

# EVALUATION OF BROILER PERFORMANCE WHEN FED NICARBAZIN: RH EFFECT

by

KRISTIN MILES

(Under the Direction of Brian Fairchild)

## ABSTRACT

Research was conducted to determine the effect of nicarbazin fed for 28 days on broiler performance and rectal temperature at two levels of RH: 35-50% and 65-75%. Nicarbazin treatment significantly decreased broiler body weights, weight gain, and feed consumption and significantly increased FCR compared to those fed the control diet ( $P < 0.05$ ). Seven days after nicarbazin was removed from the diet, there were no differences in weekly weight gain, but the nicarbazin treatment group had a significantly lower FCR compared to control birds ( $P < 0.05$ ). Elevated RH, 65-75% vs. 35-50%, was not found to influence broiler performance nor exacerbate nicarbazin related performance issues. Body temperature was not affected by nicarbazin under these environmental conditions.

INDEX WORDS: anticoccidial, relative humidity, body temperature, feed conversion

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KRISTIN MILES

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by

KRISTIN MILES

Major Professor:	Brian Fairchild
Committee:	Michael Czarick
	Adam Davis
	Laura Ellestad

Electronic Version Approved:

Ron Walcott  
Vice Provost for Graduate Education and Dean of the Graduate School  
The University of Georgia  
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## CHAPTER 1

### LITERATURE REVIEW

#### **Body temperature**

Birds are classified as homeotherms meaning that they can maintain a constant core body temperature in a narrow range despite changes in their surrounding environment (Duchamp et al., 1999; Maiorka et al., 2006; Scanes and Christensen, 2020). Chicks are classified as poikilothermic for the first few days after hatch as they are unable to fully maintain their body temperature independent of their environment (Weytjens et al., 1999; Tazawa et al., 2001; Scanes and Christensen, 2020). They become homeothermic a few days after hatch as they rapidly develop the ability to regulate their body temperature (Tazawa et al., 2001; Weytjens et al., 1999; Mujahid, 2010). The rectal temperatures of chicks increased independently of environmental temperature during the first week of life (Mission, 1977; Malheiros et al., 2000; Mujahid, 2010; Luo, 2021). Chicks can then adjust their heat production according to the environmental temperature and maintain their body temperature (Weytjens et al., 1999).

The core body temperature for birds varies between 39°C to 43°C depending on the species and age of the birds (Weaver, 2001; McWilliams et al., 2016; Scanes and Christensen, 2020). Body temperature is lower in larger bird species while smaller species have a higher body temperature (Prinzinger et al., 1991; Weaver, 2001). However, birds experience fluctuation in their body temperature throughout a 24-hour period as a component of the diurnal cycle (Freeman, 1971; Kadono and Besch, 1978). Prinzinger et al. (1991) showed that there was a

2.48°C difference in body temperature during the diurnal cycle while Kadono and Besch (1978) reported a fluctuation of 0.6°C to 1.1°C.

Birds have a high body temperature that is rarely lower than the surrounding environmental temperature. Maintaining a high body temperature requires a large proportion of their total energy consumption (McWilliams et al., 2016). Chicks typically have a lower body temperature of approximately 39.7°C that increases with age and plateaus around three weeks old (Weaver, 2001). The lower deep body temperature seen in chicks is suggested to be due to a lower thermoregulatory set point rather than a failure to maintain body temperature. This theory is supported by the negative correlation between the preferred temperature of birds and their body temperature during the first 10 days post-hatch (Tzschentke and Nichelman, 1999). Chicks are able to perform thermoregulatory behaviors such as shivering and panting at hatch, but these mechanisms are not sufficient to maintain body temperature until a few days of age (Randall, 1943). Marjoniemi and Hohlotá (1999) reported that the drop in body temperature associated with a decrease in ambient temperature decreased with age. The core body temperature of adult chickens is approximately 41.5°C but can fall within the range of 40°C to 42°C depending on the strain (Etches et al., 2008; Weaver, 2001; Scanes and Christensen, 2020). Chickens have critical body temperatures which are lethal when their body temperature falls below or exceeds them. The lower critical body temperature is 15.5°C at hatch and increases with age to 22.2°C for mature chickens (Moreng and Shaffner, 1951). Birds also have an upper critical body temperature of approximately 46.0°C to 47.8°C for chicks and 46.6°C to 47.2°C for mature chickens that will result in death (Randall, 1943; Moreng and Shaffner, 1951; Weaver, 2001).

## **Thermoneutral and thermal comfort zone**

There is a limited range of environmental temperatures in which birds can maintain homeothermy (Brown-Brandl et al., 1997). The thermoneutral zone is the range of ambient temperatures that a bird can maintain its core body temperature without the need for additional effort to produce or dissipate heat (McWilliams et al., 2016; Scanes and Christensen, 2020). Alternatively, the thermoneutral zone can be defined as the range of ambient temperatures in which the blood vessels in the skin are neither fully dilated nor constricted, the evaporation of moisture from the skin/respiratory tract is minimal, feathers are not erect, and there are not any behavioral responses to cold or hot temperatures (Brown-Brandl et al., 1997). The thermal comfort zone is defined as the range of environmental temperatures in which metabolic energy is not required or at a minimum to maintain core body temperature and is denoted as the most efficient temperature for production (Maiorka et al., 2006; Scanes and Christensen, 2020). When ambient temperatures are outside of the thermoneutral or thermal comfort zones, birds will have fluctuations in their body temperature leading to hyper- or hypothermia (Weaver, 2001; Maiorka et al., 2006). If this occurs, birds will then have to use metabolic energy to either produce or dissipate heat (Scanes and Christensen, 2020). Tzschentke and Nichelman (1999) found that as bird body temperature increased with age, the preferred ambient temperature decreased. The thermoneutral temperature for ducklings and newly hatched chicks is approximately 35°C (Mission, 1977; Barre et al., 1985; 1986; Teeter and Belay, 1996). The thermal comfort zone for chicks is between 32°C and 35°C during the first week (Malheiros et al., 2000; Maiorka et al., 2006). During the first four weeks of growth, the thermal comfort zone for birds decreases from 35°C at hatch to 24°C (Teeter and Belay, 1996). The thermoneutral zone for adult chickens is 18°C to 24°C depending on the strain of bird (Weaver, 2001; Scanes and Christensen, 2020).

## **Thermoregulation**

Thermoregulation is the ability of an animal to regulate and maintain a constant core body temperature that is usually different from the surrounding environmental temperature (McWilliams et al., 2016; Scanes and Christensen, 2020). Being that chickens are endothermic homeotherms, they rely on metabolic heat production to maintain their core body temperature within a narrow range regardless of their surrounding environment (Deeb and Cahaner, 1999; McWilliams et al., 2016). For the bird's body temperature to remain constant, their heat loss and heat gain/production must be equal, but they can lose or produce heat to induce changes in their body temperature (Freeman, 1971; Deeb and Cahaner, 1999; McWilliams et al., 2016). There has not been a single mechanism found that is solely responsible for thermoregulation in birds. Rather, it is controlled by a combination of several mechanisms (Duchamp et al., 1999). Unlike mammals, birds do not have a specialized thermogenic tissue, such as brown adipose tissue (Saarela et al., 1991; Duchamp et al., 1999). Thus, the birds need a highly accurate system to control the heat production and loss simultaneously (Maiorka et al., 2006).

## **Hormone Regulation**

Thyroid hormones are essential for the regulation of basal metabolic rate which plays an integral part in maintaining a high and constant body temperature (Decuypere et al., 2005). Thyroid hormones increase heat production at different sites through the body. Central thyroid releasing hormone (TRH) plays a role in thermoregulation by stimulating the production of thyroid hormones through the hypothalamic-pituitary-thyroid (HPT) axis (Takahashi et al., 2005). Triiodothyronine (T<sub>3</sub>) acts by binding to the nuclear and mitochondrial receptors to influence gene expression for metabolic rate and respiration modulation but the underlying mechanisms are unknown (Ruuskanen et al., 2021). Thyroxine (T<sub>4</sub>) is a prohormone to T<sub>3</sub> that is

secreted by the thyroid gland that is important for the regulation of available T<sub>3</sub> and thyroid activity (Decuyper et al., 2005; Ruuskanen et al., 2021). It has been found that when birds are exposed to temperatures lower than the thermoneutral zone, circulating T<sub>3</sub> levels increase and circulating T<sub>4</sub> levels decrease (Collins et al., 2002). When birds were injected with T<sub>3</sub> and T<sub>4</sub>, heat production and rectal temperatures increased (Freeman, 1970; Hwang-Bo et al., 1990) and the rate in which body temperature decreased when exposed to cold temperatures was decreased (Freeman, 1970). Inhibition of the conversion of T<sub>4</sub> to T<sub>3</sub> resulted in a decrease of heat production at two weeks of age (Hwang-Bo et al., 1990). Plasma T<sub>3</sub> concentrations are reported to decrease as ambient temperatures increased when exposed to diurnal ambient temperature changes and held at a constant high temperature (Klandorf et al., 1981; Yahav et al., 1996). When laying hens were exposed to higher ambient temperature, there was no change in plasma T<sub>4</sub> concentrations (Klandorf et al., 1981). Embryos that were exposed to a high temperature of 38.5°C experienced a decrease in both T<sub>3</sub> and T<sub>4</sub> immediately after hatch. This was accompanied with a decrease in chick body temperature (Yahav et al., 2004).

The hypothalamic-pituitary-adrenal (HPA) axis has also been reported to play a role in thermoregulation (Takahashi et al., 2005). Corticotropin releasing factor (CRF) works to regulate the response of the HPA axis and central nervous system to stress and environmental challenges by stimulating the release of adrenocortical hormones through adrenocorticotropic hormone (ACTH) (Etches et al., 2008). Injections of CRF increased body temperature in chicks reared in thermoneutral temperatures (Mujahid and Furuse, 2008a) and lessened the decrease in body temperature of chicks exposed to temperatures below their thermoneutral zone (Mujahid and Furuse, 2008b). Chicks that were injected with CRF and exposed to cold temperatures had an increased corticosterone level (Mujahid and Furuse, 2008b). Corticosterone is a primary

adrenocortical hormone released from the adrenal gland in response to stress (Etches et al., 2008). Corticosterone has been suggested to play a role in a bird's ability to respond and adapt to rapid changes in temperature but the exact mechanism for this link is not well understood (Ruuskanen et al., 2021). Corticosterone levels are positively correlated with body temperature (Giloh et al., 2012) thus injection of corticosterone resulted in a 0.15°C to 0.6°C increase in body temperature in two-day-old chicks (Takahashi et al., 2005). Adrenalectomized birds were shown to not be able to maintain their body temperature during cold exposure (El-Halawani et al., 1971). When birds are exposed to ambient temperatures that are below the thermoneutral zone, there is a significant increase in corticosterone levels (Shinder et al., 2002; Frigerio et al., 2004; Mujahid, 2010, Alkenaky et al., 2017). Frigerio et al. (2004) used corticosterone immune-reactive metabolites excreted in the feces as an indirect indicator of plasma corticosterone levels in graylag geese exposed to colder temperatures. Metabolites increased as ambient temperature decreased. The change in corticosterone metabolites had a delayed response of several hours after exposure suggesting that this change is a physiologic adjustment to the temperature rather than an immediate stress response (Frigerio et al., 2004). Broiler chicks that were cold-conditioned did not experience a significant increase in corticosterone as was seen in non-conditioned birds. The cold-conditioned chicks continued to have lower levels of corticosterone at Days 14 and 21 when compared to the non-conditioned chicks (Shinder et al., 2002). Corticosterone levels increase during temperatures that are above the thermoneutral zone as well (Edens and Siegel, 1976; Edens, 1978; Etches et al., 2008; Giloh et al., 2012). The increase in corticosterone levels during both hot and cold stress indicate that the increase is primarily a response to stress rather than a body temperature dependent response (Etches et al., 2008).

### Heat production in birds

Metabolic heat production is one of the primary mechanisms of thermoregulation in birds (Mujahid, 2010). Birds have an active metabolic rate that is up to two times higher than that of a similar-sized mammal and 12 to 20 times higher than that of a similarly sized reptile (McWilliams et al., 2016). Metabolic rate is kept constant when the birds are within the thermoneutral zone and increases when they are exposed to ambient temperatures outside of the thermoneutral zone. When the ambient temperature is below the thermoneutral zone, the birds upregulate their metabolism to produce heat to be able to maintain their core body temperature (Mission, 1977). Barre et al. (1985) found that the metabolic rate increased linearly in ducklings as ambient temperatures decrease below the thermoneutral zone. Smaller animals have to produce more heat to maintain their body temperature due to their more extensive heat loss from having a higher surface to body weight ratio (Marjoniemi and Hohlot, 1999). Birds also increase their metabolic rate when ambient temperatures are above the thermoneutral zone as energy is required for heat loss mechanisms such as panting and gular flutter (McWilliams et al., 2016).

### Heat loss in birds

Internal body temperature increases as heat production surpasses the rate of heat dissipation. Therefore, heat being produced through metabolism and muscular activity must be lost to prevent an increase in body temperature (Weaver, 2001). Birds have many ways in which they dissipate heat: conduction, convection, radiation, evaporation, and fecal excretion. These can then be divided into two categories: sensible and latent heat loss (Freeman, 1971; Weaver, 2001). Sensible heat loss is the process in which heat is removed from the body through conduction, convection, and radiation to the surrounding environment (Scanes and Christensen,

2020). Conduction is when heat is transferred from the bird to a cooler surface via contact (Weaver, 2001). Heat is lost through radiation when the surface (skin/feathers) of the bird is warmer than its surroundings. Convection is when heat is lost to air that comes into contact with a warmer surface, i.e., the bird's skin. Latent heat loss is the heat lost due to a phase change, i.e., evaporation of water, without a change in temperature (Weaver, 2001; Scanes and Christensen, 2020). McWilliams et al. (2016) described evaporative cooling as the loss of heat from the energy required for water to evaporate across the skin and respiratory surfaces.

Both sensible and latent heat loss in birds is dependent on multiple factors such as breed, age, body weight, activity level, nutrition, environmental temperature, and relative humidity (RH) (Simmons et al., 1997; Chepete and Xin, 2001). The proportion of latent and sensible heat loss can change depending on the same factors listed above. Sensible heat loss can range from 40% to 70% of total heat loss at thermoneutral temperatures (Weaver, 2001; Czarick and Fairchild, 2008). Laying hens lose more of their body heat through sensible heat compared to broilers who lose more heat through latent heat (Roller and Dale, 1963; Reece and Lott, 1982a,b; Simmons et al., 1997; Chepete and Xin, 2001; Genç and Portier, 2003; Czarick and Fairchild, 2008). Chepete and Xin (2001) compiled data from previous literature and found that broilers lost over 50% of their heat through latent heat loss on average.

Sensible heat is the heat that is felt by the body and is associated with an actual change in body temperature (Weaver, 2001). Birds use blood flow to transfer heat from their core to their skin to be lost to the surroundings through conduction and convection (Freeman, 1971; Shinder et al., 2007; Scanes and Christensen, 2020). Birds can control the vasoconstriction and vasodilation of their blood vessels to either promote or restrict heat loss from the skin to the environment with little energy cost (Weaver, 2001; McWilliam et al., 2016). Loss of heat via

conduction is increased when birds sit on the litter (Scanes and Christensen, 2020). Heat loss through convection and radiation is dependent on the skin temperature of the bird and more specifically the difference between the skin and the ambient air temperatures (Hai et al., 1996; Weaver, 2001; Maiorka et al., 2006; Shinder et al., 2007; Nääs et al., 2010). Smaller animals lose more heat through sensible heat loss due to having a higher surface area to body weight ratio compared to larger animals. Therefore, chicks dissipate heat at a higher rate than older birds (Marjoniemi and Hohlot, 1999; Malheiros et al., 2000). The ability for birds to lose heat through sensible heat loss pathways decreases as ambient temperature approaches bird temperature and the temperature gradient between the air and skin decreases (Freeman, 1971; Simmons et al., 1997; Weaver, 2001).

During ambient temperatures that are similar to the skin temperature of birds, heat dissipation relies on latent heat loss, more specifically evaporative heat loss (Freeman, 1971; Hillman, 1985; Hai et al., 1996; Simmons et al., 1997; McWilliams et al., 2016). While at thermoneutral temperatures or lower, the rate of evaporative heat loss is kept relatively constant (Freeman, 1971). Birds do not have sweat glands but can still lose heat through their skin. However, respiration is still their primary evaporative cooling mechanism and can be increased through increased respiration such as panting and gular flutter (Randall, 1943; Freeman, 1971; Reece and Lott, 1982b; McWilliams et al., 2016; Scanes and Christensen, 2020). This is influenced by environmental RH levels. Increased RH levels decreased the evaporative heat loss for birds due to the decreased amount of water that is able to be evaporated by the air (Freeman, 1971).

## **Factors effecting bird body temperature**

### Breeder flock age

When chicks from different age breeder flocks are reared at thermoneutral temperatures, they have similar body temperatures to one another (Weytjens et al., 1999; Luo, 2021). Luo (2021) completed trials comparing chicks from young breeder flocks (27 to 28 weeks old) to prime-age breeder flocks (36 to 39 weeks old) and prime-age breeder flocks (41 to 42 weeks old) to old breeder flocks (50 to 54 weeks old). It was found that there was not a significant difference in body temperatures between chicks based on breeder flock age when reared at a thermoneutral temperature of 34°C. Weytjens et al. (1999) compared chicks from two breeder flock ages, 25 and 60 weeks old, reared at a thermoneutral temperature of 33°C and a cold stress temperature of 20°C. The chicks from both breeder flock ages had similar rectal temperatures at the thermoneutral temperature but when the ambient temperature was decreased to 20°C, the chicks from the younger breeder flock had a more pronounced reduction in rectal temperatures.

### Feathering

Birds are well equipped to handle colder temperatures due to the insulation provided from their feathers. The feathers are the first defense that can be used to protect them from cold environments (Scanes and Christensen, 2020). The skin temperature of featherless areas of the bird contributed more to sensible heat loss than the areas covered by feathers (Richards, 1971; Nääs et al., 2010). Birds with decreased feathering have less insulation but are able to dissipate heat easier in order to prevent a large increase in body temperature. At higher ambient temperatures of 32°C fully feathered birds had the highest body temperature while naked neck birds had the lowest body temperature. The change in the body temperature that was caused by a

change in feed intake was less in birds that possessed the naked neck genes when compared to that of the fully feathered birds (Deeb and Cahaner, 1999).

### Stocking density

Stocking density has been shown to have a significant effect on body temperature and heat loss. Birds who experience a high stocking density have higher body and skin temperatures than birds in lower stocking densities (Abudabos et al., 2013; Czarick et al., 2017; Teo, 2018). At high stocking densities, the airflow at the bird level is lowered and impairs their ability to dissipate heat (Fedde et al., 2002; Czarick et al., 2017; Czarick et al., 2018). Teo (2018) found that 45-to-50-day old broilers reared at 22°C had significantly higher body temperatures at a stocking density of 7 birds/m<sup>2</sup> and 10 birds/m<sup>2</sup> when compared to 2 birds/m<sup>2</sup> and 5 birds/m<sup>2</sup>. Additionally, birds in the 7 birds/m<sup>2</sup> and 10 birds/m<sup>2</sup> treatment groups presented visual signs of being hot such as panting. When the ambient temperature was increased to 26°C, the body temperature increased in all groups but the birds in the 10 birds/m<sup>2</sup> treatment were affected more, continuing to have a significantly higher body temperature than the 2 birds/m<sup>2</sup> treatment group. Even though the author reported that the body temperatures during the study were within the normal range of broiler body temperature, the results show that stocking density can still cause a change in body temperature even when birds are reared in the thermoneutral zone. (Teo, 2018).

### Lighting

The body temperature of birds has been shown to increase during periods of light (Kadono and Besch, 1978; Weaver, 2001). The diurnal fluctuations in body temperature closely follow changes in photo period throughout the day, rising from the morning until midday, then decreasing throughout the afternoon while continuing to be low throughout the night (Lacey et al., 2000). Klandorf et al. (1981) reported that heat production in layers decreased during periods

of darkness and then rapidly increased when lights came on. Xin et al. (1996) reported that there was a 25% to 26% decrease in moisture, sensible heat, and total heat production of the birds when the lights were turned off. These fluctuations may be under the control of a biological clock synchronized to the solar day as well as influenced by fluctuations in activity that increase with light and decreased with darkness (Freeman, 1971; Kadono and Besch, 1978). Conversely, Czarick et al. (2016) found that body temperature increased during the dark period. This increase was partly attributed to the birds sitting on the litter which decreases the amount of heat that can be lost from the bird.

#### *Behavior and activity to conserve heat*

The behavior and activity levels of birds are influenced by body temperature. Birds alter their behavior to help dissipate or produce heat. Body temperature increases with increased bird activity (Prinzinger et al., 1991; Weaver, 2001). Birds will move to areas that are warmer or cooler depending on their body temperature. When birds are trying to decrease heat loss, they will reduce the amount of exposed surface area by hunching, sitting to cover the unfeathered areas, and placing their head under their wing which reduces heat loss up to 12% (Scanes and Christensen, 2020). When ambient temperature decreases, chickens will increase their feed intake and movement to increase heat production. However, in extreme cold, birds will conserve heat by fluffing their feathers and sitting in the litter for more insulation (Weaver, 2001). Shivering in response to cold ambient temperatures is shown to increase metabolic rate in birds therefore increasing heat production (Barre et al., 1985; Scanes and Christensen, 2020). The muscle contractions produce heat due to their low energy efficiency (Duchamp et al., 1999). Bird behavior is indicative of body temperature/comfort and the ultimate index for determining the most suitable environment (Mujahid and Furuse, 2009).

### Behavior and activity to lose heat

Birds that are warm will increase respiration, decrease activity level, and change their body position to expose more surface area to dissipate heat (Weaver, 2001; Scanes and Christensen, 2020). Birds are able to control their heat loss through changes in posture and behavior such as repositioning of feather/ wings to expose more skin with little energy cost. Birds will also begin to dissipate heat using gular flutter, the act of opening of their beak and vibrating the gular membranes of the throat to aid in evaporative cooling (McWilliams et al., 2016). Gular flutter may be synchronized with respiratory rate such as in chickens exposed to heat stress. Panting patterns can vary based on body temperature as well as other stimuli (Hillman, 1985), such as changes in blood pH and blood gas concentrations in response to activity and environment (Powell, 2015). However, the onset of panting is closely related to hypothalamic temperature (Richards, 1971). Small changes in body temperature are followed by a relatively large increase in respiration rate (Farny, 1965). The onset of panting in mature birds begins when the body temperature is between 41°C to 42°C. There are two phases of panting: Phase 1 and Phase 2. Phase 1 panting is when the respiration rate increases but the tidal volume decreases. The respiration rate increases with Phase 1 until it peaks when the bird's body temperature reaches approximately 44°C and transitions into Phase 2 panting. In Phase 2 panting, the respiration rate decreases and the tidal volume increases (Hillman, 1985).

### Feed intake

Body temperature is positively correlated to feed intake (Koh and Macleod, 1999). The composition as well as the amount of feed consumed influences body temperature and the bird's thermoregulation capabilities. Heat production and body temperature increases after feeding and digestion begin (Klandorf et al., 1981; Weaver, 2001). Birds who were given access to feed have

a higher body temperature than those taken off feed (Deeb and Cahaner, 1999). Mission (1982) reported that seven-day old chicks maintained their body temperature 2°C lower when they did not have access to feed compared to those that had access. Heat production increased with increased feed intake and was accompanied by increased sensible and evaporative heat loss (Wiernusz and Teeter, 1993). Early feeding of chicks can help with chick development and resistance to cold stress. When chicks are fed prestarter and prestarter with extra fat, they had a higher body temperature measured at two and three days old and had less of a change in rectal temperature when exposed to cold. Additionally, diets that are formulated with fast available energy, such as dextrose, appear to aid in resistance against cold exposure (van de Brand et al., 2010).

