Title: Improving production efficiency and carcass weight of finishing pigs housed under heat stress conditions by heat abatement with dietary betaine – **NPB #12-154**

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Industry Summary:

Heat stress has serious negative consequences for the swine industry by reducing feed intake and growth performance in pigs. Based on commercial observations, we estimate that heat stress during the summer results in a reduction of hot carcass weights of approximately 6 to 10 lbs in fixed time production systems. By using dietary heat abatement strategies, we can improve the efficiency of pork production, thus minimizing the cost of feed per unit of pork produced.

In the present studies, we determined the impact of dietary betaine as a heat abatement strategy in swine. Betaine is an osmolyte and has an important role in maintaining water homeostasis and cell integrity. Beneficial effects of betaine during heat stress have been demonstrated in poultry and rabbits. To achieve this objective, 3 experiments were conducted. In Experiment 1, effects of dietary supplementation of natural betaine (0, 0.10, 0.15, or 0.20%) on pig performance and metabolic status during thermo-neutral and heat stressed conditions was evaluated using 64 pigs. Heat stress negatively impacted daily gain, feed intake, respiration rate and rectal temperature, as expected. Betaine appeared to reduce respiration rate and rectal temperature in heat stressed pigs, but did not have major impacts on serum chemistry or growth performance. In Experiment 2, the impact of betaine during heat stressed conditions on pig performance and carcass characteristics was evaluated in 1,477 pigs fed diets with or without Paylean in a commercial production facility. Betaine reduced feed intake, but did not impact any other measures of pig growth performance or carcass characteristics. Paylean improved growth performance and carcass characteristics, resulting in an increased final carcass weight of 8.9 lbs and a reduction in feed used per unit of carcass gain of 17%. In Experiment 3, a dose titration study was conducted to evaluate the impact of betaine in late finishing pigs housed under heat stressed conditions using a total of 2,193 crossbred pigs. Betaine supplementation at 1.25, 2.50, or 3.75 lbs per ton had no effects on growth performance or carcass characteristics. Paylean increased final carcass weight by 5.35 lbs and reduced the amount of feed used per unit of carcass gain by 13.6%. Collectively, these data indicate that under practical, commercial conditions, betaine did not improve pig performance or carcass characteristics when supplemented at 1.25, 2.50, and 3.75, and 4.00 lbs/ton. These studies were conducted during heat stress and betaine was supplemented during the last phase of production, with pigs weighing approximately 200 to 210 lbs until market weight. Results from these studies do not support the use of betaine in diets for late finishing pigs housed under heat stressed conditions. Supplementation with Paylean clearly improved pig performance and carcass characteristics, resulting in an immediate net economic benefit to pork producers.

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Scientific Abstract:

Betaine is an osmolyte and has an important role in maintaining water homeostasis and cell integrity. The objective of this study was to determine the impact of dietary betaine as a heat abatement strategy in swine. Three experiments were conducted. In Experiment 1, effects of dietary supplementation of natural betaine (0, 0.10, 0.15, or 0.20%) on pig performance and metabolic status during thermo-neutral and heat stressed conditions was evaluated using 64 pigs. Heat stress reduced daily gain and feed intake and increased respiration rate and rectal temperature ($P < 0.01$), as expected. Betaine appeared to reduce respiration rate and rectal temperature in heat stressed pigs ($P < 0.05$), but did not have major impacts on serum chemistry or growth performance. In Experiment 2, the impact of betaine during heat stressed conditions on pig performance, production efficiency, carcass characteristics, morbidity and mortality in pigs fed with or without Paylean in a commercial production facility was evaluated. A total of 1,477 male and female pigs (initial body weight was 91.5 ± 0.7 kg) were used and fed diets with betaine at either 0 or 0.2% and Paylean (no Paylean or a Paylean step-up program using 4.5 g per ton, followed after 20 to 21 days by 8 g/ton) in a 2 x 2 factorial arrangement. Betaine reduced feed intake ($P < 0.03$), but did not impact any other measures of pig growth performance or carcass characteristics. Paylean improved ($P < 0.01$) final body weight (by 3.4 kg), carcass weight (by 4.0 kg), carcass yield (by 1.05%), and loin depth (by 3.5 mm) and reduced backfat depth (by 2.1 mm), subsequently increasing lean percent (by 1.4%). Supplementation with Paylean improved feed efficiency ($P < 0.001$) when considering whole body weight gain (0.328 vs. 0.292; a 12.3% improvement in gain per unit of feed), with a greater impact when considering feed efficiency based on carcass gain (0.256 vs. 0.212; a 20.7% improvement in carcass gain per unit of feed). In Experiment 3, a dose titration study was conducted to evaluate the impact of betaine in late finishing pigs housed under heat stressed conditions using a total of 2,193 crossbred pigs (initial body weight was 96 ± 1 kg). Betaine supplementation at 0.0625 0.125, 0.1875% had no effects on growth performance or carcass characteristics. Paylean increased ($P < 0.05$) final body weight (by 2.45 kg), carcass weight (by 2.40 kg), carcass yield (+0.6%), loin depth (by 2.2 mm) and reduced backfat depth (by 1.3 mm) and, therefore, lean percent (by 0.8 %). Supplementation with Paylean improved feed efficiency ($P < 0.001$) when considering whole body weight gain (0.343 vs. 0.308; an 11.4% improvement in feed efficiency), with a slightly greater impact when considering feed efficiency based on carcass gain (0.257 vs. 0.226; a 13.4% improvement in carcass gain per unit of feed intake). Collectively, these data indicate that under practical, commercial conditions, betaine did not improve pig performance or carcass characteristics when supplemented at 0.0625 0.125, 0.1875, and 0.20% during heat stress. Supplementation with Paylean clearly improved pig performance and carcass characteristics, resulting in an immediate net economic benefit to pork producers.

Introduction:

High environmental temperatures are a common challenge facing swine producers causing significant economic losses. Environmental heat stress that the animal experiences is a combination of various factors, most prominently temperature and humidity. Heat is also generated within the animal as it metabolizes nutrients. With high environmental temperatures, heat dissipation to the surrounding environment will become more difficult. In response to control body temperature, pigs will have increased blood flow to the skin surface, increased respiration rate, and display behavioral changes such as wetting of the skin, if possible, to increase evaporative heat losses. Reduced feed intake is a common response to heat stress because it reduces the heat associated with metabolism. Both short term (Heitman et al., 1958; Bond et al., 1959; Nienaber and Brown-Brandl, 2008) and long-term (Nichols et al., 1982; Nienaber et al., 1987) heat stress reduce feed intake and growth of swine. Feed intake is estimated to be reduced by approximately 0.05 to 0.08 lbs per °F above 75°F and growth rate is expected to decrease in hot environments by approximately 0.03 to 0.04 lbs/d for every °F above 75 °F (Verstegen et al., 1995; Noblet et al., 2001). The impact of temperature is dependent on the weight of the pig. Heavier pigs are more sensitive to high temperatures than younger, lighter pigs. Nutritional strategies to mitigate the negative impact of heat stress have been evaluated, many of which have focused on the use of fat as a "cool" energy source and the reduction of nutrients with a high heat increment, such as fiber and protein. In poultry, betaine has been evaluated as a heat abatement strategy in heat stressed poultry and shown to positively impact growth performance (Zulkifli et al., 2004).

Betaine, or tri-methyl glycine, is a methyl donor and osmo-regulator that occurs naturally (Kidd et al. 1997). The function of betaine as a methyl donor is well known and it can serve as a substrate for methionine synthesis, therefore providing a methionine sparing effect. The role of betaine as an osmolyte has important implications because it can maintain cell volume and integrity under challenging conditions. Indeed, betaine has been shown to improve performance in poultry when exposed to stress conditions such as coccidiosis infection (Klasing et al., 2002) and heat stress in broilers (Zulkifli et al., 2004; Farooq et al., 2005) and rabbits (Hassan et al., 2011). Maintaining osmotic balance is extremely important and it has been estimated that 30 to 60% of the maintenance requirement of visceral tissue is associated with the sodium/potassium pump. Betaine functions as an osmolyte inside the cell, maintaining water balance in the cell. Osmolytes have been demonstrated to increase the ability of cells to withstand osmotic stress by 42%, which resulted in a sparing effect on sodium/potassium pump activity of 64% (Moeckel et al., 2002). Under normal conditions (no imposed stress), betaine has been shown to reduce energy requirements by 5.5% (Schrama et al., 2003). These results suggest that betaine can reduce the maintenance energy requirements through improved osmotic regulation, which will increase energy availability for growth. This is especially evident during stressful conditions, such as heat stress. It has also been clearly established that severe heat stress can compromise gut integrity and barrier function (Hall et al., 2001; Cronje, 2005), allowing toxic components, such as endotoxins to enter the bloodstream. Betaine may play a role in minimizing damage to gut barrier function as evidenced by improved structure of the lining of the intestinal tract in poultry (Kettunen et al., 2001), improved nutrient absorption in poultry and swine (Eklund et al., 2005), and reduced gut lesions due to coccidia challenge in poultry when betaine was supplemented.

Together these data provide a compelling argument that betaine supplementation may be an effective strategy to minimize the impact of heat stress in pigs. This may be especially the case in environments that further challenge the stress response of pigs, as may be the case in commercial industry settings, where limited floor space and exposure to subclinical disease can occur. We further propose that the use of Paylean may contribute to increase the heat increment of pigs due to the additional production of metabolic heat in fast growing, lean pigs. Therefore, betaine may be more effective in heat abatement in pigs fed diets containing Paylean compared to those not fed Paylean.

