Effects of environmental enrichment strategies on behavior and production performance of broiler breeder chickens reared at elevated temperatures

A Thesis presented for the

Master of Science Degree

The University of Tennessee, Knoxville

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May 2012

Dedication

This thesis is dedicated to my parents, Rev. Dr. Julius and Mary Adeniji as well as my siblings Tobi, Tomi, and Tola for their immeasurable love, support, and encouragements.

Acknowledgements

I would like to use this medium to express my unreserved appreciation to my major professor, Dr. Mike Smith for the rare privilege to carry out this research under his supervision. I thank my committee members, Drs. Hank Kattesh, and Brynn Voy for their support and useful suggestions. I am also indebted to Dr. Arnold Saxton for his immense contribution to the statistical analysis of the data.

Furthermore, I want to thank all the faculty and staff of the department of animal science, University of Tennessee, Knoxville for accepting me in to the Master program. I am also grateful for the enormous assistance from, Eddie Jarboe, Linda Miller, and Mary Roberts. I also want to thank the graduate students for the conducive atmosphere. I am thankful for wonderful friends, Aline Vaiciunas, Karianne Chung, Oby Washington, and Elizabeth Baldwin for their assistances in the course of the research.

Lastly, I would like to appreciate the support of my dear friend Olabisi Oladele and finally I am grateful to the Almighty God for the gift of life.

Abstract

Enriched housing systems are embraced as better alternatives to bare housing systems for table egg laying hens. However, attention is presently turning to the meat type (broiler breeder) laying hens. Hence, this experiment investigated the effects of perch structures and sharp sand on behavior, physiology, and production performance of broiler breeder chickens reared under elevated temperatures.

Two hundred and eighty eight broiler breeder females and 48 males (Cobb 500) at 21-weeks-old were assigned to their respective treatments in two different rooms. One room was maintained at 23°C to mimic a thermoneutral environment while the second room cycled between 23°C and 30°C to mimic a heat stressed environment. Each room had all four treatments and the treatments were replicated three times with 12 females and two males per replicate. The four treatments were sand (S), perch (P), a combination of sand and perch (SP), and control (C). Production performances (hen-day egg production, egg weights, floor eggs, fertility, and hatchability), Physiology (cortcosterone levels, heterophil/lymphocyte ratio), and behavior parameters were determined.

The production data were analyzed using a completely randomized design (CRD) with repeated measures while the physiology and behavior parameters were analyzed using CRD split plot with sampling and a factorial in the whole plot. The results indicate that S and SP increased (P=0.0138) egg production. Enrichment S, P, and SP reduced the occurrences of floor eggs. HS environment increased (P=0.0435) bird's corticosterone levels in broiler breeder males and tended to increase (P=0.07) female corticosterone levels.

There was a trend (P=0.0792) for increased preening in the HS room. The birds in treatment P tended to preen more (P=0.0655) than those in S. The frequency of attempted mating was least (P=0.025) in treatment P. In the TN environment, broiler breeders reared in treatments S, SP, and C performed more (P=0.0029) completed mating than their counterparts in the HS room. The duration of sand use was higher (P<0.05) in the TN room and there was a trend (P=0.0543) for increased overall perch use in treatment SP than P. In conclusion enriched environments positively impact production of broiler breeders under stress condition.

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Introduction

Animal welfare in the poultry industry is an ongoing topic of interest by the consumers of poultry products. These consumers are concerned about how animals are handled before, during, and after processing. For many years, there has been much emphasis on the management of table egg laying birds (hens) because they were reared in cages that provided limited amount of space for behavioral expressions. The consuming public in the form of large food supply companies are basing their purchases of eggs on audits of production conditions. The audit has led to different types of egg branding such as cage free eggs, cage eggs, and barn eggs (Mench, 2008; Thompson, et al., 2007). However in recent time, attention has turned to the meat type birds parent stock usually referred to as broiler breeders because of the feed restriction that is usually imposed on broiler breeders. Criteria to determine how positive or negative the welfare of an animal is include behavior, production, and physiology. Positive welfare in poultry is attributed to the expression of comfort behavior, better production performance, and reduced circulating levels of stress hormones. Negative welfare in broilers is linked to stress and stressful conditions that adversely affect behavior, production and physiology.

Stress is any condition that interrupts the homeostasis of the body (Virden and Kidd, 2009). Stress is also described as any condition that generates a nonspecific response of the body to any demand made upon it (Moberg and Mench, 2000). Stress is initiated by stressors and stressors come in diverse forms such as extreme temperature variations, poor management practices (extreme beak trimming, contaminated premises, uneven feed distribution), and illness (Rosales, 1994). Although stress is said to be detrimental to the poultry industry, some activities from which birds derive pleasure also result in stress conditions. Nonetheless, it cannot be ruled out that prolonged stressful conditions of any form cause detrimental effects in its subjects (Moberg and Mench, 2000).

