Growth Performance, Carcass Characteristics, Physiological and Gut Health Effects of Feeding Diets Containing Bacitracin Methylene Disalicylate (BMD) to Heat-Stressed Finishing Pigs

A THESIS
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ABSTRACT

Heat stress affects pig growth performance, carcass composition, and immune status. The present study was conducted to evaluate the effects of heat stress and adding bacitracin methylene disalicylate (BMD, 30g/ton) to a 10% DDGS commercial diet on growth performance, carcass characteristics, physiological parameters, small intestine morphology, and hindgut volatile fatty acid (VFA) production of finishing pigs. Four groups of 32 finishing pigs (n = 128) with initial BW between 80 to 90 kg were used in this study. Pigs were randomly assigned to diets and environmental temperature treatments in a 2 x 2 factorial arrangement. Pigs were fed a control (CON) or BMD (30g/ton) diet and exposed to a constant thermal neutral temperature (23˚C) or cyclical heat stress conditions (37˚C from 10:00 to 19:00 and 27˚C from 19:00 to 10:00) in environmental chambers for a 28-d experimental period. Pigs housed under heat stress conditions had significantly lower average daily gain (ADG, $P < 0.0001$), average daily feed intake (ADFI, $P < 0.0001$), gain:feed (G:F, $P < 0.001$), and higher average daily water intake (ADWI, $P = 0.03$), compared with pigs housed in the thermoneutral environment. Supplementation of BMD in the diet did not improve growth performance of pigs. Average daily gain tended ($P = 0.07$) to be lower for pigs fed the BMD diet, while ADFI, G:F, and ADWI were not affected by dietary treatment. Carcass characteristics did not differ between dietary treatments. However, pigs assigned to the heat stress environment had lower live BW ($P < 0.0001$) and lower hot carcass weight ($P < 0.0001$) than pigs housed in

the thermal neutral environment. Dressing %, 10th rib back fat depth, loin eye area, and lean % were not affected by temperature treatment. Saliva cortisol concentration did not differ between dietary treatments during the experimental period, but the initial level was lower ($P < 0.05$) for pigs fed the BMD diet. Heat stress led to an increased ($P < 0.05$) level of saliva cortisol on d 1, but no effects were observed on the following days. Serum haptoglobin concentration was not different between dietary treatments, while heat stressed pigs showed a higher ($P < 0.05$) level of haptoglobin on d 1, and levels tended to remain higher ($P < 0.1$) on d 13 of heat stress. Cytokines IL-1β and TNF-α were not affected by heat stress, but pigs fed the BMD diet had an initial lower ($P < 0.0001$) level of serum IL-1β, and tended to be lower ($P < 0.1$) on d 13 of heat stress as compared to pigs fed CON. Small intestine morphology was not affected by temperature treatment, but pigs fed the BMD diet tended to have greater ($P = 0.07$) villi height at duodenum, and greater crypt depth at duodenum ($P = 0.09$) and jejunum ($P = 0.07$), respectively. Dietary treatment did not affect VFA production in the cecum, while pigs housed under heat stress conditions tended to have less propionate ($P = 0.08$) concentration, greater A:P ratio ($P = 0.08$), and significantly less valerate ($P = 0.02$) produced in the cecum compared with pigs under the thermoneutral environment.

Key words: heat stress, finishing pigs, antibiotic.

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CHAPTER 1

LITERATURE REVIEW

I. Thermal Environment and Swine Production

Swine are susceptible to environmental stress from both cold and heat. Thermal environment, described in terms of effective ambient temperature which combines various climatic events, has a strong influence on swine behavior, energy expenditure, and voluntary feed intake and therefore, impacts pig growth performance, body composition, immune responses, as well as hindgut fermentation status (Noblet et al., 2001).

1. Thermal Environment

- ***Thermal balance***

Thermal balance is the difference between heat gained and lost from metabolism and heat gained or lost from the environment (National Research Council, 1981). Heat balance is important for pigs to regulate their body temperature. In pigs, body heat is gained either by internal metabolism during digestive and metabolic processes or from the external environment. The net loss of heat in pigs occurs through conduction, convection, radiation from the body surface, and through evaporation from respiratory tract and skin surface (National Research Council, 1981). The relative importance of the different types of heat loss in pigs was described by Bond and co-workers (1959; Table 1-1).

These researchers indicated that radiation and convection are the major channels for pigs to lose heat under cold conditions, and comprise up to 35% and 38% of the total heat loss, respectively. However, under hot conditions when the ambient temperature is similar to the body temperature of pigs, evaporation is the main process for eliminating excess body heat. The rate of heat loss is determined by the thermal demand of the surrounding environment and the rate of heat flow through the tissues and skin (National Research Council, 1981). A pig's thermal needs vary and depend on many factors, such as age, weight, activity level, and stage of production. For example, nursery pigs need higher ambient temperatures compared to sows and boars.

Table 1-1. The partition of the total loss of heat from pigs (30-200 kg BW) at three air temperatures (Bond et al., 1959).

Air temperature	Percentage of total heat loss occurring by:			
	Radiation	Convection	Conduction[1]	Evaporation
4˚C	35	38	13	15
21˚C	27	34	11	28
38˚C	3	5	3	90

[1]Heat of warming may be included in this component.

- ***Effective ambient temperature (EAT)***

Effective ambient temperature (EAT) is an index describing the combined effects of not only actual air temperature, but also air speed, humidity, surrounding surface temperatures, and insulating effects of the surroundings (National Research Council, 1981). This concept more accurately relates to the responses of the animals to a collective thermal impact of the total environment (National

Research Council, 1981). For example, it has been shown that moisture and drafts decrease EAT, while straw bedding increases EAT (Cowart, 2001). Floor surfaces also can influence EAT (e.g. a wood floor felt warmer than a concrete floor, which was warmer than woven wire; Cowart, 2001).

Developing specific formulas to calculate the EAT for each animal species is difficult because the adaptation of animals in combating environmental stress by behavioral and physiological reactions has an impact on environmental heat demand. However, the combined effect of some selected environmental variables has been reported, such as wind-chill factors and temperature-humidity index (National Research Council, 1981).

- ***Thermoneutral zone (TNZ)***

The thermoneutral zone is used to describe the relationship between animals and their thermal environment, which was first defined by Mount (1974) as "the range of ambient temperature over which, at fixed level of food intake, body heat production is minimal and constant". The thermoneutral zone was also described by Ames (1980) from another point of view as "the optimum thermal environment from the standpoint of the animal, which is the environment that promotes maximum performance and least stress for the animal". The relationship of thermal zones and temperatures is depicted in Figure 1-1 (National Research Council, 1981). The lower and the upper margins of TNZ are called the lower (LCT) and the upper critical temperatures (UCT), respectively. Effective

ambient temperatures below the LCT cause cold stress, and those above the UCT cause heat stress.

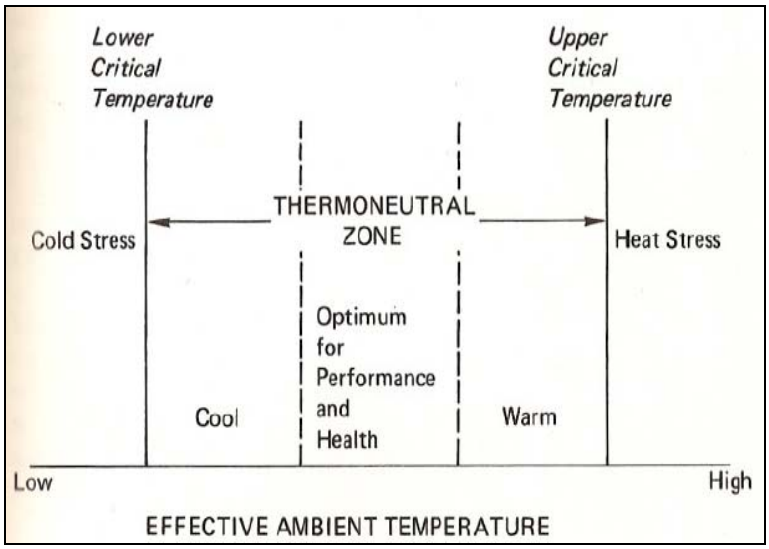


Fig. 1-1. Schematic representation showing relationship of TNZ and temperatures (National Research Council, 1981)

Environmental temperature affects both total heat loss and heat gain of pigs (Noblet et al., 2001). Below the LCT, heat loss though conduction, convection and radiation are predominant, whereas above the UCT, pigs are considered heat stressed and evaporation of moisture from the skin surface or respiratory tract is the primary mechanism to lose excess body heat (Table 1-1). In swine, because of the relatively few functional sweat glands (Ingram, 1965), pigs accelerate heat loss in a hot environment only through increasing their respiratory rate, which suggests a limited adaptation of pigs to hot conditions (Noblet et al., 2001). In addition, pigs respond to heat stress by immediately reducing feed intake to bring metabolic heat production in line with their heat dissipation capabilities. Therefore, heavy pigs with greater metabolic heat production tend to be more

susceptible to heat stress, whereas they are more capable of resisting cold stress (National Research Council, 1981).

2. Environmental Temperature and Nutrition

- ***Effects of environmental temperature on energy requirement***

Thermal environment influences maintenance energy requirements of pigs (Figure 1-2; Ames, 1980). During cold stress, maintenance energy requirement increases linearly. However, during heat stress, maintenance energy requirement increases nonlinearly as heat stress becomes more intensive. Ames (1980) explained that this nonlinear increase in maintenance energy requirement was caused by reduced efficiency of panting and sweating of swine under heat stress and the nonlinear increase in the rate of heat production resulting from the elevated core body temperature. As a result, the prediction of the effects of heat stress on maintenance energy requirement is more difficult than for cold stress.

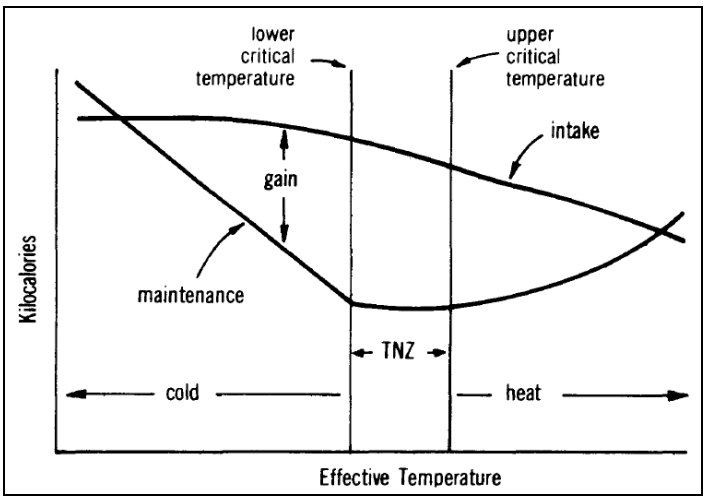


Fig.1-2. Effects of temperature on rate of intake, maintenance energy requirement, and energy retained as product (gain) (Ames, 1980).

- ***Effects of environmental temperature on swine diet composition***

The thermal environment can affect pig nutrient requirements, such as energy, amino acids, vitamins and minerals, and therefore, dietary modification of these nutrients should be considered when pigs are housed during times of either cold stress or heat stress. Decreasing dietary crude protein (CP) and increasing crude fat (e.g., beef tallow, vegetable oil) in swine diets is advantageous during heat stress, because fat has a comparatively lower heat increment than carbohydrate and protein (National Research Council, 1981). In addition, fat is more energy dense which helps offset the lower caloric intake during heat exposure due to the lowered feed intake. Stahly and colleagues (1979) reported improvements in growth performance with reduced CP and increased fat diets of heat-stressed growing-finishing pigs. Spencer et al. (2005) also discovered that when dietary CP was decreased, or fat was increased in the diet, the growth rate of late-finishing pigs was improved 7.4 or 11%, respectively, compared with that of pigs fed a high-CP, low-fat diet under heat stress conditions. In contrast, the use of fiber in the diet should be minimized during times of heat stress, because fiber increases heat increment during digestion by the pig (Spencer et al., 2005). However, in a cold environment, dietary supplementation of fiber could serve a useful role in pigs, since heat generated during the digestion and metabolism of fiber may be used to meet the animal's elevated maintenance needs, thus sparing the amount of other nutrients that must be oxidized for heat production (Stahly and Cromwell, 1986).

Because feed intake changes with changing ambient temperature, dietary concentration of some nutrients may need to be adjusted, such as amino acids and protein (Noblet et al., 2001). For example, Rinaldo and Le Dividich (1991) indicated that the lysine requirement decreases by 0.009 g/MJ ME/˚C decrease in environmental temperature between 20-12˚C, and increases by 0.005 g/MJ ME/˚C increase in environmental temperature between 20-28˚C under *ad libitum* feeding conditions. Generally, exposure of growing pigs to cold stress is associated with a reduction of dietary amino acid requirements, while heat stress induces an increase of the amino acid requirements. Additionally, increased nitrogen (N) excretion and decreased efficiency of protein utilization in growing swine housed at a high ambient temperature also suggest that dietary protein/lysine levels should be increased for pigs under heat stress (NRC, 1981). This theory was confirmed by Lopez et al. (1994) who showed that feed efficiency improved with increased dietary lysine in growing-finishing gilts reared under a hot environment rather than in a thermal neutral environment. However, inconsistent results were also reported in some studies where increased supplementation of lysine above NRC recommendations failed to improve performance of pigs under heat stress (Stahly and Cromwell, 1988; Myer et al., 1997).

3. Heat Stress and Swine Production

The definition of heat stress and its effect on pigs has been well documented (Heitman et al., 1958; Fuller, 1965; Holmes, 1973; Close and Stanier, 1984). As

shown in Figure 1-1, effective ambient temperature above the UCT induces heat stress to pigs. Mount (1974) defined UCT as "the effective ambient temperature above which total heat-production rate at a given feed intake will rise". The upper critical temperature is also described as the temperature at which heat loss is maximal for a pig with dry skin (Holmes and Close, 1977). The recommended thermal conditions and the associated UCT for swine are described in Table 1-2 (Eugene, 1952).

Table 1-2. Recommended thermal conditions for swine (Eugene, 1952).

Type and weight	Preferred range	Lower extreme[a]	Upper extreme
Lactating sow and litter	15 to 26˚C for sow 32˚C for piglets in creep area	16˚C sow area 25˚C creep area	32˚C for sow No upper limit for piglets
Prenursery, (3 to 15 kg)	26 to 32˚C	15˚C	35˚C
Nursery, (15 to 35 kg)	18 to 26˚C	5˚C	35˚C
Growing, (35 to 70 kg)	15 to 25˚C	-5˚C	35˚C
Finishing, (70 to 100 kg)	10 to 25˚C	-16˚C	35˚C
Sow or boar, (＞100 kg)	10 to 25˚C	-16˚C	92˚C

[a]Values represent lower extremes in air temperature when pigs are housed in groups.

- ***Effects of heat stress on growth performance of swine***

The level of feed or energy intake is the first factor limiting pig performance when nutritionally adequate diets are supplied (Noblet et al., 2001). Heat stress has a direct effect on feed intake, and therefore, impacts growth rate and feed conversion of pigs.

i. Feed intake

Pigs exposed to a hot ambient environmental temperature acclimate by increasing heat loss and by decreasing heat production to maintain homeothermy

(Collin et al., 2001). However, due to their limited capacity to lose body heat by evaporation, adaptation to heat stress mainly relies on the pig's ability to reduce heat production. Since eating, digestion, and nutrient absorption all generate heat, pigs reduce feed intake and increase water consumption in an attempt to lower the heat increment (Christon, 1988). In 1949, Heitman and Hughes found that decreased feed intake occurred when air temperature was higher than 21˚C for growing-finishing pigs. Figure 1-3 describes the relationship between ambient temperature, body weight, and feed intake of growing pigs (Noblet et al., 2001). Results shown in this figure illustrate that the negative effects of high ambient temperatures are more apparent with heavier pigs than with lighter pigs.

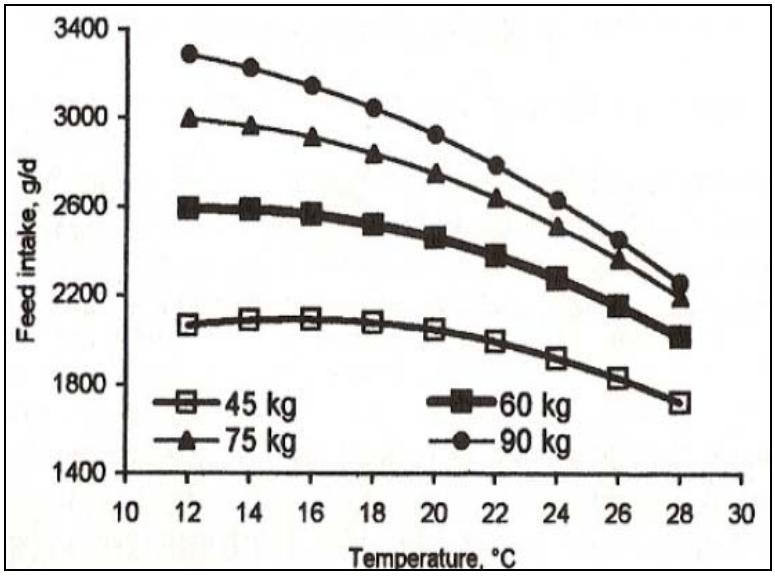


Fig. 1-3. Effect of environmental temperature and body weight on feed intake of growing pigs (Noblet et al., 2001).

ii. Growth rate and feed efficiency

When exposed to heat stress, pigs reduce feed intake to minimize their

metabolic heat production. Meanwhile, their maintenance energy requirement is increased, as shown in Figure 1-2, which eventually leads to reduced growth performance. Many researches have demonstrated average daily gain (ADG) is decreased with decreased feed intake (Christon, 1988; Kouba et al., 2001; Spencer et al., 2005). However, it should be noted that a hot ambient temperature may not have a significant effect on feed conversion because during heat stress, both feed intake and growth rate are decreased compared to these responses when pigs are housed in their TNZ (Ames, 1980; Noblet et al., 2001).

- ***Effects of heat stress on physiological response and behavior of swine***

Heat stress also causes a variety of physiological responses in pigs. Because the pig does not sweat, heat-stressed pigs have significantly increased respiration rates to accelerate heat loss, which requires increased energy expenditure (Aberle, et al., 1974; Christon, 1988).

Christon (1988) recorded a reduction in thyroid activity and decreased thyroid hormone concentration in pigs during times of heat stress. The decrease in thyroid hormone production is an apparent effort by the pig to slow its metabolism and heat production, which leads to depressed growth performance.

Plasma glucose and protein concentrations are also affected directly by high ambient temperature. A decreased plasma glucose level was found in piglets reared under high environmental temperature conditions which could be related to reduced gluconeogenesis from amino acids (Christon, 1988). The protein

degradation found in growing-finishing pigs has not been observed in younger pigs because proteins are used mainly for growth in young animals (Christon, 1988).

Heat stress also affects animal behavior by reducing activity level, including more lying and fewer standing periods, decreased appetite, and a lack of interest in normal activities (Hicks et al., 1998).

- ***Effects of heat stress on carcass composition of swine***

Effects of ambient temperature on carcass composition are highly dependent on feeding conditions. When pigs are exposed to heat stress and get harvested at the same age, carcass fatness is reduced as a result of the decreased feed intake (Sugahara et al., 1970; Nienaber and Hahn, 1983; Giles et al., 1988; Rinaldo and Le Dividich, 1991). However, if heat-stressed pigs are harvested at the same body weight, body fat is likely to increase due to a decreased maintenance energy requirement and thus, an increased availability of energy for deposition of fat (Holmes, 1971; Noblet and Le Dividich, 1982; Close and Stanier, 1984; Kouba et al., 2001). In addition, Kouba et al. (2001) provided evidence that high environmental temperature directly impacts lipid metabolism by enhancing very low density lipoprotein (VLDL) production in the liver and lipoprotein lipase (LPL) activity in adipose tissue, and thus facilitating plasma triglyceride uptake in adipose tissue resulting in greater fatness in heat-stressed pigs. However, there are also other results showing little or no effect of ambient temperature in pigs fed

to achieve similar ADG in warm temperature conditions (Verstegen et al., 1985; Le Dividich et al., 1987).