We propose that betaine can be effectively used as a heat abatement strategy in finishing pigs and that this response is larger in pigs fed Paylean. We further propose that supplemental betaine can reduce heat stress associated gut damage and, therefore, effectively improve performance of pigs housed in a health challenged environment.

Objectives:

The objective of the current research was to evaluate the impact of dietary betaine as a heat abatement strategy in finishing pigs exposed to heat stress. We hypothesized that supplemental betaine can reduce the negative impact of heat stress and that this impact is greater in pigs fed Paylean. This will result in increased production efficiency and economic return.

Materials & Methods:

Experiment 1

Animal use protocols were approved by the North Carolina State University Institutional Animal Care and Use Committee.

This experiment was conducted using 64 crossbred pigs ([Landrace x Yorkshire] x [Hampshire x Duroc]), with an average initial body weight of 39.0 ± 1.5 kg (16 barrows and 48 gilts). Pigs were assigned within weight blocks and sex to 1 of 8 treatments using an experimental animal allotment spreadsheet (Kim and Lindemann, 2007). Pigs were housed in individual pens (0.91 x 1.82 m) at the Swine Educational Unit, Raleigh, NC and had unlimited access to feed and water throughout the entire experiment. Each pen had 1 nipple water drinker and a single space feeder.

Treatments consisted of 2 environmental temperatures (thermo-neutral or heat-stressed) and 4 levels of dietary betaine (0, 0.10, 0.15, and 0.20%). Two rooms (32 pens in each room) were equipped with GT-5124LW Grower Direct (Monitrol Inc., Boucherville, Quebec, Canada) environmental control systems. Temperature in each room was set to follow a daily pattern, similar to daily fluctuations that may be experienced in commercial barns. For pigs housed under thermo-neutral conditions, temperature set points were 15, 14, 15, 15, 17, 18, 19, 20, 21, 20, 17, and 15°C, changing every 2 h throughout the day. For pigs under heat-stressed conditions, set points were 29, 28, 29, 29, 31, 32, 33, 34, 35, 34, 31, and 29°C, changing every 2 h throughout the day. Actual room temperatures and humidity were measured by data loggers (LogTag, MicroDAQ Ltd., Contoocook, NH). Two devices were placed in each room at the approximate height of the pigs and temperature and humidity were recorded every 10 min.

Within each room, pigs were fed either a control diet without betaine or diets supplemented with 0.10, 0.15, or 0.20% betaine. Each dietary treatment was replicated 8 times within each room. Although heat stress was only applied to one room compared to one control room, our main objective for this study was to provide two distinct temperatures and evaluate treatment effects within each room. Thus, the design of this study was well replicated to determine the impact of betaine on pig measurements within each room.

Feed was manufactured at the North Carolina State University Feed Mill Educational Unit. Diets were corn-DDGS-soybean meal based and contained 2.96 g standardized ileal digestible lysine/Mcal ME (Table 1). Diets were formulated to meet or exceed all nutrient concentrations suggested by NRC (2012.) One basal mix was prepared and divided into 4 batches to which betaine was added to generate the final treatments. This process ensured that diets were identical in composition with the exception of betaine content.

The experiment consisted of a diet adaptation period of 7 days, followed by a 28 d experimental period. Dietary treatments were fed during both periods. During the adaptation period, rooms were set at constant temperature of 21°C. Temperature treatments were imposed after the 7 day adaptation (designated as d 0). Body weight was measured on d -7, 0, 14, and 28 and feed consumption was calculated from the amount of feed supplied minus feed refusal measured on d 0, 14, and 28. Respiration rate and rectal temperature were measured on d 0, 1, 2, 3, 7, 14, 21, and 28. Respiration rate was measured as the number of flank movements per 30 seconds as described by Quiniou and Noblet (1999) and rectal temperature was determined by using a digital thermometer (M750 Series, GLA Agriculture Electronics, San Luis Obispo, CA). Blood samples were collected on d 3 and 28 by venipuncture into 7 mL vacuum tubes (BD Vacutainer Systems, Franklin Lakes, NJ) to obtain serum.

Chemical analysis. Blood samples were centrifuged at 2,500 x g for 20 min at 10°C to collect serum. Serum samples were analyzed for total protein, albumin, globulin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALK), Gamma-glutamyltranspeptidase (GGTP), total bilirubin, urea nitrogen, creatinine, phosphorous (P), glucose, calcium (Ca), Magnesium (Mg), potassium (K), sodium (Na), chloride (Cl), cholesterol, triglycerides, non-esterified fatty acids (NEFA), amylase, lipase, and creatine phosphokinase. All analyses were performed by Antech Diagnostics Laboratory (Lake Success, NY) using an auto analyzer (Olympus AU 5400, Melville, NY) except for NEFA. Non-esterified fatty acid concentrations were determined by a commercial kit (NEFA-HR2, Wako, Richmond, VA) according to the manufacturer's instructions.

Statistical Analysis. Data analysis was performed using the GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC) with pig as the experimental unit. The experimental design consisted of a split-plot with weight block within environmental temperature as whole plot and diet as split plot. The fitted model for pig performance, respiration rate, and rectal temperature data included environmental temperature, dietary inclusion of betaine, and the interaction of both, as the fixed effects; and weight block nested within environmental temperature as random effects. Orthogonal contrast comparisons were conducted to determine linear and quadratic effects of dietary betaine. Repeated measures of blood chemistry, and NEFA were analyzed as a split-split-plot. The data were transformed to natural log. The fitted model included environmental temperature, dietary inclusion of betaine, repeated measure of day, and their interactions, as the fixed effects; and weight block nested within environmental temperature and the interaction of the weight block and betaine levels nested within environmental temperature as random effects.

Experiment 2

This study was conducted at a commercial research farm (Hanor Company, Finish Site 727, White Hall, IL). A total of 1,477 male and female pigs (Camborough derivative x TR4 sire; initial body weight was 91.5 ± 0.73 kg) were used in a 2 x 2 factorial arrangement of treatments. Factors included: 1) supplementation with betaine (0 or 0.2%) and 2) supplementation of Paylean (no Paylean or a Paylean step-up program using 4.5 g per ton, followed after 20 to 21 days by 8 g/ton). Pigs were initially placed in pens upon arrival into the finisher barn by weight and sex. Pens were randomly allotted to dietary treatments within gender and by average pen weight blocks.

The impact of experimental treatments on pig growth performance and carcass characteristics were evaluated under heat stressed conditions. Heat stress was gradually imposed and acclimation to high temperatures was initiated 10 days before dietary treatments were imposed. Barn temperature was set at 70°F and raised by approximately 1°F per day until 88°F was reached. Temperatures were controlled by using heaters and by reducing the rate of ventilation in the barn. Ventilation in the barn was maintained by the continuous operation of a minimum ventilation fan to ensure sufficient air flow to provide good air quality. The target high temperature was approximately 88 to 90°F for 16 to18 h per day (between 6:00 and 22:00 h). The target low temperature was set at 78 to 80°F from 22:00 to 6:00. Water misters for cooling were not operable until 91°F and were used as a safety precaution only.

Experimental diets (Table 2) were provided throughout the study using an automated feeding system (Howema). This system dispensed the appropriate treatment diet to each pen and recorded the amount of feed provided. Feeding times were set according to standard operating procedures and feeders were checked daily to ensure feed was available to the pigs at all times. The accuracy of the feeding system was checked on a weekly basis by capturing and weighing feed at two locations. Feed additions were determined on a daily basis. Feed intake by pen was calculated from daily feed additions and the weight of remaining feed at each marketing cut. Feed samples were collected at the feed mill and from random sentinel feeders within the barn. Samples were analyzed to verify treatments. Pens were marketed over 3 to 4 cuts and each cut was weighed within 24 hours of loading onto the trucks for harvest. Equal numbers of pigs per treatment were placed on each truck regardless of body weight. Pigs identified for marketing were tattooed with their identifying pen number. Pigs were shipped for harvest to Triumph and carcass data were collected.

Pig weights, ADG, ADFI, and gain:feed were determined prior to the first cut for all pens and from day 0 to harvest. Carcass yield was determined using measured pig live weights at the farm and hot carcass weights from the plant. Fat-O-Meter back fat, loin depth and lean percent were determined at the packing plant. Number of dead pigs, pigs removed, down pigs, pigs dead on arrival, and stressed pigs were recorded. Ambient room temperatures and humidity were determined daily using high-low thermometers. Temperatures of the feeders were measured using an infra-red temperature gun to determine the effective temperature of the direct pig environment on a weekly basis.

Data were analyzed using the Mixed procedure of SAS. Pen was the experimental unit. The model included the effects of Paylean, betaine, and the Paylean by betaine interaction. An alpha level of 0.05 was used to assess significance among means.

