringens* or *C. septicum* following a challenge.

In both trials, broiler chickens were used as donors for fecal inoculum. Fecal material was collected over a period of 2 hours and transferred to the lab for

processing. IAMM procedures were performed as described in the previously reported methods (1). Ten replicates were included for each treatment.

In Trial 1, the challenge dose for *C. perfringens* was 2.7 x 10^4 CFU/ml, and in Trial 2, the challenge dose for *C. septicum* was 7.5 x 10^4 CFU/ml.

After a 24-hour incubation in Trial 1, serially diluted samples were plated on *C. perfringens* selective medium (TSC) consisting of an agar base (CM05878B-Preston), and the selective supplement (Oxoid # SR0088E). Plates were incubated at 37°C, under strict anaerobic conditions (90% N; 5% H_2; 5% CO_2). *C. perfringens* counts from this study are reported in log 10 CFU/ml.

In Trial2, real time PCR was performed on 20 ml of the fermentation mixture from IAMM that was centrifuged at 8,000 rpm for 10 minutes. The resulting pellet was subjected to DNA extraction using a kit from Zymo Research. DNA was quantitated using a NanoDrop and diluted to 10 ng/µL. 2 µL of DNA was used per reaction. Each reaction was run in triplicate. SsoFast Evagreen Supermix (BioRad) was used to amplify with the Universal Bacteria primers (3). iTaq Universal SYBR Green Supermix (BioRad) was used to amplify with the *C. septicum* primers (4). *C. septicum* data from this study are presented as % total bacteria DNA.

Gas Chromatography (GC) was used to determine Volatile Fatty Acid (VFA) concentrations in both studies.

All data were analyzed using ANOVA (JMP 8); the Students T test was used to compare treatment means. Means that differed at $P < 0.05$ were declared statistically significant.

Results and Discussion

Trial 1: *C. perfringens*
Compared to the Control Treatment, Original XPC significantly:

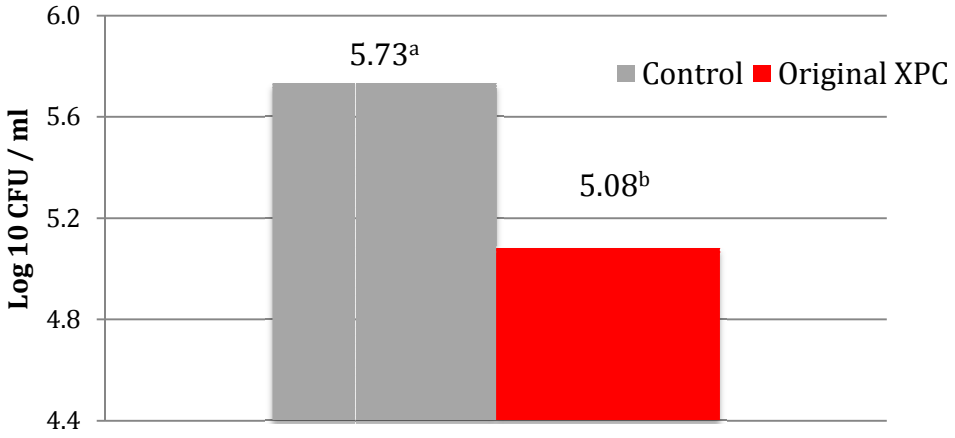
- Reduced pH (*Table 1*)
- Increased VFA concentrations by 19%
- Reduced *C. perfringens* concentrations by 0.65 log (Figure 1)

Table 1. IAMM results for *C. perfringens* challenge – Trial 1

Treatment	Control	XPC	SE
pH	6.59[b]	6.55[a]	0.005
Total VFA (mM)	39.9[a]	47.7[b]	0.60

[a,b] Means with different superscripts differ at p<0.05

Figure 1. *C. perfringens* concentration in Trial 1



a,b Means with different superscripts differ at p<0.05

Trial 2: *C. septicum*
Compared to the Control treatment, Original XPC significantly:

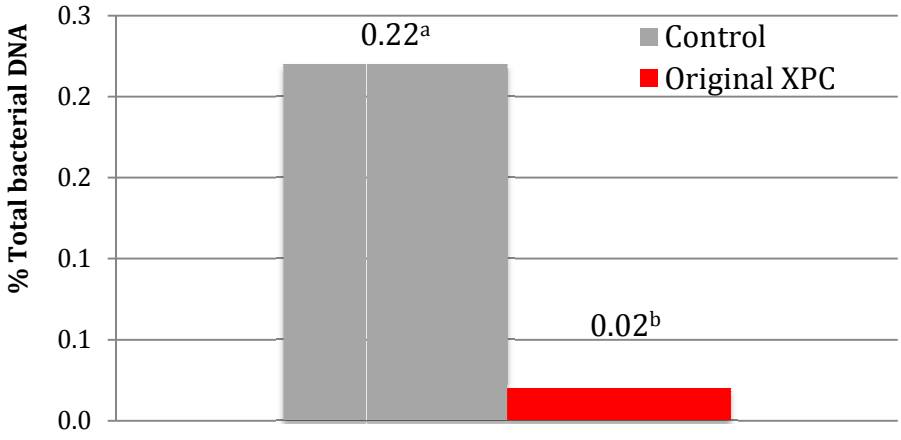
- Reduced pH (*Table 2*)
- Increased total VFA concentrations by 15%
- Reduced the proportion of total bacterial DNA accounted for by *C. septicum* to less than a tenth of that measured in the Control treatment (Figure 2)

Table 2. IAMM results for *C. septicum* challenge – Trial 2

Treatment	Control	Original XPC	SE
pH	6.71b	6.66a	0.01
Total VFA (mM)	38.4a	44.1b	1.06

a,b Means with different superscripts differ at p<0.05

Figure 2. *C. septicum* concentration in Trial 2



a,b Means with different superscripts differ at p<0.05

Observations

- Compared to the Control in Trial 1, the inclusion of Original XPC enhanced fermentation and suppressed the growth of *C. perfringens*.
- In Trial 2, the inclusion of Original XPC enhanced fermentation and suppressed the growth of *C. septicum* compared to the Control.
- The results of *in vitro* assays further demonstrate the role of Original XPC as a tool for managing the Clostridia growth *in vivo* and help explain the changes seen in clinical signs when live birds are challenged with clostridia.

One footnote to the enumeration of clostridia herein, is the advancement from culture techniques and plate counts to the use of real time PCR capabilities. While the culture of live organisms can reveal the present viable concentration of bacteria such as clostridia, at best the measurement becomes a snap shot in time. Through real time PCR, we can measure the cumulative total DNA of target organisms irrespective of viability of the organism at the time analysis. In the current study with *C. septicum*, using real time PCR demonstrated that Original XPC reduced the concentration of *C. septicum* DNA to one-tenth that in the Control treatment.

References

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