Including different forms of environmental enrichment in poultry housing has been considered in attempting to combat these stressful conditions. Environmental enrichment is defined as "the addition of biologically relevant features to animals' environment to foster natural behaviors" (Leone and Estevez, 2008). Natural behaviors include the behaviors exhibited by birds in the wild or when left alone on a free range system. The diverse forms of enrichment fall into 3 categories (1) those that yield positive results, (2) those that produce negative results, and (3) those that are almost irrelevant. Some simple enrichment strategies that had been investigated in poultry housing include visuals, audio sounds, structures, plastic materials, and smells. As birds get attracted to and become comfortable with the presence of enrichment strategies in their housing, stress in the form of fear is reduced (Brake, et al., 1994; Newberry and Blair, 1993). Enrichment also keeps the birds busy such that the rate of aggression to their counterparts is reduced. The birds' cognitive skills are also improved such that they cope better when transferred to unfamiliar environments (Meehan and Mench, 2007). Some forms of enrichment increased egg production, fertility, hatchability, as well as reduced floor egg occurrence.

Environmental enrichment strategies cannot be discussed without mentioning the European Union (EU) directive on the welfare of laying hens. The directive was put into effect in 1999, stating that conventional laying cages should be phased out by the year 2012. Research carried out in the early years of the directive was on table egg laying birds because this specie was emphasized in the directive**.** However, currently attention is beginning to turn to meat-type birds. One of the outcomes of the EU directive of 1999 on the welfare of laying hens was that the use of enriched pens has spread to other regions of the world, with different climatic conditions. This spread is partly due to similar growing welfare concerns as well as importation and exportation of poultry products. Since the directive recommends the use of enriched cages, there is a need to conduct research to evaluate the effect of other forms of environmental enrichment strategies on the behavior and production performance of meat type birds. Despite the benefits of most enrichment strategies, it is important to further evaluate the effects of other forms of enrichment strategies. This study hypothesized that 1) enriched environments will positively impact production of broiler breeders under heat stressed condition and 2) enriched environment will reduce stress level of broiler breeders subjected to heat stress.

Therefore, this research examined enrichment in the form of sand and perch because they are less expensive, made of relatively available materials, and are parts of the basis for all forms of enrichment in laying hens (Appleby and Hughes, 1995). Furthermore, these evaluations may determine if the results achieved in a thermoneutral environment will be similar to that of a heat stressed environment.

Objectives

1) To evaluate production in broiler breeders reared in environmentally enriched pens under thermoneutral and heat stress conditions.

2) To explore the links between stress indicators, behavior, and fertility in broiler breeders reared in environmentally enriched pens under thermoneutral and heat stress conditions

Literature review

Animal housing and welfare

Good health and welfare are important not only to animals but also to human beings since some diseases are zoonotic. Therefore, proper management practices need to be put in place for positive cohabitation between humans and animals. Research assessment has concluded that measures to evaluate animal welfare in poultry include behaviors, production, and physiology (Fraser, 2003; Sandøe, et al., 2003). Some of the welfare issues in the poultry industry originated as a result of general consumer concerns pertaining to unsatisfactory poultry housing systems, questionable management practices in the poultry houses, and ambiguous behaviors that were expressed by confined birds. The use of deep litter systems and conventional laying cages were the two basic forms of housing systems in the poultry industry. The deep litter system of housing was originally used on a large scale in the early years of poultry domestication. The deep litter housing is characterized by birds being reared in confinements that had insulated floor materials such as slat or litter. Furthermore, feeders, drinkers, and nest boxes (in the case of laying hens) were included in the deep litter housing. The deep litter housing provided enough space for the expression of whatever behaviors the birds' deemed fit as well as increased interactions with their counterparts, since poultry are social animals.

In the deep litter housing, there were higher occurrences of floor, dirty, and contaminated eggs as well as cannibalism, feather pecking, and foraging behavior. These poor egg conditions can be associated with direct contact of birds with their feces. Furthermore, the limitations attributed to the deep litter housing as a result of aggressive behaviors were probably due to the available spaces. As a result of the negative behaviors expressed by the birds reared on deep litter housing systems, concerns have been expressed by the producers and consumers of poultry products. As time progressed, the use of conventional laying cages were introduced and adopted as better alternative to the deep litter housing system. On the contrary, It was only a matter of time before the conventional laying cages were also deemed questionable (Tauson, 1998).

Conventional laying cages were constructed to raise birds from the chick stage to adulthood (Duff, 1969) and are made of galvanized metals and or plastic materials. Typically conventional laying cages are long with several compartments and each compartment house a small number of birds (3-5). Each conventional laying cage compartment had a metal door, a sloped floor (for eggs to roll out), a feeder, and a water trough attached. The conventional laying cages were constructed in different shapes and sizes such that the long cages with the several compartments could be stacked on each other thereby forming different levels (about 3-10 or more). The stacked cages were constructed such that the droppings from the birds in the upper level cages did not fall on the birds underneath. The benefits of conventional laying housing include better egg condition, easier management, and less contact of the birds with feces. However, boredom, movement restrictions, as well as limited behavioral expressions were some of the consequences of the conventional laying housing.