Experiment 3

This experiment was performed at a commercial research farm (Hanor Company, Finish Site 727, White Hall, IL). A total of 2,193 crossbred pigs (PIC TR-4 x Camborough product sows) were used, consisting of 1,121 barrows and 1,072 gilts with an average initial body weight of 96.0 ± 1 kg. Pigs were housed approximately 22 pigs per pen with 20 pens per treatment. Barrows and gilts were housed in separate barns. Pigs were assigned within weight blocks and sex to 1 of 5 dietary treatments. Treatments (Table 3) consisted of a negative control diet (NC), the negative control diet supplemented with either 0.0625, 0.1250, and 0.1875% of dietary betaine (Betafin®, Danisco A/S, Copenhagen, Denmark) and the negative control diet supplemented with ractopamine hydrochloride (Paylean®, Elanco Animal Health, Greenfield, IN). Paylean was included at

4.5 g for the first 21 days followed by 8 g until pigs were marketed. The Paylean diet, the NC diet and 0.1875% betaine supplemented diet were manufactured by a commercial feed mill (Hanor Company, IL) and were color coded to ensure that the proper experimental diets were fed. Diets with intermediate concentrations of betaine were mixed at the farm from the NC and 0.1875% betaine diet using a computerized feeding system (Howema, Big Dutchman, Vechta, Germany). Feed and water were provided on an ad libitum basis throughout the entire experiment.

The impact of experimental treatments on pig performance was measured under heat stressed conditions. Heat stress was initiated and maintained as described in Exp. 2. Pig performance measures, carcass characteristics and statistical analysis were conducted as described in Exp. 2.

Results:

Experiment 1

Room Temperature. During the 7 d diet adaptation period, temperature was 21°C (± 0.7°C) and humidity was 65% (± 5%) in the room that was intended to be used as the thermo-neutral room. Temperature was 21°C (± 1.5°C) and humidity 72% (± 6%) in the room that was intended to be used as heat-stressed room. Temperature and humidity data recorded by the data logging devices during the 28 day experimental period are shown in Figure 1. The mean temperature in the thermo-neutral room was 19°C, with a maximum of 24°C and a minimum of 16°C. The humidity was 68% on average with a maximum of 85% and a minimum of 38%. In the heat stressed room, the mean temperature was 32°C with a maximum of 39°C and a minimum of 28°C. Humidity ranged from 26 to 76%, with a mean of 50%.

Pig Performance. No differences in growth performance were observed during the diet adaptation period (Table 4). Average daily gain was reduced when pigs were exposed to heat-stressed conditions (0.710 vs. 0.822 kg, P = 0.007). An interaction (P = 0.110) was observed during d 15 to 28, in which betaine supplementation at 0.10 and 0.15% improved ($P < 0.104$) ADG in heat stressed pigs, but not in pigs housed under thermo-neutral conditions. Average daily feed intake was reduced when pigs were exposed to high temperatures (1.81 vs. 2.24 kg, $P = 0.002$). Feed efficiency (G:F) was greater during d 1 to 14 (0.430 vs. 0.387, $P = 0.016$) and d 1 to 28 (0.392 vs. 0.365) for pigs exposed to heat-stressed conditions. A tendency for a quadratic effect of betaine ($P = 0.072$) was observed for G:F during d 1 to 28, showing the best feed efficiency at 0.10% betaine (0.377, 0.391, 0.380, and 0.370 for 0, 0.1, 0.15, and 0.2% betaine, respectively).

Respiration rate and rectal temperature. Heat-stressed conditions increased ($P < 0.001$) respiration rate during each of the time points and for the overall heat stress period (Table 5). The increase in respiration rate due to heat stress appeared to be more pronounced during the first 3 days of heat stress, suggesting pigs were able to partially adjust to the heat stress conditions. When considering the entire 28 day heat stress period, respiration rate increased from 21.6 to 48.0 flank movements per 30 seconds for pigs fed control diets. This increase of 26.4 flank movements per 30 seconds due to heat stress was greater than the increase in flank movements per 30 seconds in pigs fed 0.10, 0.15, or 0.20% betaine (increased number of flank movements were 23.5, 20.5, and 20.2 due to heat stress). Rectal temperature increased ($P < 0.01$) when heat stress was imposed during all time points measured and over the entire 28 day period. The impact of betaine on rectal temperature during heat stress was not entirely clear, but results indicate that rectal temperature was lowest when betaine was supplemented to heat stressed pigs at 0.10% when measured on day 2, day 14, and the overall period.

Serum chemistry. Serum chemistry panels were conducted on day 3 and 28 of the heat stress study to evaluate the impact of acute and chronic heat stress, respectively. Heat stress significantly impacted many serum chemistry parameters and the impact of heat stress depended in many cases on whether it was measured during the early or late stages of heat stress (Table 6). Serum concentrations of globulin, creatinine and Mg increased during heat stress, particularly when measured on day 28. Serum concentrations of AST, GGTP, and CPK increased in response to heat stress and this response was much more pronounced on day 3, especially for AST and CPK. Heat stress decreased serum concentrations of ALT, alkaline phosphatase, and NEFA and this decrease was more evident when measured on day 28. Serum concentrations of Urea N, P, and Na decreased when measured on day 3 of heat stress, while they were not affected or reduced to a lesser extent on d 28. Serum concentration of amylase decreased due to heat stress on day 3, but increased on day 28. Serum Ca and lipase decreased and K increased due to heat stress when measured on day 3 and day 28. Creatinine concentrations decreased slightly with increasing dietary betaine when measured on day 3 of heat stress, but not on day 28. Serum concentrations of K decreased on day 3 of heat stress with increasing supplemental betaine with the lowest concentration of K at 0.15% betaine, while K was highest at 0.15% betaine when measured on day 28 of heat stress.

Experiment 2

Analyzed betaine concentrations were 0.192 and 0.186% for the control diet with betaine and the Paylean diet with betaine, respectively, confirming that the correct concentrations of betaine were present in the diets (Table 7). Temperatures were between approximately 78 to 80°F for the low and 85 to 90°F for the high in the barns with heat stress compared to approximately 68 to 70°F for cohort barns (Figure 2). Similarly, temperatures of the feeders (representing temperatures close to the pigs) generally ranged between 85 and 95°F and were slightly higher in the gilt barn compared to the barrow barn (Figure 3). Respiration rate in pigs housed under heat stress increased by 30% from the day that heat stress was gradually imposed until heat stress was fully achieved (data not shown). Feed consumption decreased and then remained relatively constant as the study progressed, despite increasing pig body weights (Figure 4). Water consumption increased dramatically during the study (data not shown).

Supplementation with betaine depressed feed intake, regardless of whether Paylean was supplemented in the diet ($P \leq 0.03$; Table 8). The depression in feed intake due to betaine resulted in reduced ADG for betaine supplemented pigs when measured until the first cut ($P \leq 0.03$), and betaine reduced ADG in pigs fed diets with Paylean when considering the entire period up to marketing ($P = 0.027$). Paylean was fed for a maximum of 44 d and improved final body weight and carcass weight by 7.6 and 8.9 lbs, respectively, compared to control pigs ($P < 0.01$; Table 9). This was due primarily to increased whole body weight, but Paylean also improved carcass yield (+1.05%), reduced backfat depth (by 2.1 mm), increased loin depth (by 3.5 mm), and subsequently increased lean percent (by 1.4%) ($P < 0.001$). Supplementation with Paylean improved feed efficiency (feed:gain) when considering whole body weight gain (3.05 vs 3.42; an 11% reduction in feed per unit of gain), with a greater impact when considering feed efficiency based on carcass gain (4.72 vs. 3.91; a 17% reduction in feed per unit of carcass gain). Betaine supplementation did not have an impact on carcass characteristics, with the exception of reducing carcass ADG when fed in combination with Paylean compared with the Paylean control treatment ($P = 0.026$).

Experiment 3

Experiment 3 was a dose-response study was conducted based on the negative impact of betaine on feed intake observed in Exp. 2, which indicated that the level of betaine used may have been too high. Similar to Exp. 2, heat stress was imposed gradually and temperatures throughout the study ranged from 30 to 35°C once the targeted level of heat stress was reached (Figure 5). The results of this study did not demonstrate any effects ($P > 0.05$) of betaine on growth performance or carcass characteristics. Supplementation with Paylean improved final body weight and carcass weight by 5.45 and 5.35 lbs, respectively, compared to control pigs ($P < 0.001$; Table 10). Paylean also improved carcass yield (+0.6%), reduced backfat depth (by 1.3 mm), increased loin depth (by 2.2 mm), and subsequently increased lean percent (by 0.8 %) ($P < 0.05$). Supplementation with Paylean improved feed efficiency (gain:feed) when considering whole body weight gain (0.343 vs. 0.308; an 11.2% improvement in feed efficiency), with a slightly greater impact when considering feed efficiency based on carcass gain (0.257 vs. 0.226; a 13.6% improvement in carcass gain per unit of feed).

Discussion:

As expected, no differences in performance were observed during the dietary adaptation period between temperature groups in Exp. 1. Once heat stress was imposed, ADFI was reduced by 25.6, 15.7, and 19.4% during day 1 to 14, 15 to 28, and the overall heat stress period, indicating that heat stress may have had a larger impact early, with partial adjustment during the latter stages of heat stress. Similarly, ADG was reduced by 14.9, 13.6, and 13.6% during day 1 to 14, 15 to 28, and the overall heat stress period. Because ADFI was impacted to a greater extent than ADG, gain:feed was actually improved due to heat stress. Respiration rate and rectal temperature were impacted by heat stress, which resulted in increased respiration rate and rectal temperature. Thus, heat stress clearly resulted in physiological changes as indicated by changes in serum chemistry. These changes in serum chemistry could be due to changes in feed intake, or be the result of physiological changes due to heat stress. The purpose of the present study was to evaluate the impact of betaine during heat stress conditions and, thus, this discussion will focus on impacts of betaine on performance and serum chemistry. Betaine affected feed intake during the first 14 days of heat stress in a quadratic manner, regardless of heat stress, with the lowest intake when betaine was fed at 0.10%. Feed efficiency was greatest when betaine was fed at 0.10% (main quadratic effect of betaine). Betaine appeared to positively impact the response to heat stress as evidenced by reduced respiration rate and rectal temperature with betaine supplementation, but the impact of betaine on serum chemistry was limited.