High ambient temperature also affects body fat distribution. An increased percentage of internal fat (viscera, leaf fat) is observed as ambient temperature is increased (Lefaucheur et al., 1991) compared with other fat depots. Lefaucheur and colleagues (1991) discovered that the activity of lipogenic enzymes in backfat was decreased while the activity of lipoprotein lipase in leaf fat was increased at a high environmental temperature. Moreover, prolonged exposure to a hot environment affects body conformation as well as induces anatomical changes (Nienaber and Hahn, 1983). Nienaber and Hahn (1983) and Lefaucheur et al (1991) proposed that because of decreased feed intake, the mass of internal organs including the liver, kidneys, and digestive tract are reduced at high environmental temperature, resulting in an improvement in carcass dressing percentage.

- ***Effects of heat stress on hindgut fermentation of swine***

The gastrointestinal (GI) tract of swine contains a species-diverse group of microflora, which are predominantly gram-positive bacteria (Savage, 1977; Mackie et al., 1999). Clearly, the intestinal microflora provide real nutritional benefits to the animal, one of which is the production of volatile fatty acids (VFA) due to the fermentative effect of these microflora. The largest population of fermentative microorganisms is present in the cecum and colon of pigs. *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* are the most abundant

cellulolytic species (Grieshop et al., 2000). Fermentation of dietary fiber, such as non-starch polysaccharides and oligosaccharides by anaerobic species in the cecum and colon of the pigs results in the production of a mixture of VFA, with a typical ratio of 60% acetate, 25% propionate, 15% butyrate (Grieshop et al., 2000). These fatty acids can provide between 5 and 28% of the total maintenance energy requirement of the pig (Grieshop et al., 2000). In addition, Sakata and Inagaki (2001) reported that VFA could stimulate gut epithelial cell proliferation and villus size, thereby increasing the absorptive surface area to the intestinal mucosa. The undissociated forms of VFA can reduce the numbers of 'undesirable' bacterial species in the cecum (Snel et al., 2002).

Kelly et al. (1967) observed a significantly reduced VFA concentration in the rumen of cattle during times of heat stress. The majority of this decline was associated with the decrease of acetate and propionate, (which declined by 50 and 72%, respectively), and thus, the Acetate : Propionate ratio increased in heat-stressed cattle. McDowell (1972) also reported reductions in ruminal concentrations of acetate and propionate in heat-stressed cattle. There are no studies that have reported results related to the influence of heat stress on hindgut fermentation status and VFA production in swine.

II. Stress and Immunity

1. The Hypothalamic-Pituitary-Adrenal (HPA) axis

The stress response in animals is a complex phenomenon including autonomic, physical, and behavioral changes as well as neuroendocrine changes. The most simplistic model of the stress response was described by Hans Selye (Selye, 1936), who was considered as the first to demonstrate the existence of biological stress. In his theory, unrelated stimuli, such as pathogen challenge or environmental stress, could all invoke a state of stress in an animal and that they all appeared to act ubiquitously by activating the hypothalamic-pituitary-adrenal (HPA) systems, which provided brain and peripheral control of stress responses (Lay and Wilson, 2001; Salak-Johnson and McGlone, 2007).

- ***Activation of HPA axis***

The HPA axis is normally considered to be comprised of three hormones: corticotrophin-releasing factor (CRF) in the hypothalamus, adrenocorticotropin (ACTH) in the anterior pituitary, and glucocorticoids in the adrenal cortex (Dunn, 2005). Following stimulation by a range of stressful stimuli (i.e., threatened homeostasis, pathogen load, physiological or psychological insult), CRF is released from the paraventricular nucleus (PVN) into the hypophysial portal blood and stimulates the synthesis of ACTH in the pituitary, which in turn travels through the peripheral circulation to the adrenal cortex and promotes the production of the glucocorticoids (Fulford and Harbuz, 2005). Glucocorticoids, with cortisol being the main glucocorticoid in swine and cattle (Lay and Wilson,

2001; Salak-Johnson and McGlone, 2007), are rapidly secreted into the systemic circulation. The secretion of cortisol is critical to both animal survival and the integrity of the HPA axis, because homeostatic dysregulation may lead to immunosuppression, neuroendocrine / autonomic dysfunction and tissue atrophy (McEwen and Stellar, 1993).

- ***Role of cortisol in the regulation of the HPA axis***

The pathways through which the stress response is mediated are extremely complex and controlled by several biofeedback mechanisms (Fulford and Harbuz, 2005). Autoregulation of the HPA axis is essential for the process of a stress response including termination of the stress response and prevention of excessive activation (Fulford and Harbuz, 2005). The negative-feedback control is mediated by the multiple regulatory actions of cortisol, as shown schematically in Figure 1-4 (Lenbury and Pornsawad, 2005). The production of cortisol has the effect of inhibiting secretion of ACTH by decreasing CRF protein synthesis. In particular, cortisol binds to its nuclear receptors and activates receptor-ligand complex, which then translocates to the nucleus and functions as a transcription factor to interfere with CRF gene transcription. The block of CRF gene transcription ultimately leads to decreased CRF protein synthesis, and thus less stimulation of cortisol secretion by the adrenal cortex (Fulford and Harbuz, 2005). The importance of cortisol to normal bodily function has also been demonstrated by studying the effects of removing the adrenal glands (Jingami et al., 1985; Akana et al., 1988; Marti et al., 1999). Jingami et al. (1985) reported an excessive

secretion of ACTH in rats after adrenal glands were removed. Akana et al. (1988) observed replacement with exogenous cortisol in drinking water or food normalized the ACTH secretion in rats with adrenal glands removed, confirming the importance of cortisol in the regulation of the HPA axis. The negative feedback inhibition of the HPA axis is important for hormonal homeostasis. Hormonal homeostasis is the maintenance level of hormones within a particular physiological range. Failure of this feedback mechanism can lead to several complications. For example, adrenal hyperplasia and hypersecretion can occur due to a reduced negative feedback (Lenbury and Pornsawad, 2005).

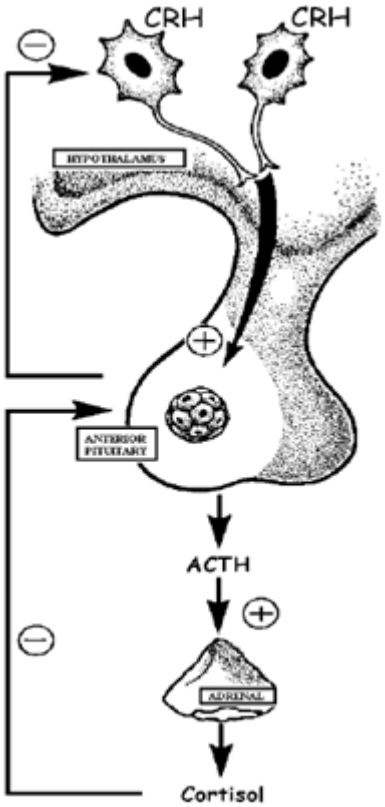


Fig. 1-4. A schematic representation of negative–feedback of HPA axis (Lenbury and Pornsawad, 2005).

2. Stress – Immune Interactions

Stress hormones (CRF, ACTH, and cortisol) which are released in response to activation of the HPA axis by stress have been shown to have a series of effects on the immune system (Salak-Johnson and McGlone, 2007). Knowledge of how these components of the HPA axis affect the immune system is increasingly constantly (Lay and Wilson, 2001). It has been widely accepted that the actions of the cortisol, the end products of activation of the HPA axis, are considered to be one of the most important communication mechanisms between stress and immune system (Fulford and Harbuz, 2005). Cytokines, which are hormone-like proteins, also serve as chemical messengers that allow the immune system to communicate with the rest of the body during times of stress (Spurlock, 1997; Johnson et al., 2001).

- ***The immunosuppressive role of cortisol during stress***

Cortisol has well defined immunosuppressive effects exerted primarily by the inhibition of macrophages, peripheral mononuclear cells, neutrophils, natural killer cells, and almost every component of the immune system (Lay and Wilson, 2001; Paez-Pereda and Stalla, 2005). Cortisol has potent therapeutic efficacy by inhibiting production of inflammatory cytokines, including interleukin-1 (IL-1), IL-2, IL-3, IL-5, IL-6, IL-8, IL-12, IL-13, tumor necrosis factor-α (TNF-α), and γ-interferon (IFN-γ; Paez-Pereda and Stalla, 2005; Wiegers et al., 2005). These immunosuppressive effects of cortisol are significant for protecting the organs

during the stress induced immune response, because a number of cytokines (IL-1, IL2, IL-6, TNF-α, IFN-γ) are toxic at high concentrations, and overstimulation of the immune-defense is harmful to internal organs, such as liver, kidney, and the intestinal tract (Munck and Naray-Fejes-Toth, 1994).

- ***The immunological role of cytokines during stress***

Cytokines are soluble polypeptide signaling proteins produced by stimulated immune cells after activation of the immune system, and exert several biological actions involved in all levels of the immune reaction, including recognition, differentiation, and cell proliferation (Besedovsky et al., 1986; Weizman and Bessler, 1999). Many studies have shown that stress increases plasma levels of cytokines (Abraham, 1991). Among the cytokines, IL-1 is one of the monokines produced predominantly by stimulated macrophages and monocytes during stress (Besedovsky et al., 1986). Interleukin-1 has immunological effects related to the control of differentiation and activation of lymphocytes and the stimulation of lymphokine production (Maizel et al., 1981).

Besodevsky et al. (1986) first discovered that stress and immune responses were controlled by an intimate negative feedback loop between the immune system and HPA axis. This discovery triggered a wealth of research to focus on the connection between these two systems resulting from a stress response. Cortisol and cytokines play an important role in regulating the stress-induced immune response (Figure 1-5; Lay and Wilson, 2001; Dunn, 2005).

When animal is challenged with a stressor or pathogen and macrophages are active, cytokines, such as IL-1 and TNF-α, are released, which activate the HPA axis to eventually cause an increase in cortisol, which in turn suppress the synthesis and release of excess cytokines (Besedovsky et al., 1986; Lay and Wilson, 2001; Paez-Pereda and Stalla, 2005). Interleukin-1 and cortisol, in this negative feedback circuit, provide a protection mechanism that prevents overstimulation of the immune defense, thereby limiting immune cell damage of tissues and autoimmunity (Dunn, 2005). This also indicates that the whole HPA axis has an essential immunoregulatory effect on continuous surveillance of immunological cells and activity in a stress response (Rey et al., 1984).

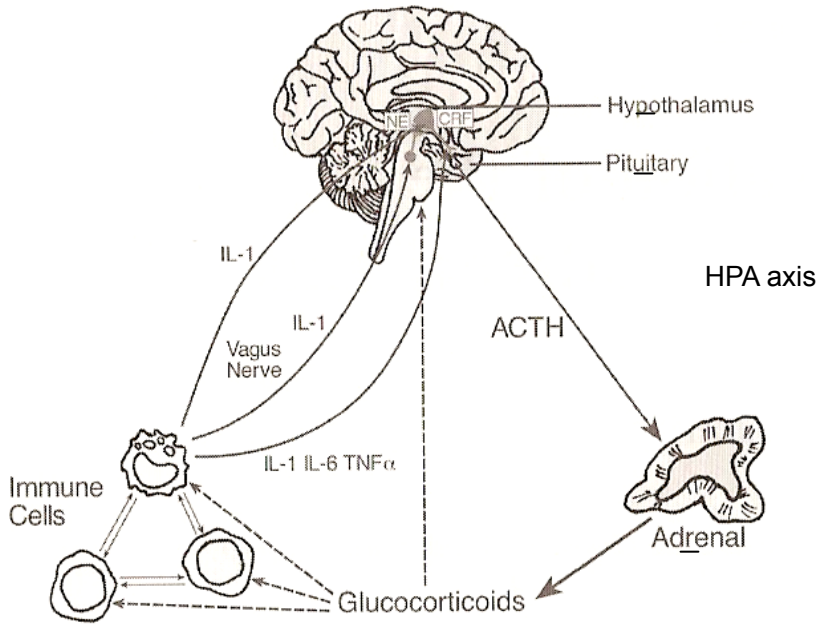


Fig. 1-5. Diagram of the relationship between the brain, the HPA axis, and immune cells (Dunn, 2005).

- ***Acute phase response and the role of haptoglobin in stress-induced immune response***

In addition to the aforementioned roles of cytokines and cortisol in an inflammatory and immune response, the synergism between these two components can induce an acute phase response. The acute phase response is an early, non-specific reaction in animals to defend against disturbances in homeostasis caused by infection, inflammation, tissue injury, or immunological disorders before specific immunity is achieved (Sanchez-Cordon, et al., 2007). The acute phase response leads to production of a number of acute phase proteins by the liver, including haptoglobin, C-reactive protein (CRP), and serum amyloid A (SAA; Black and Berman, 1999; Sanchez-Cordon, et al., 2007). Acute phase proteins are shown to have numerous roles in modulating the immune system, such as enhancing phagocytosis and lysis of macrophages, interfering with serum protease activity, and altering plasma mineral concentrations (Beisel, 1977). Acute phase proteins also promote the adaptive response of the animal for survival (Beisel, 1977). The acute phase protein concentrations in serum can be used as a physiological indicator of a physiological insult or subclinical and (or) clinical disease, and thus, are normally used for evaluating the general health status of animals and humans (Eurell et al., 1992; Grellner et at., 2002; Sanchez-Cordon, et al., 2007). Growth rate and acute phase protein level are inversely correlated in pigs (Williams et al., 1997). In pigs, haptoglobin and CRP are considered the major acute phase proteins. Grellner et al. (2002) has shown that haptoglobin is a valuable indicator of stress in swine herds (Grellner et at., 2002).

Concentrations of acute phase proteins may vary according to the severity and type of stress, infection, or inflammation (Grellner et al., 2002).

3. Effects of Heat Stress on Immune System of Swine

Assessment of the effects of heat stress on the immune system of pigs is difficult and controversial. Many factors, i.e. animal's age, gender, past experience, time and duration of heat exposure, can all affect a pig's reaction and adaptability to heat stress. Therefore it is not easy to obtain consistent measurements for selected immunological parameters and identify the most appropriate or best indicators of heat stress. For example, Morrow-Tesch et al. (1994) discovered an increased level of neutrophils and eosinophils in the peripheral circulation for nursery pigs during 4 weeks of heat stress. In contrast, results reported by Hicks et al. (1998) showed that heat stress did not alter concentrations of neutrophil, eoosinophil, lymphocyte, monocyte in the blood of nursery pigs. Cortisol of pigs has been measured and used as an indicator of hypothalamic-pituitary-adrenal (HPA) axis activation in response to the chronic heat stress (Sutherland et al., 2007). Elevated cortisol concentrations were found in serum and colostrum of heat-stressed sows at parturition and in serum of their piglets (Machado-Neto et al., 1987). Blood cytokines, such as IL-1 and TNF-α, are also used to assess the effects of heat stress on the immune system. In a human study, 17 adult patients were studied during a time of acute heat stroke. Blood IL-1α and TNF-α were significantly increased in all patients, (199 pg/ml

and 480.5 pg/ml, respectively) compared with normal control values (31.4 pg/ml and 53.7 pg/ml, respectively; Bouchama et al., 1991). However, there are no published studies to evaluate if heat stress could affect the production of cytokines in swine.

Ⅲ. The Use of Antibiotics as Growth Promoters in Swine Production

1. Introduction

An antibiotic is a substance or compound that kills or inhibits the growth of bacteria (Davey, 2000). Antibiotics are the sub-group of antimicrobial compounds that act against the growth of microorganisms, such as bacteria, fungi, viruses and parasites (Davey, 2000). Antibiotics are widely used in the livestock industry for three purposes: therapeutic use to treat sick animals; prophylactic use to prevent infection in animals; and as growth promoters to improve feed utilization and growth performance (Barton, 2000). In general, an antibiotic growth promoter is an antibiotic provided in the feed of food animals at a subtherapeutic level to enhance the animal's growth rate and production performance (Avcare, 2003). It is widely acknowledged that adding antibiotic growth promoters in the feed increases an animal's growth rate (Avcare, 2003). JETACAR (1999) stated that 'the economic benefits of antibiotics that promote growth and reduce feed requirements in intensive food-producing animal production were substantial at the time of their introduction 30 years ago'. The purpose of the following review is to describe the range and diversity of benefits gained by supplementing antibiotic growth promoters in livestock diets, especially in swine diets, and will also evaluate the potential risk for human health arising from the use of antibiotic growth promoters in livestock and poultry production.

- ***Discovery and early studies of the use of antibiotic growth promoter***

The growth-promotant effect of adding low levels of antibiotics in animal feed was first discovered in the late 1940s when Stokestad and Jukes (1950) found that chickens fed fermentation waste from tetracycline production grew more rapidly than controls. Since then, results from more studies showed growth enhancing effects, such as improved daily weight gain and feed efficiency, resulting from feeding subtherapeutic levels of a wide range of antibiotics (Avcare, 2003). Table 1-3 is a summary list of the antibiotic growth promoters registered for use in the European Union (EU), the U.S., and Australia (Barton, 2000). Since 2006, the EU has withdrawn all approved antibiotic growth promoters used in livestock and poultry production in EU member nations.

Table 1-3. Summary of antibiotics registered for use as growth promoters in Australia, the EU and the U.S. (Barton, 2000).

Class of antibiotic	Antibiotic	USA	EU	Australia
Arsenicals	Arsnilic acid	Pigs, poultry		Pigs, poultry
β-Lactams	Penicillin G	Pigs		
Glycopeptides	Avoparcin		Suspended 1997	Pigs, meat poultry, cattle
Lincosamides	Lincomycin	Pigs		
Macrolides	Erythromycin	Pigs		
	Kitasamycin			Pigs
	Oleandomycin			Cattle
	Tylosin		Suspended 1999	Pigs
	Spiramycin		Suspended 1999	
Oligosaccharides	Avilamycin		Suspended 2006	
Pleuromutilins	Tiamulin	Pigs		
Polyethers	Lasolocid			Cattle
	Monensin	Cattle	Suspended 2006	Cattle
	Narasin			Cattle
	Salinomycin		Suspended 2006	Cattle, pigs
Polypeptides	Bacitratin	Pigs, poultry, cattle	Suspended, 1999	Meat poultry
Quinoxalones	Carbadox	Pigs		

(Continued)

	Olaquindox			Pigs
Streptogramins	Virginiamycin	Pigs, poultry, cattle	Suspended, 1999	Pigs, meat poultry
Tetracyclines	Tetracycline	Pigs, poultry, cattle		
Bambermycins	Flavophospholipol		Suspended 2006	Pigs, poultry, cattle

- ***Classification and withdrawal periods of commonly used antibiotic growth promoters in swine***

As summarized in Table 1-4, the current FDA regulations for commonly used antibiotic growth promoters in swine, include class, use level, withdrawal times, and indications for use (Adapted from Feed additive compendium, 2009). There are several classification schemes for antibiotics based on bacterial spectrum (narrow vs. broad), or route of administration (injectable vs. oral; Finberg et al., 2004). However, the most widely used scheme is based on their chemical structures, as classified in Table 1-4. Antibiotics within a structural class generally have similar patterns of effectiveness, toxicity, and side-effect potential (Finberg et al., 2004). Most of the antibiotics have been approved for use at low dietary inclusion levels ranging from 5-90 g/ton of complete feed, without a withdrawal period (except for arsanilic acid and carbadox). Meeting the withdrawal times is important to avoid antibiotic residues in meat and ensure food safety. Withdrawal period for an antibiotic to clear the body varies with the type and level of antibiotic (Carlson and Fangman, 2000). Some antibiotics remain in tissues shorter than others, and thus they do not require a withdrawal time before animals are harvested (Carlson and Fangman, 2000).

Table 1-4. Summary of antibiotic growth promoters approved by FDA for using in swine diet (Adapted from Feed additive compendium, 2009).