#### Relative humidity effects on body temperature

The rectal and skin temperatures of birds reared under various RH levels show a bell-shaped curve with the lowest temperatures occurring between 60% and 65%. Hyperthermia occurred when the RH was lower or higher than this range (Yahav et al., 1995). Genç and Portier (2005) found that five- to six-week-old broilers were not affected by varying levels of RH at 25°C. However, environmental RH has a drastic impact on broiler body temperature over varying ambient temperatures (Teeter and Belay, 1996). Increased RH levels decreased the evaporative heat loss for birds due to the decreased amount of water being evaporated (Randall, 1943; Freeman, 1971). Changes in RH levels cause redistribution of heat within the body of one week old broilers which caused a decrease in body temperature. Depending on the environmental temperature, this could be beneficial or harmful for the chicks (Lin et al., 2005a).

### Body temperature during cold stress

Birds that are exposed to temperatures colder than thermoneutral will experience higher heat dissipation and if the birds can not compensate for that heat loss, their body temperature will decrease to a range that is lethal (Etches et al., 2008). Total sensible heat loss increases in birds when exposed to cold temperatures due to the increased thermal gradient between air and the bird's surface (Shinder et al., 2007). The higher rate of heat loss that chicks experience during cold temperatures is related to the increased surface to body weight ratio and the increased gradient between skin and ambient temperature (Malheiros et al., 2000). The increased temperature gradient allows for more heat to be transferred from the body to the air and the increased surface area of chicks further drives the heat loss. When chicks are reared at thermoneutral temperatures (35°C), they lost minimal heat through radiation but when the temperature is decreased to 20°C, their radiant heat loss was approximately nine times higher (Malheiros et al., 2000). To compensate for the increased heat loss during cold temperatures, birds must increase their heat production in order to maintain a constant body temperature (Mission, 1982; Tzschentke and Nichelmann, 1990).

Adult birds can regulate their body temperature easily by increasing their heat production through feed intake and increasing their metabolism (Mujahid and Furuse, 2009; Scanes and Christensen, 2020). However, chicks' inability to do the same makes them more vulnerable to the cold (Mujahid and Furuse, 2009). It has been shown that body temperature decreased when chicks are reared in temperatures lower than their thermoneutral and thermal comfort zones (Randall, 1943; Hai et al., 1996; Malheiros et al., 2000; Shinder et al., 2007; Mujahid and Furuse, 2009). There was a significant interaction effect on body temperature between age and the ambient temperature, thus suggesting that the effect of cold exposure on chicks was age-

dependent (Mujahid, 2010). Two-hour-old chicks were able to maintain sufficient heat production at 20°C for 30 minutes before their heat production declined resulting in a decrease in body temperature. However, older chicks were able to maintain sufficient heat production continuously in the same environment (Mission, 1977). Chicks had a lower body temperature for the first 24 hours of cold exposure but then returned to a normal body temperature. This was shown to be worse when combined with low RH (Hai et al., 1996).

#### Body temperature during heat stress

Heat stress occurs in all poultry during exposure to elevated RH and ambient temperature (Teeter and Belay, 1996). In ambient temperatures above their thermoneutral zone, the bird's ability to maintain body temperature is from the sufficient use of cooling mechanisms for heat loss and/or reduce their overall heat production (Yahav et al., 1995; Yahav, 2000; Scanes and Christensen, 2020). Birds tolerate a narrow range of ambient temperatures above their thermoneutral zone due to them having to rely heavily on physical mechanisms compared to chemical mechanisms to maintain body temperature (Brown-Brandl et al., 1997). In high temperature environments, bird body temperature will rise due to an increase in heat stores, but this can only happen for a short period of time before their body temperature continues to rise to a lethal range (Richard, 1971; Donkoh, 1989; Genç and Portier, 2005; Lin et al., 2005a,b; Etches et al., 2008; Alhenaky et al., 2017). Lin et al. (2005b) reported that the birds had a higher temperature gradient between the core body temperature and the skin temperature suggesting heat transmission from the core to the skin is less efficient during higher ambient temperature and RH resulting in an increased body temperature. The rate at which the ambient temperature increases also plays a role in how well a bird can cope with the stress. When ambient temperature is abruptly and rapidly increased, birds are not able to regulate their deep body

temperature as well and body temperature increases (El-Gendy and Washburn, 1995; Lacey et al., 2000).

As ambient temperature and RH increase, sensible heat production also increases (Freeman, 1971; Genç and Portier, 2005). Latent heat production increased as the dry bulb temperature increased (Genç and Portier, 2005). Wiernusz and Teeter (1993) found that birds under heat stress had a 27.3% increase in heat production. The bird's ability to lose heat through non-evaporative heat loss mechanisms is reduced due to a smaller gradient between the ambient temperature and the skin/plumage temperature (Freeman, 1971; Teeter and Belay, 1996; Lin et al., 2005a,b). As the temperature increases and sensible heat loss decreases, the birds increase their evaporative heat loss through increased panting rate (Teeter and Belay, 1996). However, this increased the energy needed for heat dissipation. The evaporative cooling of birds is less efficient with a rise in RH causing a rise in body temperature.

When RH is high, the air is more saturated with moisture and therefore cannot evaporate as much moisture from the bird's skin and respiratory system. At 35°C and 85% RH, chicks had elevated rectal temperatures during the first three days post-hatch (Hai et al., 1996). Yahav (2000) reported that body temperature of 28-day old broilers exposed to 30°C was higher when reared under 40-55% RH compared to 60-75% RH. However, the skin temperature was lower at 50% and 70%, whereas Lin et al. (2005b) showed that 35% RH treated 28-day old birds had a lower body temperature at 35°C than 60% or 85% RH which did not have an effect at 30°C.

### **Impact of environmental temperature and relative humidity on bird performance**

Environmental temperature and RH are important factors in both energy metabolism and exchange in broilers (Simmons et al., 1997; Genç and Portier, 2005; Purswell et al., 2012). At temperatures in the thermoneutral zone (21°C -24°C for adult birds), the increase or decrease of

body temperature does not require effort from the bird. At the thermal comfort zone, the birds do not have to expend metabolic energy to maintain their body temperature and is the most efficient temperature range for production (Scanes and Christensen, 2020). Maintaining the thermal environment in the thermal comfort zone to avoid heat or cold stress is important for bird health, well-being, and efficient production (Weaver, 2001; Purswell et al., 2012; Scanes and Christensen, 2020). Chicks are more sensitive to lower temperatures (Deaton, 1995) while older birds have an increased sensitivity to high temperatures and RH (Brown-Brandl et al., 1997). Relative humidity plays a key role in maintaining air and litter quality for poultry performance and welfare (Winn and Godfrey, 1967; Deaton, 1995; Czarick and Fairchild, 2012; Czarick et al., 2020).

### **Environmental temperature**

The temperature curve suggested by the Ross broiler management handbook (Aviagen, 2018) starts at 30° to 32°C at day of placement, then gradually decreases to 20°C by Day 27. While the Cobb optimum broiler development guide (Cobb, 2018) recommended starting at 33°C to 34°C and decreasing to 27°C by Day 14. Deaton (1995) provided an optimum temperature curve starting at 29.4°C during the first week, 26.7°C during the second week, 23.9°C during the third week, and 21°C for the fourth week until the birds reach market weight. More recently, Cassuce et al. (2013) reported that optimum growth occurred at the temperature curve of 31.3°C, between 26.3°C to 27.1°C, and between 22.5°C to 23.2°C for the first, second, and third week, respectively. The optimal temperature for the grow out period, three to six weeks, is approximately 21°C (Deaton, 1995; Deaton et al., 1996; Cândido et al., 2013). However, the recommended temperature curves are only meant to be used as a starting point, and the actual set temperature may vary depending on RH and bird behavior (Aviagen, 2018). Internal house

temperature varies based on the influence of seasonal and diurnal changes of the outside temperature that may exceed the heating and cooling capacity of the house. Temperatures in a curtain sided house were found to vary by approximately 12°C while the variation in a tunnel ventilated house was roughly 5°C (Xin et al., 1994). There are several other factors that play a role into the effect of temperature on bird performance such as gender, body weight, nutrition, growth rate, and genetic line (Leenstra and Cahaner, 1991; May and Lott 2001; Cassuce et al., 2013).

### Body weight

Growth rate depends on the timing of chick hatching and the environmental temperatures that birds are exposed to during the grow out period (Kempster, 1938). Moraes et al., (2002) reported that chicks grew better in the first week at a moderated temperature of 25°C rather than 35°C or 20°C. Another study found that variations in brooding temperature curves did not affect chick weight gain and suggest that the effect of temperature on gain is not as important during the brooding period (May and Lott, 2000; Zhou et al., 2019). The optimum temperature range for a constant environmental temperature was 15.5° to 26.7°C for the best performance for four- to five-week-old birds (Milligan and Winn, 1964; Deaton et al., 1973). The range of 15.5° to 26.7°C in 1960s and 1970s is broad compared to current recommendations. This may be due to differences in poultry house design and interior ambient temperature being heavily influence by the outside environmental temperature.

### FCR

FCR increases when ambient temperatures are higher (Prince et al., 1965; Deaton et al., 1978; Donkoh, 1989) or lower than optimal temperature (Deaton et al., 1978). FCR is affected by the bird's ability to maintain body temperature at their resting metabolic rate (Prince et al.,

1960, 1961). Once broilers reached a body weight of 800 grams or higher the lowest FCR was obtained at the lowest temperature tested, 23°C (May et al., 1998). FCR was the lowest for broilers reared at 31.3°C, 27.1°C, and 23.2°C for the first, second, and third week of production, respectively (Cassuce et al., 2013). As the birds got older the difference in FCR seen between different temperature regimes become smaller, thus showing that age has an effect on the way birds are affected by temperature (Leena and Cahaner, 1991). This agreed with Deaton (1995) who reported that chicks were more susceptible to colder temperatures than older birds.

### **Environmental relative humidity**

Relative humidity is a major factor that needs to be managed to maintain good air and litter quality for poultry performance and welfare. Previous recommendations for RH level for broiler production were 50% to 70% (Reece and Lott, 1982b; Deaton, 1995; Czarick and Fairchild, 2012). The Ross708 performance guide (Aviagen, 2018) recommends that the target RH should be 60-70% at chick placement and 50-60% after brooding. More recently, the recommended RH range is 40% to 60% (Cobb, 2018; Czarick et al., 2020). RH tends to be lower during the first two weeks of the flock and increases as the birds grow (Czarick and Fairchild, 2012). Variation in RH outside of the suggested range has been observed due to outside RH and improper ventilation rates with the RH level as low as 50% and as high as 90%. Xin et al. (1994) found that RH cycled throughout the day showed minimums of 35% and 54% and maximums of 76% and 74% in curtain sided houses and tunnel ventilated houses, respectively. RH levels below 40% tend to result in a dusty house leading to higher heating cost due to over ventilation (Deaton, 1995; Czarick and Fairchild, 2012). High moisture levels can occur in poultry houses and is correlated with higher incidence of disease (Winn and Godfrey, 1967). High RH can also lead to wet litter and high levels of ammonia production (Deaton, 1995; Czarick and Fairchild,

2012, Czarick et al., 2020). These factors that are affected by RH level also affect the performance and wellbeing of the birds during production.

### Performance

Several studies have reported that RH level does not affect the performance of broilers at thermoneutral temperatures (Barott and Pringle, 1949; Milligan and Winn, 1964; Prince et al., 1965; Winn and Godfrey, 1967, Zhou et al., 2019). However, other studies show decreased performance when RH is above or below the recommended range of 50% to 70% (Winn and Godfrey, 1967; Yahav, 2000). The highest gain and feed intake occurred at 60-65% RH and may be explained by maintenance energy requirement for broilers being lower at 60-65% RH. The reason for the difference in energy requirement was unknown (Yahav, 2000). Body weight was significantly lower for the high RH treatment group and was thought to be caused by the differences in the litter and air quality rather than the excess humidity alone (Weaver and Meijerhof, 1991). Winn and Godfrey (1967) investigated the effect of RH during the brooding period with temperatures starting at 32.2°C (Day 0) and decreased to 18.3°C (Day 36) at RH levels of 40%, 50%, 70%, and 90%. Bird raised within the range of 50-70% RH had the higher body weight, however, FCR was the lowest at 90% (2.02) and the highest at 40% (2.13). Conversely, Weaver and Meijerhof (1991) and Yahav (2000) found that there was no effect of varying RH levels on FCR.

### **Cold stress effect on performance and livability**

#### Body weight

Chicks reared at temperatures below thermoneutral have a significantly lower body weight during brooding (Deaton et al, 1996; Bruzual et al., 2000, Malheiros et al., 2000, Ipek and Sahan, 2006). In contrast, Cândido et al. (2016) observed that temperature treatments ranging

from thermal comfort, 33°C and 30°C, to cold stress temperatures of 27°C to 21°C, did not differ in body weight or gain of broilers chicks during the first week. Exposure to temperatures lower than the thermoneutral zone around three weeks or older resulted in body weight gain not being affected (Deaton et al., 1978, 1996; Deaton, 1995) or improving (Prince et al., 1965; Leenstra and Cahaner, 1991, Ipek and Sahan, 2006). Four-week-old 1965 strain White Plymouth Rock males reared at 12.6°C had a 5.13% higher weight gain than those reared at 23.8°C (Prince et al., 1965). Four-week-old broilers reared at 15.6°C and 8.3°C through 10 weeks of age had significantly higher weight gain than those at 26.7°C (Winn and Godfrey, 1967). Olfati et al. (2018) showed body weight was decreased when broilers were reared in cold stress conditions, 12°C, between 22 to 42 days of age. The differences between the results of the previous studies could be due to the genetics of the birds used, broilers vs. layers as well as 1960s genetics vs. modern genetics.

### Feed consumption

At ambient temperatures lower than the thermoneutral zone, birds increased their feed intake significantly due to an increase in energy requirement to maintain body temperature (Deaton, 1995; Deaton et al., 1996; Yahav et al., 1996; Prince et al., 1961, 1965; Collins et al., 2002; Cândido et al., 2018). Birds reared at 12.6°C had an 11.8% increase in feed consumption compared to birds reared at 23.8°C (Prince et al., 1965). Conversely, Hai et al. (1996) reported feed consumption was not significantly affected by environmental temperature (27°C v. 35°C) or RH (35% v. 85%) for the first 48 hours post-hatch; however, feed intake tended to be higher at 35°C. Other studies showed a decrease in feed intake when birds were exposed to cold stress conditions (Moraes et al., 2002; Olfati et al., 2018). When brooding chicks for the first seven days at 20°C, 25°C, or 35°C, the most feed was consumed at 25°C (116.2 grams) and the least at

20°C (92.7 grams) with feed intake being slightly depressed at 35°C (103.6 grams). Activity at the feeders and drinkers was decreased when chicks were brooded at 20°C compared to those at 25°C and 35°C (Moraes et al., 2002). Birds increase their feed intake during times of low ambient temperatures to increase heat production. However, if birds get too cold, they will huddle together and reduce their movement to conserve energy and heat, thus causing a decrease in feed intake. The tendency for chicks to aggregate together and sit down to reduce heat loss instead of moving to feeders could relate back to the decreased body weight observed (Malheiros et al., 2000; Moraes et al., 2002; Mujahid and Furuse, 2009).

### FCR

Chicks brooded at a cooler temperature use a greater portion of their nutrient intake to generate heat and maintain body temperature which adversely affects FCR (Prince et al., 1960, 1961; Reece and Lott, 1983; Deaton et al., 1996; Bruzual et al., 2000; Ipek and Sahan, 2006; Olfati et al., 2018). Chickens reared at ambient temperatures below the thermoneutral zone have a significantly higher FCR compared to birds raised at thermoneutral temperatures (Milligan and Winn, 1964; Reece and Lott, 1983; Ipek and Sahan, 2006; Olfati et al., 2018). Birds raised at 15.6°C had a higher FCR requiring 3% more feed than birds raised at 21.1°C and 26.7°C to achieve the same weight (Reece and Lott, 1983). Prince et al. (1961) found that there was a 12.5% improvement in FCR when the environmental temperature was increased from 7.2°C to 23.9°C. Prince et al. (1960) reported an 11.4% increase in feed efficiency when temperature is increased from 7.2°C to 18.3°C.

### Livability

Mortality rate was higher in chicks that were brooded at temperatures of 26°C to 28°C (Bruzual et al., 2000; May and Lott, 2000). It was suggested that the increased mortality may be

due to behavioral changes where the birds spent more time huddling instead of consuming feed and water. As a result, chicks in the cooler temperature treatments may have died from starvation and/or dehydration (Bruzual et al., 2000). Conversely, Deaton et al. (1996) showed that brooding chicks at 29.4°C did not show a difference in mortality compared to chicks brooded at a starting temperature of 32.2°C. Once brooding temperatures decrease as low as 26.7°C, chick livability was negatively affected during the first week as well as the grow out period. Chicks exposed to cold stress had a higher occurrence of ascites and therefore a higher incidence of ascites related mortality during the grow out period of three to six weeks of age (Deaton et al., 1996; Shinder et al., 2002; Ipek and Sahan, 2006).

### **Heat stress effect on performance and livability**

#### *Body weight*

Rearing birds at temperatures higher than the thermoneutral zone results in lower body weight and weight gain during grow out (Kempster, 1938; Prince et al., 1960, 1961; Deaton, 1995; Yahav et al., 1996; May et al., 1998; May and Lott, 2001, Alhenaky et al., 2017; Olfati et al., 2018, Koh and Macleod, 1999; Awad et al., 2020). Body weight was not affected by increased ambient temperatures during the starter period (Olfati et al., 2018; Awad et al., 2020). Studies have shown that rearing birds at temperatures greater than 5°C above thermoneutral zone (approximately 21.1°C) significantly depresses body weight and gain starting at four weeks of age (Milligan and Winn, 1964; Winn and Godfrey, 1967; Suk and Washburn, 1995). Weight gain decreased linearly as temperatures increased from 19°C to 35°C (Hurwitz et al., 1980; Donkoh, 1989). Rearing broilers at 35°C from two to four weeks of age decreased weight gain by 20% compared to those reared at 22°C (Mitchell and Carlisle, 1992). During temperature regimes mimicking high temperature fluctuations (23.9°C to 35°C) typically observed during the summer,

body weights of broilers were significantly depressed at 4 weeks of age (Deaton et al., 1978). When temperatures are cycled throughout the day, such as 10°C:30°C and 15°C:35°C, the body weight and weight gain were smaller than birds that were raised at the average temperature constantly (Yahav et al., 1996). Deaton et al. (1973) reared birds under a 24-hour cycle between 24°C and 35°C. The author reported that the growth of the birds who experienced the temperature fluctuations did not differ from those having a constant temperature of 29.4°C.

Effects of RH on body weight of birds will vary with temperature (Winn and Godfrey, 1967; Yahav et al., 1995; Zhou et al., 2019). Varying levels of RH at temperatures of 26.6°C or below did not affect performance and did not clearly define the influence of RH at low temperatures (Milligan and Winn, 1964). Body weight was significantly affected by RH levels at elevated temperatures of 31°C to 35°C resulting in a bell-shaped response with the highest body weights at 60-65% (Yahav et al., 1995; Zhou et al., 2019). Birds experienced a slow rate of gain when reared at 35°C and 90% RH and the birds were not able to acclimate. When temperature was held at 35°C and then decreased to 26.6°C, the 90% RH treatment group had a 44% improvement in body weight while the low RH treatment group only had a 26% improvement (Milligan and Winn, 1964). The effect of RH level on body weight is dependent on the ambient temperature. Alone the RH level does not have as much of an effect on growth as ambient temperature but will cause an additive effect to environmental temperature.

### Feed consumption

Feed intake of birds is significantly decreased during exposure to elevated temperatures (Prince et al., 1960; Hurwitz et al., 1980; Donkoh, 1989; Suk and Washburn, 1995; Deaton, 1995; Yahav et al., 1996; Koh and Macleod, 1999; Olfati et al., 2018; Awad et al., 2020). Feed consumption has been reported to decrease feed intake by 8.7% up to 29% when reared at

temperatures of 30°C and 35°C (Donkoh, 1989; Mitchell and Carlisle, 1992). During high temperatures of 31°C to 35°C, RHs below or above 60% to 65% caused a significant decrease in feed intake showing a bell-shaped curve with the largest feed intake at 60-65% RH (Yahav et al., 1995; Zhou et al., 2019). Birds will decrease their feed consumption to reduce the heat production that comes with digestion (Donkoh, 1989; Weaver, 2001). Because of the reduction in feed intake, growth rate is negatively affected (Weaver, 2001; Koh and Macleod, 1999).

### FCR

Birds had a higher FCR when raised at temperatures above thermoneutral (Milligan and Winn, 1964; Donkoh, 1989; Yahav et al., 1996; Alhenaky et al., 2017; Olfati et al., 2018). A study showed that FCR increased as temperatures increased until peaking at 27°C and then FCR started to decrease between 27°C and 34°C in four- to eight-week-old broilers (Hurwitz et al., 1980). During fluctuating high temperatures, typically observed during the summer, Deaton et al. (1978) reported that the birds had the same FCR as those in the lower temperatures at four to seven weeks but higher at eight weeks. The high-temperature birds would take longer to reach the same weight due to decreased feed intake. In conjunction with high ambient temperature, high RH significantly increased FCR compared to lower RH levels (Milligan and Winn, 1964; Winn and Godfrey, 1967).

### Livability

The mortality rate of broilers exposed to elevated ambient temperatures is significantly higher than that of birds reared at thermoneutral temperatures (Milligan and Winn, 1964; Winn and Godfrey, 1967; Awad et al., 2020). Milligan and Winn (1964) showed a higher mortality at 37.8°C during Weeks 4 through 8 of grow out when compared to the lower temperature treatments. Alhenaky et al. (2017) reported that acute heat stress conditions of 35°C for four

hours a day had the highest mortality of 33.3% when compared to the thermoneutral temperature regime, 20°C, and the chronic heat stress treatment, 30°C for 24 hours. When birds were exposed to a combination of high temperature and RH combinations, there was an increase in the rate of mortality when compared to low temperature and RH (Milligan and Winn, 1964; Winn and Godfrey, 1967). Acclimating birds to high temperatures can lower the mortality rate. Birds reared at high ambient temperatures had an increased mortality rate of 29% but when the birds were acclimated to heat stress the mortality rate was decreased to less than 10% (Reece et al., 1972).

### **Nicarbazin**

Nicarbazin is a commercially available anticoccidial that is commonly used in the production of poultry (Wiernusz and Teeter, 1995; Chapman, 2001). It is a synthetic compound that was synthesized in 1955 and is still found to be one of the most effective anticoccidials to date (Bacila et al., 2017). It is a highly stable ingredient when mixed in feed and is recommended to be fed at a rate of 0.0125% (125 ppm) to growing birds (Ott et al., 1956). It is normally used in shuttle programs, the use of multiple anticoccidials in a single flock at different stages of production. Typically, nicarbazin is fed for the first three to four weeks then replaced with another anticoccidial due to the adverse effects on performance and heat stress tolerance (McDougald, 1982; Chapman, 1994; da Costa et al., 2017). However, little information is known about the physiologic and biochemical mechanisms to explain the adverse effects on bird performance and heat tolerance (Beers et al., 1989; Chapman, 1994; da Costa et al., 2017).

### **Effect on body temperature**

Birds fed nicarbazin had a higher body temperature at thermoneutral temperatures (Farny, 1965; da Costa et al., 2017) and during heat stress compared to nonmedicated birds (Farny, 1965;

Keshavarz and McDougald, 1981; Beers et al., 1989; Chapman, 1994; Wiernusz and Teeter, 1995). The rectal temperature of birds treated with nicarbazin increase at a higher rate than nonmedicated birds. After 30 minutes of increased ambient temperatures, nicarbazin treated broilers had a 1.4°C increase in rectal temperature while the nonmedicated bird's rectal temperature increased by 0.83°C. After one hour of heat stress, the nicarbazin treated birds had an increase in rectal temperatures of 2.8°C, compared to 1.4°C for the nonmedicated control. When birds were acclimated to high ambient temperatures, the rate in which body temperature increased slowed to a rate that was less than non-acclimated broilers fed nicarbazin but still higher than nonmedicated controls at the first 15 minutes of heat stress (Farny, 1965). Research has shown that age also plays a role in the change in rectal temperature related to nicarbazin and heat stress. Seven- to nine-week-old broilers had an increased body temperature after 60 minutes of heat stress while four to seven weeks old birds did not have an increased body temperature until three to five hours of heat stress (Beers et al., 1989).