In Exp. 2, heat stress was clearly achieved. The temperature range imposed far exceeded the upper limit of the thermoneutral zone (approximately 62 °F for pigs weighing 210 lbs). In addition, respiration rate was increased by 30% during heat stress compared to respiration rates measured before heat stress was imposed. In this experiment, betaine tended to depress feed intake indicating that the 0.2% supplemental betaine level was excessive. Thus, a dose response curve needs to be established to define the optimal dose of supplementation. Paylean clearly improved pig performance and carcass characteristics, which will increase the amount of saleable meat by an estimated amount of at least 1.5 to 2 lbs.

In Exp. 3, a dose response study was conducted to evaluate the impact of betaine in pigs reared under heat stressed conditions. Similar to Exp. 2, heat stress was clearly achieved with temperature ranges well above the upper limit of the thermoneutral zone for pigs of approximately 210 lbs of body weight. Betaine supplementation at 0.0625, 0.1250, or 0.1875% (1.25, 2.50, and 3.75 lbs/ton, respectively) did not positively impact any of the performance or carcass measurements in the present study. Paylean improved pig growth performance and carcass characteristics.

In conclusion, results from these studies indicate that under practical, commercial conditions, betaine did not improve pig performance or carcass characteristics when supplemented at 1.25, 2.50, and 3.75, and 4.00 lbs/ton. These studies were conducted during heat stress and betaine was supplemented during the last phase of production, with pigs weighing approximately 200 to 210 lbs until market weight. Results from these studies do not support the use of betaine in diets for late finishing pigs housed under heat stressed conditions. Supplementation with Paylean clearly improved pig performance and carcass characteristics, resulting in an immediate net economic benefit to pork producers.

References

Bond, T.E., C.F. Kelly, and H. Heitman, Jr. 1959. Hog house air conditioning and ventilation data. Trans. ASAE 2:1-4.

Cronje, P. B.. 2005: Heat stress in livestock–the role of the gut in its aetiology and a potential role for betaine in its alleviation. Recent Advances in Animal Nutrition in Australia. 15:107–122.

Eklund, M., E. Bauer, J. Wamatu and R. Mosenthin. 2005. Potential nutritional and physiological functions of betaine in livestock. Nutrition Research Reviews 18:31-48.

Farooq, H. A. G., M. S. Khan, M. A. Khan, M. Rabbani, K. Pervez, and J. A. Khan. 2005. Evaluation of betaine and vitamin C in alleviation of heat stress in broilers. Int. J. Agri. Biol. 7:744-746.

Hall, D. M., G. R. Buettner, L. W. Oberley, L. Xu, R. D. Matthes, and C. V. Gisolfi. 2001: Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. American Journal of Physiology. Heart and Circulatory Physiology 280, H509–H521.

Hassan, R. A., T. A. Ebeid, A. I. Abd El-Lateif, and N. B. Ismail. 2011. Effect of dietary betaine supplementation on growth, carcass and immunity of New Zealand White rabbits under high ambient temperature. Livestock Science 135:103-109.

Heitman, H. Jr., C. F. Kelly, and T. E. Bond. 1958. Ambient air temperature and weight gain in swine. J. Anim. Sci. 17:62-67.

Kettunen, H., K. Tiihonen, S. Peuranen, M. T. Saarinen, and J. C. Remus, 2001. Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks. Comp. Biochem. Physiol. A 130:759-769.

Kidd M. T., P. R. Ferket, and J. D. Garlich. 1997. Nutritional and osmoregulatory functions of betaine. World's Poultry Science Journal 53:125-139.

Kim, B. G., and M. D. Lindemann. 2007. A spreadsheet method for experimental animal allotment. J. Anim. Sci. 85 (Suppl. 2):112.

Klasing K. C, K. L.Adler, J. C. Remus, and C. C. Calvert. 2002. Dietary betaine increases intraepithelial lymphocytes in the duodenum of coccidian-infected chicks and increases functional properties of phagocytes. Journal of Nutrition 132:2274-2282.

Moeckel, G. W., R. Shadman, J. M. Fogel, and S.M.H. Sadrzadeh. 2002. Organic osmolytes betaine, sorbitol and inositol are potent inhibitors of erythrocyte membrane ATPases. Life Sciences 71:2413-2424.

Nichols, D.A., D.R. Ames, and R.H. Hines. 1982. Effect of temperature on performance and efficiency of finishing swine. Proc., 2nd Intl. Livestock Environmental Symp., ASAE Publ. No. 3-82. ASAE, St. Joseph, Mich.:ASAE.

Nienaber, J.A., and T.M. Brown-Brandl. 2008. Heat and moisture production of Growing-Finishing barrows as affected by environmental temperature. ASABE Paper No. 84168. St. Joseph, Mich: ASABE.

Nienaber, J.A., G.L. Hahn, and J.T. Yen. 1987a. Thermal environment effects on growing-finishing swine Part I--growth, feed intake and heat production. Trans. ASAE 30(6):1772-1775.

Noblet, J., J. Le Dividich, and J. van Milgen. 2001. Thermal environment and swine nutrition. In: P. 519-544, Swine Nutrition (Lewis, A. J. and L.L. Southern, Eds.). CRC Press, Boca Raton, LA.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Quiniou, N., and J. Noblet. 1999. Influence of high ambient temperatures on performance of multiparous lactating sows. J. Anim. Sci. 77:2124-2134.

Schrama, J. W., M. J. W. Heetkamp, P. H. Simmins, and W. J. J. Gerrits. 2003. Dietary betaine supplementation affects energy metabolism of pigs. J. Anim. Sci. 81:1202-1209.

Verstegen, M. W. A., K. H. de Greef, and W. J. J. Gerrits. 1995. Thermal requirements in pigs and modeling of the effects of coldness. In: P. 123-135, Modeling growth in the pig, EAAP Publication No. 78. Wageningen Pers, Wageningen, The Netherlands.

Zulkifli, I., S. A. Mysahra, and L. Z. Jin. 2004. Dietary supplementation of betaine (Betafin®) and response to high temperature stress in male broiler chickens. Asian Aust. J. Anim. Sci. 17:244-249.

Table 1. Composition of the basal diet (as fed basis) for Exp. 1[a]

Ingredient	**%**
Corn, yellow dent	56.49
Corn DDGS, > 6 and < 9% oil	20.00
Soybean meal, 47.5% CP	17.76
Poultry fat	2.50
L-lysine HCl	0.42
DL-methionine	0.05
L-threonine	0.100
Monocalcium phosphate 21% P	0.530
Limestone	1.460
Salt	0.500
Optiphos 2000 [b]	0.0125
Market swine vitamins	0.025
Trace mineral premix	0.150
Calculated Composition	
ME, Mcal/kg	3.3
Crude Protein %	20.4
Crude Fat %	3.5
Analyzed composition, %[c]	
Moisture, %	11.4
Crude Protein, %	19.3
Crude Fat, %	6.2
Crude Fiber, %	8.8
Ash, %	4.8
Calcium, %	0.64
Phosphorous, %	0.54

[a]Diet was formulated to meet or exceed NRC (2012) requirements
[b]Provided 500 FTU/kg of phytase
[c]Analyzed by the Experiment Station Chemical Laboratories, University of Missouri.

Table 2. Composition of the experimental diets (as fed basis) for Exp. 2

Ingredient	Control	Control + Betaine	PLN Step I 4.5 g	PLN Step I 4.5 g + Betaine	PLN Step II 8.0 g	PLN Step II 8.0 g + Betaine
Corn	62.63	62.28	61.99	61.69	61.97	61.67
Corn DDGS	18	18	18	18	18	18
Soybean Meal, 47.5% CP	15	15	15	15	15	15
Fat, Choice White Grease	2.55	2.70	2.55	2.65	2.55	2.65
Limestone, Ground	1.00	1.00	1.02	1.02	1.02	1.02
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Monocalcum phosphate	0.175	0.175	0.230	0.230	0.230	0.230
Vitamin-mineral premix	0.080	0.080	0.085	0.085	0.085	0.085
L-Lysine	0.165	0.165	0.485	0.485	0.485	0.485
L-Threonine	-	-	0.160	0.160	0.160	0.160
L-Tryptophan	-	-	0.050	0.050	0.050	0.050
DL-Methionine	-	-	0.015	0.015	0.015	0.015
Paylean 9.0 g/lb	-	-	0.025	0.025	0.045	0.045
Betaine	-	0.200	-	0.200	-	0.200
Calculated Composition						
ME, Mcal/kg	3.44	3.44	3.44	3.44	3.4	3.44
Crude Protein, %	17.4	17.3	17.8	17.7	17.8	17.7
Total Lysine, %	0.877	0.876	1.128	1.127	1.128	1.127
SID Lysine, %	0.760	0.759	1.011	1.010	1.011	1.010
Calcium, %	0.581	0.581	0.601	0.601	0.601	0.601
Av Phosphorus, %	0.264	0.264	0.280	0.280	0.280	0.280
Paylean g/ton	-	-	4.5	4.5	8.0	8.0

