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Short communication

Modulation of the acute phase response following a lipopolysaccharide challenge in pigs supplemented with an all-natural *Saccharomyces cerevisiae* fermentation product☆,☆☆

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ABSTRACT

This study was designed to determine if feeding a *Saccharamyces cerevisiae* fermentation product to weaned pigs would reduce the acute phase response (APR) following an lipopolysaccharide (LPS) challenge. Pigs (n = 20; 6.4 ± 0.2 kg BW) were obtained and transported to a nursery facility. Pigs were housed individually with ad libitum access to feed and water. Pigs were weighed upon arrival and assigned to 1 of 2 treatment diets (n = 10 pigs/treatment) for 18 d: 1) non-medicated starter diet (Control); 2) Control diet + *Saccharomyces cerevisiae* fermentation product (XPC; 2 kg/MT; Diamond V Original XPC™). Pigs were anesthetized on d 7 and 14 for insertion of an intraperitoneal (IP) temperature recording device and jugular catheter, respectively. On d 15, pigs were challenged i.v. with LPS (25 μg/kg BW). Blood samples were collected at 0.5-h (serum) and 1-h (complete blood cell counts) intervals from −2 to 8 h and at 24 h relative to LPS challenge at 0 h. There was a treatment effect ($P \leq 0.002$) for serum TNF-α and IL-6 such that concentrations were greater in XPC-supplemented pigs than Control pigs (116.5 ± 5.0 vs. 90.9 ± 5.2 pg/mL TNF-α; 188.3 ± 13.4 vs. 133.4 ± 11.7 pg/mL IL-6; respectively) following LPS challenge. Administration of LPS increased IP temperature ($P < 0.01$), however, there was no effect of treatment ($P = 0.12$). Thus, feeding a *Saccharamyces cerevisiae* fermentation product to weaned pigs increased the pro-inflammatory cytokine response to an LPS challenge.

1. Introduction

The animal industry is in a progressive age of change, with new regulations and pressure from consumers and producers. Changes are occurring in housing regulations, animal care, and products used to promote growth and maintain health. Changes in the regulation of the use of antibiotics for growth promotion have led producers to seek alternatives to antibiotics in order to ensure continued growth and health of the animals under their care. Various pre-, para- and probiotic products have been demonstrated to be effective in modulating immune function (Broadway et al., 2015). One particular product, a *Saccharomyces cerevisiae* fermentation product (Original XPC, Diamond V; Cedar Rapids, IA, USA), has been used to improve performance in sows. Specifically, studies have found increased milk production (Shen et al., 2011), improved gain of litters during lactation (Kim et al., 2008; Frank and Dorton, 2013) and improved sow health when sows were fed XPC (Shen et al., 2011). While performance data has been published, there is little research on the ability of this fermentation product to modulate inflammation. Therefore, this study was designed to determine whether feeding a *Saccharomyces cerevisiae* fermentation product to weaned pigs would reduce the acute phase response following an acute lipopolysaccharide (LPS) challenge.

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2. Materials and methods

2.1. Experimental design

All experimental procedures were in compliance with the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* and approved by the Institutional Animal Care and Use Committee at the Livestock Issues Research Unit.

Twenty weaned gilts (PIC 29 × 380; 19–21 days of age; 6.4 ± 0.2 kg BW) were obtained from a university swine farm and transported (approximately 925 km) to the USDA-ARS Livestock Issues Research Unit's environmentally controlled swine nursery facility in Lubbock, TX, USA. Pigs weighed 6.3 and 6.2 ± 0.35 kg on d 0, and 9.3 and 9.2 ± 0.35 kg on d 18 for Control and XPC-supplemented pigs, respectively. Pigs were housed individually in stainless steel pens equipped with nipple waterers and stainless steel feeders allowing ad libitum feed and water intake. Although pigs were housed individually, they were able to see, hear, and touch neighboring pigs. Pigs were weighed upon arrival, assigned to 1 of 2 groups (n = 10/treatment), and fed for 18 d: 1) Control, a non-medicated starter diet; 2) Control diet + *Saccharomyces cerevisiae* fermentation product fed at 2 kg/MT (XPC; Diamond V Original XPC, Cedar Rapids, IA, USA). On d 7, pigs were anesthetized and a small incision (2–2.5 cm) was made in the lower abdominal region for the placement of an indwelling intraperitoneal (IP) temperature recording device (25.4 mm in length, 8.3 mm in diameter, 3.3 g; Star Oddi DST micro-T; MeterMall USA, Marysville, OH, USA), measuring at 5-min intervals, into the peritoneal cavity. On d 14, pigs were anesthetized and fitted with indwelling jugular vein catheters (Carroll et al., 1999). On d 15, pigs were challenged i.v. with 25 μg/kg BW LPS from E. coli O111:B4 (Sigma Aldrich, St. Louis, MO, USA). A 4.5-mL whole blood sample was collected in Starstedt tubes containing no additive (Sarstedt Inc., Newton, NC, USA) at 0.5-h intervals from −2 to 8 h, and at 24 h relative to the LPS challenge at 0 h. Samples were allowed to clot at room temperature for 30 min prior to centrifugation at 1500 g for 20 min at 4 °C. Isolated serum was stored until analyzed for cytokine concentrations. A second 4-mL sample was collected in a vacutainer containing EDTA at 1-h intervals from −2 to 8 h and again at 24 h for determination of complete blood counts using a ProCyte Dx Hematology Analyzer (IDEXX, Westbrook, ME, USA).