Animal	Drug	Class	Use level	Withdraw time	Indications for use
Swine	Arsnilic acid	Arsenicals	45-90 g/ton	5 days before slaughter	For increased rate of weight gain and improved feed efficiency in growing swine.
Swine (growing/ finishing)	Bacitracin Methylene Disalicylate	Polypeptides	10-30 g/ton	None	For increased rate of weight gain and improved feed efficiency.
Swine (growing/ finishing)	Bacitracin Zinc	Polypeptides	20 g/ton	None	For increased rate of weight gain. Feed in Type C feed*.
			20-40 g/ton	None	For improved feed efficiency. Feed in Type C feed*.
			10-50 g/ton	None	For increased rate of weight gain and improved feed efficiency.
Swine (growing)	Carbadox	Quinoxalones	10-50 g/ton	Vary by manufacturers.	For increased rate of weight gain and improved feed efficiency.
Swine	Lincomycin	Lincosamides	20 g/ton	None	For increased rate of weight gain in growing-finishing swine from weaning to market weight.
Swine	Penicillin	β-Lactams	10-50 g/ton	None	For increased rate of weight gain and improved feed efficiency.
Swine	Taimulin hydrogen fumerate	Pleuromutilins	10 g/ton	None	Increased rate of weight gain and improved feed efficiency.
Swine	Tylosin	Macrolides	10-20 g/ton	None	For increased rate of weight gain and improved efficiency - finisher feeds.
			20-40 g/ton	None	For increased rate of weight gain and improved efficiency - grower feeds.
			20-100 g/ton	None	For increased rate of weight gain and improved efficiency - starter feeds.
Swine	Virginiamycin, followed by	Streptogramins	10 g/ton	None	For increased rate of weight gain and improved feed efficiency from weaning to 120 lb.
	Virginiamycin		5 g/ton		For increased rate of weight gain and improved feed efficiency. For continuous use from weaning to market weight.

* Type C feed: A medicated feed which is intended to be a complete feed. It can be fed as the sole ration, topdressed, or free-choice. It is manufactured by diluting a Type A medicated article or a Type B or C medicated feed (Herrman and Sundberg 2002).

2. Mode of Action

The mechanism(s) by which antibiotic growth promoters improve growth performance is not well understood. Most of the antibiotic growth promoters act against gram-positive bacteria (Table 1-5) through their intrinsic antimicrobial activities, such as inhibition of cell wall synthesis, inhibition of ribosome function, and disruption of cation transfer (Avcare, 2003).

Table 1-5. Antibacterial activities of antibiotic growth promoters (Adapted from Barton, 2000)

Class of antibiotic	**Commonly used growth promoters**	**Mode of action**
Arsenicals	Arsanilic acid	DNA effects
β -Lactams	Penicillin G	Gram-positive cell-wall synthesis
Glycopeptides	Avoparcin	Gram-positive cell-wall synthesis
Lincosamides	Lincomycin	Inhibit protein synthesis in Gram-positive bacteria
Macrolides	Erythromycin, tylosin, kitasamycin, oleandomycin, spiramycin	Inhibit protein synthesis, principally in Gram-positive bacteria
Oligosaccharides	Avilamycin	Inhibit protein synthesis in Gram-positive bacteria
Pleuromutilins	Tiamulin	As for macrolides
Polyethers	Monensin, Lasolocid, Narasin, Salinomycin	Affect bacterial cell permeability; active against Gram-positive bacteris
Polypeptides	Bacitratin	Gram-positive cell-wall synthesis
Quinoxalones	Carbadox, olaquindox	Inhibit bacterial DNA synthesis and denature pre-existing DNA; active against anaerobes
Streptogramins	Virginiamycin	Inhibit protein synthesis in Gram-positive bacteria
Tetracyclines	Tetracycline	Inhibit protein synthesis; broad spectrum
Bambermycins	Flavophospholipol	Interferes with cell wall synthesis in Gram-positive bacteria

As early as 1950, Groschke concluded that antibiotics enhanced animal growth performance by modifying the intestinal flora from undesirable to desirable types. However, he did not identify the optimal microflora for the animal (maximum benefits with minimum costs) due to the lack of quantitative

analytical methods available at that time. Jukes (1955) also claimed that most of the benefits of antibiotic growth promoters are derived from the effects of manipulating the intestinal microflora, and this theory was confirmed by the fact that oral antibiotics did not have growth-promoting effects in germ-free animals (Coates et al., 1955; Coates et al., 1963). Since then, studies on the mechanism(s) of antibiotic growth promotion effects have focused on interactions between the antibiotic and intestinal microbiota. Although the exact mechanism by which antibiotics enhance growth has not been demonstrated, it is presumed that decreased competition for nutrients and a reduction in microbial metabolites that depress an animal's growth are a direct result from the effects of antibiotic growth promoters on the microflora (Visek, 1978; Anderson et al., 1999). In addition, researchers believe that antibiotic growth promoters reduce the overall numbers and (or) the numbers of species of gut bacteria (Visek, 1978; Close, 2000; Collier et al., 2003). Other effects from supplementing antibiotic growth promoters in the feed that have been observed include inhibition of microorganisms that produce toxic compounds or damage intestinal tissues, reduction of bacterial utilization of essential nutrients, improvement of synthesis of vitamins and other growth factors (Avcare, 2003). In a recent study, Collier et al. (2003) utilized PCR-denaturing gradient gel electrophoresis (DGGE) and quantitative PCR to investigate the effects of antibiotics on the microflora in growing pigs. They found that antibiotic treatments reduced species diversity and total numbers of bacteria, including lactobacilli. Results from this study provide a new approach

that showed that further use of these techniques can help us better understand the microbiological component of the mode of action of antibiotic growth promoters since most of the antibiotic growth promoters act by modifying the intestinal microflora.

3. Benefits of Using Antibiotic Growth Promoters

The benefits arising from the use of antibiotic growth promotants in livestock and poultry diets can be broadly categorized into performance improvement, disease control, environmental benefits, prevention of metabolic and fermentation disorders, and a set of other related benefits such as improved stress tolerance and improved immune status. Specific advantages of using antibiotic growth promoters in feed are summarized in Table 1-6 (Avcare, 2003).

- ***Performance improvement***

The majority of benefits from supplementation of antibiotic growth promoters are improved productivity and economic returns to producers (Taylor, 1999). The Centre for European Agricultural Studies (CEAS, 1991) reviewed studies from 1950 to 1984 which investigated the main effects of antibiotic growth promoters in growing pigs. The outcome of using four of these still used as antibiotic growth promoters in the U.S. today are summarized in Table 1-7. Results from a total of 166 studies were evaluated and ADG, feed intake, and feed efficiency responses ranged from 2.6-11.6%, 4.2-7.4%, and -0.5 to 7.1%,

respectively (Table 1-7). The Centre for European Agricultural Studies (1991) also observed that the benefits of growth promoters displayed a time-dependent trend, which were +13.3% and +8.1% for improvement of ADG and feed efficiency for the period 1950-1961, and +9.7% and +4.8% for the period 1974-1984. However, it should be noted that the number of antibiotics used was greater and their use rates were variable during these two time periods.

Table 1-6. Summary of benefits of antibiotic growth promoters (Avcare, 2003).

Benefits	**Antibiotic Growth Promoters**										
	Avilamyecin	Bacitracin	Bambermycin	Lasalocid	Monensin	Narasin	Salinomycin	Kitasamycin	Oleandomycin	Tylosin	Virginiamycin
ENVIRONMENTAL BENEFITS											
Reduced methane emission (primarily ruminants)			√	√	√	√	√			√	√
Reduced nitrogen excretion (all species)	√	√	√	√	√	√	√			√	√
Reduced phosphorus output (all species)				√	√	√	√				√
PERFORMANCE IMPROVEMENTS											
Increased rate of body weight gain	√	√	√	√	√	√	√	√	√	√	√
Lower feed requirements for each unit of gain	√	√	√	√	√	√	√	√	√	√	√
Improved carcass yield	√		√								
Improved sow performance							√				√
Improved piglet survival and growth							√				√
Increased diary cow milk production				√	√						√
Increased wool growth			√								√
DISEASE CONTROL											
Necrotic enteritis in poultry	√	√		√	√	√	√	√			√
Clostridial enteritis in pigs							√				√
Porcine proliferative enteropathy	√	√			√		√			√	√
Swine dysentery							√				√
Acute pneumonia in cattle				√	√	√	√				
Coccidiosis in calves and sheep				√	√	√	√				
Tosoplasmosis in ewes					√						
PREVENTION OF METABOLIC AND FERMENTATIVE DISORDERS											
Decreased lactic acidosis				√	√	√	√				√
Decreased laminitis				√	√	√	√				√
Decreased ketosis					√						
Decreased ruminal bloat				√	√						
OTHER BENEFITS											
Protein sparing	√	√	√	√	√	√	√			√	√
Energy sparing	√	√	√	√	√	√	√			√	√
Improved mineral absorption				√	√						√
Improved heat tolerance	√	√		√	√	√	√				√
Decreased boar taint		√									√
Reduction in antibiotic resistance and its transfer		√	√								
Improved immune status		√								√	
Drier litter and reduced foot problems in broilers	√										√
Decreased fly survival in cattle faeces				√	√						

Table 1-7. Responses of growing pigs to antibiotic growth promoters (Adapted from CEAS, 1991).

Antibiotic	#	Average daily gain (kg)			Feed intake (kg/d)			Feed conversion efficiency		
		UCT*	Treated	Change, %	UCT*	Treated	Change, %	UCT*	Treated	Improvement, %
Bacitracin	39	0.54	0.58	+2.6	1.54	1.61	+4.9	2.64	2.65	-0.5
Salinomycin	24	0.58	0.63	+7.2	1.53	1.61	+5.1	2.54	2.36	+7.1
Tylosin	39	0.51	0.57	+11.6	1.33	1.43	+7.4	2.51	2.4	+4.6
Virginiamycin	64	0.56	0.62	+9.9	1.62	1.7	+4.2	2.75	2.58	+6.2

#: Number of studies analyzed.

*UCT: untreated control animals.

A more comprehensive review of studies were completed by SOU (1997) to investigate the growth rate and feed efficiency responses to antibiotic growth promoters in piglets, growing/finishing pigs, broiler chickens, laying hens and veal calves (Table 1-8). They concluded that the response of younger animals was greater than in older pigs.

Table 1-8. Ranges of responses by livestock to supplementation of antibiotic growth promoters in the diet (SOU, 1997).

Livestock species	**% Improvement in Growth rate (ADG)**	**% Improvement in Feed Efficiency**
Piglets	16	9
Growing pigs (20-50 kg)	9	5.5
Finishing pigs (50 kg-market)	0	0
Growing-finishing pigs	2-20	1-10
Broiler chickens	3-10	3-5
Laying hens	2	1
Veal calves	7-10	4-5

- ***Disease control***

Antibiotic growth promoters control some chronic diseases in livestock, and therefore, improve productivity and economic revenue. Porcine proliferative

enteropathy (PE) is a common disease caused by *Lawsonia intracellularis*, and it is often observed in post-weaning pigs between 6 and 20 weeks of age (Avcare, 2003). Pigs having porcine PE always show diarrhea and even death if not treated in time (Avcare, 2003). Porcine PE can be prevented effectively by tiamulin or tylosin (McOrist et al., 1996). Swine dysentery (SD) is another severe muco-hemorrhagic disease primarily affecting growing-finishing pigs (Avcare, 2003), and it causes severe diarrhea, but more commonly it leads to chronic loss of body weight (Avcare, 2003). Quinosalones are shown to effectively control swine dysentery, but resistance appears to occur if treated with tylosin, lincomycin, monensin and tiamulin (Molnar, 1996). Additionally, two clinically important types of clostridial enteritis in swine, *Clostridium perfringens* Type C and A, can be prevented by strategic supplementations with appropriate antibiotic growth promoters (Avcare, 2003). *Clostridium perfringens* Type C causes fatal necrotic enteritis mostly in young piglets, and could result in up to 59% mortality in the herds (Avcare, 2003). Kyriakis et al. (1996) reported that salinomycin could be used to control *C. perfringens* Type C infection by reducing the incidence of diarrhea (51% lower) and duration of diarrhea (87% shorter) in piglets born to sows treated with 60 ppm salinomycin from two weeks before farrowing until weaning. *Clostridium perfringens* Type A causes enteritis in neonatal and weaned piglets (Avcare, 2003). The clinical signs are less dramatic than those of Type C enteritis, but piglets may express diarrhea for several days and remain in poor condition after recovery (Avcare, 2003). Kyriakis and

colleagues (1995) conducted a study on a commercial pig farm with ongoing *C. perfringens* Type A disease. They observed that weaning pigs supplemented with salinomycin at 60 ppm for an initial 30 days followed by 30 days at 30 ppm gained more weight (21% heavier) with higher gain efficiency (19% higher) than those of control pigs.

- ***Environmental benefits***

Environmental benefits resulting from the use of antibiotic growth promoters include reducing N and P excretion in manure, and therefore, reduce environmental pollution as well as increased nutrient utilization in animals (Roth and Kirchgessner, 1994; Avcare, 2003). Much of the N released by livestock is in the form of ammonia, which can affect the health of livestock (e.g. causing respiratory impairment, especially within confined spaces with suboptimal ventilation; Donham et al., 1985). Phosphorus is the most limiting nutrient for aquatic plants, and large quantities of P in the soil can enter waterways from erosion which then stimulate the growth of some toxic algae and other aquatic vegetation eventually causing eutrophication (Avcare, 2003). Lawrence (1997) noted that significant practical benefits from the use of antibiotic growth promoters include increased nutrient utilization efficiency, improved N and P retention, and decreased N and P excretion when nutrient intake meets the animal's requirements. However, the mechanisms of these effects from feeding antibiotic growth promoters are poorly understood.

4. Factors Influencing Response to Growth Promotion

The magnitude of the response to antibiotic growth promoters in animals is influenced by many factors, such as growth rate, age, stage of life cycle, and environmental conditions (Avcare, 2003). Braude et al. (1953) summarized results from many experiments and concluded that the pig's response to antibiotics was inversely related to their rate of growth **(**Figure 1-6**)**. In particular, pigs with a growth rate responded less to antibiotic treatment. Melliere et al. (1973) corroborated this concept twenty years later by reviewing performance results from 369 replicates of 4,890 healthy pigs supplemented with tylosin in the diet. They confirmed that pigs with a higher rate of gain showed less improvement of gain (Figure 1-7). The response to growth promoters also varies with the age of pigs. Hays (1979) observed that the response to growth promoters was greater in younger pigs than in more mature pigs (Table 1-9). Moreover, the growth enhancing benefits of antibiotics are more pronounced in unsanitary environments, with a higher pathogen load, compared with more sanitary, high-health environments (Cromwell, 2001). As shown in Table 1-10 (Melliere et al., 1973), field studies observed greater benefits on ADG and feed efficiency than those in the research station when tylosin was added in the diet. In addition, it is clear that the response to antibiotic growth promoters is greater for pigs during critical stages of production such as breeding, farrowing, and weaning (Hays, 1979). Environmental stresses such as crowding, shipping, mixing of animals, high or low ambient temperature also contribute to increased response to antibiotic growth

promoters (Hays, 1979).

One of the explanations of these different responses is that pigs with maximal growth performance are always provided with good quality diets which meet nutrient requirements for individual animals. The environmental conditions are optimal and stable for these pigs, and even subclinical disease is not present. As a result, supplementation of antibiotic growth promoters to these pigs may not provide notable benefits at the herd level. In contrast, pigs with low growth rates may be associated with energy or nutrient deficiencies in the diet, or presence of acute or chronic disease, combined with adverse environmental conditions. Supplementation of antibiotic growth promoters may offset nutrient deficiency and control disease, and ultimately improve growth performance of the animal.

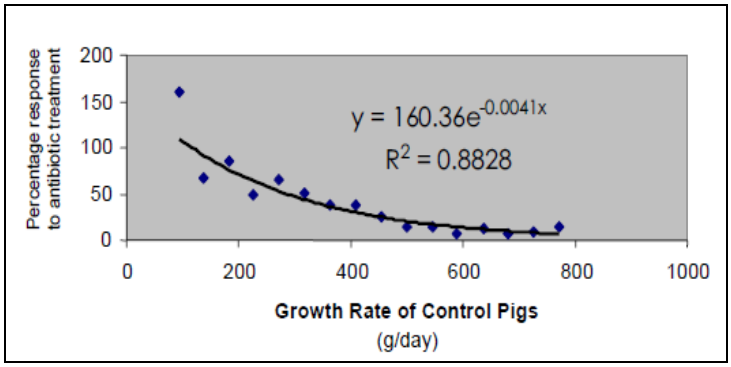


Figure 1-6. Relationship between growth rate of control and antibiotic treated pigs (Braude et al., 1953).

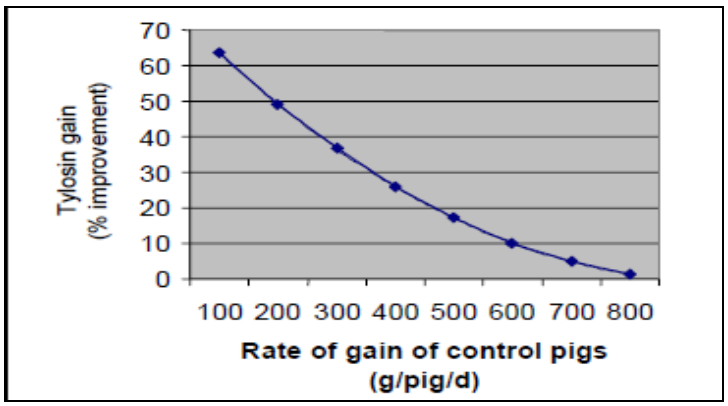


Figure 1-7. Impact of control performance of treatment effect (Melliere et al., 1973).

Table 1-9. Impact of the age of pigs on the performance response to in-feed inclusion of antibiotics (Adapted from Hays, 1979)

Antibiotic	**Average daily gain (% of improvement)**			**Average feed /gain (% of improvement)**		
	Starter	**Grower**	**Finisher**	**Starter**	**Grower**	**Finisher**
Bacitracin	9.7	5.1	2.5	3.3	2.5	2.7
Tylosin	14.8	10.9	4.6	6.0	4.2	1.5
Virginiamycin	11	10.7	5.7	5.0	6.6	3.3

Table 1-10. Effect of study location on response of finishing pigs to tylosin treatment (Adapted from Melliere et al., 1973).

Study location	**Average daily gain (g/day)**			**Feed/gain**		
	Control	**Tylosin**	**Benefit (%)**	**Control**	**Tylosin**	**Benefit (%)**
Research station	790	803	1.7	3.36	3.35	0.3
Field study	713	754	5.7	3.84	3.66	4.7

5. Use of BMD as a Growth Promoter in Swine Diets

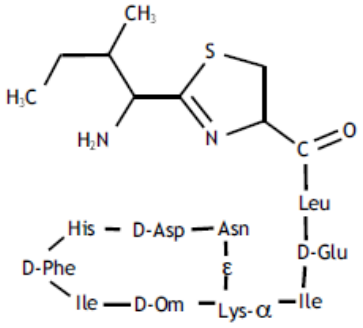
- ***Description***

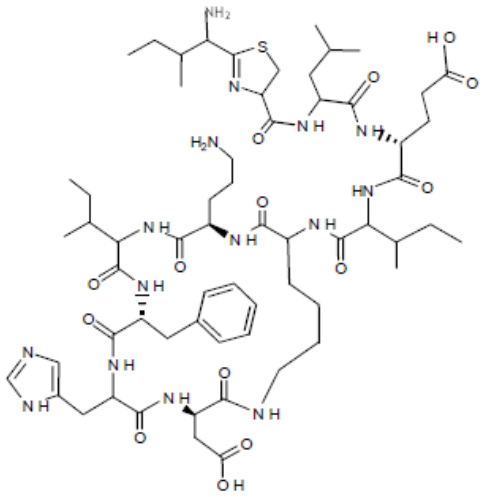
Bacitracin is a complex mixture of one or more antimicrobial polypeptides produced by certain strains of *Bacillus licheniformis* and *Bacillus subtilis*. Figure 1-8 (Avcare, 2003) provides basic information on Bacitracin such as the synonyms, chemical structure, molecular formula, and weight. Zinc bacitracin is the zinc salt complex of bacitracin and is more stable than uncomplexed bacitracin in the environment (Avcare, 2003).

Figure 1-8. Description of Bacitracin (Avcare, 2003).

Synonyms Albac
BMD

Chemical structure





Asn	asparagine	Leu	leucine
Asp	aspartic acid	Lys	lysine
Glu	glutamineOrn	ornithine	
His	histidine	Phe	phenylalanine
Ile	isoleucine		

POLYPEPTIDE STRUCTURE
(all L amino acids unless otherwise stated)

CHEMICAL STRUCTURE

Bacitracin A
Molecular formula $C_{66}H_{103}N_{17}O_{16}S$
Molecular weight 1422.719

- ***Mode of action***

Bacitracin has bactericidal effects mainly on Gram-positive bacteria by inhibiting cell wall synthesis (Butaye et al., 2003). Basically, bacitracin inhibits the transportation of the carrier for peptidoglycan synthesis by binding to the P-P-lipid between the carrier and the cell membrane. This interferes with its dephosphorylation. As a result, the carrier cannot be recycled and bacterial cell wall formation is inhibited (Avcare, 2003; Butaye et al., 2003).