Table 3. Composition of the experimental diets (as fed basis) for Exp. 3

	Betaine Levels, %				Paylean	
Ingredients	**0**	**0.063**	**0.125**	**0.185**	**4.5**	**8**
Corn	62.63	62.52	62.40	62.29	61.79	61.77
Corn DDGS	18.00	18.00	18.00	18.00	18.00	18.00
Soybean Meal, 47.5 CP	15.00	15.00	15.00	15.00	15.00	15.00
Choice white grease	2.55	2.60	2.65	2.70	2.55	2.55
Limestone, Ground	1.00	1.00	1.00	1.00	1.02	1.02
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Monocalcium Phosphate	0.18	0.18	0.18	0.18	0.23	0.23
L-Lysine	0.17	0.17	0.17	0.17	0.49	0.49
Vitamin-mineral premix	0.08	0.08	0.08	0.08	0.09	0.09
L-Threonine	-	-	-	-	0.16	0.16
L-Tryptophan	-	-	-	-	0.05	0.05
DL-Methionine	-	-	-	-	0.02	0.02
Paylean 9.0 g/lb	-	-	-	-	0.03	0.05
Betaine	-	0.062	0.126	0.188		
Red Iron Oxide	-	-	-	-	0.20	0.20
Calculated Composition						
ME Kcal/kg	3.44	3.44	3.44	3.44	3.45	3.45
Total Fat, %	7.22	7.30	7.30	7.36	7.20	7.20
Crude Protein, %	17.36	17.40	17.30	17.33	17.77	17.77
Lysine, %	0.88	0.90	0.90	0.88	1.13	1.13
SID Lysine, %	0.76	0.80	0.80	0.76	1.01	1.01
Calcium, %	0.58	0.60	0.60	0.58	0.60	0.60
Phosphorus, %	0.51	0.50	0.50	0.51	0.52	0.52
Av Phosphorus, %	0.26	0.30	0.30	0.26	0.28	0.28
Paylean g/ton	0.00	0.00	0.00	0.00	4.50	8.00
Analyzed Composition, Dry Matter basis, %						
Moisture,	11.53			11.31	11.61	11.12
Crude Protein, %	16.78			17.08	17.83	16.78
Crude Fat	6.07			6.41	6.23	6.36
Crude Fiber	12.15			12.09	11.88	11.98
Ash	3.88			4.11	4.29	4.41
Calcium	0.52			0.49	0.47	0.48
Phosphorus	0.42			0.40	0.43	0.45

Table 4. Effect of dietary supplementation of betaine on growing pig performance during thermo-neutral and heat-stressed conditions (Exp. 1).

	Ambient Conditions													
	Thermo-neutral				Heat-stressed					P value				
	Betaine Supplementation, %													
Variable	**0**	**0.10**	**0.15**	**0.20**	**0**	**0.10**	**0.15**	**0.20**	**SEM**	**Temp**	**Betaine**	**Interaction**	**Linear**	**Quadratic**
Body weight, kg														
d -7	38.8	38.8	38.7	39.0	38.5	38.7	39.6	39.9	1.487					
d 0	45.9	44.5	44.7	46.0	44.2	45.0	46.1	45.8	1.757	0.992	0.654	0.478	0.345	0.421
d 14	57.4	56.1	56.9	58.5	54.5	55.5	55.7	56.6	2.119	0.523	0.579	0.872	0.295	0.366
d 28	69.1	67.1	68.0	68.9	63.3	65.5	65.6	66.2	2.398	0.287	0.814	0.532	0.870	0.440
ADG, kg														
d -7 to 0	1.01	0.82	0.86	0.99	0.82	0.89	0.91	0.85	0.133	0.642	0.933	0.600	0.996	0.527
d 1 to 14	0.883	0.824	0.871	0.895	0.739	0.746	0.696	0.773	0.047	0.004	0.605	0.746	0.732	0.229
d 15 to 28	0.840	0.787	0.791	0.743	0.625	0.712	0.711	0.682	0.043	0.021	0.596	0.110	0.774	0.201
d 1 to 28	0.832	0.806	0.831	0.819	0.682	0.730	0.702	0.728	0.039	0.007	0.951	0.655	0.602	0.909
ADFI, kg														
d -7 to 0	1.77	1.75	1.77	1.78	1.83	1.69	1.73	1.75	0.106	0.896	0.848	0.903	0.670	0.467
d 1 to 14	2.49	2.13	2.23	2.41	1.73	1.69	1.69	1.78	0.130	0.001	0.144	0.441	0.717	0.022
d 15 to 28	2.33	2.24	2.16	2.31	1.86	1.92	1.92	1.92	0.124	0.010	0.922	0.720	0.975	0.669
d 1 to 28	2.28	2.19	2.14	2.36	1.78	1.80	1.80	1.85	0.122	0.002	0.636	0.804	0.588	0.302
G:F														
d -7 to 0	0.559	0.480	0.484	0.545	0.451	0.516	0.501	0.487	0.065	0.639	0.980	0.586	0.923	0.723
d 1 to 14	0.393	0.389	0.391	0.375	0.429	0.452	0.411	0.427	0.016	0.016	0.432	0.394	0.307	0.401
d 15 to 28	0.366	0.355	0.348	0.327	0.343	0.376	0.374	0.335	0.019	0.527	0.253	0.578	0.285	0.090
d 1 to 28	0.369	0.372	0.368	0.351	0.385	0.411	0.390	0.380	0.013	0.039	0.254	0.832	0.375	0.072

Table 5. Effect of dietary supplementation of betaine on respiration rate and rectal temperature of growing pig during thermo-neutral and heat-stressed conditions (Exp. 1)

	Ambient Conditions													
	Thermo-neutral				Heat-stressed					P values				
	Betaine Supplementation, %													
Variable	0	0.10	0.15	0.20	0	0.10	0.15	0.20	SEM	Temp	Betaine	Interaction	Linear	Quadratic
Respiration Rate, flank movements during 30 seconds														
d 0	15.2	14.1	13.7	16.6	15.1	15.7	16.9	16.1	1.331	0.351	0.644	0.411	0.380	0.358
d 1	21.7	19.7	22.8	24.2	69.5	66.7	54.0	65.5	3.592	<0.001	0.394	0.148	0.930	0.126
d 2	22.6	20.1	22.6	21.9	48.7	42.2	51.5	40.7	1.921	<0.001	0.016	0.302	0.246	0.679
d 3	19.6	21.6	22.2	24.2	63.2	57.2	55.9	58.2	3.168	<0.001	0.669	0.109	0.389	0.428
d 7	28.2	27.9	30.0	32.0	46.4	44.2	48.0	45.9	2.172	<0.001	0.312	0.576	0.184	0.330
d 14	23.6	24.6	24.7	27.3	43.3	46.1	39.7	44.4	2.250	<0.001	0.255	0.416	0.321	0.591
d 21	26.6	24.2	27.1	27.4	45.3	44.9	38.9	44.0	2.572	<0.001	0.719	0.372	0.811	0.299
d 28	13.4	14.1	14.4	15.4	45.9	33.6	28.4	33.2	2.942	<0.001	0.388	0.109	0.247	0.237
d 0 to d 28	21.6	20.9	22.5	23.9	48.0	44.4	43.0	44.1	1.224	<0.001	0.278	0.042	0.994	0.053
Rectal Temperature, °C														
d 0	39.19	39.27	39.07	39.21	39.14	38.97	39.16	39.32	0.099	0.577	0.379	0.158	0.436	0.152
d 1	39.14	39.14	39.00	39.34	39.90	39.73	39.98	39.65	0.106	<0.001	0.876	0.020	0.854	0.507
d 2	38.98	38.80	38.81	39.02	39.71	39.28	39.70	39.49	0.133	<0.001	0.147	0.337	0.579	0.067
d 3	39.06	38.71	38.81	38.84	39.54	39.51	39.58	39.56	0.125	<0.001	0.489	0.513	0.420	0.258
d 7	39.19	39.31	39.25	39.20	39.60	39.44	39.61	39.37	0.118	0.002	0.655	0.562	0.536	0.463
d 14	38.79	38.71	38.64	38.72	39.59	39.18	39.59	39.65	0.103	<0.001	0.074	0.087	0.987	0.016
d 21	39.09	38.97	39.01	39.05	39.49	39.43	39.56	39.61	0.112	<0.001	0.714	0.871	0.689	0.315
d 28	38.61	38.76	38.65	38.74	39.00	38.88	39.24	38.99	0.111	0.006	0.508	0.123	0.322	0.769
d 0 to d 28	38.97	38.96	38.91	38.99	39.49	39.20	39.55	39.46	0.064	<0.001	0.065	0.024	0.902	0.041

Table 6. Effect of dietary supplementation of betaine on blood chemistry of growing pig during thermo-neutral and heat-stressed conditions (Part I, Exp. 1)