Considering the benefits and limitations of the deep litter housing systems and conventional laying cages, it became difficult to determine with certainty how animal welfare should be defined in terms of the different housing conditions. A way to resolve these housing issues was to include environmental enrichment into the poultry housing systems. When environmental enrichment were included into the poultry housing systems, such housing systems were referred to as enriched housing or enriched housing systems. The use of enriched housing was a directive established by the European Union (EU) in 1999 that is to be fully implemented by the year 2012.

According to the EU directive of 1999, the housing requirement for hens include (1) 750 cm^2 per bird (nothing less than 600 cm^2 and should be at least 45 cm high), (2) a minimum total cage area of 2,000 cm^2, (3) a nest, (4) litter, (5) 15 cm perch per hen, and (6) 12 cm of feed trough per hen (Appleby, 2003; Bessei, 1992). Although nest and litter were the forms of enrichment strategies emphasized in the EU directive, other forms of enrichment strategies were explored for birds' benefit. The enrichment materials in the enriched housing attracted the birds and reduced aggressive pecking and fighting between pen mates. However, enriched housing is expensive to implement. These three major housing systems (deep litter, conventional laying, and enriched) had their benefits and limitations. When laying hens were placed in the three housing systems, the birds reared on a deep litter system had the highest corticosterone levels, least egg production, spent more time dust bathing and performing scratching behaviors on the litter materials. The birds in the enriched environment expressed reduced aggression, lower body weight and higher corticosterone levels compared to birds in the conventional laying cages (Pavlik, et al., 2008). Laying birds reared in conventional laying cages weighed more than those reared in enriched housing (Neijat, et al., 2011). This weight difference can be linked to a lack of exercise and the limited space available to the birds. There were no differences in the measures of production performance (overall egg production, egg weight, egg quality, body weight, and feed conversion ratio) between the conventional and enriched (perch, scratchpad, and curtained nesting area) housing systems in laying hen (Neijat, et al., 2011).

Furthermore, other aspects of welfare interest in the poultry industry encompass housing densities, behaviors (Dawkins, et al., 2004), physical conditions of health, production performance, physiological responses, behaviors, and feelings (Duncan, 1998; Jones, et al., 1996; Jones and Roper, 1997; Mills and Faure, 1990). In addition, the poultry industry identifies production performances of birds as another area for emphasis. Thus, poor production performance indicates poor welfare (Mashaly, et al., 2004). Interestingly, including environmental enrichment into poultry housing systems had been reported to greatly improve poultry welfare. For example, some forms of environmental enrichment lower physiological responses to stress conditions (Maxwell, 1993) and alarming behaviors as well as increase egg production, fertility, and hatchability in poultry (Leone and Estevez, 2008). Considering the advantages and disadvantages of the three housing systems, enriched housing is not necessarily free of faults but it is considered as a better housing system for poultry.

The EU directive on welfare of laying hens has spread to different climatic regions of the world and is currently being implemented. Moreover, poultry producers in the high temperature regions of the world would also include enrichment in their poultry housing systems. Heat stress constitutes a major concern to poultry producers in the tropics. Hence, regardless of the housing system employed heat stress may negatively influence production performance, the immune system (Bartlett and Smith, 2003), and behavioral performances in poultry (Duncan, 1998). It is important to identify the forms of environmental enrichment that are suitable for high temperature environments. Presently, the Humane Society of the United States (HSUS) and the United Egg Producers (UEP) are working to ensure a smooth, easy, and much clearer transition process from the use of conventional laying cages to the utilization of enriched cages. They insist that the hen housing specifications that are put in place by the HSUS and UEP should be fully implemented through federal legislation, such that it would cut across every state in the nation by the year 2029 (Smith, 2011).