- ***Toxicity***

Bacitracin is largely unabsorbed from the gastrointestinal tract, skin, and

mucosal surfaces (Avcare, 2003). As a result, no residues should be found in meat of animals administered bacitracin orally (Butaye et al., 2003). Results from two studies were summarized by FDA (1999) in which chickens were fed 1,100 ppm BMD for 8 weeks and laying hens were fed 1,100 ppm for 5 months. In both studies, there were no detective residues in tissues, egg yolks, or egg whites. However, these two studies did not examine if supplementation of BMD could affect health status or any physiological parameters in treated chickens. Bacitracin can be nephrotoxic if administered parenterally (Butaye et al., 2003), which means bacitracin can be only administered orally to animals.

- ***Benefits***

i. Growth improvement in swine

As early as 1953, Braude and colleagues summarized several studies of bacitracin use in pigs and they concluded that growth improvement occurs when feeding diets containing bacitracin (Table 1-11). Thirty years later, Zimmerman (1986) reviewed results from studies evaluating the use of both BMD and zinc bacitracin in grower-finisher pig diets (Table 1-12). From seven experiments using BMD, Zimmerman reported an improvement in average daily gain and feed efficiency of 1.2% and 0.4%, respectively, for pigs ranging from 26-98 kg in body weight (Table 1-12). However, these benefits were not observed when Zn Bacitracin was used in a study as shown in Table 1-12. In some studies, the response to bacitracin on pig performance has generally been greater in younger

pigs than in older pigs, as shown in Table 1-9 (Adapted from Hays, 1979).

Table 1-11. Effect of bacitracin on growth and feed efficiency in pigs (Braude et al., 1953).

Treatment	**Growth index**	**Gain efficiency**
Untreated	100	100
Bacitracin	109.0 (12)*	103.0 (10)*

*Figures in parentheses indicate number of comparisons.

Table 1-12. Response to antibiotics fed during the grower-finisher period (Adapted from Zimmerman, 1986).

Antibiotic	**Number**			**Weight (kg)**		**ADG (g)**			**G:F**		
	Exp	**Rep**	**Pigs**	**I**[3]	**F**[4]	−[5]	+[6]	%↑[7]	−	+	%↑
Bacitracin MD[1]	7	38	334	26	98	724	742	1.2	3.01	3.11	0.4
Bacitracin Zn[2]	1	4	55	31	100	695	695	0	3.36	3.42	-1.8

[1]Data were from Aherne (1982); Brumm (1983); Danielson and Crenshaw (1984); Kerr et al. (1984); Libal et al. (1983); Mahan and Corley (1982); Spurlock and Jesse (1985).
[2]Data were from Mahan et al. (1981).
[3]I: initial body weight.
[4]F: final body weight.
[5]−= untreated control pigs.
[6]+= bacitractin treated pigs.
[7]%↑= percentage improvement due to treatment.

ii. Control of clostridial enteritis in swine

In addition to growth promotion effects, bacitracin has been shown to effectively control clostridia enteritis caused by *C. perfringens* in neonatal pigs born to sows fed a level of 275 ppm BMD diet during the 14 days prior to farrowing and 21 days after farrowing with a history of clostridial scours (Table

1-13; Avcare, 2003). This study (Avcare, 2003) was conducted in swine herds in Illinois, Minnesota, Missouri and Nebraska that had natural outbreaks of clostridial enteritis. Piglet mortality resulting from clostridial enteritis was described as the percentage of pigs that died from the number born alive, and ADG and scour scores were described as litter average. Scour scores ranged from 1 to 4 representing normal to severe diarrhea.

Table 1-13. Effect of feeding BMD to pregnant sows on mortality due to *C. perfringens*, ADG and scouring of newborn piglets (Avcare, 2003).

Study	**Piglet Performance**					
	Mortality, %		**ADG, kg/day**		**Scour scores**	
	UTC[1]	BMD (275 ppm)	UTC[1]	BMD (275 ppm)	UTC[1]	BMD (275 ppm)
1	27.3	2.0	0.191	0.213	1.90	1.06
2	21.1	7.2	0.205	0.207	1.95	1.35
3	25.4	0.0	0.195	0.239	1.46	1.00
4	22.2	1.5	0.173	0.225	1.92	1.08

[1]UTC: untreated control animals.

Another study was conducted by Schultz et al. (1992), who observed significant improvements in production parameters when adding 275 ppm BMD in sow diets in a herd with subclinical clostridial infections. Compared with pigs born from untreated sows, piglets born from sows fed BMD showed 4.4 kg heavier weaning weights, 4.4% higher survival rates, and 5% higher ADG, suggesting that bacitracin could be beneficial to control clostridia enteritis disease in swine.

iii. Heat stress control in poultry

Large economic losses occur annually resulting from increased mortality and decreased production in poultry due to the high ambient temperatures. Poultry researchers have described the effects of bacitracin on decreasing heat production in broilers and layers, and improving their ability to withstand heat stress (Männer and Wang, 1991). For example, Bronsch and Männer (1988) investigated the effect of zinc bacitracin on energy metabolism in heat-stressed laying hens. They found that when the upper limit of the themoneutral zone was raised from 22.4˚C to 24˚C, or when hens were exposed to prolonged heat stress at 34˚C, inclusion of zinc bacitracin at 100 mg/kg in the diet could effectively reduce the depression of egg production, as a result of reduced energy maintenance requirement and improved energy utilization for egg production. Männer and Wang (1991) conducted two experiments with 100 and 120 hens, respectively, which were acclimatized to 20˚C and 34˚C and supplemented with diets containing zinc bacitracin at 100 mg/kg. They found that body weight gain, feed efficiency, egg number, and total egg mass of treated hens kept at a moderate temperature (20˚C) were not significantly improved. However, hens fed diets containing zinc bacitracin had a more pronounced improvement in these productivity measures than heat-stressed hens. In particular, improvements in weight gain, feed efficiency, egg number, and total egg mass were significantly improved by 66.3, 5.9, 15.4, 16.9%, respectively (Table 1-14). Additionally, supplementation with zinc bacitracin for hens acclimatized from 20˚C to 34˚C

reduced fasting heat production by 4.1% and 7.6%, respectively. However, no studies have been conducted to determine if bacitracin reduces the negative growth performance effects in heat-stressed pigs and the associated potential mechanisms of this response.

Table 1-14. Percentage improvement of zinc bacitracin treated hens over controls (Adapted from Männer and Wang, 1991).

	20˚C	**34˚C**
ADG, %	3.5	66.3
Feed efficiency, %	3.1	5.9
Egg Number, %	2.6	15.4
Total Egg Mass. %	3.3	16.9

6. Problems Associated with Antibiotic Use in Animals

The major concerns arising from the use of antibiotic growth promoters in animals are antibiotic residues in food products and bacterial resistance and associated risk to human health. A nationwide survey released in May, 2003 showed that when Americans – regardless of age, education, income level, and region – shopped for meat, almost three-quarters (74%) were concerned about the antibiotic residues in meat products. This concern was ranked just under top basic concerns such as price, flavor and food safety, indicating Americans' strong desire to buy 'antibiotic free' meat. Antibiotic resistance is a microbiological phenomenon, which is associated with the ability of a microorganism to withstand the effects of antibiotics. The use of antibiotic growth promoters is one of the contributing areas of greatest concern, because the duration of treatment may be for the whole life of the treated animals (Barton, 2000). There are three major

issues involving antibiotic resistance. First, there is a potential problem for human health because antibiotic resistant bacteria can pass through the food chain into the human body where they can develop infections (JETACAR 1999; Barton, 2000). Secondly, it is recognized that resistant bacteria are regarded as compromising the efficacy of some key human antibiotics, because many of these antibiotics are identical, or closely resemble drugs used to treat human illness, such as tylosin, spiramycin, bacitracin, and virginiamycin (Dibner and Richards, 2005). Thirdly, efficacy of antibiotic therapy may be reduced for animals colonized with resistant bacteria (Barton, 2000).

Since concerns have arisen about resistance in human pathogens resulting from the use of antibiotic growth promoters, many countries have established antibiotic-resistance surveillance programs to regulate the use of antibiotic growth promoters in livestock and poultry feeds. Avoparcin was first banned for use as an antibiotic growth promoter in Denmark in 1995 due to the concerns that glycopeptide-resistant enterococci were developed in animals fed avoparcin and that it was a potential risk to human health (Dibner and Richards, 2005). In 1997, avoparcin was banned in all EU member states by the Commission of the European Union (Dibner and Richards, 2005). In July and September 1999, other individual growth promoters were banned by the EU Commission, such as tylosin, bacitracin, and virginiamycin, because they are also used in human medicine. Furthermore, since January 1st, 2006, all antibiotics used as growth promoters were banned in EU (Pradella, 2006).

There are fewer regulatory activities regarding antibiotic growth promoter use in the United States compared to the EU. However, it is clear that the general use of antibiotic growth promoters is under scrutiny and the pressure from consumers is building to remove antibiotic growth promoters from animal feeds (Angulo, 2004). For example, Kentucky Fried Chicken (KFC) and McDonald's Corporation both claimed that they do not accept chicken meat if antibiotic growth promoters were used in their production (Dibner and Richards, 2005). These self-imposed regulations makes the practice of using antibiotic growth promoters economically impractical, and forces producers in any country that seek export markets in the U.S. and the EU to discontinue antibiotic growth promotant use in animal feed.

IV. Summary

Swine, like other homoeothermic species, are sensitive to changes in environmental temperature. In conventional growing-finishing pigs with *ad libitum* access to feed, there is an optimal range of environmental temperature that maximizes overall growth performance and carcass characteristics. Pigs under either cold stress or heat stress conditions will experience reduced growth rate and feed efficiency. Heavier pigs are particularly susceptible to heat stress due to their greater metabolic heat production, compared with lighter and younger pigs. Slower growth rate, caused by reduced feed intake and increased energy lost from panting of heat-stressed pigs has been confirmed in many studies. Heat stress also alters the pig's metabolism as it tries to minimize its heat production. One of the subsequent effects of growing-finishing pigs raised under heat-stressed conditions is that carcass composition changes (e.g. carcass fat, and increased internal carcass fat deposition in heat-stressed finishing pigs). However, reasons for the causes of body composition changes in pigs housed in hot conditions are not well understood.

It is clear that stress and the body's immune response are highly integrated. Several cytokines have the ability to activate the HPA axis and result in the elevated plasma concentration of cortisol, which provides negative feedback protecting against the overstimulation of immune-defense reaction. In addition to the immunological inhibitory effects of cortisol, cortisol can act synergistically with cytokines to initiate an acute phase protein response and thus, cause

production of a number of acute phase proteins. However, the detailed molecular mechanism for this process is poorly understood.

Another aspect based on the understanding of the relationship between stress and immunity is identifying appropriate physiological and immunological indicators of heat stress in swine. However, results from a large number of studies are conflicting, due to a number of factors including animal perception, past experience, genetics, age, and housing system. Better understanding of the impact of these factors on stress response will greatly improve our understanding of stress physiology. Also, behavioral parameters should be used together with immune and physiological parameters to demonstrate a stress response, since behavioral change is always consistent and specific to a stressor.

Antibiotic growth promoters have been used in the livestock industry for almost 60 years, during which their significant growth enhancing effects have been confirmed by many studies. Most of the antibiotic growth promoters are supplemented in the feed and at a low inclusion, and do not require a withdrawal period according to FDA regulations. It should be noted that the response of animals to antibiotic growth promoters vary depending on many factors, such as animal's age, genetics, and housing environment. Bacitracin is one of the commonly used antibiotic growth promoters in the U.S. It is clear that supplementation of bacitracin in the feed can improve the growth performance and feed efficiency in pigs. However, no studies have been conducted to determine if bacitracin reduces the negative growth performance effects in

heat-stressed finishing pigs and the associated potential mechanisms of this response.

CHAPTER 2

Growth performance, carcass characteristics, physiological and gut health effects of feeding diets containing bacitracin methylene disalicylate (BMD) to heat-stressed finishing pigs

INTRODUCTION

The effects of heat stress on feed intake and growth rate of finishing pigs are well-documented (Heitman et al., 1958; Fuller, 1965; Holmes, 1973; Close and Stanier, 1984). Heat-stressed pigs have reduced feed intake and increased water consumption in an attempt to lower the metabolic heat increment and maintain the body temperature compared to pigs housed in a thermal neutral environment (Christon, 1988). The decreased feed intake combined with increased maintenance energy requirement results in slower growth rate in heat-stressed pigs (Christon, 1988; Spencer et al., 2005). The effects of heat stress on carcass composition are inconsistent. In some studies, body fat increased in pigs housed in hot environmental temperatures (Holmes, 1971; Kouba et al., 2001), while other studies showed little or no effect of ambient temperature on carcass characteristics in pigs fed to achieve similar ADFI (Verstegen et al., 1985; Le Dividich et al., 1987). The mechanisms of these responses to heat stress are not well understood.

Thermal stress generally activates the immune system of animals (Machado-Neto et al., 1987). Following the stimulation of a stressor, the hypothalamo-pituitary-adrenal (HPA) axis is activated, resulting in the release of cortisol from the adrenal cortex into the circulation (Dantzer and Morme`de, 1983). Cortisol has immunosuppressive effects by inhibiting almost every component of the immune system, including cytokins (i.e., interleukin-1, TNF-α), to protect against overstimulation of the immune-defense reaction (Lay and

Wilson, 2001; Paez-Pereda and Stalla, 2005). Pig cortisol levels have been used as an indicator of HPA axis activation in response to heat stress (Sutherland et al., 2006). Cytokines are signaling proteins produced by stimulated immune cells after activation of the immune system (Besedovsky et al., 1986). Blood cytokines, such as IL-1 and TNF-α, are also used to assess the effects of heat stress on the immune system in humans (Bouchama et al., 1991). However, no study has been conducted to evaluate the effects of heat stress on cytokine production in swine. In addition, cortisol can act synergistically with cytokines to induce the production of a number of acute phase proteins (APP) by the liver (Sanchez-Cordon et al., 2007). Among several APPs, haptoglobin is the most widely measured APP in pigs and is a useful marker of stress and pathological challenges (Pineiro et al., 2003). Previous study in disease challenge models demonstrated that haptoglobin can increase from 2 to 20 times the level found in serum of healthy pigs (Pineiro et al., 2003). This finding supports the use of serum haptoglobin as an indicator of immune system responses, and therefore health status in pigs.

Fermentation of dietary fiber, such as non-starch polysaccharides and oligosaccharides by anaerobic microflora in the cecum and colon of pigs results in the production of a mixture of volatile fatty acids (VFA). Grieshop et al. (2000) estimated that VFA's contribute between 5 and 28% of the total maintenance energy requirement of the pig. The benefits of VFA in pigs were reported by Sakata and Inagaki (2001) showing that VFA can stimulate gut epithelial cell

proliferation and villus size, thereby may increase the absorptive ability of the intestinal tract. Results from ruminant studies have shown that VFA concentration, particularly acetate and propionate, is reduced in the rumen of cattle during the times of heat stress (Kelly et al., 1967; McDowell, 1972). However, there has been very little research conducted in swine to assess the influence of heat stress on hindgut fermentation status and VFA production.

It is widely acknowledged that including subtherapeutic doses of antibiotics in the diet of livestock improves growth performance (Taylor, 1999). Bacitracin methylene disalicylate (BMD) is an antibiotic growth promoter produced by certain strains of *Bacillus licheniformis* and *Bacillus subtilis* (Avcare, 2003). The main site of antibiotic activity of bacitracin is within the gastrointestinal tract, where bacitracin acts to modify the intestinal microflora (Huyghebaert and de Groote, 1997). Clearly, supplementation of bacitracin in the feed can improve the growth performance and feed efficiency of pigs (Braude et al., 1953; Zimmerman, 1986), but greater improvement occurs in younger and lighter pigs, rather than in more mature and heavier pigs (Hays, 1979). Poultry researchers have evaluated the effects of feeding diets containing bacitracin on decreasing heat production in broilers and layers, and showed that bacitracin improves their ability to withstand heat stress (Männer and Wang, 1991). However, no studies have been conducted to determine if bacitracin reduces the negative effects of heat-stress in pigs and the associated potential mechanisms of this response if it occurs. Therefore, the objective of this study was to evaluate growth

performance, carcass characteristics, immunological and physiological effects, and gut health of feeding diets containing BMD to heat-stressed finishing pigs.

MATERIALS & METHODS

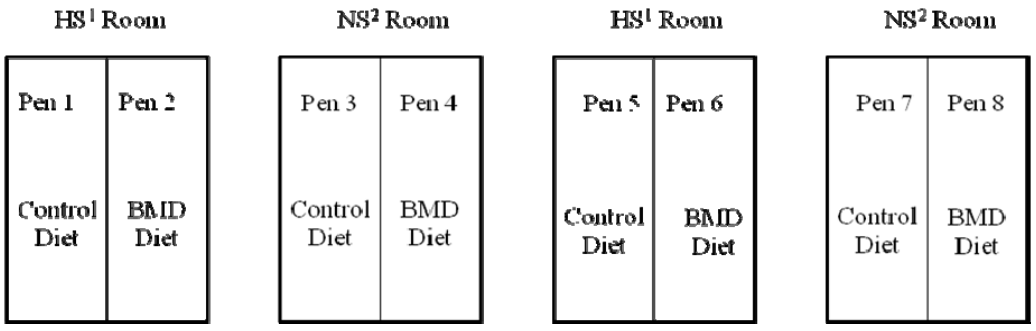
Animals and Housing

All animal use procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee (Protocol # 0711A20863). A total of 128 finishing pigs were used in this study and were terminal offspring of sows (Landrace × Yorkshire; Genetically Advanced Pigs) sired by Duroc boars (Comparts Boar Store, Nicollet, MN).

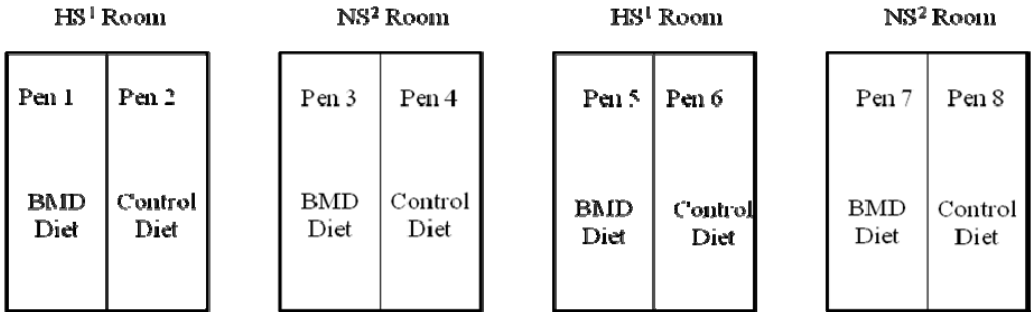
Four groups of 32 finishing pigs (16 barrows and 16 gilts / group) with initial BW from 80 kg to 90 kg were used in this study. Pigs from the first group were transported from the Southern Research and Outreach Center in Waseca, MN, whereas pigs from the second, third, and fourth groups were transported from the West Central Research and Outreach Center in Morris, MN, to the environmental chambers located at the University of Minnesota, St. Paul Swine Teaching and Research Facility. Pigs were blocked by initial BW and sex, and blocks were randomly assigned to diets and environmental temperature treatments in a 2 x 2 factorial arrangement. For each group, 4 environmentally controlled rooms were used with 2 pens per room and 4 pigs per pen. Two replicate pens of pigs (2 barrows and 2 gilts/pen) were fed a control (CON) or BMD (33 ppm) diet and were exposed to either a constant thermal neutral temperature (23˚C) or cyclical heat stress conditions (37˚C from 1000 h to 1900 h and 27˚C from 1900 h to 1000

h) from d 0 to d 28 for growth performance assessment.