		Ambient conditions																		
		Thermo-neutral				Heat-stressed														
		Betaine Supplementation, %									P value									
Variable	**Measure**	**0**	**0.10**	**0.15**	**0.20**	**0**	**0.10**	**0.15**	**0.20**	**SEM**	**Temp**	**Betaine**	**T*B**	**Measure**	**T*M**	**B*M**	**T*B*M**	**Linear**	**Quadratic**
	d 3	5.88	5.83	5.97	5.98	5.89	5.99	5.94	5.91										
Total Protein, g/Dl	d 28	6.04	5.88	5.97	6.02	6.13	6.08	6.12	5.97	0.102	0.501	0.930	0.620	0.001	0.148	0.248	0.749	0.972	0.836
	d 3	3.907	3.859	3.860	3.846	3.859	3.737	3.758	3.868										
Albumin, g/Dl	d 28	4.128	3.970	3.998	4.071	4.023	3.853	3.887	3.881	0.066	0.117	0.079	0.963	<0.001	0.128	0.569	0.225	0.140	0.031
	d 3	2.018	1.964	2.094	2.113	2.022	2.237	2.183	2.031										
Globulin, g/Dl	d 28	1.940	1.883	1.967	1.934	2.097	2.221	2.237	2.068	0.096	0.024	0.707	0.449	0.081	0.001	0.744	0.716	0.627	0.383
	d 3	26.4	27.9	30.4	29.9	41.9	36.0	35.9	34.9										
AST, U/L	d 28	22.6	22.9	32.1	26.6	26.3	28.7	27.4	27.7	3.513	0.015	0.710	0.348	<0.001	0.071	0.590	0.703	0.470	0.891
	d 3	26.8	25.6	28.0	27.7	28.0	27.0	26.0	25.5										
ALT, U/L	d 28	31.40	25.73	30.55	34.19	25.75	23.90	24.30	22.55	2.300	0.038	0.654	0.385	0.802	0.001	0.643	0.643	0.754	0.317
	d 3	163.7	183.8	178.2	171.2	145.3	131.2	137.3	172.0										
Alk Phosphatase, U/L	d 28	150.0	154.0	171.0	155.5	122.2	105.7	119.2	141.6	10.50	<0.001	0.262	0.083	<0.001	0.021	0.208	0.914	0.137	0.190
	d 3	15.3	11.6	10.5	11.1	21.8	25.9	28.0	25.8										
GGTP, U/L	d 28	37.0	33.7	33.0	36.7	44.3	46.1	48.7	43.6	6.183	0.003	0.988	0.500	<0.001	0.016	0.995	0.807	0.738	0.904
	d 3	16.3	14.3	14.6	14.8	13.1	11.8	12.9	12.9										
Urea Nitrogen, mg/dl	d 28	15.1	12.9	13.4	13.0	13.9	13.0	14.0	13.6	1.108	0.194	0.433	0.838	0.539	0.000	0.938	0.958	0.332	0.288
	d 3	0.790	0.849	0.774	0.731	0.923	0.910	0.866	0.927										
Creatinine, mg/dl	d 28	0.913	0.936	0.911	0.908	1.110	1.079	1.080	1.208	0.037	<0.001	0.706	0.179	<0.001	0.020	0.010	0.967	0.772	0.960
	d 3	9.34	9.00	8.89	8.89	8.74	8.22	8.39	8.60										
Phosphorous, mg/dl	d 28	8.38	8.36	8.02	7.98	8.01	7.77	8.26	8.28	0.205	0.047	0.337	0.121	<0.001	0.016	0.509	0.517	0.229	0.182

Table 6. Effect of dietary supplementation of betaine on blood chemestry of growing pig during thermo-neutral and heat-stressed conditions (Part II, Exp. 1)

		Ambient conditions									P value								
		Thermo-neutral				Heat-stressed													
		Betaine Supplementation, %																	
Variable	**Measure**	**0**	**0.10**	**0.15**	**0.20**	**0**	**0.10**	**0.15**	**0.20**	**SEM**	**Temp**	**Betaine**	**T*B**	**Measure**	**T*M**	**B*M**	**T*B*M**	**Linear**	**Quadratic**
	d 3	10.87	10.70	10.87	10.87	10.60	10.48	10.33	10.61										
Calcium, mg/dl	d 28	10.55	10.60	10.61	10.54	10.37	10.29	10.26	10.47	0.106	0.002	0.427	0.347	<.0001	0.279	0.703	0.641	0.968	0.109
	d 3	1.73	1.66	1.66	1.69	1.67	1.64	1.65	1.70										
Magnesium, mEq/L	d 28	1.98	1.88	1.87	1.99	2.75	2.59	2.89	2.68	0.132	0.004	0.761	0.836	<.0001	<.0001	0.956	0.840	0.851	0.351
	d 3	6.01	5.64	5.60	5.51	7.10	6.88	6.10	6.83										
Potassium, mEq/L	d 28	5.44	5.64	5.78	5.48	6.25	6.22	6.35	6.50	0.212	<.0001	0.610	0.313	0.013	0.196	0.012	0.655	0.308	0.559
	d 3	142.9	142.2	142.2	141.5	138.7	138.5	138.9	138.9										
Sodium, mEq/L	d 28	143.2	143.6	143.1	143.5	141.2	140.9	141.7	142.6	0.578	<.0001	0.914	0.323	<.0001	0.000	0.157	0.871	0.833	0.518
	d 3	102.0	101.7	101.8	101.4	102.0	101.8	102.2	102.0										
Chloride, mEq/L	d 28	100.6	101.4	102.0	101.2	101.5	102.8	102.2	102.2	0.659	0.288	0.715	0.941	0.577	0.221	0.267	0.718	0.543	0.353
	d 3	108.5	105.8	104.6	104.5	105.6	102.1	109.8	115.4										
Cholesterol, mEq/L	d 28	102.0	103.7	98.9	97.5	101.0	99.8	106.3	106.1	4.566	0.470	0.909	0.286	0.000	0.810	0.440	0.938	0.691	0.586
	d 3	34.9	32.3	27.8	30.8	36.4	33.4	29.4	34.6										
Triglycerides, mEq/L	d 28	29.83	29.43	24.27	23.52	32.64	32.86	29.39	40.56	3.431	0.060	0.141	0.331	0.099	0.067	0.913	0.427	0.204	0.304
	d 3	0.081	0.101	0.098	0.091	0.078	0.081	0.081	0.092										
NEFA, mEq/L	d 28	0.137	0.110	0.102	0.113	0.077	0.069	0.062	0.059	0.010	<.0001	0.847	0.993	0.957	0.000	0.090	0.553	0.474	0.882
	d 3	1468	1514	1329	1542	1347	1286	1523	1424										
Amylase, U/L	d 28	1537	1461	1373	1570	1588	1444	1799	1609	146.5	0.827	0.889	0.491	<.0001	<.0001	0.187	0.899	0.697	0.534
	d 3	100.1	107.8	104.2	98.2	109.4	104.0	103.4	104.9										
Glucose, mg/dl	d 28	96.2	96.0	93.3	96.2	95.4	97.9	99.6	99.6	4.471	0.285	0.974	0.872	0.000	0.997	0.693	0.317	0.872	0.702
	d 3	2.28	2.07	2.29	2.81	1.49	1.43	1.41	1.63										
Lipase, U/L	d 28	2.58	2.71	3.00	2.91	1.71	2.25	2.38	2.77	0.399	0.001	0.397	0.957	0.001	0.140	0.642	0.749	0.112	0.527
	d 3	2277	3622	2392	2474	25655	8119	5278	21057										
CPK, U/L	d 28	1428	1115	2187	1705	1607	1667	1637	3055	1738	<.0001	0.340	0.146	<.0001	<.0001	0.051	0.063	0.777	0.094

Table 7. Chemical verification of diet samples for betaine content (Exp. 2)[1]

Variable	**Control**	**Control + Betaine**	**PLN Control**	**PLN Control + Betaine**
Betaine Inclusion, lbs/ton	0	4.0	0	4.0
Betaine Inclusion, kg/MT	0	1.995	0	1.995
Samples tested, n	3	3	3	2
Betaine concentration, kg/MT	0	1.92	0	1.86
Standard Deviation	-	.08	-	.14
Range	-	1.85-2.01	-	1.76-1.96

[1]Betaine concentrations were determined by DuPont Labs. Expected Activity level was 1.86 kg/MT. The 4.5 g and 8 g/ton Paylean diet results were pooled.

Table 8. Whole-body growth response to betaine and Paylean in pigs housed under elevated ambient barn temperatures (Exp. 2)[1]

	Treatments					P-Values		
	1	2	3	4				
	Control	Control	PLN	PLN	SEM	1 vs 3	1 vs 2	3 vs 4
Betaine Inclusion, lb	0	4.0	0	4.0				
Number of pens	17	17	17	17				
Pigs placed on test	370	371	364	372				
Initial pig weight, lbs	201.8	201.9	201.9	201.9	1.6	0.952	0.971	0.998
Day 1 to 26 pig weight, lbs	250.7	248.3	259.0	255.5	1.8	0.002	0.354	0.176
ADG, lbs/d,	1.88	1.79	2.19	2.06	0.03	<0.001	0.030	0.003
ADFI, lbs/d	6.18	5.97	6.10	5.81	0.07	0.418	0.030	0.003
Feed:gain	3.30	3.34	2.78	2.82	0.03	<0.001	0.304	0.392
Day 1 to barn close								
Average market weight, lbs	269.0	266.5	277.1	273.5	1.9	0.004	0.352	0.193
Average days on feed, d	39.3	39.5	39.3	39.6	0.1	0.894	0.342	0.115
ADG, lbs/d[2]	1.74	1.69	1.95	1.82	0.04	<0.001	0.377	0.027
ADFI, lbs/d[2]	5.96	5.74	5.84	5.58	0.07	0.194	0.026	0.011
Feed:gain[2]	3.44	3.40	3.01	3.08	0.05	<0.001	0.564	0.329
Mortality/Removal, %	1.06	0.58	0.83	1.58	0.53	0.757	0.516	0.313

[1]The statistical model included sex and dietary treatment. Pen was the experimental unit for all traits. Observations greater than 3 standard deviations were considered outliers and removed from the data set. Pig days were used to calculate ADFI. ADG was measured as average pig weight out minus average pig weight in divided by days in the period. ADG did not include dead pig weights or pig days.
[2]Estimates for ADG and feed:gain were calculated using total weights, including dead pig weights. Pig days were used to calculate ADFI.