### **Changes in metabolic rate**

Birds fed nicarbazin are less tolerant of increased ambient temperature due to having a higher resting metabolic rate (Farny, 1965). Thus, toxicity of nicarbazin may be dependent on metabolic rate and related to endocrine-environment interactions (Wiernusz and Teeter, 1995). Beers et al. (1989) found that there was no difference in heart rate, blood pH, and lactate measures in seven- to nine-week-old broilers fed nicarbazin. However, four- to seven-week-old broilers fed nicarbazin had a higher heart rate compared to unmedicated controls during prolonged heat stress. After five hours of heat stress, lactate levels significantly increased in the nicarbazin fed group compared to the unmedicated group. The delayed increase in lactate production might be due to tissue hypoxia from altered blood flow that occurs during heat stress

just prior to thermal death. The study also found that broilers treated with nicarbazin had a lower  $\text{PCO}_2$  and  $\text{HCO}_3^-$  levels between 3.5 and 5 hours of heat stress caused by their inability to maintain Phase 1 respiration, when the respiration rate increases but the tidal volume decreases, as well as a higher blood pH of approximately 7.54 (control) versus 7.60 (nicarbazin) at 3.5 and 7.59 (control) versus 7.64 (nicarbazin) at 4.5 hours in four- to seven-week-old broilers (Beers et al. 1989).

### **Effect on heat production and heat loss**

Heat production of birds increased linearly as ambient temperature increased. At 24°C, unmedicated broilers had a lower heat production than the nicarbazin fed birds but heat loss did not differ. As the ambient temperature increased, the evaporative heat loss increased while the sensible heat loss decreased but these were not significantly different between the diet treatments. This suggested the nicarbazin toxicity was related to the increase in heat production (Wiernusz and Teeter, 1995). Birds fed nicarbazin had an evaporative water loss similar to control birds at 25% RH and less than control birds at 50% RH during the first 30 minutes of heat stress. After 30 minutes, the nicarbazin birds had an increased evaporative water loss compared to the control. After 60 minutes, the nicarbazin treated birds' evaporative water loss decreased which was suggested to be due to decreased respiration rate (Farny, 1965). Even though a significant difference in body temperature between groups was found, heat stress acclimated birds fed nicarbazin, did not have an increase in heat production. An increased evaporative cooling as well as a decreased respiratory efficiency and sensible heat loss was observed (Wiernusz and Teeter, 1995). The relationship between respiration rate and tidal volume was disrupted in nicarbazin treated birds causing an insufficient minute volume for adequate cooling (Farny, 1965).

## **Changes in panting**

The body temperature and change in body temperature in which panting begins is the same regardless of nicarbazin treatment, but the nicarbazin treated birds reached the temperature for the onset of panting quicker (Farny, 1965, Beers et al., 1989). In one study conducted by Farny (1965), birds fed nicarbazin increased their respiratory rate during the first 15 minutes of heat stress and by the end of 30 minutes, the birds had reached the max respiratory frequency. The birds were able to maintain their max frequency for 30 minutes before it began to decrease. The unmedicated control group were able to continue to increase their respiratory rate after the first 30 minutes. In another study, the nicarbazin-treated birds transitioned into Phase 2 respiration after four hours when their body temperature reached 45°C resulting in a decreased respiration rate. At this time the unmedicated birds were still able to maintain their body temperature under 44°C and their respiration rate continued to increase in Phase 1. Thus, it was suggested that the inability to maintain respiratory rate is due to a faster transition into Phase 2 respiration due to increased body heat rather than nicarbazin having a direct effect on the respiratory system (Beers et al., 1989). An increase in respiration requires more energy expenditure which could be a cause of the rapid increase in body temperature that was reported in earlier studies (Farny, 1965).

In a 2x2 factorial study with two levels of heat stress acclimation (acclimated and non-acclimated) and two levels of nicarbazin (0.0125% and 0%). The respiration rate during the first 10 minutes of heat stress was similar between the treatment groups. The threshold for the onset of panting was lower for acclimated birds causing them to respond quicker to heat stress and birds treated with nicarbazin were again more sensitive to heat stress than the nonmedicated controls. The acclimated birds had an increased rate after the first 10 minutes and had a higher

peak respiratory rate. However, it did not start to decrease until two hours after the onset of the heat stress. The non-acclimated nicarbazin-treated birds were only able to maintain their peak respiratory rate for 30 minutes before declining. (Farny, 1965).

## **Effect on bird performance**

### *Mortality*

It has been reported that nicarbazin treated birds experience higher mortality rates when compared to nonmedicated controls and birds fed other anticoccidials when heat stressed (Sammelwitz, 1965a; Buys and Rasmussen, 1978; McDougald and McQuiston, 1980; Keshavarz and McDougald, 1981; Harris and Macy, 1988; Beers et al., 1989; Chapman, 1994; Wiernusz and Teeter, 1995). Nicarbazin is traditionally only fed in the starter diet due to the increased mortality observed with heat stress that broilers are more susceptible to later in life. It was suggested the increased mortality in nicarbazin treated birds is related to the increase in body temperature and lower nicarbazin concentrations result in lower mortality rates (Chapman, 1994; da Costa et al., 2017). In two abstracts where Sammelwitz (1965a, b) evaluated the effect of nicarbazin on mortality at 38° to 40°C, mortality rate increases linearly with nicarbazin concentration and duration of treatment. Mortality rate increased as the nicarbazin concentration increased: 17%, 23%, 42%, 60%, 64%, 77%, 75%, and 34% when fed nicarbazin concentrations of 0, 5, 30, 62.5, 100, 125, 250, and 500 ppm. It was suggested that the decrease in mortality at 500 ppm is due to a decrease in feed consumption because such a high concentration made the feed unpalatable (Sammelwitz, 1965a). Birds fed 125 ppm nicarbazin for 12, 24, and 48 hours had an increased mortality rate of 46%, 60%, and 86%, respectively. The increase in mortality was reversed once nicarbazin was removed from the diet (Sammelwitz, 1965b).

### Performance in the absence of a coccidiosis challenge

In the absence of a challenge, there were not any significant differences in average body weight gain between unmedicated birds and birds that are fed Nicarbazin at concentrations up to 0.0125% (Newberne and Buck, 1957; McDougald and McQuiston, 1980). However, once the concentrations were more than 0.0125% there was a significant decrease in weight gain (Newberne and Buck, 1957). Even though there was not a statistical difference, McDougald and McQuiston (1980) observed a trend of nicarbazin fed birds with lower body weights than the nonmedicated group. It was discussed that it was difficult to make conclusions because the study was not set up to determine whether the cause of the trend was the nicarbazin treatment or the periods of increased ambient temperature that occurred during the trial. Adversely, other studies found that birds fed nicarbazin had significantly lower body weight gain than unmedicated birds when no coccidiosis challenge was present (Bartov, 1989a,b; Keshavarz and McDougald, 1982; Harris and Macy, 1988; da Costa et al., 2017). Mushett et al. (1958) found birds fed nicarbazin at levels of 0.01% and 0.02% had body weights that were higher or equal to that of the unmedicated control birds. However, when the concentration of nicarbazin was increased to levels of 0.04, 0.08, and 0.16% in the diet, there was less growth.

In contrast, Bartov (1989a) reported that nicarbazin did not affect the feed consumption of broilers while Bartov (1989b) only reported a decrease in feed intake for the diets with the two highest concentrations of 150 and 200 mg/kg. Mushett et al. (1958) reported that there was a depression in the appetite when nicarbazin concentration reached 0.04% or higher. Studies found that birds fed nicarbazin had a significant increase in FCR compared to unmedicated birds when no coccidiosis challenge was present (Bartov, 1989a,b; Keshavarz and McDougald, 1982; Harris and Macy, 1988; da Costa et al., 2017). Keshavarz and McDougald (1982) reported that the

results of their study indicated that the reduction in body weight gain was due to reduced feed consumption but Bartov (1989a,b) hypothesized that the reduction in body weight is due to changes in metabolism rather than a decreased feed consumption.

*Performance in the presence of a coccidiosis challenge*

In the presence of coccidiosis in layer pullets, feeding nicarbazin at a daily dose of 0.0002% for 14 days was found to depress growth by about 20% (Cuckler et al., 1956). Broilers fed nicarbazin while experiencing a coccidiosis challenge had a decreased growth and higher FCR when compared to other anticoccidial and ionophore programs (Keshavarz and McDougald, 1981; Watkins and Bafundo, 1993). Berg et al. (1956) reported that nicarbazin fed at a rate of 0.02% resulted in a significant decreased growth and poor feed conversion. However, at a rate of 0.0125%, growth was not affected but continued to have poor feed efficiency (Berg et al., 1956). When layer pullets were raised in floor pens for 11 weeks, nicarbazin was well tolerated with normal FCR in concentrations between 0.005% and 0.02% but as the concentration reached 0.04% or higher there was a trend of decreased growth (Cuckler et al., 1956). Other studies reported that birds fed nicarbazin had improved growth compared to unmedicated birds when exposed to a coccidiosis challenge (Dauguschies et al., 1998; da Costa et al., 2017). Nicarbazin-treated infected birds had a similar growth rate as noninfected and nonmedicated birds. In two of three trials, there was an improvement in the FCR when infected birds were treated with nicarbazin (Dauguschies et al., 1998). As an alternative anticoccidial treatments, Watkins and Bafundo (1993) fed Narasin, an ionophore anticoccidial, in combination with nicarbazin as a shuttle program and reported that the birds had a higher body weight and an improved FCR compared to the other ionophores or nicarbazin treatment alone.

### Nicarbazin withdrawal

Porter and Gilfillian (1955) reported that the components of nicarbazin were not detectable in the plasma, muscle, and liver 48 hours after nicarbazin was removed from the diet. In an abstract, Sammelwitz (1965b) reported that the slight adverse effects are still apparent 48 hours after nicarbazin is removed from the diet but no longer present 96 hours after withdrawal. After nicarbazin treatment was discontinued, growth was no longer depressed in turkey and broilers (Cuckler et al., 1956; Watkins and Bafundo, 1993). The birds fed nicarbazin for the first 20 days of a shuttle program showed compensatory growth with similar body weights by Day 40 when compared to birds that were only treated with ionophores (Watkins and Bafundo, 1993).

### Nicarbazin fed birds in different environments

During increased ambient temperatures, nicarbazin causes an increase in mortality and decrease in performance related to the birds' impairment to cope with heat stress (Sammelwitz, 1965a; Keshavarz and McDougald, 1981; Harris and Macy, 1988; Chapman, 1994; da Costa et al., 2017). Sammelwitz (1965a) reported that nicarbazin fed at levels as low as 30 ppm caused a significant reduction in broiler's ability to cope with heat stress of 38°C to 40°C at six to nine weeks of age based on the increase in mortality.

Due to increased sensitivity to elevated environmental temperatures and a reduced ability to cope with the higher environmental temperatures when fed nicarbazin, da Costa et al. (2017) performed a study to evaluate the performance of birds fed nicarbazin at a lower environmental temperature to avoid heat stress. Nonchallenged birds treated with nicarbazin reared at an environmental temperature lowered by 3°C had higher weight gain and lower FCR and rectal temperatures than those reared at the standard temperature curve. With a coccidiosis challenge, nicarbazin treated birds had a higher weight gain, but higher rectal temperatures in the lower

environmental temperatures. The mortality of birds fed nicarbazin was significantly higher than the nonmedicated treatment at standard environmental temperatures but not at the decreased temperature curve. That data showed that the mortality was decreased from 10.7% to 7.7% when reared at a lower environmental temperature when compared to the corresponding controls (da Costa et al., 2017).

## CHAPTER 2

### STATEMENT OF PURPOSE

Nicarbazin is a coccidiostat that is commonly used in the broiler industry. Broiler performance has been found to decrease when birds are treated with nicarbazin in the absence of a coccidia challenge. Nicarbazin has also been reported to cause an increase in bird body temperature. It has been theorized that elevated body temperatures are related to an impairment in the bird's thermoregulatory system. It is therefore not unexpected that exposure to high ambient temperatures during nicarbazin treatment has been shown to result in decreased body weight gain and feed consumption and increased FCR and mortality in broilers. To avoid potential production problems, nicarbazin is normally only fed during the colder months of the year and during the first three weeks of grow out when birds are less likely to experience heat stress.

In an effort to reduce heating costs during cold weather, RH often exceeds the level typically recommended. Latent heat loss accounts for 50% or more of a bird's total heat loss and as a result elevated RH levels can impair a bird's ability to maintain body temperature. Though there have been numerous studies on the effects of elevated ambient temperatures in combination with nicarbazin on broiler performance, the same is not true for RH.

Some poultry companies are extending the time nicarbazin is fed to 28 days to reduce the likelihood of a coccidia infection later in the grow out period. Since older birds are more sensitive to increased temperatures and easier to become heat stressed, conditions that can reduce a bird's ability to lose heat, such as high environmental humidity commonly seen during cold

weather, could compound the negative performance effects associated with nicarbazin. With limited research on the topic, it is important to understand if a high relative humidity, at commonly recommended temperatures, could negatively affect performance for birds fed nicarbazin through 28 days of age. Therefore, the objective of this study was to determine the effect of RH at two levels, 35-50% and 65-75%, on the performance and body temperature of birds fed nicarbazin for 28 days.

## CHAPTER 3

### MATERIALS AND METHODS

#### **Facilities**

The study was conducted at the University of Georgia Poultry Research Center in four 20' X 24' rooms, each with eight 5' x 4' pens arranged in two rows of four pens (Figure 1). Each pen was equipped with one drinker line and a hanging feeder. For the first seven days of the study, each pen also contained one TurboGrow 2® chick feeder (Sephnos, Celaya, GTO, Mexico). The pens had a three-inch layer of fresh fine pine shavings. Each room was equipped with two exhaust fans [9" AT09Z2CP (1,070 cfm @ 0.10") and 18" AT18ZCP (4,120 cfm @ 0.10"), Munters Corporation, Lansing, MI, United States], a 100,000 Btu/hr forced air furnace (LB White - Guardian) and two 18" circulation fans (AT18 4,360 cfm @ 0.10") (Munters Corporation, Lansing, MI, United States), which operated continuously. Light was provided by four dimmable 800 lumen, 3000k LED bulbs. The environmental temperature and lighting profiles are in Table 1.

To maintain relative humidity (RH) of 65-75%, two of the four rooms were equipped with two Auguatronics Cannon smart humidification systems (Aguair, Marietta, GA, United States). Each unit had one emitter head (2 gals/hr) mounted overhead, near the circulation fans.

Each room was equipped with a temperature and RH logger to monitor and record the environmental conditions. Two rooms were equipped with a HOBOnet Wireless RXW-THC-900 Temperature/RH sensor (Onset, Bourne, MA, United States), while the other two rooms were equipped with a S-THB-M008 12-bit Temperature/RH Smart Sensor (Onset, Bourne, MA,

United States). Sensors were connected to a HOBO RX3000 Remote Monitoring Station Data Logger (Onset, Bourne, MA, United States) with a five-minute logging interval.

Temperature/RH sensors had accuracy of  $\pm 0.4^{\circ}\text{F}$  /  $\pm 2.5\%$ .

Figure 1. Room and pen configuration.

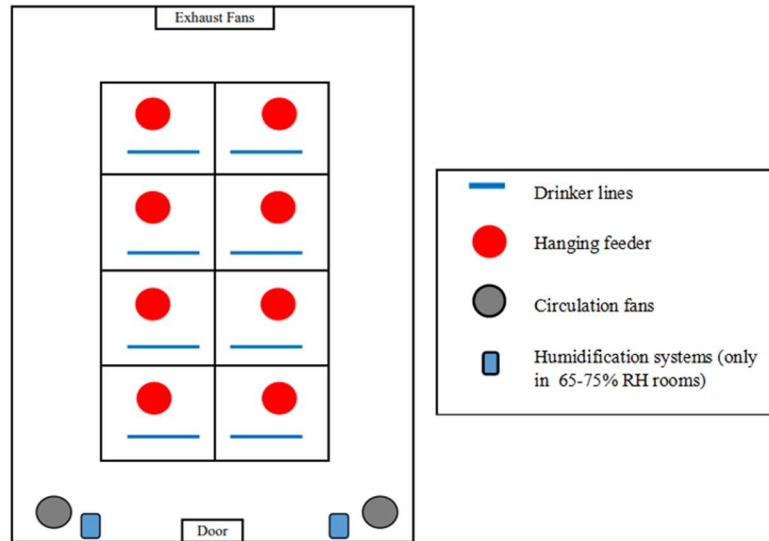


Table 1. Room temperature and lighting schedule guidelines.

Bird Age (Day)	Room Temperature ( $^{\circ}\text{C}$ )	Light Hours (L:D)	Light Intensity (lux)
0	33.8	24:0	30
3	32.2	20:4	30
7	30.0		10
14	27.8	18:6	5
21	26.1		
28	23.3	22:2	5
35	21.1		

### Environmental

Trial 1 took place from January 14th to February 18th. Trial 2 took place from March 1st to April 5th. Trial 3 took place from November 8th to December 13th. The time of year was

specifically selected due to the high probability that outside temperatures would be much lower than the targeted room temperatures which made it more likely that target room RH levels could be maintained. The RH of 65-75% was maintained through adjustments to exhaust fan runtime controlled by an interval timer and the use of the two Auguatronincs Cannon humidification systems (Aguair, Marietta, GA, United States). In the 30-50% RH rooms, the target RH was maintained through adjustments made to exhaust fan runtime.

### **Husbandry**

On day of hatch and upon arrival at the UGA Research Center, 864 chicks (Ross708 male) were weighed and separated into nine weight categories: <32, 32-34, 35-37, 38-40, 41-43, 44-46, 47-49, 49-51, and >51 grams. Chicks were then randomly selected from each weight range to be placed in a pen to ensure the starting pen weight was similar for each of the 32 pens. Chicks were assigned to one of four different treatment groups:

- 30-50% RH fed control diet
- 30-50% RH fed Nicarbazin diet
- 65-75% RH fed control diet
- 65-75% RH fed Nicarbazin diet

Each pen had 27 chicks resulting in a stocking density of 0.75 ft<sup>2</sup>/bird. The chicks were raised in an absence of a coccidiosis challenge.

All birds were fed starter crumble diet from 0 to 21 days of age and grower pellet diet through 35 days. Two treatment diets were utilized and fed from 0 to 28 days of age. The first treatment diet contained Nicarb 25% premix (Phibro Animal Health Corporation™, Teaneck, NJ, United States) at a rate of 125 ppm (113 g/ton) for the starter diet and 99 ppm (90g/ton) for the grower diet. The control diet did not have nicarbazin. On Day 28, all birds were fed the

control grower diet for the remainder of the trial. Both diets were formulated equally per the specifications of a local integrator (Table 2). All feed was made at the University of Georgia Feed Mill. The nicarbazin treatment feed was sent for testing by Phibro Animal Health Corporation to ensure the diets contained the correct concentration of nicarbazin. Feed and water were given *ad libitum* during the trial.

Table 2. Starter and grower diet formulations

<b>Ingredient</b>	<b>Starter Diet</b>	<b>Grower Diet</b>
	<b>%</b>	
Corn	58.166	62.118
Soybean Meal	37.225	32.900
Soybean Oil	1.590	2.130
Phosphate-DEFL	1.000	0.875
Limestone	0.633	0.625
Methionine- DL	0.329	0.291
Salt	0.253	0.258
Vitamins Premix	0.250	0.567
L-Lysine HCL	0.175	0.163
Sodium Bicarbonate	0.110	0.125
Threonine-L	0.93	0.088
Choline- CL 60%	0.075	0.075
Trace Mineral Premix 572	0.070	0.075
Quantum Blue-5G	0.030	0.030
Econase-XT (AB Vista)	0.002	0.002
Nicarb Premix 25% <sup>1</sup>	0.0005	0.0004
<b>Calculated analysis</b>		
ME (kcal/kg)	2562.4	3105.4
Crude Protein (%)	22.1	20.4
Lys (%)	1.18	1.07
Met (%)	0.621	0.566
Cys (%)	0.264	0.248
TSAA (%)	0.885	0.813
Threonine (%)	0.767	0.706
Available Phosphorus (%)	0.441	0.411
Calcium (%)	0.881	0.822

<sup>1</sup>Diets formulated the same apart from the absence of nicarbazin in the control diet.

All birds in a pen were weighed together to obtain a total bird pen weight. The total bird pen weight was divided by the number of birds in the pen to obtain an average bird weight and in

turn an average weight gain measurement. Feeders were weighed in order to calculate feed consumption and FCR. These measurements were collected at Days 0, 7, 14, 21, 28, and 35 and analyzed by week. One bird per pen was selected at Day 21 to be euthanized and necropsied for a coccidiosis check. Mortality was recorded daily and necropsied.

### **Rectal Temperatures**

Rectal temperatures, from five birds per pen, were taken weekly starting at Day 7. The rectal temperatures were measured using the Thermistor Temperature Instrument TM99A (Cooper-Atkins®, Middlefield, CT, United States). The probe was inserted 2.5 centimeters into the cloaca for birds up to 14 days of age (Lu, 2019). From Day 21 to Day 35, the probe was inserted 3 to 5 centimeters into the cloaca (Donkoh, 1989; Hai et al., 1996; Shinder et al., 2007). The instrument had a resolution of 0.1°C and an accuracy of  $\pm 0.2^\circ\text{C}$ .

### **Trials**

Trial 2 was the same as Trial 1 with the exception of RH room assignments and the chicks placed. RH room assignments were changed to eliminate any possible room effects during the study. Ross708 female chicks were used rather than Ross708 males due to chick availability.

Trial 3 was the same as Trial 2 with the exception of the number of replicates and the chicks placed. Six 20' X 24' rooms, each with eight 5' x 4' pens, increasing the number of pens from 32 to 48. The RH of three rooms was maintained at 65-75% using one Auguatronincs Cannon smart humidification system (Aguair, Marietta, GA, United States). Each unit was connected to two emitter heads (2 gals/hr) mounted overhead, near the circulation fans. 1,296 Ross708 male chicks were placed.

## **Data Analysis**

The data from Trials 1 through 3 were arranged as a split-plot design. There were two main effects each having two levels: Diet (Control, Nicarbazin) and RH (30-50%, 65-75%). The main plot consisted of RH nested within the room assignment. The subplot consisted of the two diets treatments which were randomly assigned within each room. Individual pens were used as the experimental unit. For Trials 1 and 2, the main effects had a n=16 and the interaction effects had a n=8. The main effects for Trial 3 had a n=24 and the interaction effect had a n=12. Data were analyzed using the ANOVA procedure (JMP Pro, ver. 15). The means of variables that were statistically significant at  $P \leq 0.05$  were separated using Student's t test.