Pigs were fed their respective experimental diets for a 14-day adaptation period at the thermal neutral temperature (23˚C) prior to imposing environmental temperature treatments. Room temperature and humidity were monitored daily during the experimental period. The specific design for each group is shown in Figure 2. All pigs were housed in 2.7 m×1.2 m solid concrete floor pens containing one self-feeder and a nipple drinker in a watering bowl with a water meter in each pen. The light schedule of environmental rooms was controlled and consisted of 16 hours of light and 8 hours of dark (2200 h to 600 h) using an automatic timer. All pigs were allowed *ad libitum* access to feed and water throughout the trial and were monitored for health on a daily basis.



Design for Group 1 and Group 4



Design for Group 2 and Group 3

Figure 2. Experimental design for specific group.

[1]HS: Heat Stress (37˚C from 1000 h to 1900 h and 27˚C from 1900 h to 1000 h)

[2]NS: Non-Heat Stress (23˚C)

Pigs continued to be housed and fed their respective treatments until harvest which varied for each group (total experimental days for Group 1: 38 d; Group 2: 37 d; Group 3: 28 d; Group 4: 37 d) based on availability of the abbatoir.

Dietary Treatments

The two experimental diets were comprised of corn, soybean meal, and 10% DDGS (CON) and BMD (30 g/ton) diet (Table 2-1). All diets were formulated on a standardized ileal digestible amino acid basis, and nutrient levels of the diets met or exceeded NRC (1998) nutrient requirements for pigs with 325 g lean gain/d. The DDGS used in this study was obtained from a modern ethanol plant (Golden Grain Energy, Mason City, IA) and the values of standardized ileal digestible (SID) amino acids (AA) for this DDGS source are shown in Table 2-2 (Urriola et al., 2007).

Feed samples from each manufactured batch were collected and frozen at -20 °C until laboratory analysis were performed. Samples from each batch of each experimental diet (8 batches/dietary treatment totally) were pooled and sent to commercial laboratories for proximate analysis (Minnesota Valley Testing Laboratory, New Ulm, MN), AA analysis (Experiment Station Chemical Laboratory, University of Missouri-Columbia), and BMD analysis (Alpharma Animal Health, Chicago, IL).

Growth Performance Measurements

Individual pig BW, pen feed disappearance, and pen water consumption were measured on d 0, 7, 14, and 28 to calculate the average daily gain (ADG), average daily feed intake (ADFI), gain:feed (G:F) and average daily water intake (ADWI) for wk 1, 2, 3-4, and overall for the experimental period. Additionally, pen feed intake and water disappearance were measured daily during the first 7-d of the experimental period to determine the profile of daily pen feed intake and water consumption for wk 1 of the experimental period.

Saliva, Blood Sample Collection, and Rectal Temperature Measurement

Saliva samples from group 2, 3, and 4 were collected from every pig on four collection days: d -1, d 1, d 13, and d 27. Saliva was collected between 1030 h to 1200 h starting from Pen 1 to Pen 8 on all collection days. Salivette with a cotton wool swab (SARSTEDT, Aktiengesellschaft and Co, Numbrecht, Germany) was used for saliva collection. Each pig was allowed to chew the cotton wool swab while it was clipped to a flexible thin metal rod for approximate 3 min until the swab was thoroughly moistened. Approximately 0.6 mL of saliva was obtained from each swab. The salivette with moistened cotton swabs were put into the refrigerator (4˚C) immediately after collection and were centrifuged at 400 x g for 5 min to extract saliva and then frozen at -20˚C until cortisol analysis was performed.

Blood samples from the 4 groups were collected from one randomly selected

barrow per pen on the same day after saliva collection, and all samples were collected from the same pigs on each collection day. Pigs were restrained by using a nose snare. Blood samples were collected via jugular vein puncture using a vacuum tube (Vacutainer, Becton Dickinson, Rutherford, NJ) and a 20 gauge needle. After collection, all blood samples were stored at 4˚C overnight before centrifugation at 2000 x g for 20 min. Serum was removed, and aliquots were put into 1.5 ml microfuge tubes and kept at -20˚C until laboratory analysis could be conducted for haptoglobin, IL-1β and TNF-α. Blood samples obtained on d -1 were also sent to Veterinary Diagnostic Laboratory (College of Veterinary Medicine, University of Minnesota) for PRRS, mycoplasma and SIV (H1/H3) serology detection to characterize health status of the pigs used in this study.

Rectal temperature was measured on d 1 and 27 in the afternoon from 32 pigs in Group 4 by inserting an electronic digital probe thermometer approximately 5 cm into the rectum. The thermometer was held for approximately 15-20 sec until a stable reading was achieved.

Cortisol Analysis

Salivary cortisol concentration was analyzed using the solid phase cortisol radioimmunoassay kit (Coat-A-Count TKCO, Diagnostic Products Corporation, Los Angeles, CA) modified by Ruis et al. (1997). Phosphate buffer solution (0.01 M, pH 7.2) was used to prepare a serial dilution of supplied human serum for the test standard cortisol concentrations of 0.000, 0.3125, 0.625, 1.25, 2.5, 5,

10, 20, 40, and 80 ng/ml. Each sample and standard were analyzed in duplicate aliquots of 200 μL. One mL ^{125}I of cortisol was added to each tube and mixed with samples or standards, and then incubated for 45 min at 37˚C which allowed the binding of cortisol with its antibody at the bottom of the tube. Following incubation, unbound ^{125}I was aspirated and the remaining radioactivity was counted using a gamma counter (Packard Instrument Company, Meriden, CT). The assay was conducted in two batches. The intra-assay coefficient of variation was 6.1% and the inter-assay coefficient of variation was 8.3%. The recovery percentage of cortisol from the saliva using this method has been reported to be 102% (Anil et al., 2007), and the minimal detectable sensitivity of this assay is 0.31 ng/ml (Anil et al., 2007).

Haptoglobin Analysis

The serum haptoglobin concentration was measured using the Porcine Haptoglobin Measurement Kit (TP801, Tridelta Development Ltd, Wicklow, Republic of Ireland) which was single radial immunodiffusion test plates. The standards that were provided with the kit had concentrations of 250 μg/ml and 1000 μg/ml. Each sample and standard was analyzed in duplicate following the manufacturer's instructions. For each test sample, 5 μL of diluted serum (1:4) was pipetted into the porcine specific test well and incubated in a humidified box at room temperature for 48 h. During the incubation, the sample diffused radially from the well into the gel agar plate, where a specific precipitate occurred

between porcine haptoglobin and the specific rabbit antiserum to porcine haptoglobin, and thus, a visible precipitate ring was formed. After 48 h, the plates were removed from the incubator and the diameter of the precipitate rings was measured. The values of each diameter precipitate ring were plotted on the vertical axis of semi-logarithmic graph paper provided by the kit, and the haptoglobin concentration was determined from the horizontal axis. The assay was conducted in four batches. The intra-assay coefficient of variation was 8.0% and the inter-assay coefficient of variation was 1.3%. The assay sensitivity for haptoglobin was 50 μg/ml according to the kit manufacturer.

Circulating Cytokines Analysis

Serum concentrations of circulating IL-1β and TNF-α were assayed using commercial ELISA kits specific for porcine IL-1β and TNF-α (both from Quantikine; R&D Systems, Minneapolis, MN) following the manufacturer's instructions. Generally, 100 μL and 50 μL of samples were used for IL-1β and TNF-α analysis, respectively. Standards provided by the kits and serum samples were pipetted into the wells. Any porcine IL-1β or TNF-α present in the samples was bound by a specific immobilized monoclonal antibody coated at the bottom of the microplate. After washing away any unbound substances, 100 μL of enzyme-linked polyclonal antibody specific for porcine IL-1β or TNF-α was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, 100 μL of substrate solution was added to the wells. When the enzyme

reaction yielded a yellow product from the original blue colored product, 100 μL of the stop solution was added. The intensity of the color was measured by a microplate reader (Synergy HT, Bio-Tek, Vermont, USA), which was in proportion to the amount of IL-1β and TNF-α bound in the initial step, and the sample concentrations were determined using one standard curve. Each sample and standard was analyzed in duplicate. The assay sensitivity for IL-1β and TNF-α in this single assay were 10 pg/ml and 5 pg/ml, respectively.

Small Intestinal Morphology

Small intestinal tissues were collected at harvest from each pig in group 4. Samples from group 1, 2 and 3 failed to provide measurable villi or crypts, since most of the villous enterocytes were sloughing off, and in some sections villous tips were completely necrotic/autolytic, due to the heat (carcass scalding) used during the harvest process. In group 4, after the pigs were euthanized, the viscera were removed and put on ice immediately. The small intestine was separated from the stomach at the pyloric junction, and from the large intestine at the ileo-cecal junction. A 6-cm section of the small intestine was cut longitudinally at 25, 50, and 75% of the small intestine length which represented the duodenum, jejunum, and ileum sections of the small intestine, respectively. All sections were flushed with cold saline to remove digesta, and two ends were tied by string. A 5 cc amount of 10% neutral buffered formalin was injected into each section, and all samples were stored in labeled containers containing 10%

neutral buffered formalin.

Tissue processing was conducted by the Cancer Center Histopathology Core (CCHC; University of Minnesota, MN). Small intestine samples of each section were fixed using standard paraffin embedding procedures provided by CCHC. Samples were then sectioned at 6 μm intervals, stained with eosin and azur A, and mounted on glass slides. Villus height and associated crypt depth were measured at 10 x magnification using an Olympus BX40, Model-U-DO microscope (Olympus Optical Co., LTD, Japan) equipped with Spot, InSight Color Model 3.2.0 digital camera and imaging software (Diagnostic Instruments Inc., Sterling Heights, MI).

Villus height was measured from the tip of the villus to the opening of the crypt mouth. Crypt depth was measured from the crypt mouth to the muscularis. Four photos were taken for each slide and the average values from 10 measurements of the most regular villi, and their associated crypts were used to calculate the average length measurements. In addition, villus height:crypt depth ratio (VCR) was calculated.

Carcass Measurements

At the end of each trial, pigs were harvested at the University of Minnesota Meat Laboratory on the St. Paul campus in one day. All pigs were weighed individually one day prior to harvest to obtain a live BW. For the first three groups, hot carcass weight was measured on-line immediately after harvest with

the head, skin and feet remaining on the carcass, and hot carcass weight was adjusted to a head-off, skin-on basis by multiplying by 0.95 (NPPC, 2000). The 10^{th} rib backfat depth was measured using a ruler in the cooler with the skin-on one day after harvest. For the fourth group, the procedure was different since we collected small intestine samples immediately after pigs were sacrificed. Hot carcass weight was measured on-line with head-off, skin-off, feet-off, and the head, skin and feet of each carcass were weighed together in a container. Hot carcass weight was adjusted to a head-off, skin-on basis by adding the weight of head, skin, feet and then multiplying by 0.95 (NPPC, 2000). The 10^{th} rib backfat depth for these carcasses was measured the following day after skin removal, and was adjusted to a skin-on basis by adding 2.54 mm to the fat depth of skinned carcass (NPPC, 2000). Dressing percentage was calculated using the hot carcass weight which was adjusted to a head-off, skin-on basis. The BW obtained at the barn one day prior to harvest was used as the live weight when calculating the dressing percentage. The percentage carcass lean was determined using the 10^{th} rib backfat depth and loin eye area measured by using a plastic grid directly on the cross-sectional surface and by using the following equation [8.588 – 0.862 x 10^{th} rib backfat depth (mm) + 0.00466 x loin eye area (mm^2) + 1.025 x hot carcass wt. (kg)] (NPPC, 2000).

Digesta Sample Collection and Volatile Fatty Acid (VFA) Analysis

In order to analyze VFA concentrations, digesta samples from the cecum were

collected at harvest from 32 pigs (one pig/pen) for analysis of VFA concentration. Samples were collected from the same pigs used for saliva and blood sample collection. For each sample, approximate 30 mL of digestive fluid was strained through 4 layers of cheesecloth into a container. Two 5 mL subsamples were transferred into two 15-mL centrifuge tubes containing 1 mL 25% meta-phosphoric acid, and mixed well. All tubes were stored at -20˚C until VFA analysis was conducted.

Before the analysis, all samples were thawed at room temperature and collected by centrifugation at 5,000 x g for 5 min. The supernatant fluid was transferred into a new 15 mL centrifuge tube and frozen. The centrifugation process was repeated three times until a clarified digestive fluid was obtained.

The VFA were analyzed using a gas chromatograph (model 6890; Hewlett Packard, Palo Alto, CA) with a 4% Carbowax 20M80/120 Carbopack B-DA column (Supelco, Bellefonte, PA) using oxalic acid as the internal standard. One mL distilled water was added to 0.5 mL digesta sample, mixed, and 1 mL of this mixture was transferred to a new test tube. The pH of this solution was adjusted to 6 to 7 by adding 4N KOH to the solution. After that, 0.1 mL of 0.3 M oxalic acid was added to the solution so that the final sample contained 0.03% oxalic acid. All samples were analyzed by gas chromatograph in one batch. The average value of two samples from the same pig was used for statistical analysis.

Statistical Analysis

All data including growth performance, carcass characteristics, physiological parameters, small intestinal morphology, and VFA concentration were analyzed using the Proc Mixed Procedure of SAS 9.1 (SAS Inst. Inc., Cary, NC) to evaluate the main effects of environmental temperature and dietary BMD and any two-way interactions. The pen was used as the experimental unit for these analyses. The statistical model included fixed effects of dietary treatment, temperature treatment, and diet x temperature interactions. Group was used as a covariate in all these analyses if it was significant ($P < 0.05$). Initial BW on d 0, and live BW at harvest were used as the covariates in analysis of growth performance and carcass characteristics, respectively. The initial level of serum haptoglobin, IL-1β, TNF-α, and saliva cortisol on d -1 were used as covariates in analysis of these physiological parameters. The unstructured (UN) option was used to fit a variance-covariance matrix in the model for repeated measures in time. The slice option of SAS was used to separate main effects within different time periods. All results are reported as least square means and comparisons among treatments were performed using the Tukey adjustment option of SAS. The significance level chosen was $\alpha = 0.05$. Treatment effects were considered significant if $P < 0.05$, while values between $0.05 < P < 0.10$ were considered statistical trends.

Table 2-1. Composition and nutrient levels of experimental diets (as-fed basis).

	Diet[1]	
Item	**CON**	**BMD**
Ingredient, %		
Corn	76.31	76.31
Soybean Meal (46% CP)	11.53	11.53
DDGS	10.00	10.00
Limestone	1.06	1.06
Dicalcium phosphate	0.40	0.40
Salt	0.30	0.30
Vitamin/trace mineral permix[2]	0.25	0.25
L-Lys HCl	0.15	0.15
BMD	0.00	33 ppm
Analyzed composition:		
ME, kcal/kg[3]	3,309	3,309
Crude protein, %	14.17	13.59
Crude fat, %	3.92	3.47
Crude fiber, %	2.54	2.27
Lys, %	0.77	0.76
Met, %	0.23	0.21
Thr, %	0.49	0.46
Trp, %	0.16	0.21
Calcium, %	0.73	0.75
Phosphorus, %	0.45	0.44
Avail P, %[4]	0.18	0.18
Ca:Avail P[4]	4.01	4.12
Salt, %	0.48	0.43
Sodium, %	0.15	0.15
BMD	0.00	31.5 ppm

Indent footnotes

[1]CON = control diet, BMD = diet containing 30g/ton BMD.

[2]Vitamin-trace mineral premix that supplied the following nutrients per kilogram of feed: 5512.5 IU of vitamin A as retinyl acetate; 1,378 IU of vitamin D_3; 27.6 IU of vitamin E as DL-α-tocopherol acetate; 2.2 mg of vitamin K as menadione dimethylpyrimidinol bisulfite; 5.0 mg of riboflavin; 27.6 mg of niacin; 16.5 mg of pantothenic acid as D-calcium pantothenate; 248.1 mg of choline as choline chloride; 0.03 μg of vitamin B_{12}; 1.10 mg of pyridoxine; 0.83 mg of folic acid; 0.55 mg of thiamine; 1.10 mg of biotin; 1.10 mg of iodine as ethylenediamine dihydroiodide; 0.15 mg of selenium as sodium selenite; 42.4 mg of zinc as zinc oxide; 25.5 mg of iron as ferrous sulfate; 2.7 mg of copper as copper sulfate; and 9.0 mg of manganese as manganese oxide.

[3]ME values were calculated with NRC (1998) for corn and SBM and 3330 kcal/kg for DDGS.

[4]Calculated with analyzed Ca and P and availability of P in DDGS from Whitney and Shurson (2001).

Table 2-2. Total and standardized ileal digestible (SID) amino acid (AA) values of DDGS.

	AA[1] (as is)	
	Total AA	SID AA
Lys,%	0.85	0.48
Met,%	0.50	0.40
Phe,%	1.27	1.00
Thr,%	0.99	0.63
Try,%	0.22	0.12
Ile,%	0.98	0.70
Arg,%	1.19	0.92
Val,%	1.30	0.92
His,%	0.72	0.54
Leu,%	3.02	2.51
Cys,%	0.48	0.34

[1]AA values and digestibility from Urriola et al. (2007).

RESULTS & DISCUSSION

Thermal Stress

To mimic summer heat stress conditions in many parts of the U.S., the temperature of heat-stressed rooms was set at 37˚C from 1000 to 1900 h during the day time, and reduced to 27˚C from 1900 to 1000 h during the night time. Temperature and humidity of each room were monitored daily throughout the experimental period by a thermometer and humidistat located in each room. The average daily low and high temperature, and average daily low and high relative humidity were: 22˚C, 24˚C, and <20%, 27% for the non-heat stressed rooms; and 26˚C, 40˚C, and <20%, <20% for the heat-stressed rooms, respectively. Specifically, the low and high temperature, and average daily low and high relative humidity for each group are shown in Table 2-3. The desirable temperature ranges for finishing (55 to 110 kg) pigs reared in groups and provided *ad libitum* access to feed is 10 to 24˚C (Pond and Maner, 1984). The upper critical temperature (UCT) for growing-finishing pigs has been reported by NRC (1981) to be 35˚C. In the current study, pigs housed in hot rooms were considered to be heat-stressed, because the designed air temperature (37˚C) for the heat-stress rooms during the day exceeded the desirable temperature range (10-24˚C), and was even higher than the upper extreme for finishing pigs (35˚C) (Eugene, 1952; National Research Council, 1981).

Heat stress for finishing pigs can also be indicated by increased rectal temperature (Becker et al., 1992). Previous studies have confirmed that pigs

housed in a heat stress environment express elevated rectal temperature which might prevent heat dissipation (Holmes, 1973, 1974; Christon, 1988). In this study, rectal temperature of each pig was measured on d 1 and d 27 for Group 4 (32 pigs; Figure 2-1). On d 1, which was 24 h after heat stress conditions were imposed, pigs housed in the heat stressed conditions tended to have increased body temperature ($P < 0.1$) compared with pigs housed in the thermal neutral environment. On d 27, which was 4 wks after the heat stress treatment was imposed, pigs housed in the heat stress environment had significantly higher rectal temperature ($P < 0.01$) compared with pigs housed under thermal neutral environment. The increased rectal temperature for pigs housed in the heat stressed environment suggested that the environmental conditions imposed to create heat stress in this study were sufficient. No effects of dietary treatments and diet × temperature interaction on rectal temperature were observed in this study.

Overall Growth Performance

During the 14-d adaptation period when all pigs were housed under a thermal neutral environment, there were no dietary treatment effects on ADG, ADFI and G:F, as shown in Table 2-4. For the duration of 28-d experimental period when heat stress was imposed, pigs housed in heat stress conditions had significantly lower ADG ($P < 0.0001$), ADFI ($P < 0.0001$), G:F ($P = 0.001$) and higher ADWI (average daily water intake) ($P = 0.03$) compared with pigs housed in a thermal

neutral environment (Table 2-5). Pigs fed the BMD diet tended to have lower ADG (P = 0.07) compared with those fed control diet (CON). However, there was no dietary treatment effect on ADFI, G:F and ADWI. Initial BW on d 0 was not affected by dietary treatment. However, final BW on d-28 was lower (P < 0.0001) for pigs housed under heat stress conditions, and those fed BMD diet tended to have lower (P = 0.07) final BW on d-28 compared with the CON. There were no interactions between diet x temperature observed for any of the above parameters.