Table 9. Carcass performance response (fixed time) to betaine and Paylean in pigs housed under elevated ambient barn temperatures (Exp. 2)[1]

	Treatments					P-Values		
	1	2	3	4			1 vs	3 vs
	Control	Control	PLN	PLN	SEM	1 vs 3	2	4
Betaine Inclusion, lb	0	4.0	0	4.0				
Number of pens	17	17	17	17				
Pigs harvested	361	363	341	365				
Maximum days on feed, d	43.9	43.5	43.5	43.9	0.3	0.313	0.231	0.295
Average days on feed, d	39.3	39.5	39.3	39.6	0.1	0.894	0.342	0.115
Average market weight, lbs	269.0	266.5	277.1	273.5	1.9	0.004	0.352	0.193
Hot carcass weight, lbs	198.7	197.6	208.2	205.9	1.5	<0.001	0.593	0.290
Carcass yield, %	74.0	73.7	74.9	74.9	0.16	<0.001	0.325	0.993
Initial carcass weight, lbs[2]	149.3	149.3	149.5	149.3	1.2	0.930	1.00	0.943
Carcass gain, lbs[2]	49.4	48.2	58.8	56.5	0.8	<0.001	0.283	0.043
Carcass ADG, lbs/d[2]	1.26	1.22	1.50	1.43	0.02	<0.001	0.252	0.026
Carcass feed:gain[2]	4.74	4.70	3.90	3.92	0.04	<0.001	0.495	0.837
FOM Fat Depth, mm[3]	22.2	22.6	20.4	20.2	0.3	<0.001	0.268	0.464
FOM Loin Depth, mm[3]	59.8	60.2	63.5	63.4	0.4	<0.001	0.397	0.925
FOM Lean, %[3]	51.8	51.7	53.1	53.2	0.12	<0.001	0.734	0.576

[1]The statistical model included sex and dietary treatment. Pen was the experimental unit for all traits. Observations greater than 3 standard deviations were considered outliers and removed from the data set.
[2]Average daily carcass gain was calculated using hot carcass weight minus initial carcass weight divided by average days on feed, where average days was calculated using pig days during marketing divided by total head count shipped to the packing plant plus 26 days. Initial carcass weight was calculated as initial weight at the start of the study multiplied by 0.74. Carcass ADG did not include dead pig weights.
[3]Hot carcass weight was used as a covariate in the model for these traits.

Table 10. Growth and carcass response to betaine and Paylean in pigs housed under elevated ambient barn temperatures (Exp. 3)[1]

	Betaine, %						P values			
Variable	**0**	**0.0625**	**0.1250**	**0.1875**	**Paylean**	**SEM**	**Betaine**	**Linear**	**Quadratic**	**Paylean**
Pen initial body weight, kg	2111	2106	2116	2098	2068	25.2	0.911	0.711	0.726	0.072
Pig initial body weight, kg	95.8	95.8	95.7	95.7	95.8	0.78	0.970	0.767	1.000	0.776
Pen total feed intake, kg	1690	1688	1717	1681	1677	27.6	0.743	0.981	0.489	0.541
Average daily feed intake, kg	2.47	2.47	2.49	2.46	2.49	0.04	0.972	0.924	0.747	0.707
Pen weight gain, kg	521	506	521	523	566	18.6	0.821	0.748	0.500	0.012
Average daily gain, kg	0.760	0.744	0.752	0.766	0.843	0.02	0.877	0.766	0.457	0.001
Gain:feed	0.308	0.304	0.303	0.313	0.343	0.01	0.658	0.668	0.244	<0.001
Days to market	31.5	31.2	31.5	31.7	31.3	0.57	0.864	0.795	0.515	0.740
Hot carcass weight, kg	88.5	87.7	87.8	87.6	90.9	0.57	0.498	0.209	0.502	<0.001
Back fat depth, mm	20.5	19.8	20.2	19.7	19.2	0.36	0.434	0.207	0.843	0.041
Loin depth, mm	59.5	58.6	58.6	57.2	61.7	0.61	0.069	0.014	0.612	<0.001
Lean, %	52.6	52.7	52.6	52.7	53.4	0.21	0.996	0.964	0.900	0.001
Carcass yield, %	74.2	73.8	74.0	74.1	74.8	0.21	0.557	0.789	0.204	0.001

Figure 1. Temperature and humidity for the experimental period for the thermo-neutral (TN) and heat-stressed (HS) rooms (Exp. 1).

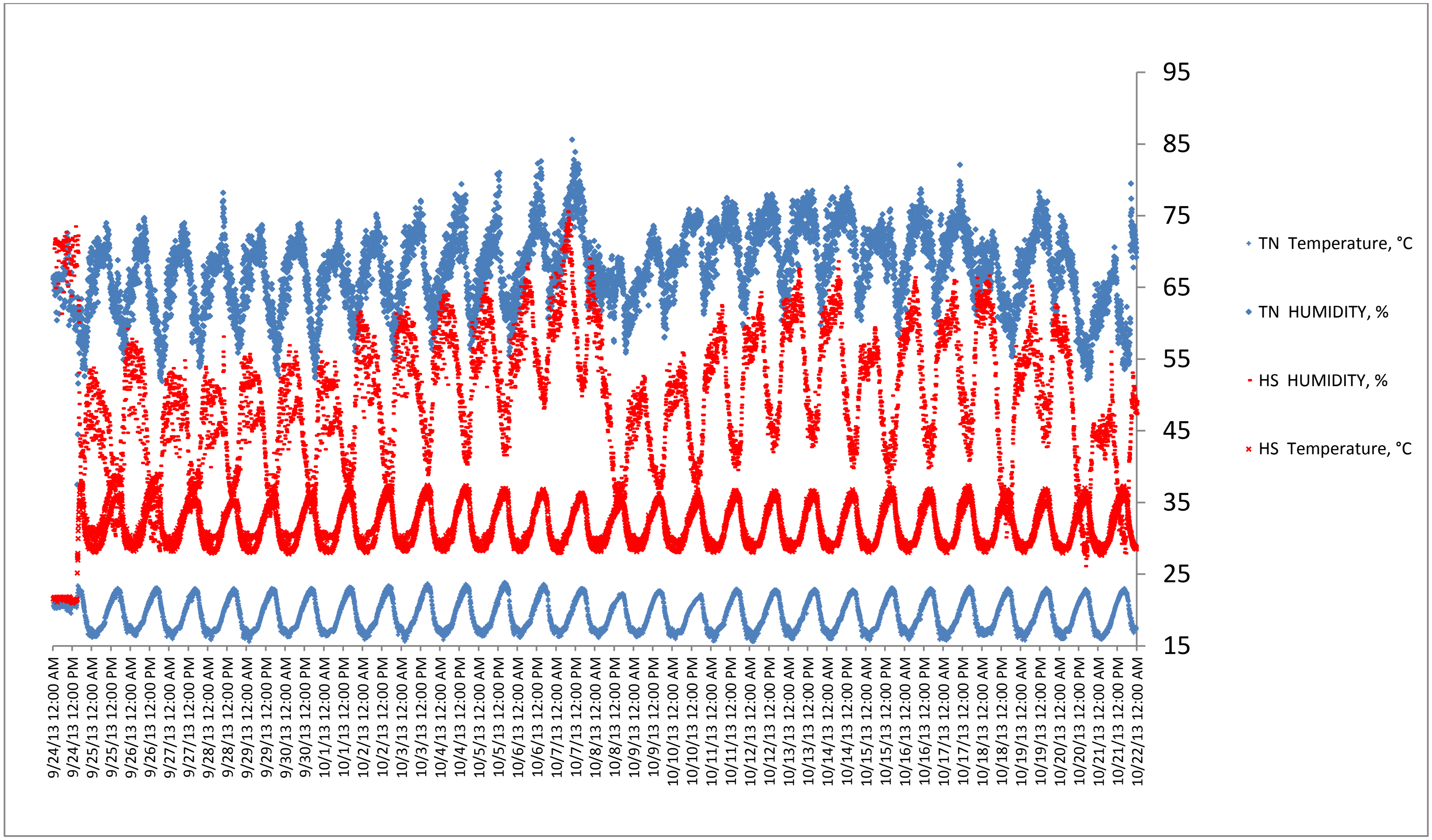

Figure 2. Barn temperatures of the heat-stressed barns and cohort control barns during the experimental period (Exp. 2).

