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Effects of supplementation with betaine and superdosed phytase on semen characteristics of boars during and after mild heat stress

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ABSTRACT

The purpose of this experiment was to evaluate the effects of betaine and superdosed phytase on boar reproduction during mild heat stress. Twenty-seven mature (36 wk old), crossbred boars [Duroc × (Landrace × Yorkshire)] were randomly allocated to treatment and were fed 2.6 kg/d of 1 of 3 corn, soybean meal diets: control (CNT; 250 phytase units/kg *Escherichia coli* phytase; n = 9), betaine (BET; 250 phytase units/kg *E. coli* phytase and 0.6% betaine; n = 9), and betaine and superdosed phytase (BP; 2,500 phytase units/kg *E. coli* phytase and 0.6% betaine; n = 9). The experiment was split into 4 environmental phases (4 wk/phase) consisting of pre-heat stress (26°C), heat stress (30.2°C), post-heat stress 1 (16.7°C), and post-heat stress 2 (17.5°C). Semen was collected weekly from each boar and was evaluated for semen quantity and quality parameters. Total motility, progressive motility and percentage of morphologically normal sperm were reduced in the heat stress period ($P < 0.01$) with no effects from the dietary treatments ($P \geq 0.27$). Total sperm did not differ among treatments ($P = 0.99$). Percent distal droplets increased from the pre-heat stress to heat stress period for CNT ($P < 0.01$), but the increase was not statistically different for BET ($P = 0.97$) or BP ($P = 1.00$). This suggests that supplementation with betaine alone or with phytase may potentially reduce the effects of heat stress on specific morphological abnormalities, though total normal morphology did not differ.

Key words: betaine, heat stress, phytase, semen quality, swine

INTRODUCTION

Heat stress (**HS**) has been estimated to cost the swine industry over $300 million annually (St-Pierre et al., 2003), having a significant effect on pigs in all stages of production, including grow-finish (Pearce et al., 2014), sows (Tompkins et al., 1967; Edwards et al., 1968; Omtvedt et al., 1971; van Wettere et al., 2012), and boars (McNitt and First, 1970; Wettemann et al., 1979; Cameron and Blackshaw, 1980). Due to global climate change, heat stress will continue to plague the swine industry, where the extremes in ambient temperatures are predicted to continue to increase and worsen (Russo et al., 2014).

Betaine, a naturally occurring methylamine, has been shown to improve reproductive characteristics in sows under heat stress conditions during gestation (van Wettere et al., 2012) and lactation (Cabezon et al., 2016a). Though there is limited data in the boar, one recent study showed that betaine supplementation to boars during summer months tended to increase total sperm production, and the authors concluded that betaine supplementation could improve reproduction in boars during times of elevated temperature (Cabezon et al., 2016b). However, further research is needed to confirm this finding. Betaine acts as both an osmolyte and an antioxidant in animals, which may improve reproductive performance in times of stress. Phytase, the enzyme responsible for breaking down phytate, has been shown to also increase total sperm production in boars when supplemented at superdosed levels (levels above that needed to cleave nutrients from phytate; Stewart et al., 2018). Phytase has been shown to improve the use of minerals, such as P, Ca, Cu, Mg, and Zn, in pigs (Lei et al., 1993; Adeola, 1995; Adeola et al., 1995; Walk et al., 2013). Zinc, in particular, has been shown to be a critical mineral for sperm maturation, motility, and survival (Hidiroglou and Knipfel, 1984; Roy et al., 2013). Inositol (released from phytate) may be important for sperm maturation in the epididymis (Pruneda et al., 2007). Therefore, the purpose of this study was to investigate the use of supplemental betaine and superdosed phytase in boar diets on sperm production and semen quality estimates during and after heat stress.

One author works for AB Vista, the company which provided the betaine and phytase supplements for the project. This author did not contribute to study design, data collection, or data interpretation. The other authors declare no conflict of interest.