## CHAPTER 4

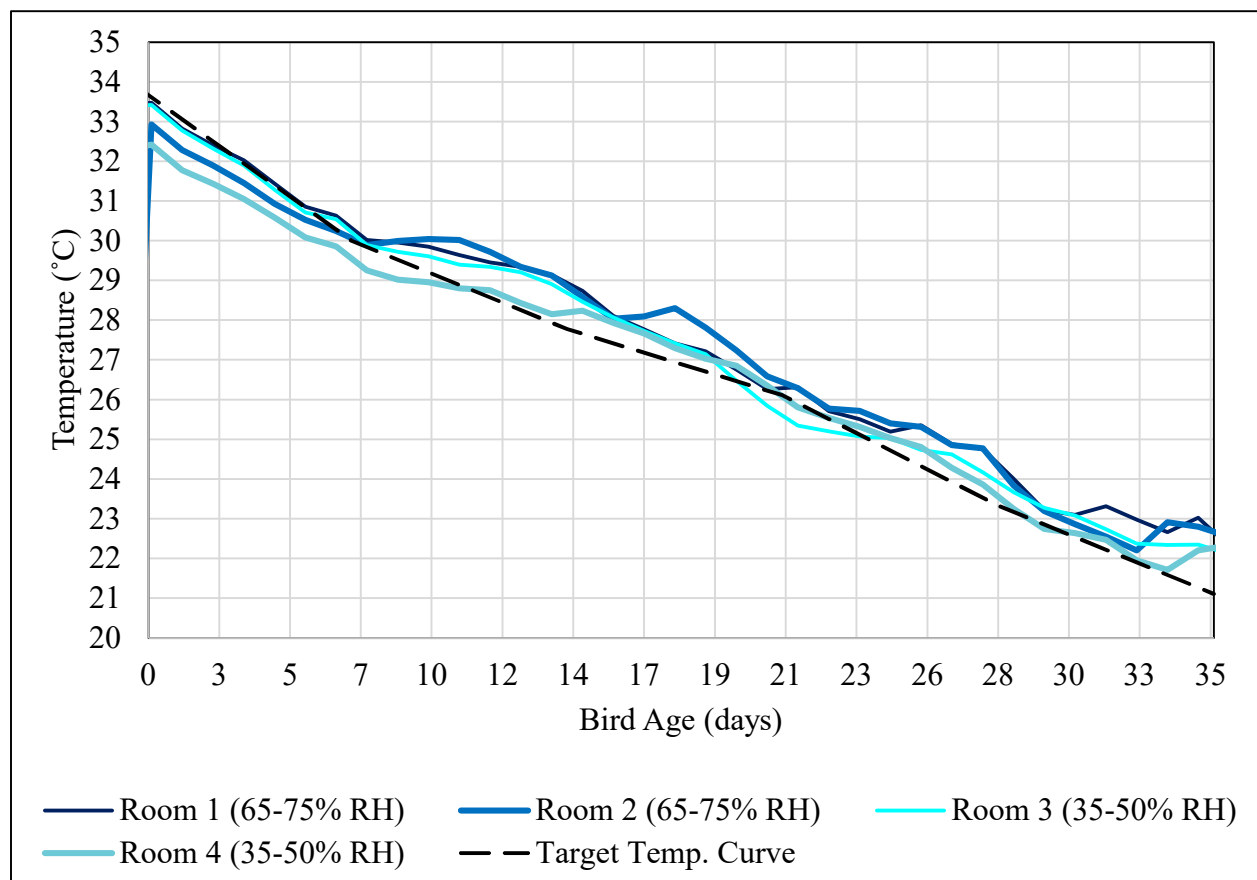
### RESULTS

#### Trial 1

#### Temperature

The room temperatures were set to follow the target temperature curve described in the materials and methods (Table 1). With the exception of three days, the air temperature in all the rooms were able to be maintained with a daily variation of  $\pm 0.4^{\circ}\text{C}$  in each room (Figure 2).

**Figure 2:** Average daily temperatures recorded in rooms maintained at two RH levels throughout Trial 1 compared to an industry standard target temperature curve.<sup>1</sup>



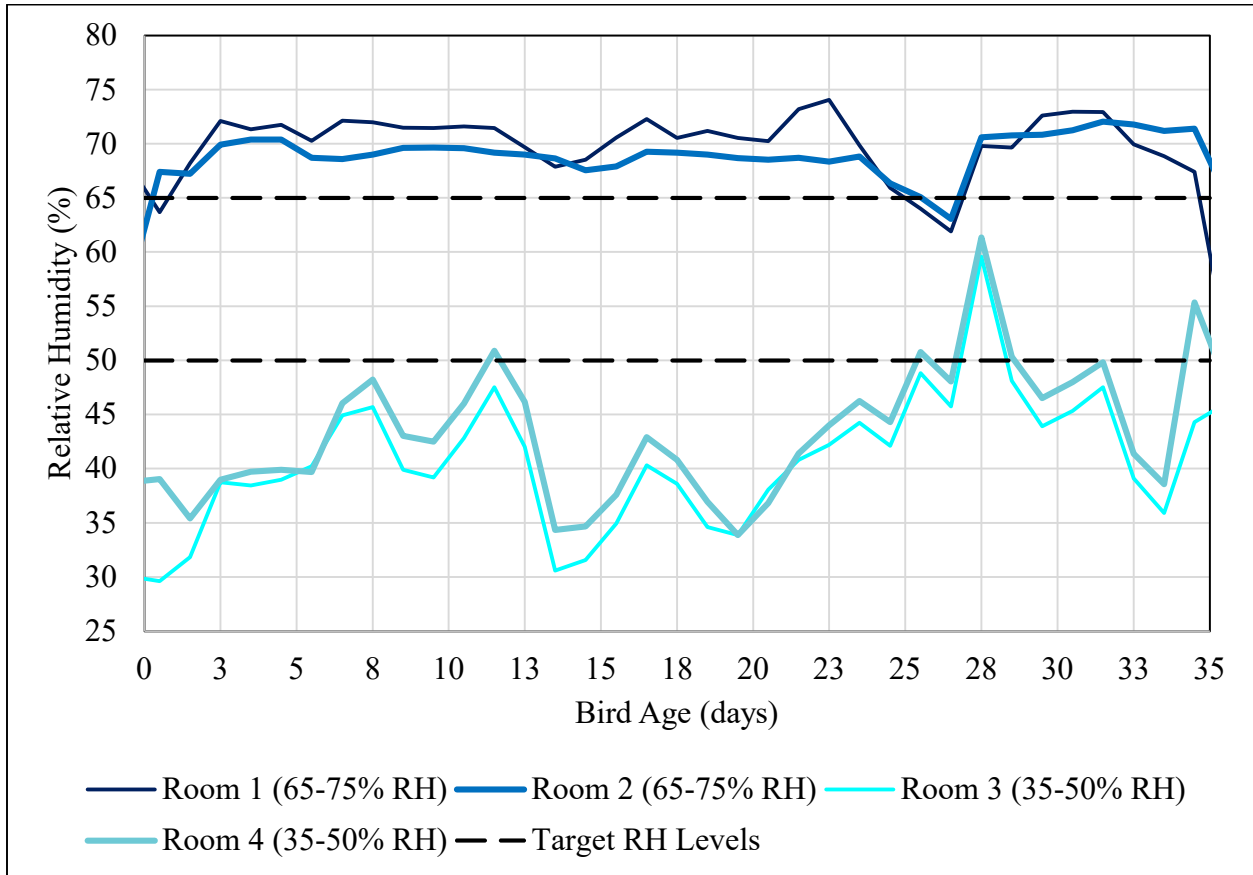
<sup>1</sup>Average daily S.D. of room temperatures was  $\pm 0.4^{\circ}\text{C}$  within each room.

## **Relative humidity**

The target relative humidity (RH) levels for the study were 35-50% (control) and 65-75% (treatment). With the exception of five days during the trial, the RH was maintained within the targeted ranges (Figure 3). The daily variation in RH maintained was at  $\pm 2\%$  in the treatment RH rooms and  $\pm 3\%$  in the control RH rooms. There were days where the target RH levels were not achievable. Between Days 25 and 29 and Days 34 and 35, the outside temperature and RH increased and as a result the room environmental controllers activated exhaust fans to maintain target room temperature. The increased room air exchange rate and decreased heating system operation resulted in the RH in the control rooms increased above the target range. In the treatment rooms, the humidification systems could not maintain the RH at the target with the higher air exchange rates resulting in a RH below the target range.

There was more variation in the control RH rooms (SD of 7.1%) throughout the trial compared to the treatment RH rooms (SD of 5.9%). The control RH rooms were heavily influenced by the outside temperature and RH. This is due to RH being controlled by increasing room air exchange rate. When the outside temperature and RH were high due to warm and rainy conditions, then the room RH would increase. When outside temperature and RH were low, the room RH would decrease. The control rooms did not have humidification systems so the only way RH could be increased was to reduce the air exchange which was limited due to the concern of creating a possible CO<sub>2</sub> difference between rooms. In contrast, the treatment RH rooms had humidification units within the rooms which allowed for more control in RH levels.

**Figure 3:** Average daily RH levels recorded for rooms maintained at two RH levels throughout Trial 1.<sup>1</sup>



<sup>1</sup>Average daily S.D. of the RH was  $\pm 2\%$  in the 65-75% RH rooms and  $\pm 3\%$  in the 35-50% RH rooms.

### Mortality

The average overall mortality rate of Trial 1 was 1.8%. Neither diet nor RH had a significant effect on mortality throughout the trial (Table 3).

**Table 3: Relationship of nicarbazin and RH to mortality of broilers 0 t 35 days of age in Trial 1.<sup>1</sup>**

<b>Treatment</b>	<b>N<sup>2</sup></b>	<b>%</b>
<b>Diet</b>		
Control	16	1.9
Nicarbazin	16	1.8
SEM		0.5
P-value		0.8631
<b>Relative Humidity</b>		
35-50%	16	2.0
65-75%	16	1.6
SEM		0.2
P-value		0.3132
<b>Interaction</b>		
35-50%-C	8	2.3
35-50%-N	8	1.8
65-75%-C	8	1.4
65-75%-N	8	1.8
SEM		0.7
P-value		0.6842

<sup>1</sup> Significant difference were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

### **Body weight**

At placement, the chicks were individually weighed and placed to ensure uniform weights between treatments. By Day 7, the broilers fed nicarbazin weighed significantly ( $P < 0.05$ ) less (164g) than those fed the control diet (178g). The significant lower body weight was observed throughout the nicarbazin treatment. At Day 35, broilers fed nicarbazin continued to weigh significantly less than those fed the control diet (2,371g vs. 2,570g) after nicarbazin was removed from the diet at 28 days of age. An interaction occurred between diet and RH at Day 14. The birds fed the control diet and reared under treatment RH had a slightly higher body weight than those fed control diet and raised under the control RH but not significant (503g vs. 484g).

In addition, the body weight of the birds fed nicarbazin diet reared under the control or treatment RH levels were similar (436g vs. 437g). The interaction occurred due to the slight increase in body weight of birds fed control diet and reared under treatment RH compared to the birds fed control diet reared under control RH which was not seen in the treatment diet groups (Table 4).

**Table 4: Effect of nicarbazin and RH on bird body weight (g) of broilers 0 through 35 days of age in Trial 1.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0	7	14	21	28	35
<b>Diet</b>							
Control	16	42.8	178	494	997	1697	2570
Nicarbazin	16	43.0	164	437	864	1489	2371
SEM		0.1	1.0	4.2	10.9	11.7	17.2
P-Value		0.1092	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
<b>Relative Humidity</b>							
35-50%	16	42.8	171	460	921	1593	2469
65-75%	16	42.9	171	470	939	1594	2472
SEM		0.1	0.4	5.1	14.2	11.1	17.9
P-Value		0.4518	0.3735	0.3160	0.4613	0.9624	0.9132
<b>Interaction</b>							
35-50%-C	8	42.8	177	484 <sup>a</sup>	987	1695	2563
35-50%-N	8	42.9	165	436 <sup>b</sup>	856	1490	2375
65-75%-C	8	42.9	179	503 <sup>a</sup>	1007	1698	2577
65-75%-N	8	43.0	164	437 <sup>b</sup>	872	1489	2368
SEM		0.1	1.4	5.9	15.4	16.5	24.4
P-Value		0.8911	0.5461	<b>0.0435</b>	0.7970	0.8865	0.6498

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

<sup>a-b</sup>Means within a column with different superscripts differ significantly ( $P < 0.05$ ).

### Body weight gain

Birds fed nicarbazin gained significantly ( $P < 0.05$ ) less weight than the birds fed the control diet for the first four weeks. During Week 5, all birds were fed the same control diet. During this period, there was not a significant difference in body weight gain between the birds fed the control diet and those previously fed the nicarbazin diet (873g vs. 882g). However, when

comparing the overall gain from all five weeks, the birds treated with nicarbazin gained significantly less weight than the birds fed the control diet (2,328g vs. 2,527g). An interaction occurred between diet and RH during Week 2. The birds fed the control diet and reared under treatment RH had a slightly higher body weight gain than those fed control diet and raised under the control RH but was not significant (324g vs. 307g). Similarly, there was not a significant difference between the body weight gain of the birds fed nicarbazin diet reared under the control or treatment RH levels (272g vs. 273g) but the numerical difference seen was smaller than that of the control diet groups. The interaction occurred due to the slight increase in body weight gain of birds fed control diet and reared under treatment RH compared to the birds fed control diet reared under control RH which was not seen in the treatment diet groups (Table 5).

**Table 5: Effect of nicarbazin and RH on average body weight gain (g) of broilers 0 through 35 days of age in Trial 1.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0-7	8-14	15-21	22-28	29-35	0-35
<b>Diet</b>							
Control	16	135	316	504	700	873	2527
Nicarbazin	16	121	270	427	626	882	2328
SEM		1.0	4.0	7.4	6.4	14.7	17.2
P-Value		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.5026	<b>&lt;0.0001</b>
<b>Relative Humidity</b>							
35-50%	16	128	290	461	671	876	2426
65-75%	16	129	298	470	654	879	2429
SEM		0.5	5.0	9.4	3.0	18.6	18.0
P-Value		0.4694	0.3370	0.5834	0.5834	0.9394	0.9161
<b>Interaction</b>							
35-50%-C	8	134	307 <sup>a</sup>	503	708	868	2520
35-50%-N	8	122	272 <sup>b</sup>	419	635	885	2332
65-75%-C	8	136	324 <sup>a</sup>	504	691	878	2534
65-75%-N	8	121	273 <sup>b</sup>	435	617	879	2325
SEM		1.4	5.6	10.4	9.0	20.8	24.4
P-Value		0.5424	<b>0.0364</b>	0.2828	0.2828	0.5383	0.6505

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

<sup>a-b</sup>Means within a column with different superscripts differ significantly ( $P < 0.05$ ).

### Feed consumption

The broilers fed nicarbazin consumed significantly ( $P < 0.05$ ) less feed than those fed the control diet throughout the duration of the study (3,305g vs. 3,486g). The RH level did not have an effect on feed consumption, nor was there an interaction effect between RH and diet (Table 6).

**Table 6: Effect of nicarbazin and RH on average feed consumption (g) of broilers 0 through 35 days of age in Trial 1.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0-7	8-14	15-21	22-28	29-35	0-35
<b>Diet</b>							
Control	16	140	392	666	1013	1309	3486
Nicarbazin	16	131	364	608	973	1265	3305
SEM		1.3	5.7	11.6	14.3	17.6	36.6
P-Value		<b>0.0005</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0448</b>	<b>0.0072</b>	<b>&lt;0.0001</b>
<b>Relative Humidity</b>							
35-50%	16	138	370	628	992	1286	3382
65-75%	16	133	386	646	993	1288	3408
SEM		1.1	7.2	15.4	15.0	22.4	45.0
P-Value		0.0656	0.2613	0.5041	0.9803	0.9634	0.7239
<b>Interaction</b>							
35-50%-C	8	143	382	652	1030	1317	3487
35-50%-N	8	133	358	605	954	1255	3278
65-75%-C	8	136	403	680	995	1302	3485
65-75%-N	8	129	369	612	991	1274	3332
SEM		1.8	8.1	16.4	20.2	25.0	51.7
P-Value		0.4243	0.3547	0.2181	0.0728	0.2833	0.4519

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

## FCR

Nicarbazin fed broilers had a significantly higher FCR than those fed the control diet throughout the first four weeks (Table 7). After the nicarbazin was removed from the diet at Day 28, the nicarbazin treated birds had a significantly lower FCR than the control diet group (1.43 vs. 1.52). During the first week, the chicks reared under the control RH conditions had a significantly higher FCR than those in the treatment RH conditions (1.09 vs. 1.04). Significant interactions occurred during Weeks 3 and 4. The FCR of the birds fed the control diet and reared under treatment RH was slightly higher, yet not statistically different compared to those fed control diet and raised under the control RH (1.35 vs. 1.32) during Week 3. In addition, the FCR for the nicarbazin-treated birds reared under treatment RH was slightly lower, yet not statistically

different than those reared under control RH levels (1.44 vs.1.41). The interaction occurred due to the numerical increase in FCR of birds fed control diet and reared under control RH compared to treatment RH and the slight decrease in FCR for birds fed nicarbazine and reared under the control RH compared to treatment RH. During Week 4, the birds fed nicarbazine and raised under treatment RH have a significantly higher FCR (1.61) than those fed the control diet at both RH levels (1.46 and 1.47). The FCR for birds fed nicarbazine and reared under control RH (1.51) was not statistically different from any other treatment group.

**Table 7: Effect of nicarbazine and RH on feed conversion ratio (g/g) of broilers 0 through 35 days of age in Trial 1.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0-7	8-14	15-21	22-28	29-35	0-35
<b>Diet</b>							
Control	16	1.04	1.24	1.33	1.46	1.52	1.40
Nicarbazin	16	1.09	1.34	1.43	1.56	1.43	1.44
SEM		0.01	0.02	0.02	0.02	0.01	0.01
P-Value		<b>0.0011</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0002</b>	<b>0.0002</b>	<b>0.0067</b>
<b>Relative Humidity</b>							
35-50%	16	1.09	1.28	1.38	1.48	1.48	1.41
65-75%	16	1.04	1.30	1.38	1.54	1.47	1.43
SEM		0.00	0.01	0.02	0.03	0.01	0.01
P-Value		<b>0.0162</b>	0.4900	0.9839	0.2923	0.8136	0.5501
<b>Interaction</b>							
35-50%-C	8	1.07	1.25	1.32 <sup>b</sup>	1.46 <sup>b</sup>	1.54	1.41
35-50%-N	8	1.10	1.32	1.44 <sup>a</sup>	1.51 <sup>ab</sup>	1.42	1.42
65-75%-C	8	1.00	1.24	1.35 <sup>b</sup>	1.47 <sup>b</sup>	1.50	1.40
65-75%-N	8	1.07	1.36	1.41 <sup>a</sup>	1.61 <sup>a</sup>	1.45	1.45
SEM		0.01	0.02	0.02	0.03	0.02	0.01
P-Value		0.2024	0.2778	<b>0.0340</b>	<b>0.0436</b>	0.0686	0.0747

<sup>1</sup>Significant differences were determined at P<0.05 by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

<sup>a-b</sup>Means within a column with different superscripts differ significantly (P<0.05).

## Rectal temperature

Nicarbazin treatment and differences in RH did not cause a significant change in bird rectal temperature (Table 8).

**Table 8: Effect of nicarbazin and RH on average rectal temperature (°C) of broilers 7 through 35 days of age in Trial 1.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days				
		7	14	21	28	35
<b>Diet</b>						
Control	16	40.9	40.9	41.2	41.2	41.3
Nicarbazin	16	41.0	41.0	41.1	41.2	41.3
SEM		0.05	0.04	0.03	0.03	0.03
P-Value		0.5608	0.3438	0.1526	0.2767	0.4751
<b>Relative Humidity</b>						
35-50%	16	41.0	41.0	41.2	41.2	41.3
65-75%	16	40.9	40.9	41.1	41.2	41.4
SEM		0.07	0.04	0.03	0.04	0.04
P-Value		0.8736	0.9235	0.2951	0.2929	0.1637
<b>Interaction</b>						
35-50%-C	8	40.9	40.9	41.2	41.2	41.3
35-50%-N	8	41.0	41.0	41.2	41.3	41.3
65-75%-C	8	40.9	40.9	41.2	41.2	41.4
65-75%-N	8	40.9	41.0	41.1	41.2	41.4
SEM		0.08	0.05	0.04	0.05	0.05
P-Value		0.3351	0.6828	0.8712	0.1767	1.0000

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. 5 birds were randomly selected from each pen to obtain rectal temperature measurements.

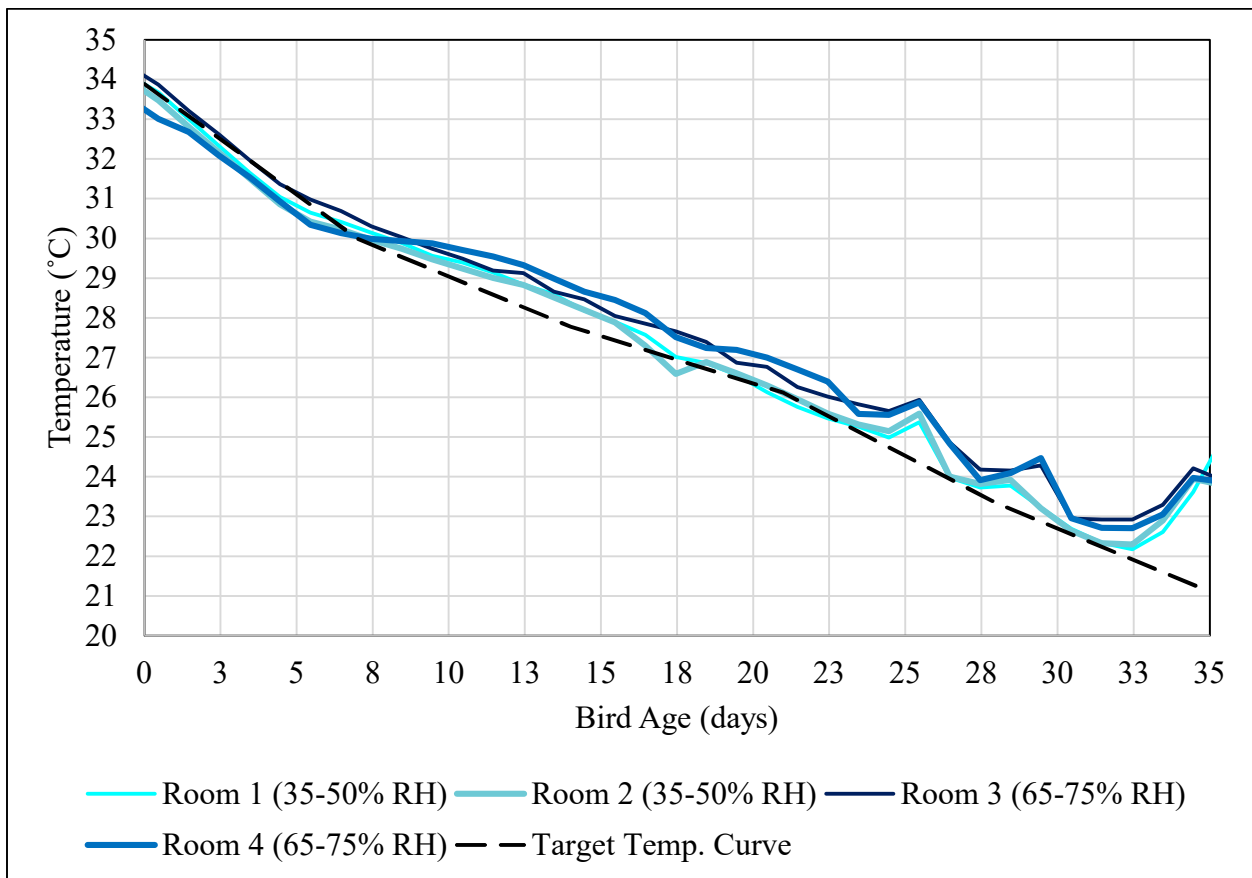
## Trial 2

### Temperature

The room temperatures were set to follow the target temperature curve described in the materials and methods (Table 1). With the exception of three days, the air temperature in all of the rooms (Figure 4) was able to be maintained within  $\pm 0.5^\circ\text{C}$  of the daily average temperature of the rooms. Between Day 32 and 35 there was a higher average variation of  $1.1^\circ\text{C}$  above the

average daily temperatures which was due to an increased outside temperature influencing the room temperatures. On Days 25, 29 and between Days 33 and 35, the temperatures in the room increased approximately 0.7°C to 1.3°C above the target temperature curve due to increased outside temperatures.