Over the entire experimental period, pigs reared under the heat stress conditions grew 31% slower, ate 23% less feed per day, and gained 9% less BW per unit of feed intake than pigs reared under the thermal neutral environment. These detrimental effects of heat stress on growth performance of finishing pigs are in agreement with several published studies (Stahly and Cromwell 1979; Christon, 1988; Kouba et al., 2001; Spencer et al., 2005). The reductions in ADFI are not linear and are dependent on BW, with high temperatures having a more negative effect on heavier pigs than lighter weight pigs (Quiniou et al., 2000). Le Bellego et al. (2002) observed a 15% reduction in ADFI for 24 to 59 kg growing pigs housed in 29˚C vs. 22˚C environment, whereas Stahly et al. (1979) observed greater (19%) reduction in feed consumption in growing pigs in higher environmental temperature (35˚C). In the current study, a 23% reduction in ADFI was observed for 96 to 121 kg finishing pigs kept in 37˚C vs. 23˚C, confirming that heavy pigs are susceptible and likely to have a great reduction in

ADFI during heat stress.

In the present study, the negative effect that heat stress had on G:F is in contrast to results from some studies where heat stress had no significant effect on feed efficiency (Stahly et al., 1979; Ames, 1980; Xin and DeShazer, 1991; Lopez et al., 1991; Le Bellego et al., 2002). These discrepancies in feed efficiency responses as a result of being subjected to heat stress conditions could be related to the level of reduction in feed intake. In a study by Le Bellego et al. (2002), a 15% reduction in feed intake was associated with a similar feed efficiency between pigs housed under heat-stressed and thermoneutral conditions. In contrast, Collin et al. (2001) and Nienaber et al. (1987) both reported a lowered feed efficiency which was associated with a 25% and 30% feed intake reduction for pigs housed under environmental temperatures of 33˚C and 30˚C, respectively. Nichols et al. (1982) and Nienaber et al. (1987) concluded that the reduced feed efficiency response observed in heat-stressed pigs was at a higher temperature than the reduced feed intake response observed (30˚C vs 25˚C). In addition, decreased efficiency of energy and other essential nutrient utilization in heat-stressed pigs may explain the reason for the observed reduction in feed efficiency (Stahly and Cromwell, 1979). The reduction in efficiency of energy utilization and feed utilization was more apparent for heat-stressed pigs at a high level of feeding, *ad libitum* vs. restricted-fed (Le Dividich et al., 1987; Christon, 1988).

The growth promotion benefits of supplementation with subtherapeutic doses

of antibiotics to pig diets have been well documented (CEAS, 1991; SOU, 1997; Taylor, 1999). However, in the current study, adding 30 g/ton BMD to the diet failed to improve ADG, ADFI or G:F of finishing pigs under either heat-stress or thermoneutral conditions. Actually, pigs fed the BMD diet tended to have lower ADG and lower final BW. The magnitude of response to antibiotic growth promoters is influenced by many factors, such as health status of pigs, age of pigs, and environmental conditions (Avcare, 2003).

In this study, one pig/pen for a total of 32 pigs was tested and found to be free of Porcine Reproductive and Respiratory Syndrome (PRRS), Swine Influenza Virus (SIV), and mycoplasma at the beginning of the experiments. Consequently, pigs used in this experiment did not have major health problems. Pigs with high health status and high growth rate have a minimal, if any, response to antibiotic growth promoters compared to pigs with poor health status and low growth rate (Avcare, 2003). One of the explanations for this response is that pigs with maximal growth performance are always provided with good quality diets which meet nutrient requirements for individual animals, and no subclinical disease is present. As a result, supplementation of antibiotic growth promoters to these pigs may not provide notable benefits at the herd level. In contrast, pigs with low growth rate may be associated with low energy or nutrient deficient diets, or presence of acute or chronic health problems. Supplementation of antibiotic growth promoters may offset nutrient deficiencies and control disease, and therefore improve the animal's growth performance.

Additionally, the response to growth promoters also varies with the age of animals. Pigs used in this study were late-finishing pigs weighing from 96 kg to 121 kg. In a study by Hays (1979), supplementation of bacitracin in the diet improved ADG by 9.7, 5.1, and 2.5% for nursery, growing, and finishing pigs, respectively, indicating that the response to growth promoters was greater in younger and lighter pigs than in more mature and heavier pigs.

The growth enhancing benefits of antibiotics are more pronounced in unsanitary environments with a high pathogen load compared with more sanitary, high-health environments (Cromwell, 2001). For example, in a report by Melliere et al. (1973), greater advantages in ADG (5.7 vs. 1.7% improvement) and feed efficiency (4.7 vs. 0.3% improvement) were observed in field studies compared with studies conducted on research stations when tylosin was added in swine diets. Cromwell (2001) concluded that responses to antibiotic growth promoters under farm conditions may be twice as great as those observed on a research station, where the facilities are generally cleaner with less disease load and a more comfortable environment. In this study, pigs were housed in a secluded research station, which provided a very high-health environment and reduced pathogen exposure. Also, the production conditions of the source herds where these pigs were raised have high biosecurity and thus, low immune system challenge. The sanitary environment in this study could be one reason that BMD did not significantly improve growth performance of pigs. In general, factors that influence the response of pigs to antibiotic growth promoters are extremely

complicated, and thus can lead to inconsistent results.

Growth Performance by Week and Day

Growth performance was measured during wk 1, wk 2, and wk 3 and 4 combined of the experimental period to evaluate the acute and chronic responses to temperature and dietary treatments on growth. Figure 2-2 shows the weekly ADG among treatments. Pigs housed under heat-stress conditions had lower ($P < 0.0001$) ADG during wk 1 (44% less), wk 2 (31% less), and wk 3-4 (25% less), compared with those housed under thermal neutral conditions. The gradual decrease in the reduction of ADG during the experimental period suggests that ADG of heat stressed pigs was most affected during the first wk of the heat-stress period. During wk 3 and 4, the heat stressed pigs appeared to be more able to cope with the high temperatures, which probably reflects an adaptation to chronic heat stress developed in these pigs. This pattern of ADG responses over time is inconsistent with results from a previous study where heat stress was more apparent at heavier weights as the pigs increased in body fat (Lopez et al., 1991). However, this discrepancy in results between these studies could be due to the differences in independent variables (i.e., pig weight, degree of fatness, health status, diet, temperature and humidity compared, etc.) inherent in each study. Pigs fed the BMD diet tended to have lower ($P = 0.08$) ADG compared with pigs fed CON during the first wk of heat stress. However, there were no significant effects of dietary treatments during wk 2 and wk 3-4.

As shown in Figure 2-3, ADFI for heat-stressed pigs was less ($P < 0.0001$) than that for the non-heat stressed pigs during wk 1 (22% less), wk 2 (23% less), and wk 3 and 4 (24% less). The similar differences in ADFI between heat stressed and non-heat stressed pigs indicated that heat stress could continuously and effectively impact the feed intake of pigs. No notable effects of dietary treatments on weekly ADFI were observed.

There were no effects of dietary treatments on G:F (Figure 2-4). The effects of heat stress on feed efficiency were different among weeks. During wk 1, pigs housed under hot conditions had reduced ($P < 0.01$) G:F, which was 27% lower than that of pigs housed in the thermal neutral environment. However, during wk 2, there was only a trend ($P < 0.1$) for heat-stressed pigs to have a 10% lower G:F compared with pigs housed under the thermal neutral temperature. During wk 3 and 4, G:F was similar between pigs housed under the thermal neutral and heat stress environments. The gradual decrease in the difference in G:F between heat-stressed and non-heat stressed pigs over the length of the experimental period was caused by the decreased difference in ADG and unchanging ADFI. The similar G:F during wk 3 and 4 suggests that heat stressed pigs might be able to adjust to chronic high temperatures over time. There were no overall time effect on G:F observed in this study.

Dietary BMD did not alter pigs' ADWI during any time period (Figure 2-5). As expected, pigs under heat stress had elevated ($P < 0.01$) water consumption compared with that of pigs housed under the thermal neutral environment during

wk 1, 2, and 3-4, with 38%, 37%, and 50% more water intake, respectively. According to Bond et al. (1959), evaporation of moisture from the respiratory tract is the primary mechanism used by pigs to lose excess body heat in a hot environment. Therefore, heat-stressed pigs were observed to drink more water to compensate for excess water loss caused by the increased respiration in the hot environment (Lopez et al., 1991; Zdzislaw et al., 1995). In this study, heat-stressed pigs had the highest water consumption during wk 3 and 4, which was 50% more than that of non-heat stress pigs. This increase may indicate a mechanism by which pigs adjust to heat stress.

Feed intake and water consumption were also recorded daily during the first wk of heat stress to determine daily pattern of ADFI and ADWI. Average daily feed intake for heat-stressed pigs was reduced ($P < 0.05$) from d 1 to d 7, compared with that of pigs in the thermal neutral environment (Figure 2-6). This result is in agreement with those of Stahly and Cromwell (1979) and Lopez et al. (1991), who stated that initial heat stress caused pigs to decrease their feed intake in an effort to reduce the burden of heat increment. The effect of heat stress on ADWI was not notable on the first day, but starting on d 2, ADWI was higher ($P < 0.01$) in heat-stressed pigs, indicating that pigs do not appear to respond to heat stress by increasing the water demand immediately (Figure 2-7). Brown-Brandl and colleagues (1997) found a decrease in feed intake for 84 kg, high-lean-growth barrows when temperature rose from 18 to 24˚C, but they did not observe an increase in water consumption until the air temperature reached 32˚C. These

observations support the notion that when the ambient temperature rises above the thermal neutral zone, feed intake is suppressed before the demand for drinking water is increased by heat stress. Dietary BMD did not impact daily ADFI and ADWI during the first week, although a trend (P = 0.09) was observed for pigs fed BMD to have lower ADWI on d1.

Carcass Composition

Dressing percentage, 10th rib backfat depth, loin eye area and lean yield percentage were not influenced by either temperature or dietary treatments (Table 2-6). Slaughter weight of pigs housed in hot conditions was reduced by 7% ($P <$ 0.0001) compared with pigs housed in the cooler environment, with an average of 116.5 and 125.8 kg, respectively. This also resulted in a 6% reduction ($P <$ 0.0001) in hot carcass weight when comparing heat-stressed pigs with non-heat stressed pigs. There were no diet x temperature interactions observed for these carcass characteristics.

Previous studies evaluating the effect of high ambient temperature on carcass characteristics have shown inconsistent results (Christon, 1988; Becker et al., 1992; Lopez et al., 1994). One of the reasons for this inconsistency may be due to the different feeding conditions during the time of heat stress. In the study of Holmes (1971), backfat was increased in pigs exposed to 33˚C, with similar levels of feed intake at both heat stress and non-heat stress temperatures. Similar observations were also reported by other studies indicating that body fat was

likely to increase due to an increased availability of energy for deposition of fat (Holmes, 1971; Noblet and Le Dividich, 1982; Close and Stanier, 1984; Kouba et al., 2001). However, there are also other results showing little or no effect of ambient temperature on carcass characteristics in pigs fed to achieve similar ADFI (Verstegen et al., 1985; Le Dividich et al., 1987). In contrast to similar feed intake levels for heat stressed and non-heat stressed pigs, results from other studies observed reduced or unchanged carcass fatness when pigs were slaughtered at the same age, due to the decreased level of feed intake (Sugahara et al., 1970; Nienaber and Hahn, 1983; Giles et al., 1988; Rinaldo and Le Dividich, 1991), which was similar to the results observed in the current study. Additionally, prolonged exposure to a hot environment has been shown to induce pig's anatomical changes (Nienaber and Hahn, 1983). Because of decreased feed intake, the mass of internal organs including the liver, kidneys, and digestive tract has been found to be reduced at high environmental temperatures, resulting in an improvement in carcass dressing percentage (Nienaber and Hahn, 1983; Lefaucheur et al., 1991). However, these results are not consistent with the observations in the current study where carcass dressing percentage was not influenced by heat stress.

Immunological and Physiological Parameters

- *Salivary Cortisol*

Changes in concentrations of circulating cortisol, in response to an activated

hypothalamic-pituitary-adrenal (HPA) axis, often have been used as major physiological indicators of stress in pigs (Ruis et al., 1997). Following the stimulation of a stressor, the HPA axis is activated, resulting in elevated corticotrophin-releasing hormone (CRH) and increased synthesis of ACTH in the pituitary, which in turn stimulates the release of cortisol from the adrenal cortex into the blood (Dantzer and Mormede, 1983). Heat stress can increase plasma cortisol concentration in pigs (Becker et al., 1985; Machado-Neto, et al., 1987; Hicks, et al., 1998). However, plasma cortisol concentration can be changed rapidly in response to a stressor (Sorrells et al., 2007). Based on this response, saliva, instead of blood, was collected for cortisol measurements in this study. Collection of saliva for cortisol analysis is an effective technique which causes minimal pain and distress to pigs with no need for restraint (Parrott et al., 1989; Ekkel et al., 1996). In pigs, cortisol concentrations in saliva are between 5 and 10% of those in plasma and lag at least 15 min behind plasma cortisol changes (Parrott et al., 1989; Cook et al., 1996; Sorrells et al., 2007). Therefore, the salivary cortisol concentration may be less affected by acute stress than plasma cortisol. Additionally, in the current study, all saliva samples were collected in the morning between 1030 h and 1200 h from Pen 1 to 8 to control potential effects of circadian rhythms on cortisol measures. Previous studies demonstrated that cortisol concentrations of pigs are higher in the morning, especially late morning, than in the afternoon and evening in both blood (Klemcke et al., 1989; Griffith and Minton, 1991; Janssens et al., 1995;) and saliva (Ekkel et al., 1996).

Salivary cortisol concentrations on d -1 (baseline), which was one day before the heat stress conditions were imposed, were 21% lower ($P = 0.02$) for BMD-fed pigs compared with those fed CON (Figure 2-8). On d 1, since heat stress was imposed for 24 h, a dramatic increase in saliva cortisol was observed in pigs in the heat stress environment, which was shown to be 71% higher ($P = 0.02$) than that of pigs in the thermal neutral environment. However, on d 13 and d 27, saliva cortisol concentration did not differ between temperature treatments. There were no effects of dietary BMD on salivary cortisol concentration on d 1, 13 and 27.

The elevated cortisol level observed in heat stressed pigs is consistent with several published studies (Becker et al., 1985; Machado-Neto, et al., 1987), indicating that the HPA axis is activated by heat stress. The HPA axis plays an important immunomodulatory role during a stress response (Bailey et al., 2003). Physiological concentrations of endogenous cortisol have positive effects on various aspects of the immune response (Wilckens and De Rijk, 1997). However, many factors, such as length of time that the stressor is applied, could influence the response of circulating cortisol concentration to heat stress in pigs, and thus, lead to inconsistent results (Ruis et al., 1997). In a recent study by Sutherland et al. (2006), the plasma cortisol concentration was greater in nursery pigs kept at 24˚C compared with those kept at 32˚C on d 14. Suppressed cortisol levels, along with lowered ACTH concentrations in chronically heat-stressed pigs were also observed in other studies (Marple et al., 1972; Heo et al., 2005), suggesting that reduced cortisol and ACTH concentrations following chronic heat stress

might be due to decreased adrenal responsivenss to ACTH, increased turnover of plasma cortisol, or both. Moreover, Pitman et al. (1988) pointed out that repeated exposure to the same stressor could reduce the responsiveness of the HPA axis to stress. In the present study, the cortisol level of heat-stressed pigs appeared to peak on d 1, and then returned to a similar level as those of non-heat stressed pigs on d 13 and d 27, suggesting that cortisol could be used as an indicator for acute heat stress, but may not effectively indicate chronic heat stress in finishing pigs.

- *Serum Haptoglobin*

During stress and immune system activation, various acute phase proteins (APP) are produced by the liver (Sanchez-Cordon et al., 2007). Changes in concentrations of APPs in the serum can be used as a physiological indicator of a physiological insult or subclinical and/or clinical disease, and thus, are normally used for evaluating the general health status of animals and humans (Eurell et al., 1992; Grellner et at., 2002; Sanchez-Cordon, et al., 2007). Among several APPs, haptoglobin is the most widely measured APP in pigs and is a useful marker of stress and pathological challenges (Pineiro et al., 2003). Results from previous studies have shown that in disease challenge models, haptoglobin can increase from 2 to 20 times the levels found in serum of healthy pigs (Pineiro et al., 2003), which indicates that serum haptoglobin could be a useful measure of immune system status.

In this study, the serum haptoglobin concentration was measured from one pig per pen for each group on d -1 (baseline), 1, 13 and 27. On d 1 after heat stress was imposed for 24 h, pigs housed under the heat stress conditions had a elevated (P = 0.04) level of serum haptoglobin, which was 47% higher than that of pigs housed under the thermal neutral environment (Figure 2-9). However, on d 13, which was two weeks after heat stress was imposed, the concentration of serum haptoglobin in heat-stressed pigs was decreased, and there was only a trend for those pigs to have higher haptoglobin concentration (P = 0.06) compared with pigs in the thermal neutral environment. On d 27, which was 4 wk after heat stress, serum haptoglobin concentration did not differ (P = 0.20) between temperature treatments. Initial, and d 1, 13 and 27 serum haptoglobin concentrations did not differ between dietary treatments.

Although elevated serum haptoglobin concentrations have been shown to be an effective indicator of several stressors in pigs, such as pathological challenges (Francisco et al., 1996; Knura-Deszczka et al., 2002), diseases caused by viruses (Asai et al., 1999; Amory et al., 2007), housing stress (Sorrells et al., 2007), social stress (Sutherland et al., 2006), and shipping stress (Hicks et al., 1998), little research has been conducted to evaluate if measuring serum haptoglobin is an effective means of assessing immune status of swine in response to heat stress. In the current study, the highest level of serum haptoglobin was observed on d 1 in heat-stressed pigs, indicating that acute heat stress could effectively activate the immune system. During the following days, serum haptoglobin in these pigs

gradually returned to levels similar to those of the CON pigs, suggesting a reduction in the immune system activation. Decreased levels of serum haptoglobin, together with decreased levels of salivary cortisol found on d 13 and 27, suggest that pigs might adapt to chronic and repeated heat stress. This statement can be supported by results from a previous study by Beisel (1977), who reported that haptoglobin could promote the adaptive response of an animal for survival. In addition, elevated serum haptoglobin in pigs under heat stress conditions on d 1 and 13 was consistent with the suppressed growth rate observed in this study, confirming the previous conclusion that serum haptoglobin concentration is negatively correlated with growth in pigs (Eurell et al., 1992; Williams et al., 1997).

- *Serum Interleukin-1β and TNF-α*

When an animal is challenged with a stressor or pathogen, cytokines, such as IL-1 and TNF-α, are released by the immune cells (Paez-Pereda and Stalla, 2005). Results from one previous study have shown that stress could increase plasma levels of cytokines (Abraham, 1991). However, there are no published studies to evaluate if heat stress could affect the production of cytokines in swine.

In this study, serum IL-1β and TNF-α concentration were measured from one pig per pen of each group on d -1 (baseline), 1, 13, and 27 to evaluate the effects of heat stress and dietary BMD on circulating cytokine concentration. Only 8 pigs fed the CON diet and 5 pigs fed the BMD diet had detectable levels of serum

IL-1β throughout the entire experimental period (data not shown). This suggests that pigs fed the BMD diet may have had a lower incidence of detectable level of serum IL-1β. The values of these detectable serum IL-1β concentrations were also analyzed by PROC MIXED procedure of SAS, as shown in Figure 2-10. Pigs fed the BMD diet had lower ($P < 0.0001$), and tended to have lower ($P < 0.1$) serum IL-1β concentration, compared with CON on d -1 and d 13, respectively. The concentration of IL-1β did not differ between dietary treatments on d 1 and 27. Also, as shown in Figure 2-11, dietary BMD did not have an impact on TNF-α on any sampling day. The lower level of IL-1β for pigs fed BMD indicated that BMD appears to prevent or reduce immune system activation, and thus, suggests that BMD appears to have immunomodulatory role in pigs.

As shown in Figure 2-10 and Figure 2-11, heat stress did not alter serum IL-1β and TNF-α concentration on d -1, 1, 13, and 27. This result is inconsistent with results from a previous human study by Bouchama et al. (1991), who observed significantly elevated level of blood IL-1β and TNF-α in acute heat stroke patients. Additionally, serum IL-1β and TNF-α might also be expected to increase in heat-stressed pigs due to the increased haptoglobin level observed in this study, since it has been demonstrated that acute phase proteins rise in pigs in response to cytokine release (Williams et al., 1997). However, this is not apparent in our results. One possible explanation of unchanged IL-1β and TNF-α in response to heat stress is the immunosuppressive effect of cortisol that may inhibit the production of inflammatory cytokines.