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MATERIALS AND METHODS

Animals and Experimental Design

All procedures were approved by the Purdue University Animal Care and Use Committee before initiation of the study. At approximately 36 wk of age (approximately 180 kg), 27 boars were trained for semen collection using an artificial sow. Following semen collection training, semen was collected weekly for a minimum of 10 wk before initiation of the experiment. All animals were fed a boar-specific diet to meet or exceed NRC (2012) requirements for breeding boars. The present study used 3 treatment diets and 4 environmental phases in a factorial design. Boars were fed 2.6 kg/d of the following diets: (1) control [**CNT**; 250 phytase units (**FTU**) of phytase per kg, Quantum Blue, AB Vista, Marlborough, UK], (2) CNT plus betaine supplement (**BET**; 0.63% of 96% betaine, Vistabet, AB Vista), or (3) CNT with betaine and superdosed phytase (**BP**; 0.63% of 96% betaine, Vistabet, AB Vista; 2,500 FTU of phytase per kg, Quantum Blue, AB Vista). Diet composition is summarized in Table 1. Supplements were included in the diet at the expense of corn. Phytase supplements were added to the diets in a premix with an inclusion rate of 0.2% of the total diet containing corn plus either 0.005% (CNT and BET) or 0.05% (BP) phytase product. Feed samples were collected from each batch of feed made (4 batches per diet) and sent to the University of Arkansas Center for Excellence for Poultry Science Central Analytical Lab for proximate and mineral analyses. For the proximate analysis, DM, ash, fat, and protein followed AOAC laboratory protocols 934.01, 923.03, 920.39c, and 990.03, respectively (AOAC International, 2012). Acid detergent fiber and NDF were analyzed based on Ankom Technology Methods 12 and 13, respectively (Ankom Technology, 2017a,b). Feed mineral analyses were performed following AOAC laboratory protocol 968.08 adapted for inductively coupled plasma mass spectrometry (AOAC International, 2012). Feed samples were sent to AB Vista for phytase activity analysis by an ELISA kit (Quantiplate kit; method AP181, Rev. 12–28–11; Envirologix, Portland, ME) and to the Purdue University Bindley BioScience Center for betaine concentration. For betaine analysis, 50 mL of distilled water was added to 50 mg of feed for extraction; samples were rocked for 30 min and centrifuged at 3,220 × *g* for 10 min. Supernatant (1 mL) was collected, and 10 μg of deuterium-3 betaine was added for analysis. Betaine analysis was performed using liquid chromatography coupled mass spectrometry as previously described (Cabezon et al., 2016b).

Boars were limit fed (2.58 ± 0.01 kg/d) the CNT diet for a 3-wk period before the environmental phases of the experiment (wk 1), at which point treatment diets were fed. All boars were randomly allocated to dietary treatment and blocked by location in the barn, with one boar per treatment represented in each block (9 blocks). Boar stalls were 0.7 by 2.1 m in size with a feeder located in the front, and a water nipple was shared between adjacent stalls with ad libitum access to water. The barn was designed with 3 negative pressure exhaust fans that were thermostatically controlled and located on the north half of the barn with 5 ceiling inlets located on the southern half of the barn. The other 2 fans were set to turn on when barn temperature exceeded 31°C, with the middle fan used to keep constant air flow in the barn. Boar stalls were arranged with half of the boars on the southern half of the barn facing north and the other half on the northern side of the barn facing south. This experiment was conducted in the fall and winter in central Indiana with wk 1 corresponding to October 3, 2016. Due to the timing of the study, thermostatically controlled (set to 29°C) propane heaters were needed to induce the heat stress. At the center of the barn, 2 large propane heaters (LB White Company, Onalaska, WI) were suspended over the dorsal edge of the southern boar stalls, with the heating exhaust being directed outward at each corner on the southern half of the barn. Two semen collection pens and a large alleyway was located behind the southern row of boars with approximately 3 m from the back of the boar stalls to the wall. Ambient room temperatures and relative humidity (**RH**) were recorded every 30 min during the trial period using data loggers (EL-USB-2; DATAQ Instruments Inc., Akron, OH) that were suspended at boar height (1 m off the ground) at each row end, with one suspended in the middle of the central alleyway dividing the north and south rows approximately 2 m off the ground. The present experiment consisted of 4 environmental phases: pre-heat stress (**PreHS**; wk 1–4; 26.0 ± 0.7°C with 55.6 ± 2.2% RH), heat stress (**HS**; wk 5–8; 30.2 ± 0.7°C with 39.0 ± 2.2% RH), post-heat stress 1 (**Post1**; wk 9–12; 16.7 ± 0.3°C with 56.8 ± 1.0% RH), and post-heat stress 2 (**Post2**; wk 13–16; 17.5 ± 0.3°C with 55.4 ± 1.0% RH). The 3-wk period before feeding dietary treatments was used as a covariate for statistical analyses of all parameters measured, when all boars were being fed the CNT diet (wk −2 to 0; 22.2 ± 0.7°C with 67.8 ± 2.5% RH). The post-heat stress phase was split into 2 periods (Post1 and Post2) due to the latent effects of heat stress on sperm motility, morphology, and concentration (Wettemann et al., 1976; Wettemann and Desjardins, 1979; Malmgren, 1989).

Respiration rates were estimated by trained technicians 2 d/wk at 1400 h. Respiration rates were counted by 2 trained technicians twice per time period and averaged for analysis. Rectal, scrotal skin, and ear skin temperatures were recorded 2 d/wk at 0600 and 1400 h. Rectal temperatures were measured with a digital rectal thermometer inserted a minimum of 2.5 cm into the rectum and were recorded after a reading was stable for 5 s. Scrotal and ear skin temperatures were measured with an infrared thermometer (TG54 IR Spot Thermometer; FLIR, Nashua, NH) approximately 15 cm from the skin at the center of each testicle on the scrotum and at the center of the base of the ear. Body weights were recorded, and blood was collected via ear vein catheterization at wk −1, 5, 9, and