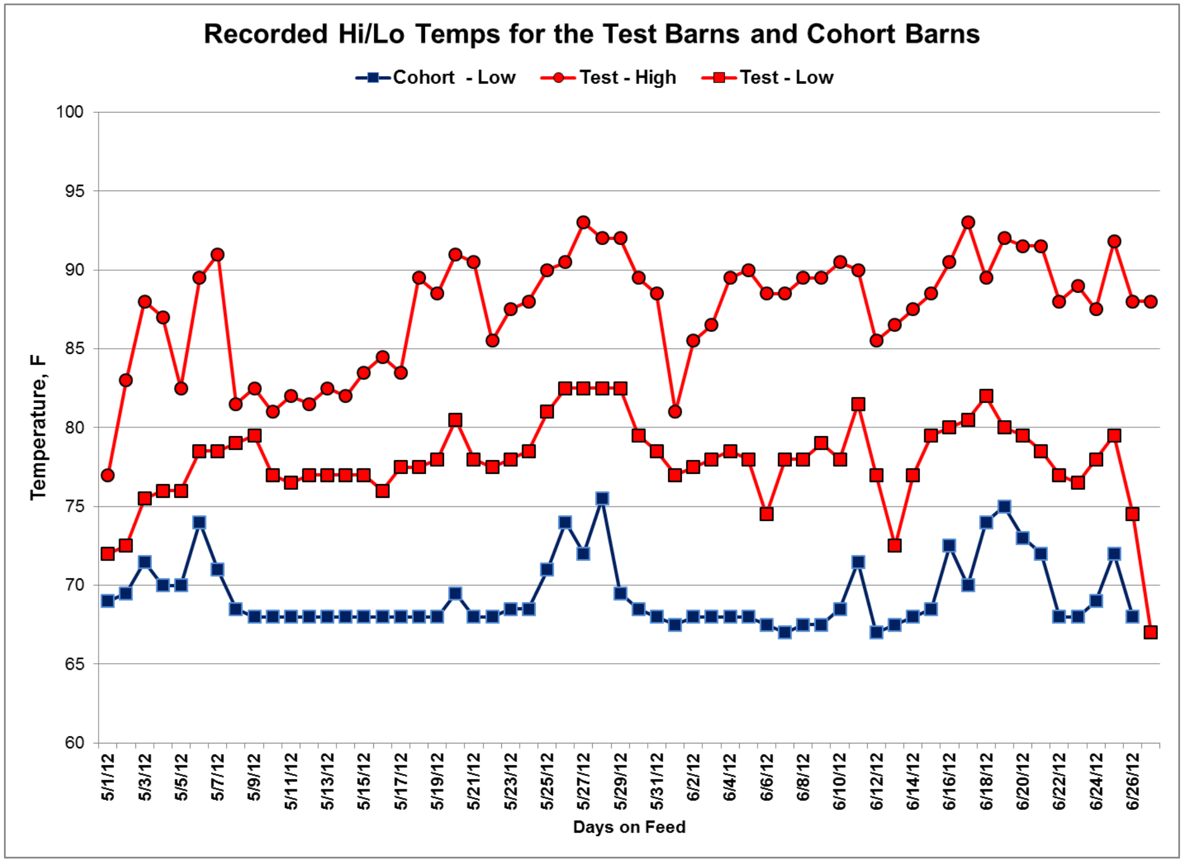

Figure 3. Test barn feeder temperatures for the barrow and gilt barns during the experimental period (Exp. 2).

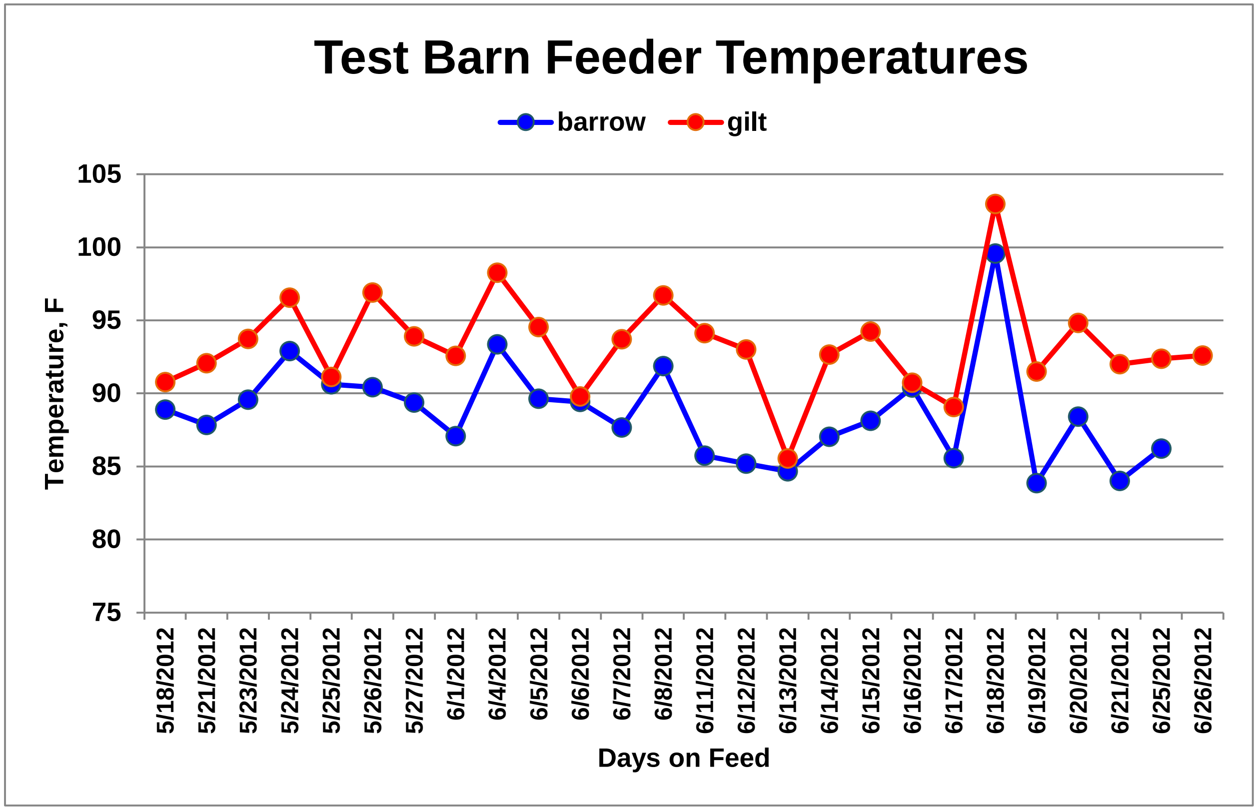

Figure 4. Effects of betaine and Paylean (PLN) on feed intake of pigs during the experimental period (Exp. 2)

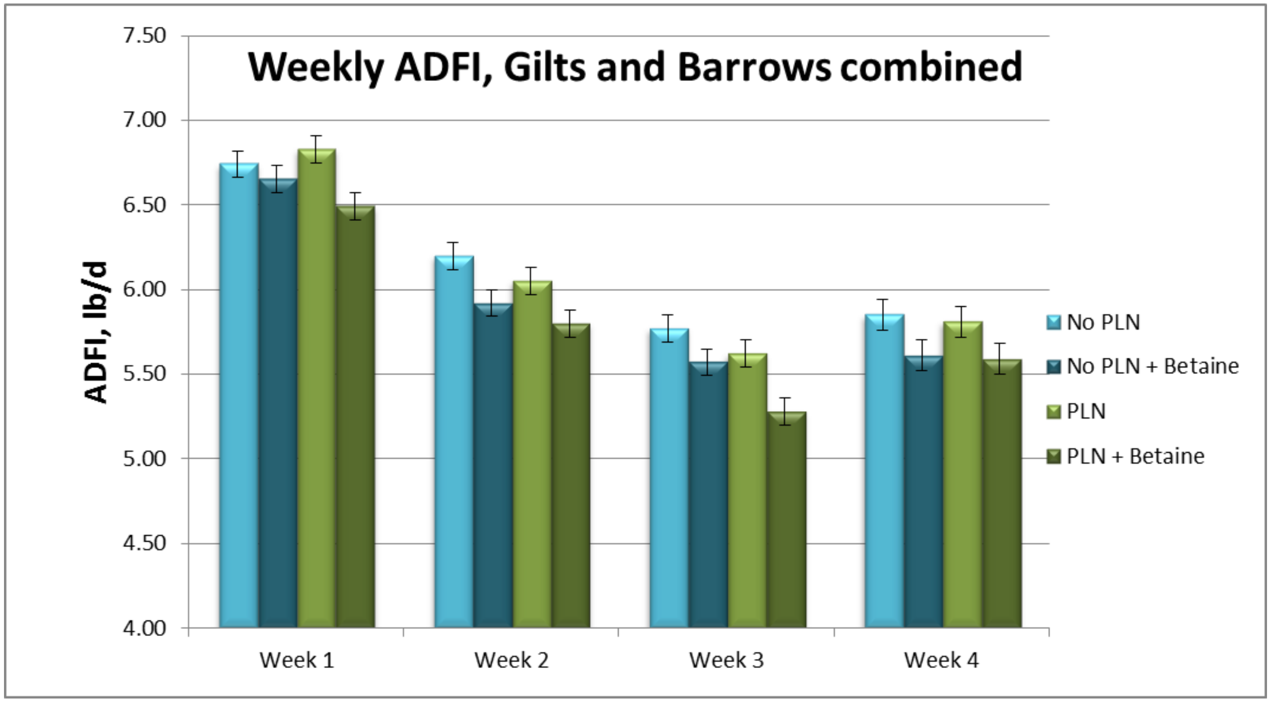

Figure 5. Barn temperatures of the heat-stressed barns and cohort control barns during the experimental period (Exp. 3).