**Figure 4:** Average daily temperatures recorded in rooms maintained at two RH levels throughout Trial 2 compared to an industry standard target temperature curve.<sup>1</sup>



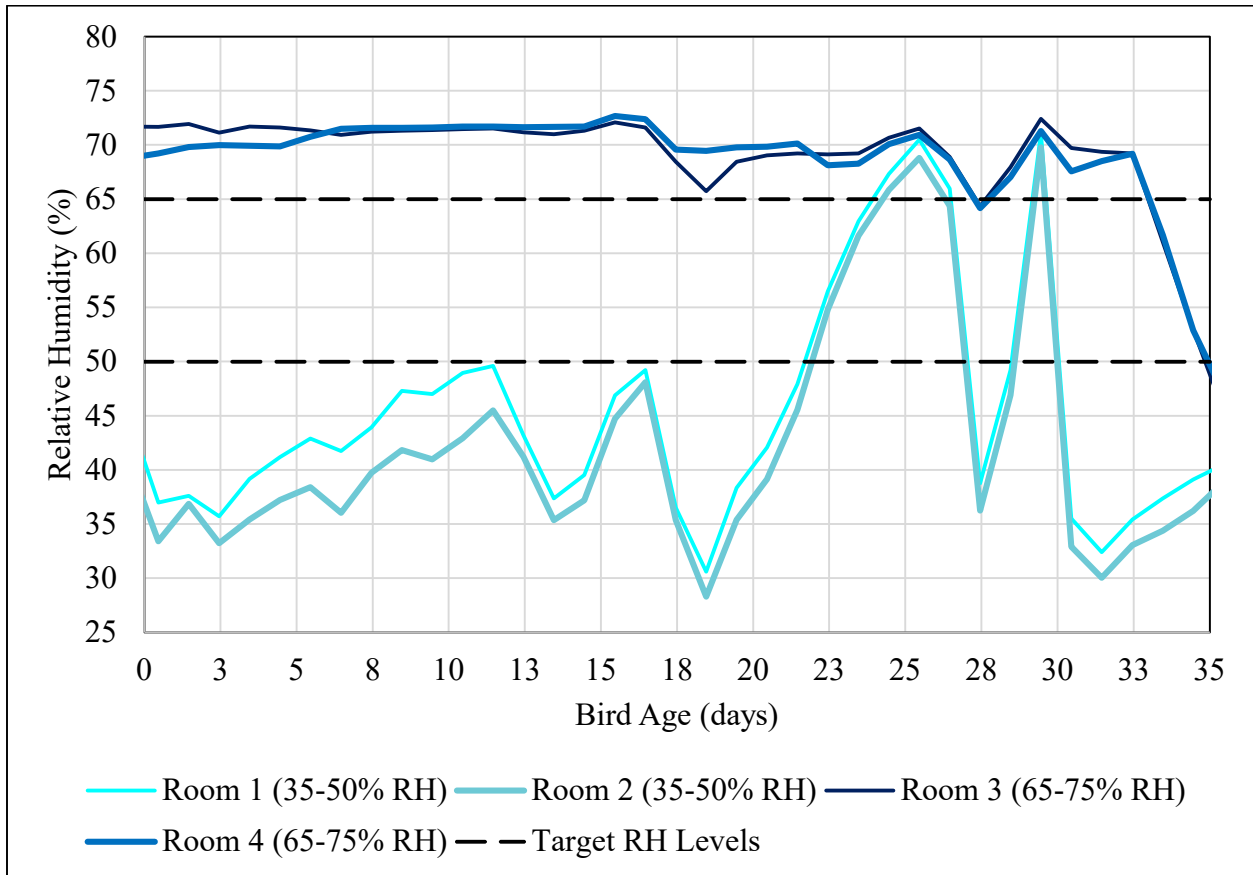
<sup>1</sup>Average daily S.D. of room temperatures was  $\pm 0.5^{\circ}\text{C}$  for Rooms 1 (35-50% RH),  $\pm 0.4^{\circ}\text{C}$  for Room 3 (35-50% RH) and Room 2 (35-50% RH), and  $\pm 0.03^{\circ}\text{C}$  for Room 4 (65-75% RH) temperatures.

### Relative humidity

The target RH levels for the study were 35-50%, control, and 65-75%, treatment. With the exception of eight days during the trial, the RH was maintained within the targeted ranges. The daily variation in RH maintained was  $\pm 0.5\%$  in treatment RH rooms and  $\pm 3\%$  in the control RH rooms (Figure 5). There were days where the target RH levels were not achievable. Between

Days 22 and 27 and Days 29 and 30, the outside temperature and RH increased and as noted previously, the control over the rooms was adversely affected. Between Days 33 and 35, another period of increased outside temperature occurred but the RH did not increase as observed in Trial 1. Only the treatment RH rooms were negatively affected as the humidifier systems could not maintain the RH with the increased air exchange rates. There was more variation in the control RH rooms (S.D. of 11.9%) throughout the study compared to the treatment RH rooms (S.D. of 7.6%) for the same reason as mentioned in Trial 1.

**Figure 5:** Average daily RH levels recorded for rooms maintained at two RH levels throughout Trial 2.<sup>1</sup>



<sup>1</sup>Average daily S.D. of RH was  $\pm 0.5\%$  for 35-50% RH rooms and  $\pm 3\%$  for 65-75% RH rooms.

## Mortality

The overall average mortality rate of Trial 2 was 10.4%. This was higher than that of Trial 1, 1.8%. There was a higher incidence of mortality due to omphalitis that lasted for the first three weeks. This is most likely related to problems during hatching (Table 9).

**Table 9: Relationship of nicarbazin and RH to mortality of broilers 0 to 35 days of age in Trial 2.<sup>1</sup>**

<b>Treatment</b>	<b>N<sup>2</sup></b>	<b>%</b>
<b>Diet</b>		
Control	16	9.5
Nicarbazin	16	11.3
SEM		1.4
P-value		0.2222
<b>Relative humidity</b>		
35-50%	16	10.4
65-75%	16	10.4
SEM		0.9
P-value		0.5528
<b>Interaction</b>		
35-50%-C	8	8.3
35-50%-N	8	12.5
65-75%-C	8	10.6
65-75%-N	8	10.2
SEM		2.0
P-value		0.2222

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

## Body weight

As in Trial 1, the chicks were individually weighed and placed to ensure uniform weights between treatments at placement. By Day 7 the broilers fed nicarbazin weighed significantly ( $P < 0.05$ ) less (162g) than those fed the control diet (171g). The significant decrease body weight continued throughout the nicarbazin treatment. At Day 35 broilers fed nicarbazin continued to weigh significantly less than those fed the control diet (2,245g vs. 2,398g) after nicarbazin was

removed from the diet at 28 days of age. There was a significant difference in body weight at placement for the control and treatment RH levels. The RH level did not have an effect on the body weight (Table 10).

**Table 10: Effect of nicarbazine and RH on bird body weight (g) of broilers 0 through 35 days of age in Trial 2.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0	7	14	21	28	35
<b>Diet</b>							
Control	16	46.5	171	445	921	1626	2398
Nicarbazin	16	46.4	162	416	853	1476	2245
SEM		0.0	1.8	6.3	11.6	11.6	23.9
P-Value		0.1611	<b>0.0040</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
<b>Relative Humidity</b>							
35-50%	16	46.5	165	427	893	1550	2343
65-75%	16	46.4	167	434	882	1552	2300
SEM		0.0	1.6	7.9	14.7	10.0	28.7
P-Value		<b>0.0460</b>	0.5417	0.6203	0.6513	0.8657	0.3986
<b>Interaction</b>							
35-50%-C	8	46.6	170	437	920	1614	2409
35-50%-N	8	46.4	161	417	866	1485	2277
65-75%-C	8	46.4	171	453	923	1638	2388
65-75%-N	8	46.4	162	414	841	1467	2212
SEM		0.1	2.5	8.9	16.4	16.3	33.8
P-Value		0.2721	0.9199	0.0948	0.1726	0.2640	0.3934

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

### Body weight gain

As seen in Trial 1, birds fed nicarbazine gained significantly ( $P < 0.05$ ) less weight than the ones fed the control diet for the first four weeks. During Week 5, there was not a significant difference in body weight gain between the birds fed the control diet and those previously fed the nicarbazine diet (772g vs. 769g). However, when comparing the overall gain from all five weeks, the birds treated with nicarbazine gained significantly less (2,198g vs. 2,352g). As seen in Trial 1, the RH level did not have an effect on body weight gain (Table 11).

**Table 11: Effect of nicarbazin and RH on average body weight gain (g) of broilers 0 through 35 days of age in Trial 2.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0-7	8-14	15-21	22-28	29-35	0-35
<b>Diet</b>							
Control	16	124	275	476	704	772	2352
Nicarbazin	16	115	254	438	623	769	2198
SEM		1.8	5.3	6.5	6.3	15.7	23.9
P-Value		<b>0.0043</b>	<b>0.0003</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.7966	<b>&lt;0.0001</b>
<b>Relative Humidity</b>							
35-50%	16	119	262	466	657	793	2297
65-75%	16	120	267	448	670	748	2253
SEM		1.5	6.6	7.8	5.2	19.7	28.7
P-Value		0.5186	0.6543	0.2540	0.2064	0.2410	0.3995
<b>Interaction</b>							
35-50%-C	8	123	267	483	694	795	2362
35-50%-N	8	115	256	449	619	792	2231
65-75%-C	8	125	282	470	715	750	2341
65-75%-N	8	116	252	427	626	745	2166
SEM		2.5	7.5	9.2	8.9	22.2	33.8
P-Value		0.8969	0.0634	0.5248	0.5334	0.9433	0.3912

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

### Feed consumption

During the first week, neither nicarbazin nor RH level caused a significant difference in feed consumption. During Week 2, nicarbazin caused a significant ( $P < 0.05$ ) decrease in feed consumption compared to the birds fed the control diet (347g vs. 368g). This lower feed intake continued through the end of Week 5 (1,284g vs. 1,226g). The overall feed consumed for broilers fed nicarbazin was significantly less than those fed the control diet (3,178g vs. 3,340g) similar to the results of Trial 1. The RH level did not have an effect on feed consumption, nor was there an interaction effect between RH and diet (Table 12).

**Table 12: Effect of nicarbazin and RH on average feed consumption (g) of broilers 0 through 35 days of age in Trial 2.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0-7	8-14	15-21	22-28	29-35	0-35
<b>Diet</b>							
Control	16	131	368	667	990	1284	3340
Nicarbazin	16	130	347	623	938	1226	3178
SEM		2.4	4.5	13.4	7.8	14.5	32.4
P-Value		0.8023	<b>0.0020</b>	<b>0.0041</b>	<b>0.0007</b>	<b>0.0149</b>	<b>0.0024</b>
<b>Relative Humidity</b>							
35-50%	16	130	356	647	975	1292	3308
65-75%	16	130	359	643	953	1218	3211
SEM		2.4	4.8	16.2	5.2	13.4	30.7
P-Value		0.9921	0.6608	0.8782	0.0936	0.0593	0.1531
<b>Interaction</b>							
35-50%-C	8	130	367	670	1005	1318	3389
35-50%-N	8	130	344	624	945	1267	3227
65-75%-C	8	131	368	663	975	1250	3292
65-75%-N	8	129	350	622	930	1186	3130
SEM		3.4	6.4	18.9	11.0	20.6	45.8
P-Value		0.7074	0.6522	0.8577	0.5924	0.7459	0.9990

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

## FCR

Nicarbazin fed broilers had a significantly ( $P < 0.05$ ) higher FCR during Weeks 1 (1.17 vs. 1.09) and 4 (1.51 vs. 1.41). After the nicarbazin was removed from the diet at Day 28, the nicarbazin treated birds had a significantly lower FCR than the control group (1.60 vs. 1.67) as previously seen in Trial 1. There was not a significant difference between birds fed nicarbazin or control diets in FCR during Weeks 2 and 3. The RH level did not have an effect on FCR, nor was there an interaction effect between RH and diet (Table 13).

**Table 13: Effect of nicarbazin and RH on feed conversion ratio (g/g) of broilers 0 through 35 days of age in Trial 2.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0-7	8-14	15-21	22-28	29-35	0-35
<b>Diet</b>							
Control	16	1.09	1.38	1.41	1.41	1.67	1.47
Nicarbazin	16	1.17	1.39	1.45	1.51	1.60	1.49
SEM		0.02	0.02	0.02	0.02	0.02	0.01
P-Value		<b>0.0081</b>	0.6139	0.1922	<b>0.0002</b>	<b>0.0229</b>	0.1304
<b>Relative Humidity</b>							
35-50%	16	1.11	1.40	1.40	1.49	1.63	1.49
65-75%	16	1.14	1.37	1.45	1.43	1.64	1.47
SEM		0.02	0.02	0.02	0.02	0.02	0.00
P-Value		0.3532	0.4781	0.1580	0.1457	0.8736	0.1548
<b>Interaction</b>							
35-50%-C	8	1.10	1.42	1.39	1.45	1.66	1.48
35-50%-N	8	1.19	1.38	1.42	1.53	1.61	1.50
65-75%-C	8	1.08	1.34	1.43	1.37	1.68	1.45
65-75%-N	8	1.15	1.41	1.48	1.49	1.60	1.49
SEM		0.03	0.03	0.03	0.02	0.03	0.01
P-Value		0.6154	0.0644	0.7095	0.3418	0.6653	0.4756

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

### Rectal Temperature

There was a significant ( $P < 0.05$ ) increase in body temperature on Day 28 for the birds fed nicarbazin. However, the difference between the average body temperature was approximately  $0.1^\circ\text{C}$  which is not likely to be of biological significance. RH level did not cause a significant difference in body temperature, nor was there an interaction effect between diet and RH level (Table 14).

**Table 14: Effect of nicarbazin and RH on average rectal temperature (°C) of broilers 7 through 35 days of age in Trial 2.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days				
		7	14	21	28	35
<b>Diet</b>						
Control	16	40.9	41.0	41.0	41.0	41.2
Nicarbazin	16	40.9	41.0	41.1	41.1	41.2
SEM		0.03	0.03	0.03	0.03	0.03
P-Value		0.4868	0.6698	0.0867	<b>0.0459</b>	0.5482
<b>Relative Humidity</b>						
35-50%	16	41.0	41.0	41.0	41.0	41.2
65-75%	16	40.9	41.0	41.1	41.1	41.2
SEM		0.02	0.03	0.04	0.03	0.02
P-Value		0.1372	0.2951	0.7455	0.2318	0.7327
<b>Interaction</b>						
35-50%-C	8	41.0	41.0	41.0	41.0	41.2
35-50%-N	8	41.0	41.0	41.1	41.0	41.2
65-75%-C	8	40.9	41.0	41.1	41.0	41.2
65-75%-N	8	40.9	41.0	41.1	41.1	41.2
SEM		0.04	0.04	0.04	0.04	0.04
P-Value		0.4868	0.6698	0.0867	0.7296	0.7634

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student  $t$ 's test. Significant P-values are bolded.

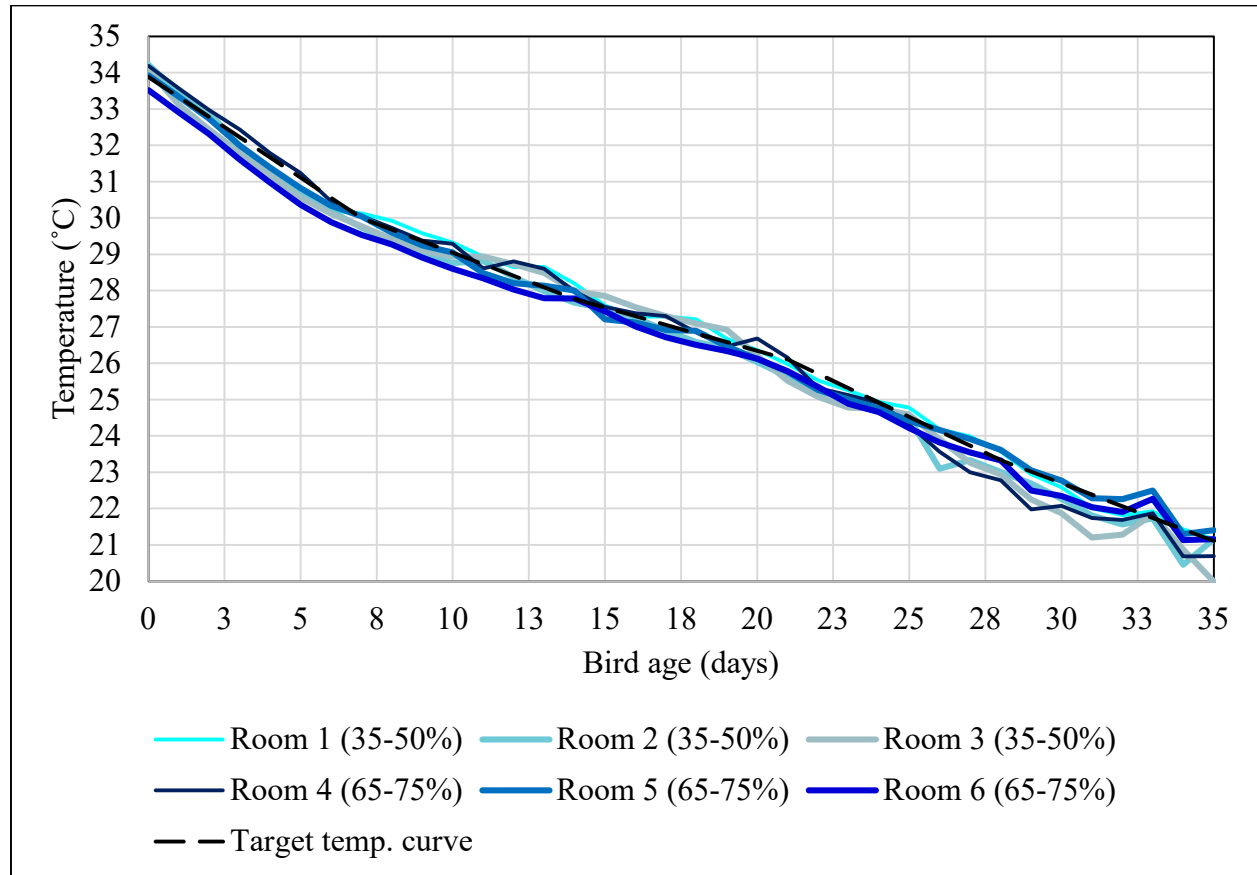
<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. 5 birds were randomly selected from each pen to obtain rectal temperature measurements.

### **Trial 3**

#### **Temperature**

The room temperatures were set to follow the target temperature curve described in the materials and methods (Table 1). The temperature of the rooms (Figure 6) was able to be maintained within  $\pm 0.3^\circ\text{C}$  of the average daily room temperature in Room 1 (control RH) and Room 6 (treatment RH),  $\pm 0.4^\circ\text{C}$  in Room 3 (control RH) and Room 5 (treatment RH), and within  $\pm 0.5^\circ\text{C}$  of Room 2 (control RH) and Room 4 (treatment RH).

**Figure 6:** Average daily temperatures recorded in rooms maintained at two RH levels throughout Trial 3 compared to an industry standard target temperature curve.<sup>1</sup>



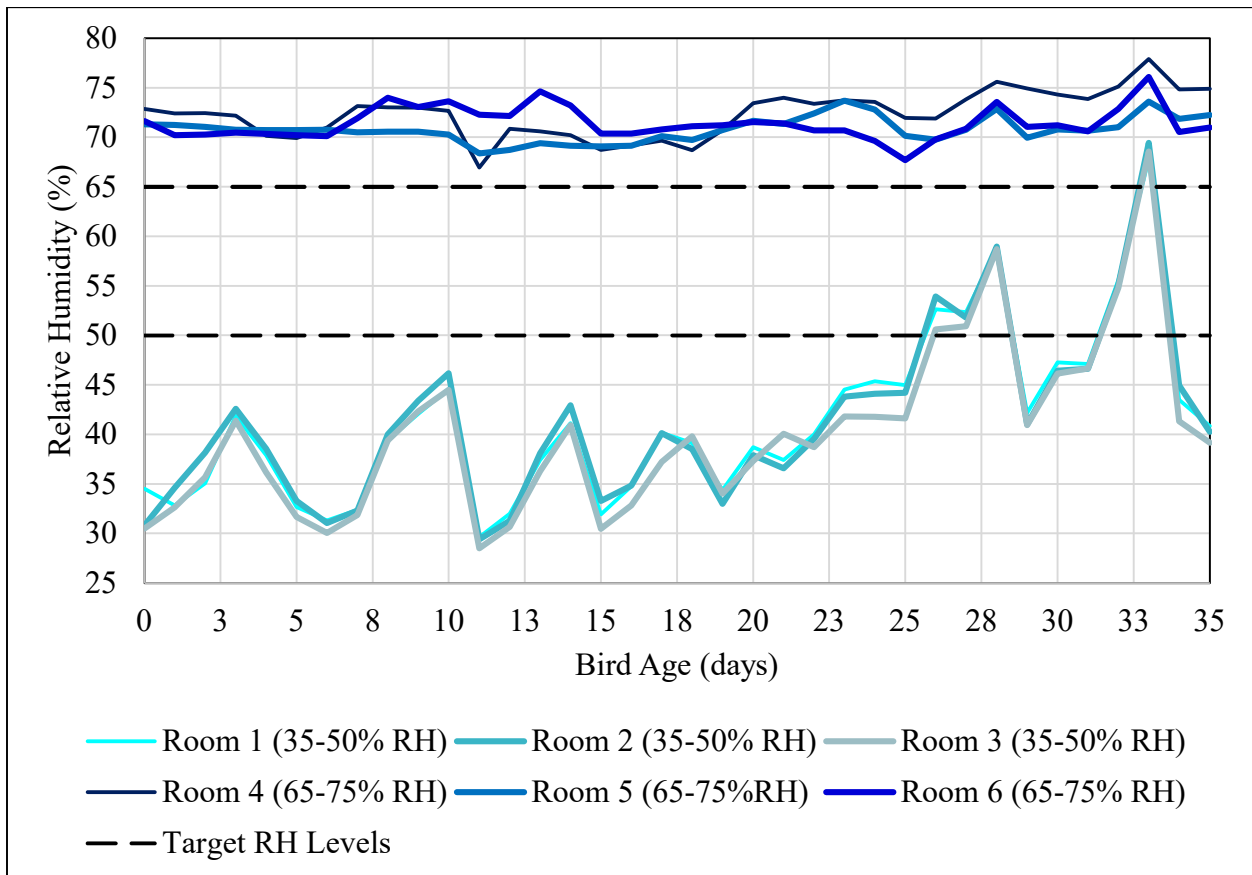
<sup>1</sup>Average daily S.D. of room temperatures was  $\pm 0.3^{\circ}\text{C}$  for Room 1 (35-50% RH) and Room 6 (65-75% RH),  $\pm 0.4^{\circ}\text{C}$  in Room 3 (35-50% RH) and Room 5 (65-75% RH), and  $\pm 0.5^{\circ}\text{C}$  of Room 2 (35-50% RH) and Room 4 (65-75% RH).

### Relative humidity

The target RH levels for the study were 35-50% (control) and 65-75% (treatment). With the exception of eight days during the trial, the RH was maintained within the targeted ranges. The variation in RH maintained was  $\pm 2\%$  in the treatment RH rooms and  $\pm 4\%$  in Room 2 the treatment RH rooms (Figure 7). There were periods where the control RH levels were not achievable. Between Days 26 and 29 and Days 31 and 34, the outside temperature and RH increased, thus the control room RH levels increased similarly to Trials 1 and 2. Unlike in previous trials, the air exchange rates did not exceed the capacity of the humidification units and

did not affect the RH of the treatment RH rooms. There was more variation in the control RH rooms (S.D. of 9.6%) throughout the study compared to the treatment RH rooms (S.D. of 3.1%) as discussed in previous trials.

**Figure 7:** Average daily RH levels recorded for rooms maintained at two RH levels throughout Trial 3.<sup>1</sup>



<sup>1</sup>Average daily S.D. of RH was  $\pm 4\%$  for the 35-50% RH rooms and  $\pm 2\%$  for the 65-75% RH rooms.

### Mortality

The average mortality rate for Trial 3 was 9.0%. There was less mortality due to omphalitis for Trial 3 compared to Trial 2. Nicarbazin treatment resulted in significantly higher mortality than the control diet treatment (13.1% vs. 4.8%) (Table 15).