Broiler Breeder Production

Broiler breeders are relied on for further production of day old broiler chicks typically known as “meat type” birds. Broilers are known for the huge amount of flesh they accumulate within a short period of time. They have their genetic make-up continually improved for efficient growth response, especially when fed without restriction. Broiler breeders are reared on deep litter housing systems with wood shavings or other forms of litter material on the floor as means for insulation. Commercial broiler breeders are not raised in conventional laying cages which appear to be controversial to the consumers. Nonetheless, welfare concerns still apply to broiler breeders in other areas such as feed restrictions, skin lesions, and aggressive behaviors. These areas of welfare concerns are related in that feed restriction may lead to the expression of aggressive fighting and pecking between conspecifics, with a target on the skin and other body parts of the birds. Therefore, feed restriction can be described as a possible foundation on which skin lesions and aggressive behaviors are built (King, 2001). When the feed of broiler breeders was rationed, birds ate faster in order to consume as much feed as possible before there was another long wait for the next meal. Continuous feed restriction sometimes results in survival of the fittest and as a result, birds subconsciously develop the habit of moving very fast to obtain feed (Petherick and Rutter, 1990; Savory, et al., 1993). When feed restrictions lasted for a short period of time, birds kept themselves busy and explored the locations of their environment that had enrichment materials or structures (Jones, et al., 2004; Nicole and Guilford, 1991). As feed restrictions progressed for longer periods of time, birds became more aggressive with an increased tendency to peck, and fight with their counterparts (Jones, et al., 2004; King, 2001). Interestingly, the occurrence of aggressive behavior was reduced in a larger flock compared to a smaller flock especially at appropriate housing densities (Estevez et al, 2003). Furthermore, in a large group size it would be impossible for birds to recognize all pen mates and as a result reduce aggressive behavioral expression. De Jong et al, (2003) reported that when broiler breeders were subjected to feed restrictions at different levels (90%, 70%, 50%, 35%, 25% of ad-libitum intake), the behaviors affected were lying down and idle periods.

Over the years, further research found that feed restriction can also be beneficial to broiler breeders. Feed restriction reduces the incidences of illness such as prolapse and leg problems that occur in broiler breeders as a result of high rate of muscle accumulation (Bessei, 1992; Weeks, et al., 2000). Feed restriction increased the rate of egg production and prolonged production capacity (Robinson, et al., 1991). In broiler breeder females, unrestricted feeding leads to rapid development of the ovarian follicles. Ovarian follicles of chicken occur in single hierarchy due to normal ovulatory cycle. However, unrestricted feed consumption disrupts the single hierarchy thereby becoming multiple hierarchies (Robinson and Wilson, 1996). One of the effects of the multiple hierarchies is the increased production of egg yolks that pair up to form double yolked eggs. Double yolked eggs are poorly shaped and as a result, are not good to set. If unrestricted feed is prolonged, early reduction in egg production results (Robinson, et al, 2003). Feed restriction also improved fertility (Cerolini, et al., 1995) in breeder birds. Considering these benefits of feed restriction, it is therefore advantageous to restrict feed consumption in broiler breeder birds for better health and production performance in their adult life. Broiler breeders are kept for about 60 weeks or more and by including environmental enrichment to their housing, feed restriction would be more bearable. When birds are allured to the enrichment structures in their environment, the impact of aggression to their counterpart and the rate of mortality are reduced (Gvaryahu, et al., 1994). The enrichment structures that are made available in the poultry environment keep the birds busy and increase foraging behaviors in broiler breeders. Enrichment in the form of perch structures acts as a shield for birds away from aggressive counterpart. Adding brightly colored moving light, intermittently projected on the floor of the broiler house as well as scattering whole wheat on the floor of the pen engage broiler breeder (Bizeray, et al., 2002). Including sand into the environment of broiler breeders also ameliorate the effects of feed restriction since birds could consume and forage on sand without adverse effects.

Production Performances

Increased rate of egg production is of great importance to the poultry industry particularly the broiler breeder sector. The addition of enrichment strategies in the form of nest box, scratch pad, and perch had no effects on egg production and egg weight but increased the occurrence of dirty eggs (Tactacan et al., 2009). However, birds reared in enriched cages (perch, nest box, and sand as a dust bathing material) laid significantly higher number of eggs compared to the control (Pohle and Cheng, 2009). Zhao et al., (2009) reported that including 4 - 6% of sand into the diet of laying hens increased the rate of egg production. However, increased egg production was absent when sand was added to the feed of laying hens at 2, 4, and 8% inclusion levels. Similarly, (van dar Meulen, et al., 2008) found that adding 0, 10, 20, 25, and 30% sand inclusion levels did not affect egg production.

Eggs that are not laid in the nest boxes (floor eggs) constitute major concerns to the broiler breeder production because of the higher risk of contamination. Nest box is a form of enrichment that reduces the occurrences of broken eggs (Appleby and Hughes, 1995). There is a higher preference for metal littered nest boxes compared to wood littered nest, and a metal nest with rubber mat (Holcman, et al., 2007). Furthermore, birds demonstrate a high preference for the lower nest compared to the nest boxes that were located higher. The incidences of floor eggs decrease with age in laying birds and in most cases, floor eggs are usually laid by the same birds (Cooper and Appleby, 1996). There is a higher occurrence of floor egg laying in broiler breeders compared to commercial laying hens (Sheppard and Jewitt, 2002). The strain of birds also influences the rates of floor eggs even in the presence of nest boxes (Singh, et al., 2009). Adding cover panels (Leone and Estevez, 2008) and perch structures to poultry housing reduced the incidence of floor eggs in laying hens (Gunnarsson, et al., 1999) and broiler breeder hens (Brake, 1987). Moreover, floor egg numbers were reduced in the laying birds that actually perched (Appleby, et al., 1983).