Hindgut Volatile Fatty Acid (VFA)

The influence of heat stress on VFA levels in the rumen have been well demonstrated in several ruminant studies (Kofb and Pfander, 1965; Kelly, 1967; McDowell, 1972; Knapp et al., 1991), but there has been little research conducted in swine. The total VFA level was not affected by environmental temperature treatments (P = 0.51) in the current study (Table 2-7), which was in inconsistent with the hypothesis in this study that the total VFA concentration in the cecum may be decreased for pigs under heat stress conditions due to a decrease in voluntary feed intake. The molar proportion of propionate tended (P = 0.08) to be decreased by 12% for pigs in the heat stress treatment compared with the non-heat stress treatment. This decrease in propionate in pigs assigned to the heat stress treatment also led to a trend (P = 0.08) for a 19% increase in the A:P ratio (Acetate : Propionate ratio). This observation is consistent with the results of a previous ruminant study by Kelly (1967), who observed a decrease in propionate and an increase in the A:P ratio in Holstein cows as the temperature increased from 1.6 to 37.7˚C. However, these results were inconsistent with those reported by Weldy et al. (1964), who observed a decreased A:P ratio due to the lack of significant change in ruminal propionate concentration at 31.2˚C compared with that of cattle kept under prevailing ambient conditions (2.2–25.6˚C). The decreased level of propionate and increased A:P ratio observed in this study suggests that heat stress might alter the microbiological ecology in the cecum resulting in a decreased gram-negative population, thereby

shifting the acetate to propionate ratio toward less propionate production. As shown in Table 2-7, the molar valerate proportion was decreased by 21% when comparing the heat stress with non-heat stress treatments, with the average of 0.60 and 0.76 mol/100 mol, respectively. This result was inconsistent with results from several published ruminant studies that showed that valerate proportion was unaffected by heat stress (Kelly, 1967; McDowell, 1972; Knapp et al., 1991). These differences in responses might be due to the differences in gut microflora populations and diet composition between cattle and pigs. Further research related to the mechanisms of how heat stress affects hindgut fermentative status in swine is needed in order to better understand the results observed in this study.

In the present study, inclusion of 30 g/ton dietary BMD did not influence total VFA or molar proportions of VFAs in the cecum of finishing pigs suggesting that adding 30 g/ton BMD in the diet may not effectively modify the population and activity of intestinal microflora, such as *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* which are main cellulolytic species producing VFAs in swine (Grieshop et al., 2000). However, in a recent study by Gong et al. (2008), the authors observed that supplementation of 50 g/ton bacitracin in the diet could markedly alter the composition of the microbiota in the cecal digesta, including *Ruminococcus,* in 3-d-old chicks and 42-d-old chickens. One of the reasons for these inconsistent responses may be due to different species and age of animals. Besides, in the same study, Gong et al. (2008) found that the impact of bacitracin on microflora composition was more dramatic in 3-d-old chicks than 42-d-old

chickens. It appears that the influence of bacitracin on the microbiota is particularly pronounced when the animals were young and their microbiota is in the process of development. Additionally, previously reported studies proposed that antibiotic growth promoters might have effects in reducing the overall species of gut bacteria (Visek, 1978; Close, 2000; Collier et al., 2003). One study by Collier et al. (2003) supported this statement by using quantitative PCR-DGGE and found that antibiotic treatments reduced species diversity and total numbers of bacteria in pigs. However, in a recent study by Pedroso et al. (2006) using the same method, dietary bacitracin, or avilamycin had no significant effect on the diversity of total tract microbiota, but only altered the composition of small intestinal bacterial microbiota in broiler chickens. Similarly, Gong et al. (2008) found that supplementation of broiler diets with bacitracin allowed the establishment of a distinct bacterial community in the ileum and cecum without reducing the overall numbers of the ileal and cecal microbiota. The observations in the study of Pedroso et al. (2006) and Gong et al. (2008) were somewhat consistent with the results of the present study showing that inclusion of 33 ppm BMD in the diet did not alter total VFA production. However, it should be pointed out that only one pig/pen with a total of 8 pigs/treatment were randomly chosen for VFA analysis. Further research involving more animals in different ages, weights, and production stages will be helpful to better understand the effects of antibiotic growth promoters, particularly BMD, on hindgut VFA production.

Small Intestine Morphology

Villus height, crypt depth, and villus height:crypt depth ratio (VCR) are general measures of small intestinal morphology. Pigs fed the BMD diet tended to have 10% longer ($P = 0.07$) villi and 10% greater ($P = 0.09$) crypt depth in the duodenum than pigs fed CON (Table 2-7). A villus height to crypt depth ratio (VCR) was calculated using villi height and crypt depth measurements from pigs from each treatment. Villus height to crypt depth ratio did not differ between dietary treatments in the duodenum. When villi height, crypt depth, and VCR were measured in the jejunum, pigs fed BMD tended to have a 16% greater crypt depth ($P = 0.07$) than those fed CON. However, villus height and VCR did not differ between dietary treatments. In the ileum, dietary treatments did not alter villi height, crypt depth, and VCR.

The effects of dietary bacitracin on villus height and crypt depth have been controversial. In a recent study by Yang et al. (2007), chickens fed Zn bacitracin for 35-d had significantly higher jejunal villi than the control group. However, in another study by Leeson et al. (2005), villus height of broiler chickens was not affected by dietary bacitracin, whereas bacitracin significantly decreased duodenal villi crypt depth. No data have been published regarding the effects of feeding bacitracin under heat stress conditions on villus height and crypt depth in swine. Moran (1982) suggested that longer villi are associated with larger absorptive surface in pigs. In this study, villus height in duodenum tended to increase in pigs fed BMD, indicating that BMD might be beneficial to villi development in

pigs. However, the mechanism of this effect is unknown. Results from previous studies have suggested that under the influence of bacitracin, the enterococci count increased as coliform bacteria were inhibited in the intestine, and thus, stimulating the activities of digestive pancreatic enzymes such as amylase, chymotrypsin, and lipase (Engberg et al., 2000), causing increased capability of nutrients metabolism (Männer and Bronsch, 1987). Based on this statement, one possible interpretation of the benefits of increased villi height observed in this study is that bacitracin may modify the intestinal environment (e.g., nutrition and microflora), thereby inducing the observed intestinal morphological responses.

In this study, villus height, crypt depth, and VCR of the duodenum, jejunum and ileum of pigs exposed to heat stress were found to be unaffected by environmental temperature. In our review of the literature, no study has been reported that has evaluated the effect of heat stress on the villi height and crypt depth of the small intestine of pigs. In a previous study in quail, Sandikci and colleagues (2004) reported shorter villi height in the duodenum, jejunum, and ileum exposed to heat stress than those of the control group. The authors claimed that the decrease in height of the villi of quail under heat stress conditions could be the result of decreased feed intake, since decreased villus height was also found in chickens after a 3-d starvation period (Shamoto and Yamauchi, 2000) or feed withdrawals (Tarachai and Yamauchi, 2000) . However, we did not observe a reduction in villus height due to heat stress of finishing pigs in the current study.

SUMMARY

In conclusion, heat stress markedly affected pig growth performance by reducing feed intake, growth rate, feed efficiency, and increasing water consumption. Supplementation of 30 g/ton BMD in the diet did not improve growth performance of finishing pigs in either heat stress or non-heat stress conditions, suggesting that factors, such as animal's health status, age, weight, and housing environment may influence the efficiency of antibiotic growth promoters. In this study, late-finishing pigs with high health status were used, and the study was conducted in a sanitary research station environment. Therefore, we did not observe a response to subtherapeutic growth promoting antibiotics as well as moderately immune challenge in pigs. Carcass characteristics were not affected by heat stress or dietary BMD. Attempts to identify appropriate physiological and immunological indicators of heat stress have been difficult and controversial. In this study, elevated serum haptoglobin and salivary cortisol concentrations on d 1 of pigs exposed to heat stress suggested that a combination of these two parameters may be a reliable indicator of acute heat stress in pigs. Additionally, serum haptoglobin and salivary coritisol were correlated negatively with growth rate, suggesting that an activated immune response may be a detriment to growth. Total VFA production was unaffected by heat stress, but the molar proportions of propionate and valerate appeared to decrease, indicating that heat stress may result in a shift of microflora population in the cecum. Inclusion of BMD tended to

increase villi height and crypt depth in the duodenum, and increase crypt depth in the jejunum, suggesting a potential benefit to nutrient absorption and morphological development. However, the mechanism(s) of this effect needs further investigation.

Table 2-3. The low and high temperature and average daily low and high relative humidity for each group.

	Non-heat stressed rooms				Heat-stressed rooms			
	Temperature, ºC		Humidity, %		Temperature, ºC		Humidity, %	
	Low	High	Low	High	Low	High	Low	High
Group 1	21.4	23.6	<20.0	26.0	25.6	40.0	<20.0	<20.0
Group 2	22.3	24.2	<20.0	28.0	26.4	40.0	<20.0	<20.0
Group 3	22.5	24.4	<20.0	26.5	26.5	40.0	<20.0	<20.0
Group 4	21.8	23.8	<20.0	27.5	25.5	40.0	<20.0	<20.0
Overall	22.0	24.0	<20.0	27.0	26.0	40.0	<20.0	<20.0

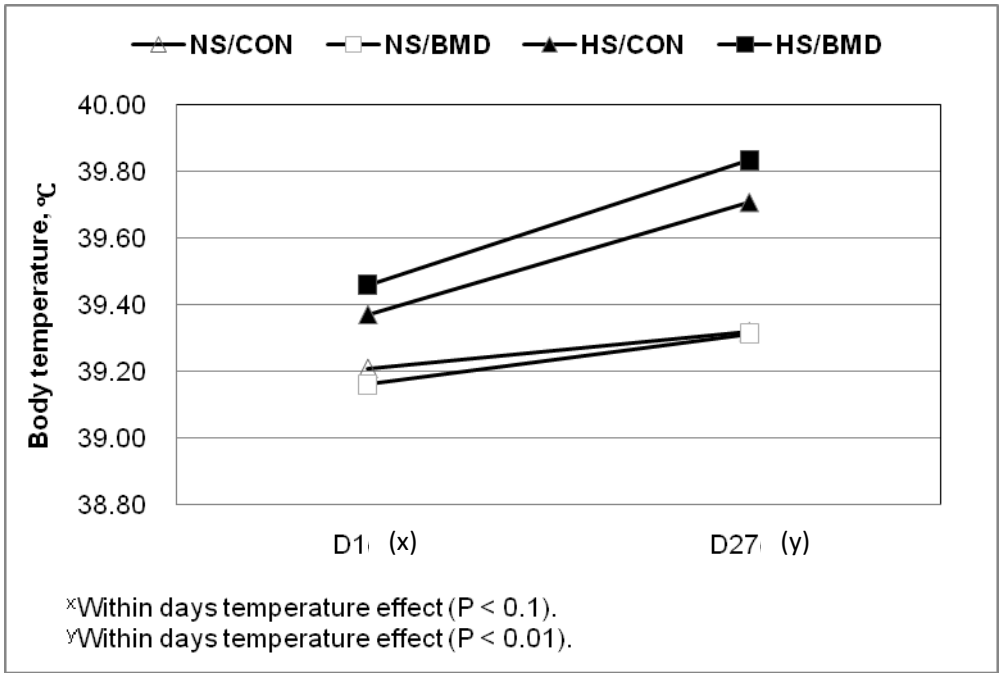


Figure 2-1. Effects of environmental temperature and dietary BMD on body temperature on d 1 and d 27 of the experimental period.

Table 2-4. Effects of environmental temperature and dietary BMD on growth performance during the adaptation period (least square means).

	Diets		PSE	P values
Items	CON[1]	BMD[2]		Diet
ADG, kg	0.84	0.88	0.03	0.25
ADFI, kg	2.62	2.72	0.05	0.20
Gain:feed	0.32	0.32	0.01	0.81

[1]CON = control diet without BMD.
[2]BMD = diet containing 31.5 ppm BMD.

Table 2-5. Effects of environmental temperature and dietary BMD on overall growth performance during the experimental period (least square means).

	Thermal Neutral (23˚C)		Hot (27-37˚C)		PSE	P values		
Items	CON[1]	BMD[2]	CON[1]	BMD[2]		Diet	Temp	Diet*Temp
Initial wt at d 0, kg	96.5	97.0	96.2	96.9	0.6	0.28		
Final wt at d 28, kg	121.0	118.3	112.8	112.0	1.0	0.07	<.0001	0.33
ADG, kg	0.87	0.77	0.58	0.55	0.03	0.07	<.0001	0.31
ADFI, kg	3.21	2.93	2.36	2.36	0.11	0.22	<.0001	0.21
Gain:feed	0.27	0.26	0.24	0.24	0.01	0.42	0.001	0.68
ADWI[3], L	6.67	5.31	7.91	8.11	0.90	0.53	0.03	0.39

[1]CON = control diet without BMD.

[2]BMD = diet containing 31.5 ppm BMD.

[3]ADWI = average water intake.

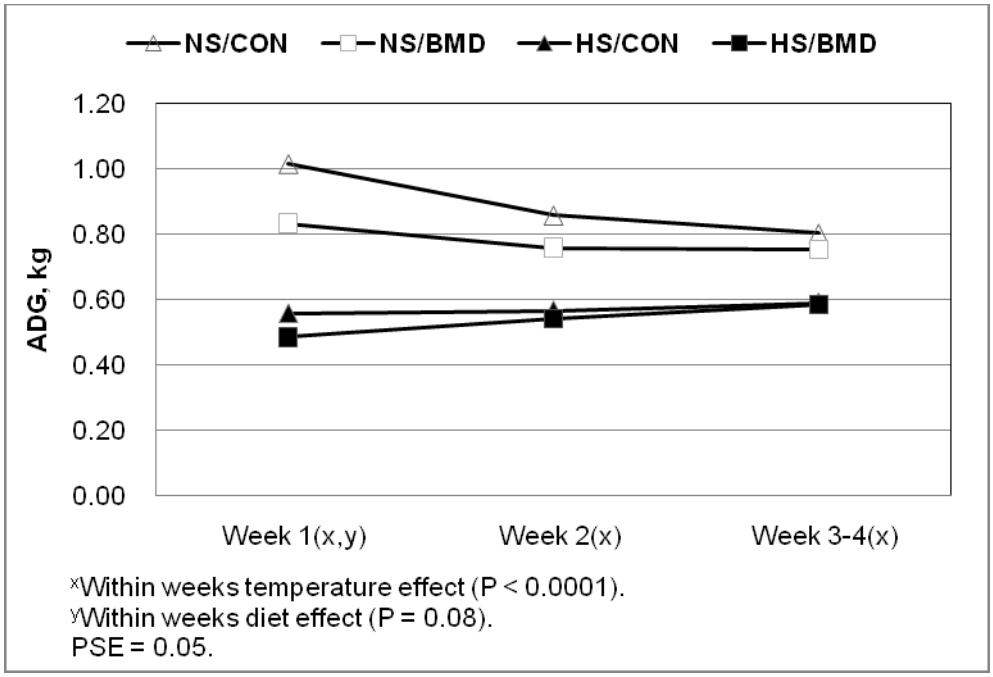


Figure 2-2. Effects of environmental temperature and dietary BMD on weekly ADG.

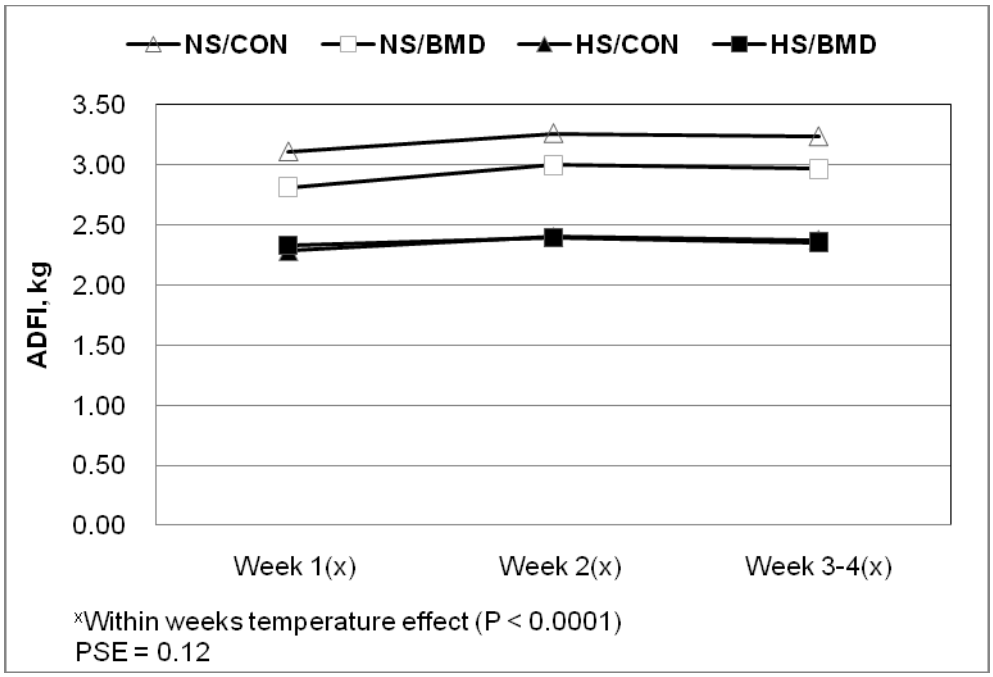


Figure 2-3. Effects of environmental temperature and dietary BMD on weekly ADFI.

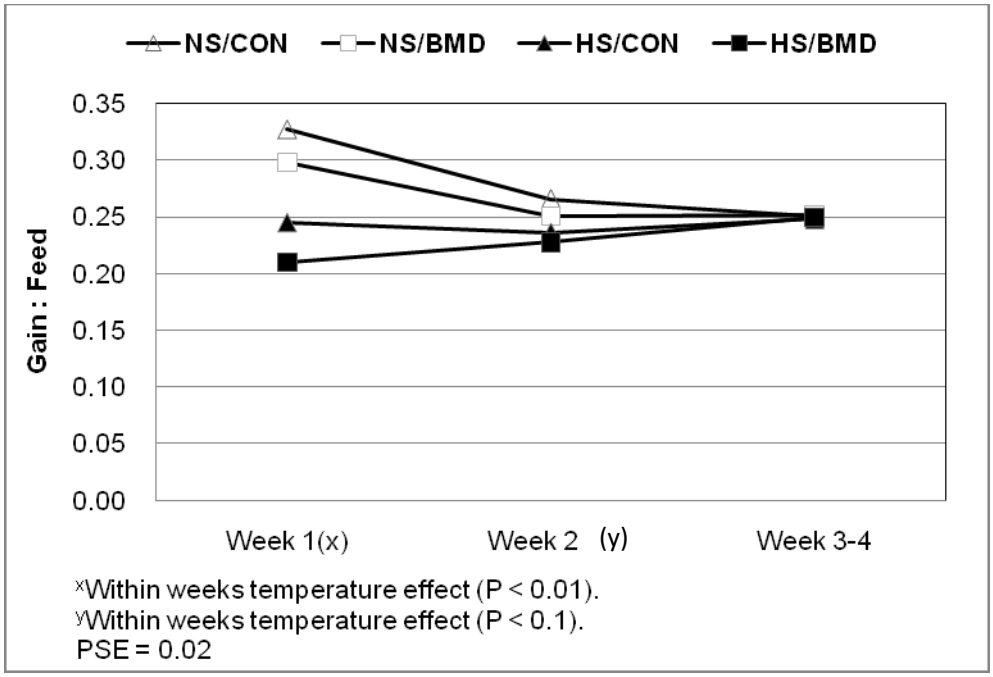


Figure 2-4. Effects of environmental temperature and dietary BMD on weekly G:F.