**Table 15: Relationship of nicarbazin and RH to mortality of broilers 0 to 35 days of age in Trial 3.<sup>1</sup>**

<b>Treatment</b>	<b>N<sup>2</sup></b>	<b>%</b>
<b>Diet</b>		
Control	24	4.8
Nicarbazin	24	13.1
SEM		2.5
P-value		<b>0.0073</b>
<b>Relative Humidity</b>		
35-50%	24	9.3
65-75%	24	8.6
SEM		3.1
P-value		0.8077
<b>Interaction</b>		
35-50%-C	12	5.6
35-50%-N	12	11.7
65-75%-C	12	4.0
65-75%-N	12	14.5
SEM		3.6
P-value		0.5016

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student  $t$ 's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

### **Body weight**

Day 7, broilers fed nicarbazin weighed significantly ( $P < 0.05$ ) less (172g) than those fed the control diet (182g). The body weight was significantly lower throughout the nicarbazin treatment as previously seen in Trials 1 and 2. At Day 35, broilers fed nicarbazin continued to weigh significantly less than those fed the control diet (2,357g vs. 2,551g) after nicarbazin was removed from the diet at Day 28. The RH level did not have an effect on the body weight (Table 16) as seen in previous trials.

**Table 16: Effect of nicarbazine and RH on bird body weight (g) of broilers 0 through 35 days of age in Trial 3.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0	7	14	21	28	35
<b>Diet</b>							
Control	24	41.9	182	520	1066	1786	2551
Nicarbazin	24	41.8	172	454	910	1554	2357
SEM		0.0	1.5	2.9	8.8	10.0	18.6
P-Value		0.8406	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
<b>Relative Humidity</b>							
35-50%	24	41.9	175	483	981	1661	2448
65-75%	24	41.8	179	491	992	1678	2460
SEM		0.0	1.9	3.3	11.1	11.2	20.9
P-Value		0.1795	0.2987	0.1792	0.6338	0.3574	0.7218
<b>Interaction</b>							
35-50%-C	12	41.9	180	517	1064	1780	2552
35-50%-N	12	41.9	170	450	904	1543	2345
65-75%-C	12	41.9	184	523	1068	1791	2549
65-75%-N	12	41.8	173	459	916	1565	2370
SEM		0.0	2.2	4.1	12.4	14.1	26.3
P-Value		0.2580	0.6053	0.6880	0.6588	0.6328	0.5550

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student  $t$ 's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

### Body weight gain

Similar to Trials 1 and 2, birds fed nicarbazine gained significantly ( $P < 0.05$ ) less weight than the ones fed the control diet for the first four weeks. During Week 5, the bird previously fed nicarbazine had a significantly higher body weight gain than those fed the control diet continuously (804g vs. 765g). However, when comparing the overall gain from all five weeks, the birds treated with nicarbazine gained significantly less (2,315g vs. 2,509g). The RH level did not have an effect on body weight gain (Table 17).

**Table 17: Effect of nicarbazin and RH on average body weight gain (g) of broilers 0 through 35 days of age in Trial 3.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0-7	8-14	15-21	22-28	29-35	0-35
<b>Diet</b>							
Control	24	141	337	546	720	765	2509
Nicarbazin	24	130	283	456	644	804	2315
SEM		1.5	2.4	7.4	5.4	11.3	18.6
P-Value		<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0254</b>	<b>&lt;0.0001</b>
<b>Relative Humidity</b>							
35-50%	24	134	308	501	677	787	2406
65-75%	24	137	312	501	686	782	2418
SEM		1.9	2.8	9.5	4.9	11.0	20.9
P-Value		0.2939	0.3323	0.9673	0.2905	0.7561	0.7210
<b>Interaction</b>							
35-50%-C	12	139	336	547	717	771	2510
35-50%-N	12	129	279	455	638	802	2303
65-75%-C	12	143	338	543	723	759	2508
65-75%-N	12	131	286	457	649	805	2338
SEM		2.2	3.4	10.5	7.6	16.1	26.3
P-Value		0.6300	0.3904	0.7355	0.7848	0.6470	0.5520

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

### Feed consumption

Birds fed nicarbazin had a significantly ( $P < 0.05$ ) lower feed intake than those fed the control diet for the first four weeks of the trial. During the fifth week, there was not a significant difference between the feed consumption of the birds fed the control diet and those previously fed nicarbazin (1,257g vs. 1,233g). The overall feed consumption was significantly less for the birds that were treated with nicarbazin (3,260g vs. 3,463g). During the first week of the trial, the birds reared under the treatment RH level had a significantly lower feed intake than those reared under the control RH level (142g vs. 156g). However, this statistical difference did not continue through the following weeks. The overall feed consumption was not affected by RH level (3,364g vs. 3,329g). During the third week, an interaction occurred between diet and RH. When

fed the control diet, bird under the treatment RH consumed slightly less than those under the control RH (691g vs. 706g). In contrast, when birds were fed the nicarbazin diet, feed consumed was slightly increased in those reared in the treatment RH compared to the control RH (654g vs. 638g). The interaction occurred due to the slight increase in feed consumption of birds fed control diet and reared under control RH compared to treatment RH and the slight decrease in feed consumption for birds fed nicarbazin and reared under the control RH compared to treatment RH (Table 18).

**Table 18: Effect of nicarbazin and RH on average feed consumption (g) of broilers 0 through 35 days of age in Trial 3.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0-7	8-14	15-21	22-28	29-35	0-35
<b>Diet</b>							
Control	24	153	391	699	1017	1257	3463
Nicarbazin	24	145	366	646	946	1233	3260
SEM		2.5	2.9	6.4	4.8	16.3	24.8
P-Value		<b>0.0060</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	0.2366	<b>&lt;0.0001</b>
<b>Relative Humidity</b>							
35-50%	24	156	377	672	979	1257	3364
65-75%	24	142	380	673	984	1233	3329
SEM		3.1	3.2	7.9	2.3	18.0	25.2
P-Value		<b>0.0283</b>	0.4725	0.9707	0.1908	0.3997	0.3841
<b>Interaction</b>							
35-50%-C	12	162	390	706 <sup>a</sup>	1019	1264	3487
35-50%-N	12	151	364	638 <sup>b</sup>	939	1250	3241
65-75%-C	12	144	393	691 <sup>a</sup>	1015	1250	3438
65-75%-N	12	140	368	654 <sup>b</sup>	953	1216	3220
SEM		3.6	4.1	9.1	6.8	23.0	35.1
P-Value		0.2721	0.8609	<b>0.0169</b>	0.3329	0.6309	0.6969

<sup>1</sup>Significant differences were determined at P<0.05 by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

<sup>a-b</sup>Means within a column with different superscripts differ significantly (P<0.05).

## FCR

Nicarbazin fed broilers had a significantly higher FCR than those fed the control diet during Weeks 2, 3 and 4 of this trial. After the nicarbazin was removed from the diet at Day 28, the nicarbazin treated birds had a significantly lower FCR than the control group (1.56 vs. 1.64) as seen in Trials 1 and 2. During the first week, the chicks reared under the control RH conditions had a significantly higher FCR than those in the treatment RH conditions (1.18 vs. 1.04) similarly to Trial 1. There was an interaction between RH and diet during Week 3. The birds fed the control diet and reared under treatment RH had a slightly lower FCR than those fed control diet and raised under the control RH (1.27 vs. 1.29). While the nicarbazin-treated groups reacted inversely to the control. The nicarbazin-treated birds reared under control RH had a slightly lower FCR than those reared under treatment RH levels (1.41 vs.1.44). The interaction occurred due to the slight decrease in FCR of birds fed control diet and reared under control RH compared to treatment RH and the slight increase in FCR for birds fed nicarbazin and reared under the control RH compared to treatment RH (Table 19).

**Table 19: Effect of nicarbazin and RH on feed conversion ratio (g/g) of broilers 0 through 35 days of age in Trial 3.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days					
		0-7	8-14	15-21	22-28	29-35	0-35
<b>Diet</b>							
Control	24	1.10	1.16	1.28	1.51	1.64	1.43
Nicarbazin	24	1.13	1.30	1.42	1.58	1.56	1.47
SEM		0.02	0.01	0.01	0.02	0.01	0.01
P-Value		0.1996	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0109</b>	<b>0.0012</b>	<b>0.0007</b>
<b>Relative Humidity</b>							
35-50%	24	1.18	1.23	1.36	1.55	1.60	1.46
65-75%	24	1.04	1.23	1.35	1.54	1.59	1.44
SEM		0.02	0.00	0.01	0.03	0.01	0.01
P-Value		<b>0.0035</b>	0.7607	0.9175	0.7525	0.6614	0.2877
<b>Interaction</b>							
35-50%-C	12	1.17	1.16	1.29 <sup>b</sup>	1.51	1.64	1.43
35-50%-N	12	1.19	1.30	1.41 <sup>a</sup>	1.59	1.56	1.48
65-75%-C	12	1.02	1.16	1.27 <sup>b</sup>	1.52	1.64	1.42
65-75%-N	12	1.07	1.30	1.44 <sup>a</sup>	1.56	1.55	1.46
SEM		0.02	0.01	0.01	0.03	0.02	0.01
P-Value		0.5067	0.7343	<b>0.0455</b>	0.4574	0.8358	0.2877

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. Pens contained 27 birds.

<sup>a-b</sup>Means within a column with different superscripts differ significantly ( $P < 0.05$ ).

### Rectal temperatures

Nicarbazin caused birds to have significantly ( $P < 0.05$ ) higher rectal temperatures on Day 14 and 21 (Table 20). In contrast, the birds fed the control diet had significantly higher rectal temperatures on Day 28. Interactions occurred on Day 14 and 21 between the diet and RH treatments. On both Day 14 and 21, the birds fed nicarbazin and reared under the control RH level had significantly higher rectal temperatures those fed control diet and reared under the control RH level (40.9°C vs. 40.8°C and 41.0°C vs. 40.9°C). The birds reared under the treatment RH level and fed the control or nicarbazin did not have rectal temperatures that differed from the

other treatment groups. As seen in Trial 2, the differences between the average rectal temperatures were approximately 0.1°C which is likely not of biological significance.

**Table 20: Effect of nicarbazin and RH on average rectal temperature (°C) of broilers 7 through 35 days of age in Trial 3.<sup>1</sup>**

Treatment	N <sup>2</sup>	Days				
		7	14	21	28	35
<b>Diet</b>						
Control	24	40.9	40.8	40.9	41.0	41.1
Nicarbazin	24	40.9	40.9	41.0	40.9	41.0
SEM		0.03	0.03	0.02	0.04	0.03
P-Value		0.4178	<b>0.0364</b>	<b>0.0179</b>	<b>0.0155</b>	0.2034
<b>Relative Humidity</b>						
35-50%	24	41.0	40.8	40.9	40.9	41.0
65-75%	24	40.9	40.8	41.0	40.9	41.1
SEM		0.04	0.03	0.03	0.05	0.03
P-Value		0.0565	0.7149	0.3545	0.4303	0.1469
<b>Interaction</b>						
35-50%-C	12	41.0	40.8 <sup>b</sup>	40.9 <sup>b</sup>	41.0	41.0
35-50%-N	12	41.0	40.9 <sup>a</sup>	41.0 <sup>a</sup>	40.9	41.0
65-75%-C	12	40.9	40.8 <sup>ab</sup>	41.0 <sup>ab</sup>	40.9	41.1
65-75%-N	12	40.8	40.8 <sup>ab</sup>	41.0 <sup>ab</sup>	40.8	41.1
SEM		0.04	0.04	0.03	0.06	0.04
P-Value		0.1094	<b>0.0177</b>	<b>0.0179</b>	0.7199	0.7972

<sup>1</sup>Significant differences were determined at  $P \leq 0.05$  by Student t's test. Significant P-values are bolded.

<sup>2</sup>Values are means of 16 pens for main effects and 8 pens for interaction effect. 5 birds were randomly selected from each pen to obtain rectal temperature measurements.

<sup>a-b</sup>Means within a column with different superscripts differ significantly ( $P < 0.05$ ).

## CHAPTER 5

### DISCUSSION

#### **Mortality**

##### **Nicarbazin treatment**

In Trials 1 and 2, nicarbazin did not cause a significant difference in mortality. This is contradictory to what has been previously reported (Sammelwitz, 1965a, b; Harris and Macy, 1988; Buys and Rasmussen, 1978; McDougald and McQuiston, 1980; Keshavarz and McDougald, 1981; Beers et al., 1989; Wiernusz and Teeter, 1995; da Costa et al., 2017). da Costa et al. (2017) hypothesized that the mortality seen in their study may be related to an increase in body temperature at the thermoneutral temperature regime. In some studies, the birds fed nicarbazin were exposed to increased ambient temperatures resulting in an increased mortality (Sammelwitz, 1965a,b; Harris and Macy, 1988; Buys and Rasmussen, 1978; Beers et al., 1989; Wiernusz and Teeter, 1995). The ambient temperatures during the current study were maintained in the thermoneutral zone and there was no significant increase in body temperature that affected mortality.

In Trial 3, the nicarbazin treatment groups had a significantly higher mortality rate. The increase in mortality only occurred in Rooms 3 and 5 on one occasion during the trial. In the fourth week of Trial 3, Room 3 (control RH) received 24 hours of light for four days while Room 5 (treatment RH) received 24 hours of light for 2 days. The other four rooms still received six hours of dark as described in the lighting program in the materials and methods (Table 1). When lights were returned to the original light program of six hours of dark, there was an

increase in mortality in the nicarbazin pens during the first dark period. A possible reason for the increased mortality in the nicarbazin pens in Rooms 3 (control RH) and 5 (treatment RH) is an excessive increase in body temperature during the dark period. Czarick et al. (2016) reported that the body temperature of birds increased during the dark period which was attributed to them sitting down onto the litter reducing the surface area exposed to the air and their heat loss. Nicarbazin increases a bird's metabolic rate and impairs their ability to dissipate heat which results in them having a more rapid increase in body temperature than unmedicated birds (Farny, 1965; Weirnuusz and Teeter, 1995). This can further increase body temperature during dark periods in birds fed nicarbazin to the point of death.

### **Body weight and body weight gain**

#### **Nicarbazin treatment**

During the period that birds were fed nicarbazin, body weight gain was significantly depressed, agreeing with previous research studies (Cuckler et al., 1956; Newberne and Buck, 1957; Harris and Macy, 1988; Watkins and Bafundo, 1993; da Costa et al., 2017). In Trials 1 and 2, once nicarbazin was removed from the diet, there was no significant difference in weight gain between the nicarbazin and control diet groups. In Trial 3, the birds previously fed nicarbazin had a significantly higher body weight gain after nicarbazin was removed from the diet. Sammelwitz (1965b) reported that the effects of nicarbazin on mortality were fully reversed 96 hours after nicarbazin was removed from the diet. This nicarbazin withdrawal time may be applicable to the adverse effect on the growth rate as well.

During Week 5 of the current study the treatment birds gained weight at a higher rate, over the previous week's weight gain, than the control group in all three trials (percent increase over the previous week: treatment: 40.9%, 23.4%, and 24.8% vs. control: 24.7%, 9.7%, and

6.3% for Trials 1, 2, and 3 respectively). Although, the nicarbazin treated birds gained weight at a higher rate after nicarbazin was removed in Trials 1 and 2, compensatory growth was only observed during Trial 3. Even with the increase growth rates seen in all three trials, the average body weight of the treatment birds was still significantly lower than the control birds at 35 days of age.

The nicarbazin treatment regime used in the current study is not commonly used. Nicarbazin is typically only fed until Day 21, especially when birds are only raised to a market age of 35 days. The four-week treatment regime has been used in limited operations growing birds six to nine weeks of age. It appears that one week off of nicarbazin feed is not enough time for birds to increase their body weight to be similar to the control birds. If the research from Sammelwitz (1965b) is applied, it takes 96 hours for the residual effect of nicarbazin to stop. In the current study, this means that within the period of one week, the birds only had three to four days without nicarbazin affecting their growth during the final week of the study. Three to four days may not be long enough for compensatory growth to occur in all of the trials. Further studies rearing birds to six weeks or older are required to determine if there is a trend of compensatory growth after nicarbazin is removed from the diet.

### **RH treatment**

Relative humidity (RH) level did not have an effect on body weight or body weight gain of the birds when raised at thermoneutral environmental conditions, which agrees with previous studies that examined the influence of RH on poultry performance (Barott and Pringle, 1949; Milligan and Winn, 1964; Prince et al., 1965; Winn and Godfrey, 1967).

A possible explanation for the treatment RH not having an effect on body weight gain is that the RH was not high enough to cause a heat stress at the temperatures used in the current

study. Weaver and Meijerhof (1991) did not show negative effects on body weight gain of broilers during elevated RH levels at thermoneutral temperatures at Days 14 and 28 but did at Day 42 when birds are more prone to heat stress. When in combination with increased ambient temperature, elevated RH can exacerbate the adverse effects of high ambient temperatures on performance (Milligan and Winn, 1964, Winn and Godfrey, 1967; Yahav et al., 1995, Yahav et al., 2000). Even though the treatment RH was 10% to 15% higher than typically recommended, 40% to 60% (Czarick et al., 2020), it did not appear to adversely affect weight gain at the temperature profile used in the current study.

### **Diet and RH interaction**

An interaction between diet and RH level occurred for body weight and body weight gain during the second week of Trial 1. The results indicated the treatment RH caused a slight increase in body weight within the control diet treatment. This interaction in body weight and weight gain did not repeat during Trial 1 nor did it occur in Trials 2 and 3 thus providing insufficient evidence to prove that this interaction effect is biologically significant.

### **Feed consumption and FCR**

#### **Nicarbazin treatment**

As previously reported, nicarbazin depressed feed intake for the four-week treatment period in broilers in all three trials. Though feed intake in the treatment birds continued to be significantly lower than the control group after nicarbazin was removed in Trials 1 and 2, this was likely due to their smaller size.

Nicarbazin was found to increase feed conversion, which agrees with previous studies (Harris and Macy, 1988; da Costa et al., 2017). After nicarbazin is removed from the diet, the birds in the nicarbazin treatment group become more efficient in converting feed into body

weight gain compared to the control diet group. Watkins and Bafundo (1993) showed that birds fed nicarbazin for the first 20 days of a shuttle program had similar FCR and body weights to birds fed ionophores at 47 days of age. During the fifth week of the current study, the FCR of birds fed nicarbazin was significantly lower than the birds fed the control diet, but the final body weight of the treatment birds was still significantly smaller. This difference could be attributed to the short nicarbazin withdraw period of one week in the current study compared to the withdraw period, approximately four weeks, of the study conducted by Watkins and Bafundo (1993).

### **RH treatment**

RH level did not have an effect on feed consumption except for the first week of Trial 3 nor was there a significant difference in overall feed consumption for all three trials. These results agree with previous research completed in thermoneutral environmental conditions. Prince et al. (1965) reported that varying levels of RH have been shown to not influence feed consumption when ambient temperature is maintained within the thermoneutral zone. Zhou et al. (2019) showed that RH levels in combination with temperatures ranging from thermoneutral, 20°C, to moderate, 26°C, at 28 to 42 days of age did not cause a significant effect on feed consumption.

During the first week of Trial 3, the birds reared in the treatment RH had a significantly lower feed intake than those reared in the control RH conditions. Similar responses to increased RH have been reported in mature birds when RH was in combination with high ambient temperatures (Yahav et al., 1995; Zhou et al., 2019). When birds are under any form of thermal stress, they will decrease their feed intake to reduce their total heat production (Donkoh, 1988; Weaver, 2001). Digestion is a major source of heat production and therefore will only intensify the effects of thermal stress (Wiernusz and Teeter, 1993).

During the first week of Trials 1 and 3, the chicks reared under the treatment RH had a significantly lower FCR than those reared in the control RH conditions. These results are similar to those reported by Winn and Godfrey (1967). It was reported that, during brooding, chicks exposed to higher RH levels, 90%, had a better FCR than those exposed to low RH levels, 40%. The Ross708 performance guide (Aviagen, 2018) recommends that the RH level be higher during brooding at 60% to 70% and then decreased to 50% to 60% after brooding. This may be due to chicks' reliance on external factors to maintain body temperature. High levels of RH limit the amount of heat that can be lost through latent heat loss (Freeman, 1971), therefore possibly decreasing the chicks' overall heat loss. If the chicks are losing less heat, then they will not need to produce as much heat to maintain body temperature, in turn, decreasing maintenance requirements. A high maintenance requirement will increase the amount of energy from feed intake that is used for maintenance and decrease the amount used for growth resulting in an increased FCR. This is similar to what is seen during cold stress (Prince et al., 1960; Prince et al., 1961; Reece and Lott, 1983; Deaton et al., 1996; Bruzual et al., 2000; Ipek and Sahan, 2006; Olfati et al., 2018). By decreasing the need for extra heat production, the maintenance requirement will be lower, and the birds will be able to use the feed for growth instead. The same significant difference in FCR during Week 1 of Trials 1 and 3 was not seen during Trial 2 which may be attributed to the high occurrence of omphalitis and rate of mortality during this trial.

After the first week, the FCR was not affected by RH. This could be due to chicks gaining better control over thermoregulation throughout the first week of life. It is well documented that chicks are better able to regulate their body temperature independent of their surroundings after the first week post hatch (Tazawa et al., 2001; Weytjens et al., 1999; Mujahid, 2010; Scanes and Christensen, 2020).

## **Diet and RH interaction**

An interaction between RH and diet for feed consumption occurred during the third week of Trial 3. The treatment RH caused a slight, although not significant, decrease in feed consumption in birds fed the control diet. In contrast, birds fed the nicarbazin diet and reared in the treatment RH had a slight, although not significant, increase in feed consumption compared to birds fed nicarbazin and reared in the control RH. The slight difference seen between the control diet group reared under the treatment RH is similar to that seen during increased ambient temperatures while the response in the nicarbazin fed birds reared under the treatment RH was the opposite (Yahav et al., 1995; Zhou et al., 2019). This interaction in feed consumption was not seen during Trials 1 or 2 nor was it repeated at any other point in Trial 3.

The same interaction seen in feed consumption was also observed in FCR during the third week of Trial 3. The treatment RH caused a slight decrease in FCR for birds fed the control diet. In contrast, birds fed the nicarbazin diet and reared in the treatment RH had a slight increase in FCR compared to birds fed nicarbazin and reared in the control RH. Conversely, there was an interaction during Week 3 of Trial 1 that showed the treatment RH caused a slight, although not significant, increase in FCR in birds fed the control diet. Birds fed the nicarbazin diet and reared under the treatment RH had a slight, although not significant, decrease in FCR. These contradicting interactions provide no conclusive evidence that the interaction between nicarbazin and RH level has a determined effect on FCR.

During Week 4 of Trial 1, birds fed nicarbazin and raised under treatment RH have a significantly higher FCR than those fed the control diet at both RH levels. This is a similar response as those reported in studies on nicarbazin treatment while under heat stress. Keshavarz and McDougald (1981) reported that 28 to 51-day old broilers fed nicarbazin and reared at

32.2°C had a significantly higher FCR than birds fed nicarbazin and exposed to 21.1°C and birds fed control diet at either temperature. McDougald and McQuinston (1980) also found that birds fed nicarbazin had an increased FCR during heat stress when compared to birds that are treated with other anticoccidials as well as unmedicated birds. The birds fed nicarbazin and reared under the treatment RH in Trial 1 may have experienced a high effective temperature due to the high RH resulting in an increased FCR during Week 4. This interaction was not observed in Trials 2 and 3.

### **Rectal temperatures**

#### **Nicarbazin treatment**

Trial 1 did not show a significant difference in rectal temperatures between diet groups. On Day 28 of Trial 2 and Days 14 and 21 of Trial 3, the birds fed nicarbazin had a significantly higher rectal temperature than those fed the control diet which does agree with previous research (Farny, 1965; Beers et al., 1989; Wiernusz and Teeter, 1995; Keshavarz and McDougald, 1981; da Costa et al., 2017). On Day 28 of Trial 3, the birds fed the control diet had significantly higher rectal temperatures than those fed nicarbazin. This observation differs from the majority of research focused on nicarbazin and body temperature. da Costa et al. (2017) showed that body temperature was not significantly affected during the first two weeks of nicarbazin treatment but at 21 days of age, the 125 ppm nicarbazin treatment had a significantly higher rectal temperature than the other treatments (0.1°C-0.2°C) which was similar to that seen in the current study (0.1°C). A difference of 0.1°C to 0.2°C is negligible when compared to the variation in bird body temperature that occurs naturally throughout their diurnal cycle. Prinzing et al. (1991) used reported body temperature data from 61 different avian species and reported a

variation up to 2.48°C in body temperature throughout the diurnal cycle. Kadono and Besch (1978) reported a 0.6°C to 1.1°C fluctuation in the body temperature of domestic fowl.