Improved egg fertility and hatchability are necessary for further production of chicks. In one study, eggs collected from broiler breeder houses at five hour intervals hatched better than those picked up at one hour intervals (Kirk et al., 1980). Broiler breeder males express almost no courtship behaviors and as a result aggressively force the broiler breeder females to mate. This forceful mating causes damage to different parts of the females' skin (Jones, 2002) and could be the reason why broiler breeder females spend more time on the slat portion of their housing (Jones and Prescott, 2000). In light of this, adding environmental enrichment into broiler breeder environment ameliorates the occurrence of mating concerns. Enrichment continuously attracts the female birds to the floor of the pen where the males are predominantly located for mating to take place. Broiler breeder females seek refuge within the perch, away from forceful and aggressive mating demonstrated by the males. Birds that feel threatened may jump on the perch or stay within the perch, especially in the case of four cornered or round perch structures (Appleby, 2003; Appleby, et al., 1992b). In addition, including enrichment in the form of a cover panel into broiler breeder environment improved fertility and hatchability (Leone and Estevez, 2008).

Heat Stress

The effects of heat stress are very expensive to the poultry industry especially with regards to broiler breeder birds. To the poultry farmer, heat stress affects every stage of production by reducing growth rate, reducing number of eggs laid, and increasing susceptibility to disease. Great amounts of resources are put into reducing the detrimental effects of heat stress to the barest minimum since it cannot be totally avoided. Prolonged heat stress conditions are injurious to broiler breeders especially in regions of the world where high environmental temperature is the norm. The average body temperature for broiler breeders is 41°C and the optimum temperature for growing broilers ranges between 18 - 22°C (Charles and Walker, 2002). Cycling and constant high temperature environments both negatively affect the production performances of broiler breeders. Birds reared in a heat stress environment (32°C) experienced significant decrease in body weight, egg production, egg weight, shell weight, and shell thickness (Beaumont, et al., 1998). Furthermore, the mortality rate was increased in constant high temperature environment compared to the cycling high temperature environment. Beaumont et al., (1998) also found that birds raised in a high temperature environment had decreased body weight gain heritability without significant alteration in feed efficiency heritability. When three lines of broilers were reared in Fall, body weight gain and feed consumption were similar across the 3 different chicken lines. However, reduced weight gains were experienced in the same broiler lines during the summer (Yalçin et al. 1997; Settar et al., 1999). Beaumont et al. (1998) reported that when birds were exposed to high environmental temperature, the feed efficiency of the birds remained the same but the heritability of body weight gain was decreased.

When birds are exposed to heat stress, the different poultry systems (hormonal, immune) have initial mechanisms to cope with the stress until it gets out of control. The physiological responses of the body to different types of stressful conditions follow similar mechanism, usually referred to as the Hypothalamic-Pituitary-Adrenal pathway (HPA). According to the HPA (Siegel, 1971) mechanism, the presence of stress is detected by the cortex of the brain, which sends a specifically sympathetic stimulation to the hypothalamus. The hypothalamus sends hormonal signals to the pituitary, and the pituitary sends hormonal signals to the adrenal gland in the form of adrenocorticotropic hormone (ACTH) to release glucocorticoid. The glucocorticoid of interest in poultry is corticosterone. If the stressor persists and corticosterone levels remain elevated then the deleterious effects of corticosterone become evident. Heat stress increases the susceptibility to diseases and dietary electrolyte imbalances in broilers. Borges et al., (2004) reported that elevated temperature in birds (broiler breeder males) results in hemodilution. In turn, the birds experienced decreased levels of blood Na, K, Cl, and lymphocyte when exposed to high temperature as well as increased blood bicarbonate, and heterophils.

Moreover, prolonged heat stress (Lin, et al., 2005) results in economic losses (St-Pierre, et al., 2003), impaired production performance (McDaniel, et al., 1995), altered meat quality (Akit, et al., 2005), reduced fertility in broiler breeder males (McDaniel, et al., 1995), and at very extreme levels, negatively affects digestibility of proteins, fats and starches (Bonnet, et al., 1997). In order to combat the detrimental effects of heat stress and other forms of stress, poultry have been subjected to genetic selection for heat tolerance traits (Deeb and Cahaner, 2002; Settar, et al., 1999; Yalcin, et al., 1997), several corresponding nutritional strategies (Cahaner, et al., 1995), different feeding regimen, and incorporation of environmental enrichment strategies (Lin, et al., 2005). Including enrichment strategies in the form of perch structures reduces the effect of heat stress such that there is better air flow beneath the birds when they roost on the perch structure and hence, better cross ventilation (LeVan, et al., 2000).