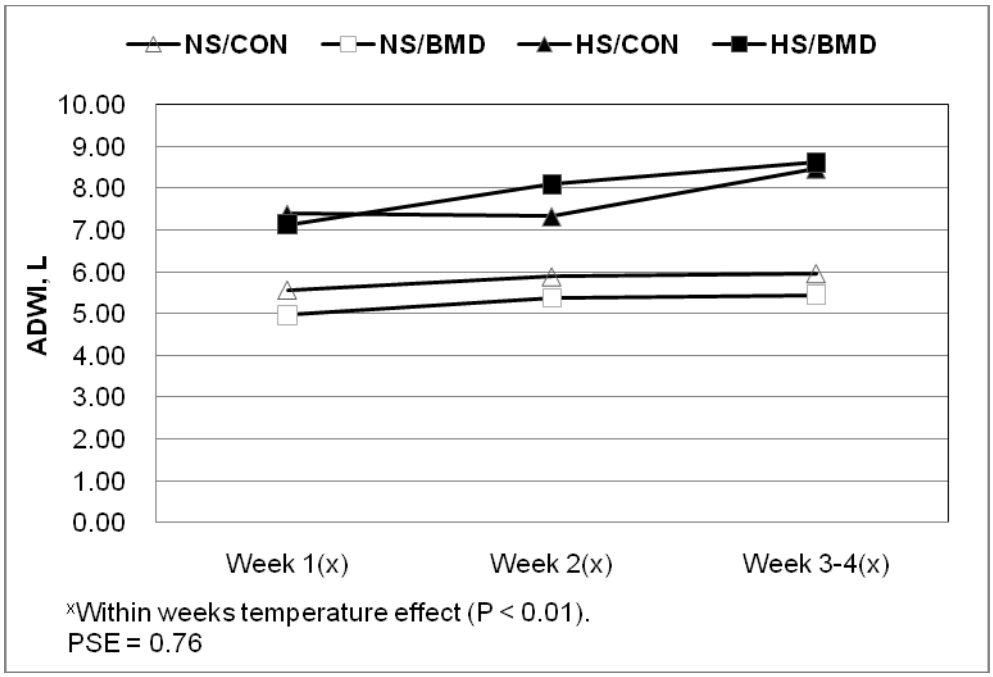


Figure 2-5. Effects of environmental temperature and dietary BMD on weekly ADWI.

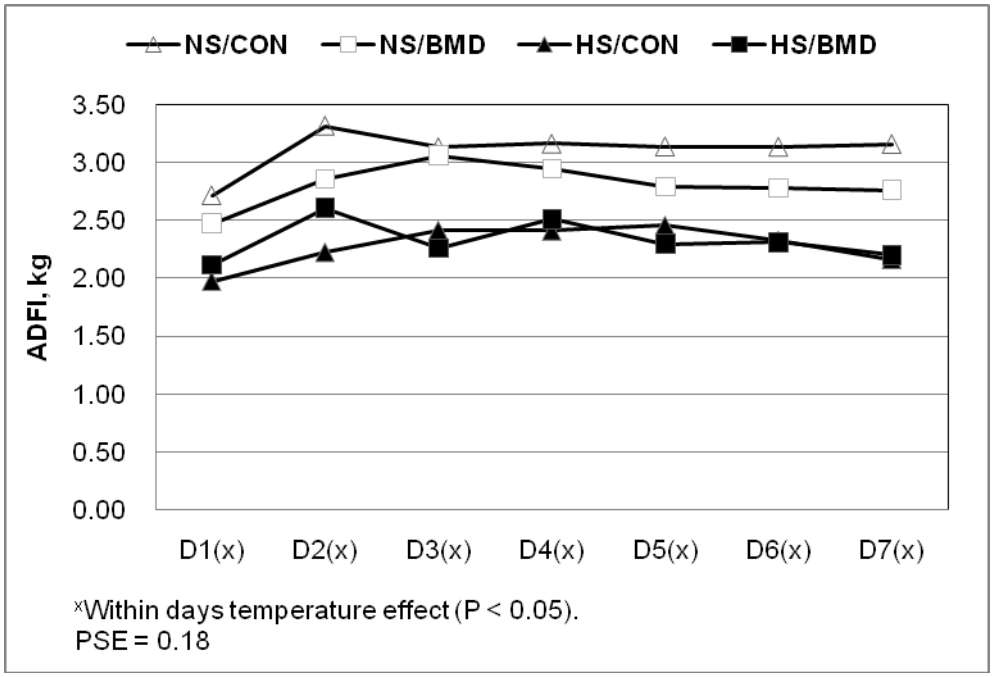


Figure 2-6. Effects of environmental temperature and dietary BMD on daily ADFI during the first week of the experimental period.

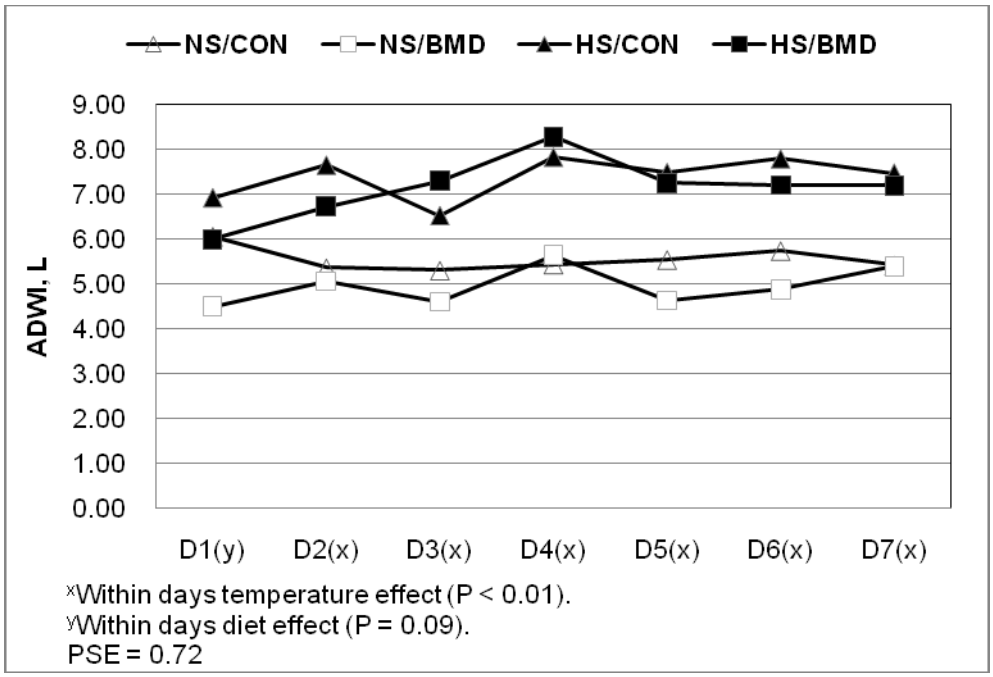


Figure 2-7. Effects of environmental temperature and dietary BMD on daily ADWI during the first week of the experimental period.

Table 2-6. Effects of environmental temperature and dietary BMD on carcass characteristics (least square means).

Items	Thermal Neutral (23˚C)		Hot (27-37˚C)		PSE	P values		
	CON[1]	BMD[2]	CON[1]	BMD[2]		Diet	Temp	Diet*Temp
Slaughter wt, kg	125.9	123.8	116.9	116.1	1.1	0.18	<.0001	0.53
Hot carcass wt, kg	97.5	96.1	91.1	90.6	0.7	0.16	<.0001	0.54
Dressing percentage	77.5	77.6	78.0	78.0	0.3	0.80	0.19	0.87
10^{th} rib backfat depth, cm	1.65	1.59	1.68	1.64	0.05	0.45	0.69	0.79
Loin eye area, cm^2	39.27	39.48	38.26	38.72	0.61	0.59	0.16	0.84
Lean, %	54.8	55.2	54.8	55.1	0.5	0.44	0.89	0.97

[1]CON = control diet without BMD.

[2]BMD = diet containing 30 g/ton BMD.

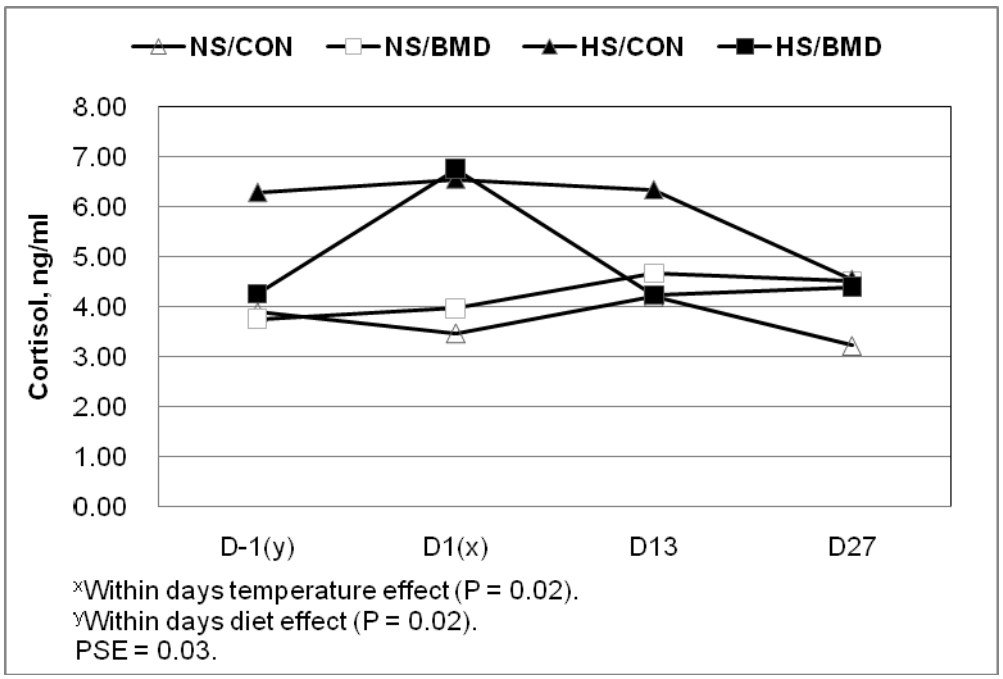


Figure 2-8. Effects of environmental temperature and dietary BMD on saliva cortisol concentration.

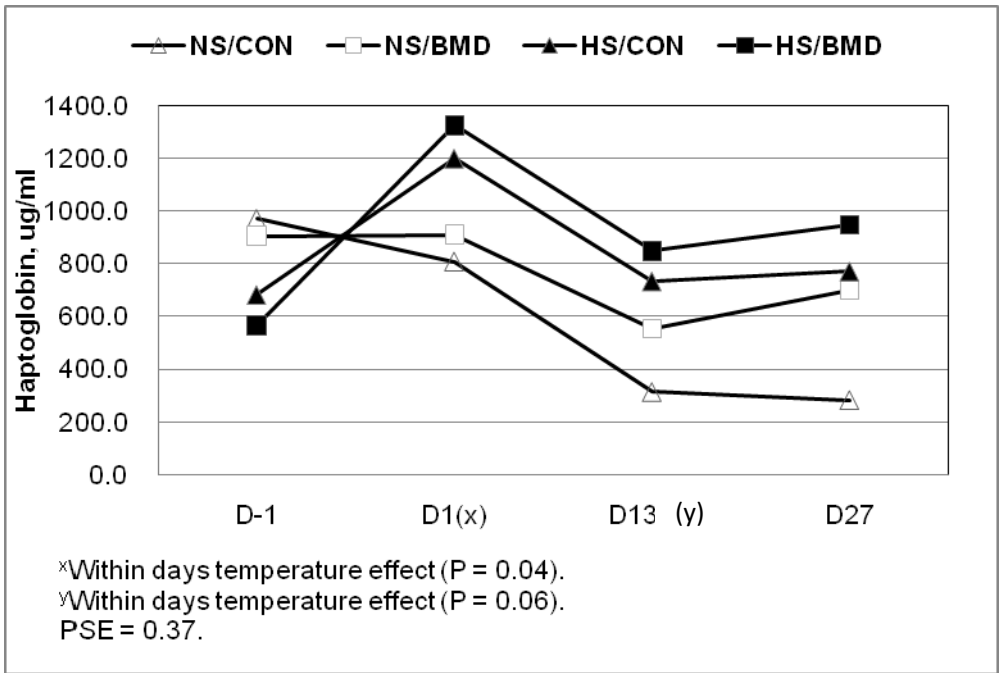


Figure 2-9. Effects of environmental temperature and dietary BMD on serum haptoglobin concentration.

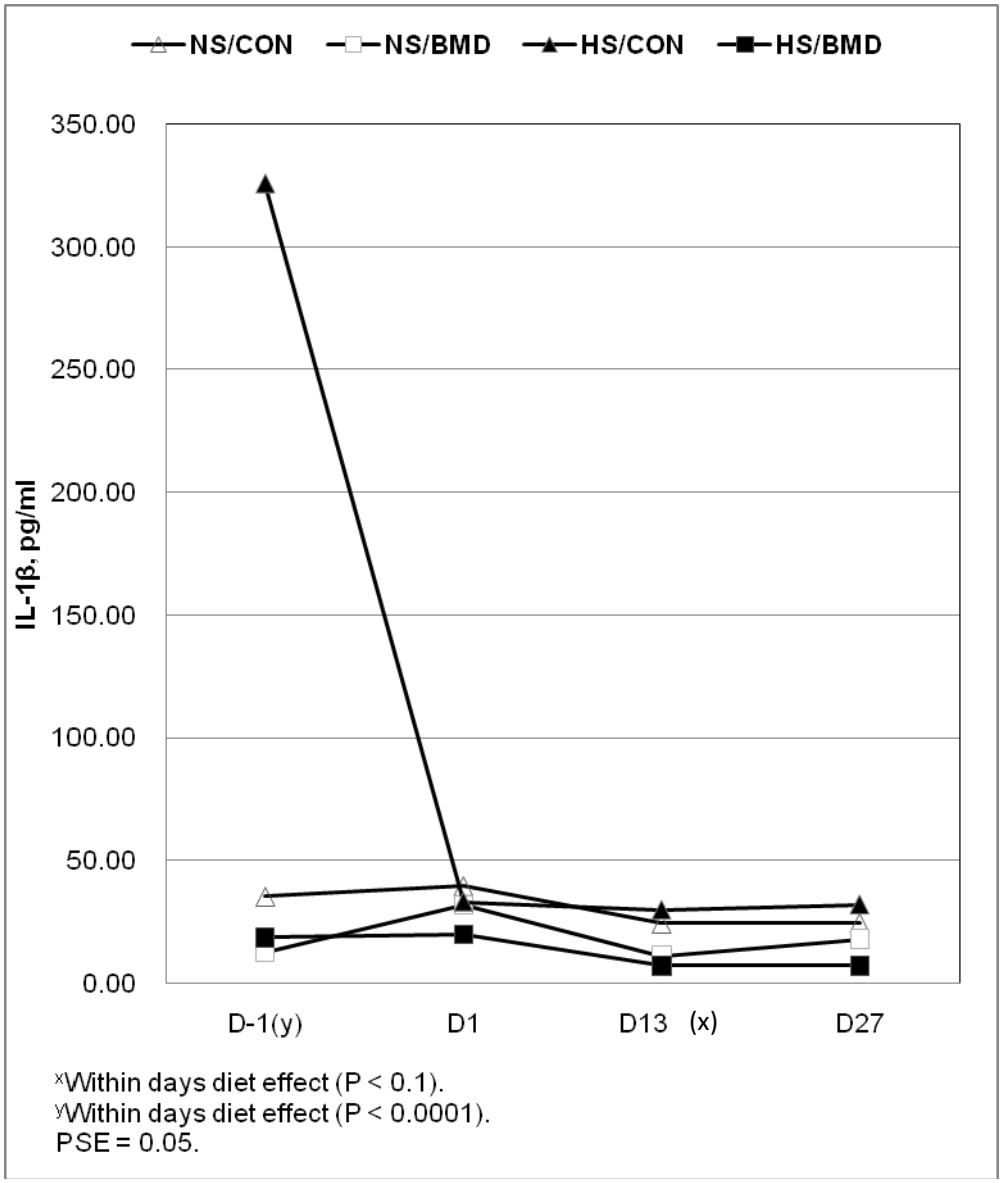


Figure 2-10. Effects of environmental temperature and dietary BMD on detectable serum IL-1β concentration.

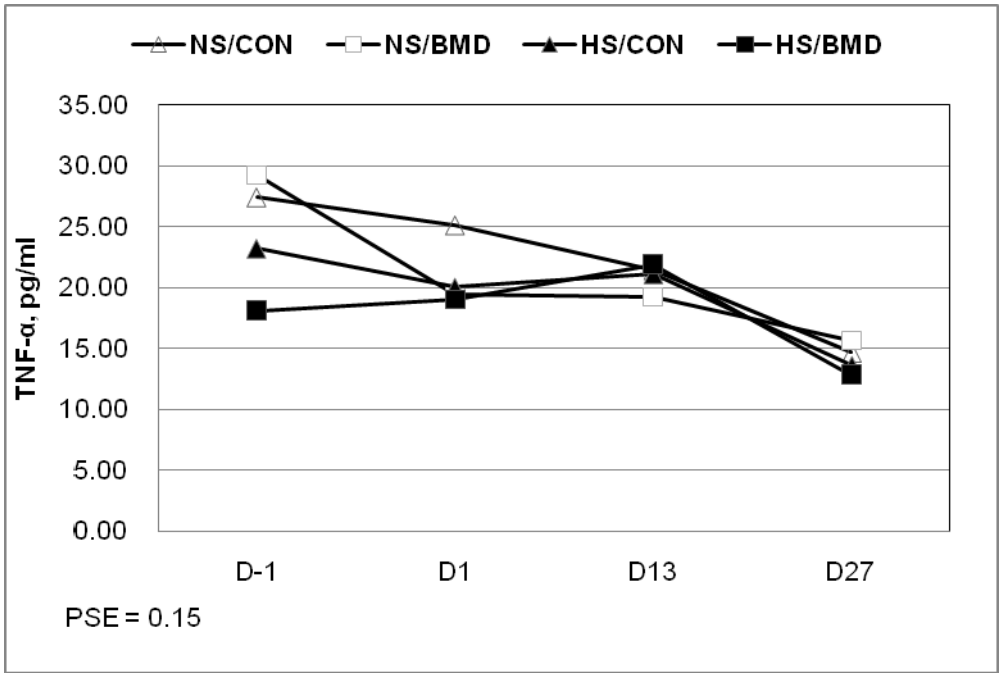


Figure 2-11. Effects of environmental temperature and dietary BMD on serum TNF-α concentration.

Table 2-7. Effects of environmental temperature and dietary BMD on cecal VFA concentrations and proportions (least square means).

	Thermal Neutral (23˚C)		Hot (27-37˚C)		PSE	P values		
VFA	**CON[1]**	**BMD[2]**	**CON[1]**	**BMD[2]**		**Diet**	**Temp**	**Diet*Temp**
Total, m*M*	38.5	36.1	35.4	36.1	2.3	0.71	0.51	0.49
Individual, mol/100mol								
Acetate	68.6	67.9	72.7	70.2	0.02	0.45	0.14	0.67
Propionate	20.6	20.8	17.2	19.2	0.03	0.41	0.08	0.47
Butyrate	8.3	9.5	8.1	8.4	0.01	0.18	0.23	0.36
Valerate	0.68	0.84	0.57	0.62	0.08	0.15	0.02	0.55
A:P ratio*	3.41	3.37	4.35	3.75	0.37	0.39	0.08	0.44

[1]CON = control diet without BMD.

[2]BMD = diet containing 30 g/ton BMD.

*A : P ratio = acetate : propionate ratio.

Table 2-8. Effects of environmental temperature and dietary BMD on small intestine morphology (least square means).

	Thermal Neutral (23˚C)		Hot (27-37˚C)		PSE	P values		
Items	**CON[1]**	**BMD[2]**	**CON[1]**	**BMD[2]**		**Diet**	**Temp**	**Diet*Temp**
Duodenum (upper 25% of small intestine)								
Villi height, µm	417	477	431	456	17	0.07	0.84	0.36
Crypt depth, µm	288	336	284	294	13	0.09	0.15	0.22
VCR*	1.46	1.42	1.52	1.55	0.10	0.96	0.39	0.72
Jejunum (50% of small intestine)								
Villi height, µm	474	493	511	455	34	0.61	0.98	0.33
Crypt depth, µm	235	309	254	261	17	0.07	0.42	0.11
VCR*	2.02	1.60	2.04	1.75	0.22	0.17	0.71	0.78
Ileum (lower 75% of small intestine)								
Villi height, µm	382	407	447	387	35	0.55	0.65	0.29
Crypt depth, µm	226	253	218	219	15	0.38	0.23	0.41
VCR*	1.70	1.78	1.87	1.79	0.24	0.99	0.72	0.75

[1]CON = control diet without BMD.

[2]BMD = diet containing 30 g/ton BMD.

*VCR=villus height : crypt depth ratio.

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