The majority of previous nicarbazin related body temperature research was conducted under heat stress conditions (Farny, 1965; Beers et al., 1989; Wiernusz and Teeter, 1995; Keshavarz and McDougald, 1981). Wiernusz and Teeter (1995) reported that nicarbazin did not cause an elevated body temperature at thermoneutral temperatures but instead impeded the bird's ability to lose heat during times of thermal stress. The inability to dissipate adequate heat during high ambient temperatures would cause an imbalance in heat production and heat loss, therefore, increasing body temperature. Preliminary research (data not reported) showed that the body temperatures of two-week old broilers fed nicarbazin did not differ from those of birds fed a control diet at thermoneutral temperatures (30°C). When ambient temperature was increased 2.8°C, the birds fed nicarbazin had a greater increase in body temperature than the birds fed the control diet resulting in a higher body temperature. Once the heat treatment was discontinued, the body temperature of both nicarbazin treated birds and control fed birds decreased to a similar resting body temperature, thus agreeing with the conclusion of Wiernusz and Teeter (1995). The lack of significant differences, statistically and biologically, in body temperature during thermoneutral temperatures of the current study in combination with the increased mortality attributed to stress during Trial 3 could support the results and conclusion provided by the preliminary research and Wiernusz and Teeter (1995). A heat stress was not applied during the current study that would indicate the need for increased heat loss however, the changes in lighting that occurred during Trial 3 may have created an increase in body temperature during the dark period. This increase in body temperature would require an increase in heat dissipation to maintain normal body temperature. The reduced heat dissipation caused by nicarbazin

treatment may cause the bird's body temperature to increase to the point that was lethal. Even though increased body temperature may have been the cause of the mortality during that time period, the nicarbazin fed birds did not have an elevated body temperature at any point afterwards which would agree with the results of the previously mentioned preliminary research (data not reported).

### **RH treatment**

The hypothesis was that RH could possibly induce a similar stress response in birds fed nicarbazin that is seen during elevated temperatures due to RH's effects on the bird's ability to lose or retain heat through latent heat loss, but this was not observed in the current study. The effect of RH on body temperature usually has a compounding effect with ambient temperatures. Genç and Portier (2005) found that body temperature was not significantly affected by varying levels of RH when six-week-old broilers are reared at thermoneutral temperatures. The ambient temperature in the current study was maintained in the thermoneutral zone throughout the trial which could explain why there was not a significant difference in body temperatures observed.

### **Diet and RH interactions**

On Days 14 and 21 of Trial 3, birds fed nicarbazin and reared under the control RH had significantly higher rectal temperature than birds fed the control diet and reared under the control RH level. The birds reared under the treatment RH level, regardless of diet treatment, did not have a significantly different rectal temperature from the two diet groups under the control RH level. A nicarbazin diet group having an increased rectal temperature agrees with previous research (Farny, 1965; Beers et al., 1989; Wiernusz and Teeter, 1995; Keshavarz and McDougald, 1981; da Costa et al., 2017). However, the difference between the highest and lowest rectal temperatures of the current study was approximately 0.1°C, which is not

biologically significant. Similar differences in rectal temperature were seen in Trials 1 and 2 however, there were no interactions. This could be due to the increased sample size in Trial 3 (n=8 vs. n=12). The larger a sample size, the easier it is to have a significance for small differences even though it is not biologically meaningful.

## CHAPTER 6

### CONCLUSION

The objective of this study was to determine the effect of nicarbazin fed for 28 days on the performance and body temperature of broilers under relative humidity (RH) levels of 35-50% and 65-75%. The conclusions were:

1. Birds fed nicarbazin for 28 days weighed approximately 13% less than birds fed a control diet.
2. Total weight gain was depressed by approximately 8% at 35 days of age when birds were fed nicarbazin for the first 28 days.
3. Nicarbazin caused an approximate 8-point increase in FCR at 28 days while the treatment birds had a 3-point increase, on average, in overall FCR at 35 days of age.
4. An average RH of roughly 70% when compared to 42%, was not found to influence weekly broiler weight, gain, feed consumption, or rectal temperature
5. An average RH of 70%, compared to 42%, did not exacerbate performance issues related to nicarbazin.
6. Nicarbazin does not have an effect on bird body temperature at the commonly recommended temperatures and relative humidity.

Varying levels of RH did not appear to exacerbate the negative effects of nicarbazin that was observed on bird performance. This is not to say that RH does not need to be monitored closely when nicarbazin is fed. RH still needs to be maintained due to its known influence on air and

litter quality as well as its compounding effect with ambient temperature that influences bird health and performance.

## REFERENCES

- Abudabos, A.M., E.M. Samara, E.O.S. Hussein, M.Q. Al-Ghadi, and R.M. Al-Atiyat. 2013. Impacts of stocking density on the performance and welfare of broiler chickens. *Italian Journal of Animal Science*. 12:66-71.
- Alhenaky, A., A. Abdelqader, M. Abuajamieh, and A.R. Al-Fataftah. 2017. The effect of heat stress on intestinal integrity and *Salmonella* invasion in broiler birds. *Journal of Thermal Biology*. 70:9-17.
- Aviagen, R.P.B., 2018. Ross broiler management handbook. Aviagen, Huntsville, AL, USA. Accessed June 2021. [https://aviagen.com/assets/Tech\\_Center/Ross\\_Broiler/Ross-BroilerHandbook2018-EN.pdf](https://aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf)
- Awad, E.A., M. Najaa, Z.A. Zulaihka, I. Zulkifili, and A.F. Soleimani. 2020. Effects of heat stress on growth performance, selected physiological and immunological parameters, caecal microflora, and meat quality in two broiler strains. *Asian-Australasian Journal of Animal Science*. 33:778-787.
- Bacila, D.M., V. Feddern, L.I. Mafia, G.N. Scheuermann, L. Molognoni, and H. Daguer. 2017. Influence of anticoccidial drugs on losses of broiler chickens from heat stress and coccidiosis. *Poult. Sci*. 99:31-40
- Barott, H.G., and E.M. Pringle. 1949. Effect of environment on growth and feed and water consumption of chickens (II)—The effect of temperature and humidity of environment during the first eighteen days after hatch. *Journal of Nutrition*. 37:153-162.
- Barre, H., A. Geloën, J. Chatonnet, A. Dittmar, and J.L. Rouanet. 1985. Potentiated muscular thermogenesis in cold-acclimated muscovy duckling. *American Journal of Physiology*. 249:R533-R538.
- Barre, H., J. Nedergaard, and B. Cannon. 1986. Increased respiration in skeletal muscle mitochondria from cold-acclimated ducklings: uncoupling effects of free fatty acids. *Comparative Biochemistry and Physiology. B.*, 85:343-348.
- Bartov, I. 1989a. Lack of interactive effect of nicarbazin and dietary energy-to-protein ratio on performance and abdominal fat pad weight of broiler chicks. *Poult. Sci*. 68:1535-1539.
- Bartov, I. 1989b. Lack of effect of dietary factors on nicarbazin toxicity in broiler chicks. *Poult. Sci*. 65:145-152.
- Beers, K.W., T.J. Raup, W.G. Bottje, and T.W. Odom. 1989. Physiological responses of heat-stressed broilers fed nicarbazin. *Poult. Sci*. 68:428-434

- Berg, L.R., C.M. Hamilton, and G.E. Bearnse. 1956. Nitrofurazone and Nicarbazine as Growth Stimulants and Coccidiostatic Agents for Young Chicks. *Poult. Sci.* 35:1394-1396
- Brown-Brandl, T.M., M.M. Beck, D.D. Schulte, A.M. Parkhurst, and J.A. Deshazer. 1997. Physiological responses of tom turkeys to temperature and humidity change with age. *Journal of Thermal Biology.* 22:43-52.
- Bruzual, J.J., S.D. Peak, J. Brake, and E.D. Peebles. 2000. Effects of Relative Humidity During the Last Five Days of Incubation and Brooding Temperature on Performance of Broiler Chicks from Young Broiler Breeders. *Poult. Sci.* 79:1385-1391.
- Buyts, R.W., and S.B. Rasmussen. 1978. Heat stress mortality in nicarbazine fed chickens. *Journal of the South African Veterinary Association.* 49:127-128
- Cândido, M.G.L., I.D.F.F. Tinôco, F.D.A.D.C. Pinto, N.T. Santos, and R.P. Roberti. 2016. Determination of thermal comfort zone for early-stage broilers. *Journal of Brazilian Association of Agricultural Engineering.* 36:706-767.
- Cassuce, D.C., I.D.F.F. Tinôco, F.C. Baêta, S. Zolnier, P.R. Cecon, and D.D.F.A. Vieira. 2013. Thermal comfort temperature update for broiler chickens up to 21 days of age. *Eng. Agric. Jaboticabal.* 33:28-36.
- Chapman, H.D. 1994. A review of the biological activity of the anticoccidial drug nicarbazine and its application for the control of coccidiosis poultry. *Poultry Sci. Rev.* 5:231-243.
- Chapman, H.D. 2001. Use of anticoccidial drugs in broiler chickens in the USA: analysis for the years 1995 to 1999. *Poult. Sci.* 80:572-580.
- Chepete, H.J., and H. Xin. 2001. Heat and moisture production of poultry and their housing systems- a literature review. Pages 319-335 in *Livestock Environment VI: Proceedings of the 6th International Symposium (21-23 May 2001, Louisville, Kentucky, USA)* ed. Richard R. Stowell, Ray Bucklin, and Robert W. Bottcher.
- Cobb, 2018. Optimum broiler development guide. Aviagen, Siloan Spring, AR, USA. Accessed June 2021. <https://www.cobb-vantress.com/assets/Cobb-Files/management-guides/95e52e5b32/optimum-broiler-development.pdf>
- Collins, A., J. Buyse, P. van As, V.M. Darras, R.D. Malheiros, V.M.B. Moraes, G.E. Reyns, M. Taouis, and A. Decuyper. 2002. Cold-induced enhancement of avian uncoupling protein expression, heat production, and triiodothyronine concentrations in broiler chicks. *General and Comparative Endocrinology.* 130:70-77.
- Cuckler, A.C., C.M. Malanga, and W.H. Ott. 1956. The antiparasitic activity of nicarbazine. *Poult. Sci.* 35:98-109
- Czarick, M., and B. D. Fairchild, 2012. Relative Humidity...The Best Measure of Overall Poultry House Air Quality. University of Georgia Cooperative Extension Service Poultry Housing Tips 24(2). Accessed June

2021. <https://www.poultryventilation.com/system/tdf/vol24n2.pdf?file=1&type=node&id=4925&force=>
- Czarick, M., and B.D. Fairchild. 2008. Poultry housing for hot weather climates. Pages 48-79 in *Poultry Production in Hot Climates*. CBA International. ed. N.J. Dagher. Oxfordshire, UK.
- Czarick, M., B.D. Fairchild, and C. Mou. 2020. DOL 53 Ammonia Sensor. University of Georgia Cooperative Extension Service Poultry Housing Tips 32(1). Accessed Sept. 2021. <https://www.poultryventilation.com/system/tdf/vol32n1.pdf?file=1&type=node&id=5122&force=>
- Czarick, M., M. Hicks, and B. Fairchild. 2016. Are Birds Cooler at Night When the Lights Go Out? University of Georgia Cooperative Extension Service Poultry Housing Tips 28(3). Accessed November 2021. <https://www.poultryventilation.com/system/tdf/vol28n3.pdf?file=1&type=node&id=4966&force=>
- Czarick, M., M.L. Teo, and B. Fairchild. 2017. Bird Density and Its Influence on Effective Temperature. University of Georgia Cooperative Extension Service Poultry Housing Tips 29(5). Accessed November 2021. <https://www.poultryventilation.com/system/tdf/vol29n5.pdf?file=1&type=node&id=4980&force=>
- Czarick, M., M.L. Teo, and B. Fairchild. 2018. Density Can Have More of an Affect on Body Temperatures Than Air Temperature. University of Georgia Cooperative Extension Service Poultry Housing Tips 30(6). Accessed November 2021. <https://www.poultryventilation.com/system/tdf/vol30n6.pdf?file=1&type=node&id=5093&force=>
- da Costa, M.J., K.W. Bafundo, G.M. Pesti, E.A. Kimminau, and H.M. Cervantes. 2017. Performance and anticoccidial effects of nicarbazin-fed broilers reared at standard or reduced environmental temperatures. *Poult. Sci.* 96:1615-1622
- Dauguschies, A., U. Gässlein, and M. Rommel. 1998. Comparative efficacy of anticoccidials under the conditions of commercial broiler production and in battery trials. *Poult. Sci.* 76:163-171
- Deaton, J. W. 1995. Environmental Factors Involved in Growth and Development in World Animal Science, Production-System Approach. Pages 119-138 in: *Poultry Production*, ed. Dr. Peter Hunton, Elsevier Publishers, New York, NY.
- Deaton, J.W., F.N. Reece, and J.L. McNaughton. 1978. The Effect of Temperature During the Growing Period on Broiler Performance. *Poult. Sci.* 57:1070-1074.
- Deaton, J.W., F.N. Reece, L.F. Kubena and J.D. May. 1973. The effect of temperature during the growing period on broiler performance. *Poult. Sci.* 52:1993-2106. (Abstr.)

- Deaton, J.W., S.L. Branton, J.D. Simmons, and B.D. Lott. 1996. The Effect of Brooding Temperature on Broiler Performance. *Poult. Sci.* 75:1217-1220.
- Decuypere E., P. Van As, S. Van der Geyten, V.M. Darras. 2005. Thyroid hormone availability and activity in avian species: A review. *Domestic Animal Endocrinology.* 29:63-77.
- Deeb, N., and A. Cahaner. 1999. The Effects of Naked Neck Genotypes, Ambient Temperature, and Feeding Status and Their Interactions on Body Temperature and Performance of Broilers. *Poult. Sci.* 78:1341-1346.
- Donkoh, A. 1988. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. *International Journal of Biometeorology.* 33:259-265.
- Duchamp C., F. Marmonier, F. Denjean, J. Lachuer, T.P.D. Eldershaw, J.L. Rouanet, A. Morales, R. Meister, C. Bénistant, D. Roussel, and H. Barré. 1999. Regulatory, cellular and molecular aspects of avian muscle nonshivering thermogenesis. *Ornis Fennica.* 76:151-165.
- Edens, F.W. 1978. Adrenal Cortical Insufficiency in Young Chickens Exposed to a High Ambient Temperature. *Poult. Sci.* 57:146-1750.
- Edens, F.W., and H.S. Siegel. 1976. Modification of Corticosterone and Glucose Responses by Sympatholytic Agents in Young Chickens During Acute Heat Exposure. *Poult. Sci.* 55:1704-1712.
- El-Gendy, E., and K.W. Washburn. 1995. Genetic Variation in Body Temperature and its Response to Short-Term Acute Heat Stress in Broilers. *Poult. Sci.* 74:225-230.
- El-Halawani, M.E., W.O. Wilson, and R.E. Burger. 1971. Cold Acclimation of Adrenalectomized Male Leghorn Chickens. *Poult. Sci.* 50:402-407.
- Etches, R.J., T.M. John, and A.M. Verrinder. 2008. Behavior, physiological, neuroendocrine, and molecular responses to heat stress. Pages 48-79 in *Poultry Production in Hot Climates.* CBA International. ed. N.J. Dagher. Oxfordshire, UK.
- Farny, D.G.O. 1965. Heat stress studies in nicarbazin treated chickens. Ph.D. Diss. Univ. Delaware, Newark.
- Feddes, J.J.R., E.J. Emmanuel, and M.J. Zuidhof. 2002. Broiler Performance, Bodyweight Variance, Feed and Water Intake, and Carcass Quality at Different Stocking Densities. *Poult. Sci.* 81:774-779.
- Freeman B.M. 1971. Body Temperature and Thermoregulation. Pages 1115-1151 in *Physiology and Biochemistry of the Domestic Fowl.* Academic Press Inc. ed. D.D. Bell and W.D. Weaver. NY.
- Freeman, B.M. 1970. Thermoregulatory mechanisms of the neonate fowl. *Comparative Biochemistry and Physiology.* 33:219-230.

- Frigerio, D., J. Dittami, E. Möstl, and K. Kotrschal. 2004. Excreted corticosterone metabolites co-vary with ambient temperature and air pressure in male Greylag geese (*Anser anser*). *General Comparative Endocrinology*. 137:29-36.
- Genç, L., and K.M. Portier. 2005. Sensible and Latent Heat Productions from Broilers in Laboratory Conditions. *Turk. J. Vet. Anim. Sci.* 29:635-643.
- Giloh, M., D. Shinder, and S. Yahav. 2012. Skin surface temperature of broiler chickens is correlated to body core temperature and is indicative of their thermoregulatory status. *Poult. Sci.* 91:157-188.
- Hai, L., D. Rong, G. Xionghong, and Z. Ziyi. 1996. The effects of thermal environment on the growth of neonatal chicks: I. The development of thermoregulation. *J. Anim. Physiol. a. Anim. Nutr.* 75:200-206
- Harris, G.S., and L.B. Macy. 1988. Effect of nicarbazin and maxiban™ on broiler performance when fed to different age broiler males subjected to environmental stress. *Poult. Sci.* 67:17. (Abstr.)
- Hillman, P. E., N. R. Scott, and A. Van Tienhoven. 1985. Physiological adaptations to hot and cold environments. Pages 27-28 in *Stress Physiology in Livestock, Volume III: Poultry*. M.K. Yousef, ed. CR C Press, Inc. Boca Raton, FL.
- Hurwitz, S., M. Weiselberg, U. Eisner, I. Bartov, G. Riesenfeld, M. Sharvit, A. Niv, and S. Bornstein. 1980. The Energy Requirements and Performance of Growing Chickens and Turkeys as Affected by Environmental Temperature. *Poult. Sci.* 59:2290-2299.
- Hwang-Bo, J., T. Muramatsu, and J. Okumura. 1990. Research Note: Age Dependency of Triiodothyronine-Induced Thermogenesis in Young Chicks: Inhibition by Propylthiouracil. *Poult. Sci.* 69:1599-1601.
- Ipek, A., and U. Sahan. 2006. Effects of Cold Stress on Broiler Performance and Ascites Susceptibility. *Asian-Australasian Journal of Animal Science.* 19:734-738.
- Kadono, H., and E.L. Besch. 1978. Telemetry Measured Body Temperature of Domestic Fowl at Various Ambient Temperatures. *Poult. Sci.* 57:1075-1080.
- Kempster, H.L. 1938. The Influence of Summer Temperatures on the Rate of Growth of Chickens. *Poult. Sci.* 17:259-263.
- Keshavarz, K., and L.R. McDougald. 1981. Influence of Anticoccidial Drugs on Losses of Broiler Chickens from Heat Stress and Coccidiosis. *Poult. Sci.* 60:2423-2428
- Klandorf, H., P.J. Sharpe, and M.G. Macleod. 1981. The Relationship between Heat Production and Concentrations of Plasma Thyroid Hormones in the Domestic Hen. *General and Comparative Endocrinology*, 45:513-520.

- Koh, K., and M.G. Macleod. 1999. Effects of ambient temperature on heat increment of feeding and energy retention in growing broilers maintained at different food intakes. *Brit. Poul. Sci.* 40:511-516.
- Lacey, B., T.K. Hamrita, M.P. Lacey, G.L. van Wicklen, and M. Czarick. 2000. Monitoring deep body temperature biotelemetry responses of broilers using. *J. Appl. Poultry Res.* 9:6-12.
- Leenstra, F., and A. Cahaner. 1991. Genotype by Environment Interactions Using Fast-Growing, Lean or Fat Broiler Chickens, Originating from The Netherlands and Israel, Raised at Normal or Low Temperature. *Poult. Sci.* 70:2028-2039.
- Lin, H., H. F. Zhang, H. C. Jiao, T. Zhao, S. J. Sui, X. H. Gu, Z. Y. Zhang, J. Buyse, and E. Decuypere. 2005a. Thermoregulation Responses of Broiler Chickens to Humidity at Different Ambient Temperatures. I. One Week of Age. *Poult. Sci.* 84:1166-1172.
- Lin, H., H. F. Zhang, R. Du, X. H. Gu, Z. Y. Zhang, J. Buyse, and E. Decuypere. 2005b. Thermoregulation Responses of Broiler Chickens to Humidity at Different Ambient Temperatures. II. Four Weeks of Age. *Poult. Sci.* 84:1173-1178.
- Lu, T.L. 2019. Effect of air movement on chick body temperature during brooding. M.S. Thesis Univ. Georgia, Athens.
- Luo, Y.C. 2021. The Effects of Breeder Age and Chick Hatch Size on Body Temperature for Day 0-7 . M.S. Thesis Univ. Georgia, Athens.
- Maiorka, A., F. Dahlke, and M.S.F. de Azevedo Morgulis. 2006. Broiler adaption to post-hatch period. *Ciência Rural.* 36:701-708.
- Malheiros, R. D., K M. B. Mows, L. D. G. Brunp, E. B. Malheiros, R. L. Furlan and M. Macari. 2000. Environmental temperature and cloacal and surface temperatures of broiler chicks in first week post-hatch. *J. Appl. Poultry Sci.* 9:111-117.
- Marjoniemi, K., and E. Hohlot. 1999. Environmental temperature and cloacal and surface temperatures of broiler chicks in first week post-hatch. *Physiological and Biological Zoology.* 72:484-492.
- May, J.D., and B.D. Lott. 2000. The effect of environmental temperature on growth and feed conversion of broilers to 21 days of age. *Poult. Sci.* 79:669-671.
- May, J.D., and B.D. Lott. 2001. Relating Weight Gain and Feed:Gain of Male and Female Broilers to Rearing Temperature. *Poult. Sci.* 80:581-584.
- May, J.D., B.D. Lott, and J.D. Simmons. 1998. The effect of environmental temperature and body weight on growth rate and feed:gain of male broilers. *Poult. Sci.* 77:499-501.
- McDougald, L.R. 1982. Chemotherapy of coccidiosis. Pages 373-428 in *The Biology of the Coccidiosis*, e.d. P.L. Long,

- McDougald, L.R., and T.E. McQuiston. 1980. Mortality from heat stress in broiler chickens influenced by anticoccidial drugs. *Poult. Sci.* 59:2421-2423
- McWilliams, S., E. Adkins-Regan, and C. Vleck. 2016. Bird Physiology. Pages 215-264 in *The Cornell Lab of Ornithology Handbook of Bird Biology* third edition. John Wiley & Sons. ed. I.J. Lovette and J.W. Fitzpatrick. West Sussex, UK.
- Milligan, J.L., and P.N. Winn. 1964. The Influence of Temperature and Humidity on Broiler Performance in Environmental Chambers. *Poult. Sci.* 43:817-824.
- Mission, B.H. 1977. The relationships between age, mass, body temperature and metabolic rate in the neonatal fowl (*Gallus domesticus*). *Journal of Thermal Biology.* 2:107-110.
- Mission, B.H. 1982. The thermoregulatory responses of and starved 1-week-old chickens (*Gallus domesticus*). *Journal of Thermal Biology.* 7:189-192.
- Mitchell, M.A., and A.J. Carlisle. 1992. The effects of chronic exposure to elevated environmental temperature on intestinal morphology and nutrient absorption in the domestic fowl (*Gallus domesticus*). *Comp. Biochem. Physiol.* 101A:137-142.
- Moraes V.M.B., R.D. Malheiros, R.L. Furlan, L.D.G. Bruno, E.B. Malheiros, and M. Macari. 2002. Effect of Environmental Temperature During the First Week of Brooding Period on Broiler Chick Body Weight, Viscera and Bone Development. *Brazilian Journal of Poultry Science.* 4:1-8.
- Moreng, R.E., and C.S. Shaffner. 1951. Lethal Internal Temperatures for the Chicken, from Fertile Egg to Mature Bird. *Poult. Sci.* 30:255-266.
- Mujahid, A. 2010. Acute cold-induced thermogenesis in neonatal chicks (*Gallus gallus*). *Comparative Biochemistry and Physiology, Part A.* 156:34-41.
- Mujahid, A., and Furuse. 2008a. Central administration of corticotropin-releasing factor induces thermogenesis by changes in mitochondrial bioenergetics in neonatal chicks. *Neuroscience.* 155:845-851.
- Mujahid, A., and Furuse. 2008b. Homeothermy in neonatal chicks exposed to low environmental temperature with or without intracerebroventricular administration of corticotropin-releasing factor. *FEBS Letters.* 582:3052-3060.
- Mujahid, A., and Furuse. 2009. Behavioral responses of neonatal chicks exposed to low environmental temperature. *Poult. Sci.* 88:917-922.
- Mushett C.W., F.V. Washko, K.L. Kelley, and A.J. Bloom. 1958. A Hematologic-Pathologic Study in Chickens Fed Graded Levels of Nicarbazin. *Poult. Sci.* 37:580-586
- Nääs, I.A., C.E.B. Romanini, D.P. Neves, G.R. do Nascimento, and R.A. Vercellino. 2010. Broiler surface temperature distribution of 42-day old chickens. *Sci. Agric.* 67:497-502.