Environmental Enrichment

The addition of environmental enrichment in broiler breeder housing systems aim at stimulating birds in confinement to express behaviors as though they were in the wild or left alone on free range (Leone and Estevez, 2008). Including enrichment strategies in the form of nest boxes, dust baths, and perches, encourage birds in confinement to express nesting, dust bathing, and perching behaviors respectively. These behaviors are important to birds in confinement because they would naturally express these behaviors in the wild, or when reared on free range housing systems. Hence, nest boxes, dust baths, and perch structures are described as the basis for all forms of enrichment (Appleby and Hughes, 1995). Previously researched enrichment strategies in the poultry industry occurred in visual, auditory, olfactory, and tactile forms. The inclusion of enrichment in the poultry house, depending on the form of enrichment, attracts the attention of the birds but in most cases, the interest is short lived.

Visual Enrichment

Visual forms of enrichment, such as computer screen savers and televised images, attract poultry. Research showed that chicks demonstrated higher preference for screen savers that displayed images compared to a blank screen (Clarke and Jones, 2000), and moving images instead of non-moving ones (Bolhuis, 1999; Ten

Cate, 1989). When televised chicken images were projected in the house of adult chickens, there were behavioral imitation and solidarity in the spectator counterparts (Clarke and Jones, 2001; Keeling and Hurnik,1993; McQuoid and Galef, 1993). The incorporation of visual forms of enrichment progressively attracted the attention of birds, especially those exposed to several images (Jones, et al., 1998). The interests of the birds fade away within few weeks on exposure to the same images over time. The projected images need to be changed frequently so as to sustain the interest of the birds. The benefit of the visual form of enrichment is that birds show less fear when transferred to unfamiliar environments without the visual form of enrichment (Clarke and Jones, 2000).

Audio Enrichment

Audio enrichment include sounds, however, the results were inconsistent. Birds react to sounds differently and are at alert in the case of strange sounds. Audio enrichment in the poultry house occurs in the form of music, water- hose, and aircraft. When hens were exposed to music, water-hose, and train sounds at an intensity range of 90-100 dB, the birds preferred the location of their environment with less sound (McAdie et al. 1993). Furthermore, sound at 90 dB in the form of background noises plus truck, train, and aircraft playbacks for 60 minutes also increased H/L ratio in 36-week-old birds compared to the control birds (McAdie, et al., 1993).

Music is an auditory form of enrichment. It reduces circulating heterophil to lymphocyte (H/L) ratio in laying hens and reduces fear in the form of strange and sudden noises to chicken (Anonymous, 1994; Gross and Siegel, 1983; Jones, 1989; Maxwell, 1993). However playing of country and classical/jazz for eight hours reduced

H/L ratio, increased feeding, preening, and shaking of head in laying hens (Ladd, et al., 1992). The incorporation of music into enrichment structures and filial imprinting were found to increase feed consumption in broiler chicks, especially when the music was played intermittently (Gvaryahu, et al., 1989). Unfortunately, the positive increase in feed consumption was absent when birds were raised in heat stress rooms with similar enrichment strategies. There are speculations that playing music, depending on the type of music, may also calm the farm workers down to perform their duties to the birds (Jones, 2002). The intensity of different sounds also affects the reaction of poultry birds. Exposing birds to sounds from fans and chicken vocalization at 104 dB for 30 seconds increased their circulating H/L ratio (Gross, 1990).

Olfactory Enrichment

For several years, the sense of smell was described as poorly developed in birds (Fischer, 1975; Wood-Gush, 1971). However, further research found behavioral responses to olfaction in chickens (Fluck, et al., 1996; Jones and Black, 1979; Jones and Gentle, 1985; Jones and Roper, 1997; Marples and Roper, 1996; Rogers, 1995; Stattelman, et al., 1975; Vallortigara and Andrew, 1994). Olfactory form of enrichment is described as the inclusion of odorants into poultry environment particularly to reduce fear. Thus, the transfer of chicks to new environments with familiar odorants ‘re-assure’ the chicks of safety (Jones and Roper, 1997). When birds were transferred to new environments that had the option of clean wood shaving location and soiled substrate locations, birds expressed preference for the familiar soiled substrate locations, typical of their initial environments (Jones and Faure, 1982; Jones and Gentle, 1985). Burne and Rogers (1995) found that when chicks were reared in an environment that had soiled litter from adult hens, the chicks grew accustomed to the odor. Furthermore, when the same chicks were transferred to a new environment, the birds preferred the familiar odor tube to an unscented tube.