2.2. Serum analysis

Serum cytokine concentrations (TNF-α, IFN-γ, and IL-6) were determined in duplicate by a custom porcine 3-plex sandwich-based chemiluminescence ELISA kit (Searchlight-Aushon BioSystems, Inc., Billerica, MA, USA). The intra-assay coefficients of variation were less than 6.1% and inter-assay coefficients of variation were less than 8.3% for all assays.

2.3. Statistical analysis

Prior to analysis, IP temperature data was averaged into 1-h intervals. All data were analyzed using the MIXED procedure of SAS specific for repeated measures (SAS Inst. Inc.). Treatment, time and their interaction were included as fixed effects with pig within treatment as the subject. Specific treatment comparisons were made using the PDIFF option in SAS, with $P \leq 0.05$ considered significant. All data are presented as the LSM ± SEM.

3. Results

Prior to the challenge, baseline IP temperature values were greater ($P < 0.001$) in the XPC-supplemented pigs (39.39 ± 0.006 °C) than Control pigs (39.18 ± 0.006 °C). Therefore, the change in IP temperature relative to baseline values was analyzed. An increase ($P < 0.001$) in IP temperature was observed in both groups immediately following the challenge; however, there was no treatment ($P = 0.12$; 0.38 vs. 0.39 ± 0.02 °C for Control and XPC) nor a treatment x time interaction ($P = 0.99$) for IP temperature.

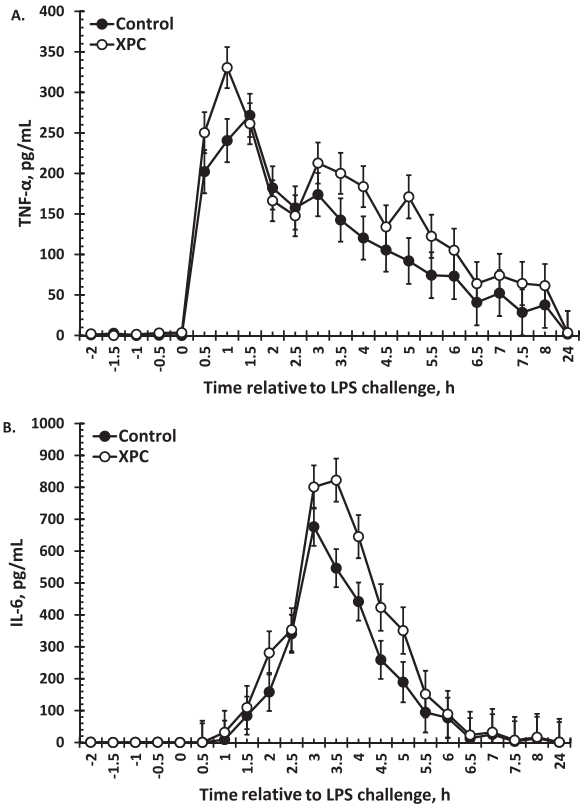

Fig. 1. Effect of treatment with *Saccharomyces cerevisiae* fermentation product (Original XPC, Diamond V, Cedar Rapids, IA USA) on the serum A) TNF-α, and B) IL-6 response to an i.v. lipopolysaccharide (LPS; 25 μg/kg BW) challenge in weaned pigs. Data presented as LSM ± SEM.

Serum concentrations of TNF-α increased ($P < 0.001$) in response to the LPS challenge, and were greater ($P < 0.001$) post-challenge in XPC-supplemented pigs (142 ± 4 pg/mL) compared to Control pigs (111 ± 6 pg/mL; Fig. 1A). Similarly, serum IL-6 concentrations also increased ($P < 0.001$) within 1.5 h of the LPS challenge, and were greater ($P = 0.002$) post-challenge in XPC (230 ± 17 pg/mL) as compared to Control pigs (163 ± 15 pg/mL; Fig. 1B). There was no treatment ($P = 0.21$; 2.23 vs. 2.28 ± 0.3 pg/mL for Control and XPC) effect for serum concentrations of IFN-γ. There were no treatment x time interactions for serum cytokines ($P \geq 0.49$).

There was no treatment effect for red blood cells, hemoglobin, hematocrit, platelets, monocytes, or eosinophils ($P \geq 0.22$), although all parameters were influenced by time ($P < 0.001$; data not shown). Total white blood cells were greater ($P = 0.04$) in Control pigs than XPC-supplemented pigs prior to the LPS challenge, and remained greater post challenge ($P < 0.001$; Fig. 2). Relative to time, total white blood cells decreased ($P < 0.001$) within 1 h following LPS administration and remained below baseline values through 7 h post-challenge. Similar to white blood cells, circulating neutrophil concentrations were greater in Control pigs (7.1 ± 0.3 × 10^3 cells/μL) than XPC-supplemented pigs (4.6 ± 0.3 × 10^3 cells/μL) prior to the challenge ($P = 0.01$). Concentrations of neutrophils decreased ($P < 0.001$) following administration of LPS, and remained below baseline values through 6 h post-challenge. Post-challenge neutrophils remained greater in Control (6.8 ± 0.3 × 10^3 cells/μL) pigs compared to XPC pigs (4.2 ± 0.3 × 10^3 cells/μL; $P < 0.001$). Lymphocyte concentrations were not affected by treatment prior to the challenge ($P = 0.39$;); however, Control pigs had greater ($P = 0.04$) post-challenge lymphocyte concentrations

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