- Newberne, P.M., and W.B. Buck. 1957. Studies on drug toxicity in chicks: 3. The influence of various levels of nicarbazin on growth and development of chicks. *Poult. Sci.* 36:304-312
- Olfati, A., A. Mojtahedin, T. Sadeghi, M. Akbari, and F. Martinez-Pastor. 2018. Comparison of growth performance and immune responses of broiler chicks reared under heat stress, cold stress and thermoneutral conditions. *Spanish Journal of Agricultural Research.* 16:1-7.
- Ott, W.H., S. Kuna, C.C. Porter, A.C. Cuckler, and D.E. Fogg. 1956. Biological studies on nicarbazin, a new anticoccidial agent. *Poult. Sci.* 35:1355-1367
- Porter, C.C., and J.L. Gilfillan. 1955. The absorption and excretion of orally administered nicarbazin by chickens. *Poult. Sci.* 34:995-1001.
- Powell, F.R. 2015. Respiration. Pages 325-330 in *Sturkie's Avian Physiology* sixth edition. Elsevier Inc. e.d. C.S. Scanes. Waltham, MA.
- Prince, R.P., J.H. Whitaker, L.D. Matterson, and R.E. Luginbuhl. 1965. Response of Chickens to Temperature and Relative Humidity Environments. *Poult. Sci.* 44:73-77.
- Prince, R.P., L.M. Potter, and W.W. Irish. 1961. Response of Chickens to Temperature and Ventilation Environments. *Poult. Sci.* 40:102-108.
- Prince, R.P., W.C. Wheeler, W.A. Junnila, L.M. Potter and E.P. Singesen. 1960. Effect of temperature on feed consumption and weight gain in broiler production.
- Prinzinger, R., A. Preßmar, and E. Schluecher. 1991. Body temperature in birds. *Comp. Biochem. Physiol.* 99:499-506.
- Purswell, J.L., W.A. Dozier III, H.A. Olanrewaju, J.D. Davis, H. Xin, and R.S. Gates. 2012. Effect of temperature-humidity index on live performance in broiler chickens grown from 49 to 63 days of age. Ninth International Livestock Environmental Symposium. .
- Randall, W.C. 1943. Factors influencing the temperature regulation of birds. *American Journal of Physiology.* 139:56-63.
- Reece, F.N., J.W. Deaton, and L.F. Kubena. 1972. Effects of High Temperature and Humidity on Heat Prostration of Broiler Chickens. *Poult. Sci.* 51:2021-2025.
- Reece, F.N., and B.D. Lott. 1982a. The effect of environmental temperature on sensible and latent heat production of broiler chickens. *Poult. Sci.* 61:1590-1593.
- Reece, F.N., and B.D. Lott. 1982b. Heat and moisture production of broiler chickens during brooding. *Poult. Sci.* 61:661-666.
- Reece, F.N., and B.D. Lott. 1983. The Effects of Temperature and Age on Body Weight and Feed Efficiency of Broiler Chickens. *Poult. Sci.* 62:1096-1908.

- Richards, S.A. 1971. The significance of changes in the temperature of the skin and body core of the chicken in the regulation of heat loss. *J. Physiol.* 216:1-10.
- Roller, W.L., and A.C. Dale. 1963. Heat loss from leghorn layers at warm temperatures. *Transactions of the ASAE.* 6 (2): 0136-0139.
- Ruuskanen, S., B.Y. Hsu, and A. Nord. 2021. Endocrinology of thermoregulation in birds in a changing climate. *Molecular and Cellular Endocrinology.* 519:1-12.
- Saarela, S., J.S. Keith, E. Hohtola, and P. Trayhurn. 1991. Is the "mammalian" brown fat-specific mitochondrial uncoupling protein present in adipose tissues of birds?. *Comp. Biochem. Physiol.* 100B:45-49.
- Sammelwitz, P. H. 1965a. Factors related to nicarbazin induced heat stress mortality in broilers. *Poultry Science* 44:1412. (Abstr.)
- Sammelwitz, P. H. 1965b. Heat stress mortality in broilers. *Poultry Science* 44:1412. (Abstr.)
- Scanes, C.G., and K.D. Christensen. 2020. Pages 78-280 in *Poultry Science* fifth edition. Waveland Press Inc. Long Grove, IL.
- Shinder, D., D. Lugar, M. Rusal, V. Rzepakovsky, V. Bresler, and S. Yahav. 2002. Early age cold conditioning in broiler chickens (*Gallus domesticus*): thermotolerance and growth responses. *Journal of Thermal Biology.* 27:517-523.
- Shinder, D., M. Rusal, J. Tanny, S. Druyan, and S. Yahav. 2007. Early age cold conditioning in broiler chickens (*Gallus domesticus*): thermotolerance and growth responses. *Poult. Sci.* 86:2200-2209.
- Simmons, J.D., B.D. Lott, and J.D. May. 1997. Heat loss form broiler chickens subjected to various air speed and ambient temperatures. *Applied Engineering in Agriculture.* 13:665-669.
- Suk, Y.O., and K.W. Washburn. 1995. Effects of Environment on Growth, Efficiency of Feed Utilization, Carcass Fatness, and Their Association. *Poult. Sci.* 74:285-296.
- Takahashi, H., M. Iigo, K. Ando, T. Tachibana, D.M. Denbow, and M. Furuse. 2005. Regulation of body temperature by thyrotropin-releasing hormone in neonatal chicks. *Developmental Brain Research.* 157:58-64.
- Tazawa, H., K. Moriya, A. Tamura, T. Komoro, R. Akiyama. 2001. Ontogenetic study of thermoregulation in birds. *Journal of Thermal Biology.* 26:281-286.
- Teeter, R.G., and T. Belay. 1996. Broiler management during acute heat stress. *Animal Feed Science Technology.* 58:127-142.
- Teo, M.L. 2018. The effect of different stocking densities on body temperature of broiler chickens. M.S. Thesis Univ. Georgia, Athens.

- Tzschentke, B., and M. Nichelmann. 1999. Development of avian thermoregulatory system during the early postnatal period: development of the thermoregulatory set-point. *Ornis Fennica*. 76:189-198.
- van den Brand, H., R. Molenaar, I. van der Star, and R. Meijerhof. 2010. Early feeding affects resistance against cold exposure in young broiler chickens. *Poult. Sci.* 89:716-720.
- Watkins, K.L., and K.W. Bafundo. 1993. Effect of anticoccidial programs on broiler performance. *Poult. Sci.* 2:55-60
- Weaver W.D. 2001. Poultry Housing. Pages 101-112 in *Commercial Chicken Meat and Egg Production*. Springer Science +Business Media. ed. D.D. Bell and W.D. Weaver. NY.
- Weaver, W.D. Jr., and Meijerhof, R. 1991. The Effect of Different Levels of Relative Humidity and Air Movement on Litter Conditions, Ammonia Levels, Growth, and Carcass Quality for Broiler Chickens. *Poult. Sci.* 70:746-755.
- Weytjens, S., R. Meijerhof, J. Buyse, and E. Decuypere. 1999. Thermoregulation in chicks originating from breeder flocks of two different ages. *J. Appl. Poultry Res.* 8:139-145.
- Wiernusz, C.J., and R.G. Teeter. 1993. Feeding Effects on Broiler Thermobalance During Thermoneutral and High Ambient Temperature Exposure. *Poult. Sci.* 72:1917-1924.
- Wiernusz, C.J., and R.G. Teeter. 1995. Nicarbazin effects on broiler thermobalance during high ambient temperature stress. *Poult. Sci.* 74:577-580.
- Winn, P.N., and E.F. Godfrey. 1967. The effect of humidity on growth and feed conversion of broiler chickens. *International Journal of Biometeorology*. 11:39-50.
- Xin, H., I. L. Berry, G. T. Tabler, and T. L. Barton. 1994. Temperature and humidity profiles of broiler houses with experimental conventional and tunnel ventilation systems. *Applied Engineering in Agriculture*. 10:535-542.
- Xin, H., J. Sell, and D.U. Ahn. 1996. Effects of Light and Darkness on Heat and Moisture Production of Broilers. *Transactions of the ASAE*. 39:2255-2258.
- Yahav, S. 2000. Relative humidity at moderate ambient temperatures: Its effect on male broiler chickens and turkeys. *British Poultry Science*. 41:94:100.
- Yahav, S., A. Straschnow, I. Plavnik, and S. Hurwitz. 1996. Effects of diurnally cycling versus constant temperatures on chicken growth and food intake. *British Poultry Science*. 37:43-54.
- Yahav, S., R. Sasson, and D. Shinder. 2004. The effect of thermal manipulations during embryogenesis of broiler chicks (*Gallus domesticus*) on hatchability, body weight and thermoregulation after hatch. *Journal of Thermal Biology*. 29:245-250.

- Yahav, S., S. Goldfeld, I. Plavnik, and S. Hurwitz. 1995. Physiological responses of chickens and turkeys to relative humidity during exposure to high ambient temperature. *Journal of Thermal Biology*. 20:245-253.
- Zhou, Y., X. M. Li, M. H. Zhang, and J. H. Feng. 2019. Effect of relative humidity at either acute or chronic moderate temperature on growth performance and droppings' corticosterone metabolites of broilers. *J. Integr. Agr.* 18:152–159.

## APPENDIX

### **Nicarbazin palatability and aversion study**

#### **Introduction**

A decrease in feed consumption has been well documented during the use of nicarbazin in broilers but there has been little research done to determine the cause. It has been speculated that broilers eat less feed due to a decrease in palatability due to taste or a learned aversion due to changes in metabolism.

Taste, smell, texture, and vision are immediately used to determine the palatability of feed, typically during the first encounter with the feed. Animals typically decrease feed consumption after one negative experience related to taste (Rozin and Vollmecke, 1986). In birds, this has been seen for diets that were high in salt. When chickens were given a diet with a 5% sodium chloride concentration, there was an immediate decrease in feed intake within the first hour that continued through the entire eight-hour measurement period (Gentle, 1971). Little research has been published that investigates whether nicarbazin induces a similar response. Sammelwitz (1965) reported that birds fed a feed with a nicarbazin concentration of 500 ppm caused a decrease in feed intake due to the feed being unpalatable. Mushett et al. (1958) attributed the linear decrease in body weight to a linear decrease in feed palatability as the nicarbazin concentration increased. However, neither of these studies measured for palatability. Domingues et al. (2014) reported that nicarbazin treated laying hens had a significantly lower number of taste buds than the hens of the control treatment. The alteration of the number of taste buds could influence the perception of taste and therefore decrease the palatability of the feed.

Birds will sample feeds that are accessible and will associate the internal ‘feeling’ induced by that feed with the sensory properties of the feed. Using this association, the birds will learn to differentiate between feeds that caused a positive metabolic response and those that caused a negative metabolic response therefore ‘learning’ which foods to avoid. A learned aversion has been observed with feeds of varying protein levels and/or amino acid imbalances. (Forbes and Shariatmadari, 1994). Birds fed a diet with a high level of protein had a significant decrease in feed consumption from five to nine weeks of age. It was a linear decrease over the four-week experiment period rather than an immediate decrease in feed intake (Shariatmadari and Forbes, 1993). A decrease in feed due to a learned aversion would take longer to be detected compared to palatability because it is a change in feed intake that is ‘learned’ over time through the association of metabolic changes. An increase in metabolism has been reported in birds fed nicarbazin (Farny, 1965; Bartov, 1989; Wiernusz and Teeter, 1995) which could affect feed consumption. Bartov (1989) reported that nicarbazin significantly decreased body weight gain but did not affect feed consumption. Bartov (1989) pointed out that this response was similar to those reported by May (1980) during the supplementation of T<sub>3</sub> which is known to increase metabolism.

The objective of this study was to determine if the decrease in feed consumption was due to palatability or learned aversion.

## **Materials and Methods**

Two trials were conducted to evaluate whether or not the broilers had a palatability issue or an aversion to eating feed with Nicarbazin. Trial 1 utilized 16 pens of 8 Ross708 females that were randomly assigned to two treatment diets, 90 ppm nicarbazin grower diet or control diet (Table 2). The pens were 5’ x 2’ and contained one hanging feeder and one drinker line. At 21

days of age, the feed in eight of the 16 pens was changed from the control diet to the nicarbazin diet. The remaining eight pens continued to be fed the control diet. Feed was weighed every four hours for the first 24 hours after the feed change. The feed was then weighed every 12 hours for the following two days. Nicarbazin was fed from Day 21 to 24 for a total of 76 hours.

Trial 2 utilized 24 5' x 4' pens of 12 Cobb500 by-product males assigned to two treatment diets, 90 ppm nicarbazin grower diet or control diet (Table 2). The pens contained one hanging feeder and one drinker line. At 23 days of age, the birds were individually weighed and sorted based on body weight. Birds were then randomly selected from each weight range to ensure the starting pen weight was similar for each of the 24 pens. Three days were given for acclimation to avoid a stress effect. At 26 days of age, 12 of the 24 pens were switched to the nicarbazin treatment feed. The remaining 12 pens remained on the control diet throughout the rest of the trial. Feed was weighed every 12 hours for 120 hours after the initial feed switch.

The differences in broiler strain, bird age, bird number, pen size, and pen number between the two trials were due to time, space, and bird availability. Measurement times were changed, and the sampling period was extended from 76 hours in to 120 hours from Trial 1 to Trial 2. These changes were made based on the results seen in Trial 1.

Feed consumption per bird per hour was calculated for each time measurement interval. The data were analyzed using a one-way ANOVA procedure (JMP Pro, ver. 15) with diet as the main effect. The means of variables were determined to be statistically significant at  $P \leq 0.05$  and were then separated using HSD Tukey test.

## **Results and Discussion**

In Trial 1, there was a significant ( $P < 0.05$ ) decrease in feed consumption for nicarbazin treated birds during 28 to 40 and 64 to 76-hour measurement periods (5.5g vs. 6.0g and 6.7g vs.

7.2g) (Table 21). During Trial 2, the birds fed nicarbazin had a significantly ( $P < 0.05$ ) decreased feed intake between 48 and 60 hours after nicarbazin treatment began (9.5 vs. 10.2g), but there was no statistical or numerical discernable pattern to which diet treatment group had consumed more feed (Table 23). The response in feed intake seen in the current study was not similar to that of the palatability of an increased salt concentrations (Gentle, 1971). The lack of a significant difference in the first 12 to 24 hours suggests that the decreased feed consumption observed with nicarbazin treatment is not related to palatability.

There was not a linear and/or consistently significant decrease in feed intake after the initial significant decrease occurred. These results differ from those seen with birds fed high protein diets (Shariatmadari and Forbes, 1993). Conclusive evidence that the birds developed a learned aversion to the nicarbazin feed was not provided.

In Trial 1, birds fed nicarbazin had a significantly lower total feed intake (348g vs. 369g), body weight (1,459g vs. 1,544g), and body weight gain (649g vs. 714g) than the control birds (Table 22). In Trial 2, the birds fed nicarbazin had a significantly lower body weight (2,193g vs. 2,265g) and body weight gain (868g vs. 945g) at the end of the 120-hour period (Table 24) similar to Trial 1. Unlike Trial 1, there was not a significant difference in total feed consumption between the control and nicarbazin diet groups (988g vs. 973g). There was not a significant difference in FCR during Trial 1 but in Trial 2, the nicarbazin treated birds had a significantly higher FCR than the control birds. The increased FCR for nicarbazin birds in Trial 2 could suggest that the effect of nicarbazin interferes with metabolic processes which supports previous claims (Farny, 1965; Bartov, 1989; Wiernusz and Teeter, 1995). More studies on palatability and metabolism need to be conducted in order to determine the cause of decreased feed intake in nicarbazin fed birds.

## **Conclusion**

The objective of the current study was to determine whether the decrease in feed consumption typically observed with nicarbazin treatment is caused by a decrease in palatability or a learned aversion. The decrease in feed consumption caused by nicarbazin was not proven to be related to palatability or learned aversion.

## **Future Research**

More research on palatability/aversion and metabolism related to nicarbazin needs to be completed in order to determine the cause of the decrease in feed consumption. If this study was to be repeated, it would need to use younger birds within the age range of 1 to 3 weeks of age in which nicarbazin is typically used and with a larger number of birds per pen. It would be helpful to conduct two different types of trials. One where only one diet is provided to the birds, nicarbazin and control, and the second trial type would be a preference style test where the birds could choose between the diets. The preference study is the most common study used to evaluate the palatability of feed. Blood tests to evaluate measures related to metabolism such as hormones T<sub>3</sub> and T<sub>4</sub> would be beneficial in determining if the decreased feed intake was due to a learned aversion due to metabolism.

**Table A1: The effect of nicarbazine on average feed consumed (per bird per hour, g) of broilers in Trial 1.<sup>1</sup>**

Treatment	N <sup>2</sup>	Hours after feed change									
		0-4	4-8	8-12	12-16	16-20	20-28	28-40	40-52	52-64	64-76
<b>Diet</b>											
Control	8	4.8±0.3	4.7±0.2	5.4±0.2	5.6±0.2	5.9±0.2	3.1±0.1	6.0±0.2	7.6±0.2	6.1±0.1	7.2±0.1
Nicarbazin	8	4.8±0.3	4.6±0.2	5.6±0.1	5.1±0.1	5.7±0.2	3.1±0.1	5.5±0.1	7.1±0.2	5.8±0.1	6.7±0.1
P-Value <sup>3</sup>		0.9218	0.9262	0.3550	0.0770	0.3937	0.7975	<b>0.0377</b>	0.0610	0.1418	<b>0.0162</b>

<sup>1</sup>Birds were 21 days old at the time of nicarbazine inclusion into the diet.

<sup>2</sup>Values are means of 8 pens for main effects. Pens contained 8 birds.

<sup>3</sup>Significant differences were determined at P≤0.05 by Student t's test. Significant P-values are bolded.

**Table A2: The effect of nicarbazine on body weight, weight gain, total feed consumption (g), and FCR in Trial 1.<sup>1</sup>**

Treatment	N <sup>2</sup>	D21 BW	D25 BW	BWG	TFC	FCR
<b>Diet</b>						
Control	8	830	1544	714	369	0.71
Nicarbazin	8	810	1459	649	348	0.74
SEM		13.4	21.4	12.0	6.4	0.01
P-Value <sup>3</sup>		0.2938	<b>0.0140</b>	<b>0.0021</b>	<b>0.0385</b>	0.1463

<sup>1</sup>Birds were 21 days old at the time of nicarbazine inclusion into the diet.

<sup>2</sup>Values are means of 8 pens for main effects. Pens contained 8 birds.

<sup>3</sup>Significant differences were determined at P≤0.05 by Student t's test. Significant P-values are bolded.

**Table A3: The effect of nicarbazin on average feed consumed (per bird per hour, g) in Trial 2.<sup>1</sup>**

Treatment	N <sup>2</sup>	Hours after feed change									
		0-12	12-24	24-36	36-48	48-60	60-72	72-84	84-96	96-108	108-120
<b>Diet</b>											
Control	12	9.4±0.1	9.2±0.7	9.4±0.4	9.9±0.4	10.2±0.3	8.8±0.3	9.9±0.2	9.7±0.5	11.6±0.3	10.4±0.3
Nicarbazin	12	9.9±0.2	8.2±0.4	9.6±0.2	9.9±0.5	9.5±0.1	8.5±0.5	9.4±0.3	10.2±0.4	10.9±0.3	10.3±0.2
P-Value <sup>3</sup>		<b>0.0419</b>	0.2039	0.6496	0.9453	<b>0.0260</b>	0.5894	0.1214	0.4599	0.1541	0.7565

<sup>1</sup>Birds were 23 days old at the time of nicarbazin inclusion into the diet.

<sup>2</sup>Values are means of 12 pens for main effects. Pens contained 12 birds.

<sup>3</sup>Significant differences were determined at P≤0.05 by Student t's test. Significant P-values are bolded.

**Table A4: The effect of nicarbazin on body weight, weight gain, total feed consumption (g), and FCR in Trial 2.<sup>1</sup>**

Treatment	N <sup>2</sup>	D23 BW	D31 BW	BWG	TFC	FCR
<b>Diet</b>						
Control	12	1320	2265	945	988	1.05
Nicarbazin	12	1325	2193	868	973	1.12
SEM		5.5	20.7	18.9	11.0	0.02
P-Value <sup>3</sup>		0.5597	<b>0.0225</b>	<b>0.0091</b>	0.3330	<b>0.0238</b>

<sup>1</sup>Birds were 23 days old at the time of nicarbazin inclusion into the diet.

<sup>2</sup>Values are means of 12 pens for main. Pens contained 12 birds.

<sup>3</sup>Significant differences were determined at P≤0.05 by Student t's test. Significant P-values are bolded.

## References

- Bartov, I. 1989. Lack of interactive effect of nicarbazin and dietary energy-to-protein ratio on performance and abdominal fat pad weight of broiler chicks. *Poult. Sci.* 68:1535-1539.
- Domingues, C.H.de F., S. Sgavioli, M.F.F.M. Praes, D.M.C. Castiblanco, P.C.A. Marchizeli, A.A. Pereira, K.F. Duarte, and O.M. Junqueira. 2014. Use of Nicarbazin, Salinomycin and Zinc Oxide as Alternative Molting Methods for Commercial Laying Hens. *Brazilian Journal of Poultry Science.* 16:25-30.
- Farny, D.G.O. 1965. Heat stress studies in nicarbazin treated chickens. Ph.D. Diss. Univ. Delaware, Newark.
- Forbes, J.M. and F. Shariatmadari. 1994. Diet selection for protein by poultry. *World's Poultry Science Journal.* 50:7-24.
- Gentle, M.J. 1971. Taste and its importance to the domestic chicken. *Br. Poult. Sci.* 12:77-86.
- May, J.D. 1980. Effect of dietary thyroid hormone on growth and feed efficiency of broilers. *Poult. Sci.* 59:888-892.
- Mushett C.W., F.V. Washko, K.L. Kelley, and A.J. Bloom. 1958. A Hematologic-Pathologic Study in Chickens Fed Graded Levels of Nicarbazin. *Poult. Sci.* 37:580-586
- Rozin, P. and T.A. Vollmecke. 1986. Food likes and dislikes. *Ann. Rev. Nutr.* 6:433-456
- Sammelwitz, P. H. 1965. Factors related to nicarbazin induced heat stress mortality in broilers. *Poultry Science* 44:1412. (Abstr.)
- Shariatmadari, F., and J.M. Forbes. 1993. Growth and food intake responses to diets of different protein contents and a choice between diets containing two concentrations of protein in broiler and layer strains of chicken. *Bri. Poult. Sci.* 34:959-970.
- Wiernusz, C.J., and R.G. Teeter. 1995. Nicarbazin effects on broiler thermobalance during high ambient temperature stress. *Poult. Sci.* 74:577-580.