Chicks express preference for some odor such as Vanillin compared to others such as garlic (Jones and Roper, 1997). The exposure of chicks to geranium oil odor increased preening, pecking, vocalization, and movements (Jones and Gentle, 1985). The transfer of chicks to new environments increases fear and chicks disperse in the new environment until fear is reduced (Gallup,1983; Jones and Mills, 1999; Vallortigara, et al., 1990). When chicks get attracted to certain odors, they easily identify and associate with familiar smell in the presence of alternative odorants in a totally different environment. Jones et al. (2002) reported that when previously exposed chicks to Vanillin were moved to a new environment, the chicks moved to the section of the room with Vanillin. Furthermore, the chicks ate in the presence of Vanillin and demonstrated a trend of increased preening behavior as well as pecked at the environment more frequently. Two different chicks were reared in separate environments that had Vanillin and both were transferred to a new environment that also had Vanillin. One would expect initial aggressive pecking or fighting between the strangers in an unfamiliar environment instead, the birds moved closer to each other and stayed together in a ten minute observation (Jones and Redman, 2002). Introducing new birds into an existing flock leads to aggressive fighting or pecking by the resident and new birds. Therefore, it would be important to further examine the effect of the olfactory form of enrichment on aggressive behavior in older birds upon introduction of new birds in an existing flock.

Chicks adjusted faster to sudden change in the form of their feed (from mash to pellet) when the feeder was treated with a familiar odorant (Jones, 2000).

Enrichment structures.

Other structures used as enrichment include toys (Lindberg and Nicol, 1994), rubber tubing, colored plastic keys, table-tennis balls, strings (Jones, et al., 2002) chains, beads (Jones and Rayner, 2000), nest boxes (Appleby and Hughes, 1995), and cover panels (Leone and Estevez, 2008).

Perch structure

Perching is usually referred to as roosting. It is a natural behavior of birds both in the wild (Collias and Collias, 1967) and in confinement, as long as elevated structures are available. Perch structures usually vary in height, shape, and material. The shape of the perch can be in the form of squares, circles, rectangles, or simply length of materials supported on both sides. Apart from perch structures, birds utilize other forms of elevated structures or surfaces in their housing as a means to perch. Other surfaces in the chicken house that are perched on by birds include the horizontal female feeders and handles of nest boxes. Some of the factors influencing the use of perch structures by birds in confinement include perch height, perch materials, and the time of its introduction into the bird’s housing. Female birds perch more in the morning, whereas male birds perch more in the afternoon, and both males and female birds perch most at night (Appleby et al., 1983). Birds reared on rectangular perch structures showed reduced foot pad problems compared to circular perch structures (Appleby, 1998; Duncan, et al., 1992; Faure and Jones, 1982a). Further research found that early introduction of perch structures to the poultry environment gave birds the opportunities to explore and be familiar with the perch use faster. (Gunnarsson, et al., 2000).

Another factor that influenced the use of perch structures in poultry is genetics (Faure and Jones, 1982b). Genetics was confirmed to influence the use of perch after comparing the effects of sex, strain, and type of perch materials on three different strains of domestic birds. Results from the experiment found that birds were well distributed in the pen especially in areas with perches. Lower perches were preferred by chicks and broiler breeders instead of higher ones. Some breeds of birds perched more than others. There were no significant differences in the use of different perch materials by broiler birds (wood and wire). Moreover, square perches were preferred by birds compared to lengths of wood.

Upon significant reduction in the bursa weight and bursa to body weight ratio, Heckert, et al. (2002) reported that the inclusion of perch structures into older broiler birds' housing could be a threat to their immune system. Nonetheless, some of the benefits of perch structures in poultry housing systems include the increase in explorative ability of birds and the increase in physical activities which in turn decreases associated leg problems that are typical to broilers (Kestin, et al., 1992; Thorp, 1992). Perch also improves bone strength (Appleby, et al., 1992b), and maximizes the use of different locations in the poultry houses where the perch structures are placed (LeVan, et al., 2000; Newberry and Shackleton, 1997). Furthermore, perch structures also serve as refuge for attacked birds from aggressive mates (Appleby and Hughes, 1991), increases the space available to the birds

(Gunnarsson, et al., 2000), and also reduces the incidence of floor eggs (Appleby, et al.,1983).

Sand

In the wild or a free range system, birds may have access to sand and carry out several activities on sand. Some of these activities include eating the sand, foraging on the sand, and dust bathing in the sand. These activities are also expressed by birds reared in confinement (Arnould, et al., 2004; Shields, et al., 2004), particularly as they age (Shields, et al., 2005). In the absence of sand, birds still express these behaviors on other litter material. In confinement, the use of sand had been explored as a substitute litter material for broiler birds. Thus, results have shown that sand can replace wood shavings as litter material. Furthermore, after slaughtering and processing the carcass of chicken, it was reported that broiler breeders reared on sand as litter material showed significantly lower abdominal fat yield in comparison to those raised on pine shavings. Upon evaluating the different types of bedding, birds reared on sand as litter material harbored less harmful micro-organisms such as coliform bacteria, which include *E. coli* and aerobic bacteria (Bilgili, et al., 1999), as well as lower body temperature, (Gernat, 2009).

The frequent pecking and scratching of sand when added to the environment of poultry blunt out their beak and claw lengths thereby reduces the rate of damage to other birds (Fickenwirth, et al., 1985). Adding sand to poultry feed improves the digestibility of certain feed ingredients such as brewer’s dry grain (BDG) (Leone and Estevez, 2008). Including high levels of sand into layers’ diet (about 20-30%) diluted the feed and increased feed consumption (Van Der Meulen, et al., 2008). Sand is relatively available and biologically has a lower percentage of moisture content when compared to pine shavings. When given a choice, broiler birds prefer sand as bedding instead of wood shavings (Gernat, 2009), pine wood, rice hull, and recycled paper (Shields, et al., 2005). Sand emits more ammonia since it's an organic material (Miles, et al., 2011). There is also a buildups of nutrients in broiler housing systems that have sand as litter material (Bowers, et al., 2003). The nutrient build up in the sand can be beneficial when used as fertilizer.

In an experiment carried out by Vestergaard et al. (1997), laying birds were placed in two different cages containing either sand or wire floor. After some months, the birds were swapped between the two treatments such that the birds in the sand were later housed in the wire floor house and those initially on the wire floor were later housed on sand. The research lasted for 2.5 years. Findings showed that birds initially housed in cages with sand and later deprived of sand, experienced significant increase in corticosterone levels. Furthermore, the birds initially reared on wire floor and transferred to the cages with sand did not experience any change in their corticosterone level.

Enriched cages are being sold, but are relatively expensive. Some forms of enrichment are not in constant use by the birds for as long as they are included in their environment (Jones, et al., 2000; Mench, 1994; Newberry, 1995; Sherwin, 1995), while others initiate the expression of aggressive behaviors.

Behavior

Poultry welfare, defined from the psychologist perspective, encompasses poultry behavior. Behavior is a fast and positive indicator of an animal's condition. Poultry behavior can be determined by recording, observing, and analyzing birds that are expressing their natural behavior. A good understanding of poultry behavior is important when considering welfare in the production and management of poultry. Poultry behavior can be better explained by examining birds in their natural habitat compared to the birds reared in confinement. It is noteworthy that the expression of some behaviors by birds in the wild does not necessarily mean that it is important and should be expressed in confinement (Craig and Swanson, 1994). The EU directive of 1999 on the welfare of laying hens was passed due to the inability of birds to express natural behaviors and the restriction of the birds' movement (Appleby, 2003). This indicates that consumers of poultry products pay great attention to poultry behaviors (Jones, 2002). Some poultry behaviors include comfort, mating, and aggression. Some individual behaviors include nesting, roosting, scratching, dust bathing, and preening.

Nesting Behavior

Nesting behaviors are expressed in birds especially the laying birds. Nesting behavior is typical when birds are reared in deep litter systems. To perform nesting behavior, birds identify cool, calm, and isolated locations of their environment. Birds then scratch the litter to form a concave shape on the litter (Petherick and Rushen, 1997). Nesting behavior in birds is activated by abrupt hormonal fluctuation and usually occurs prior to egg laying (Wood-Gush, 1975; Wood-Gush and Gilbert, 1973). Nesting behavior occurs regardless of the environmental temperature (Duncan, 1998) and hens locate nest site at the time of egg laying (Cooper and Appleby, 2003; Follensbee, et al., 1992). In the deep litter housing system, poultry producers include nest boxes into the poultry housing for the birds to lay their eggs. The nest box serves as an enrichment structure which mimics the natural environment where birds lay their eggs. Birds reared in the cages also express nesting behaviors, but are characterized by restlessness, escape behavior, and stereotypical pacing (Appleby 1992; Baxter, 1994; Duncan, 1970; Hughes, 1983; Sherwin, 1992; Wood-Gush, 1972; Yue and Duncan, 2003).

Perching and Roosting Behavior

Perching behavior is a means by which birds protect themselves from predators especially at night. Interestingly, birds display perching behavior at different times of the day either in confinement or when left alone in the wild. Perching behavior is so important for birds such that they go through ordeals to access perch structures (Baxter, 1994; Olsson and Keeling, 2002; Olsson and Keeling, 2000).

Scratching Behavior

Scratching is another behavior of birds that is expressed both in wild and in confinement. In the wild, birds explore their environment by scratching the ground in search for food. Birds naturally scratch the ground even when placed on solid floors such as wood or concrete. Studies have shown that birds spend more than 50% of their active time expressing foraging behavior (Savory 1978; Dawkins 1989). In the battery cage system, hens still scratch the floor of the cage even in the presence of feed (Dawkins, 1989; Duncan and Wood-Gush, 1972a; Duncan, 1972).

Dust bathing Behavior

Dust bathing is characterized by birds crouching or rolling in the litter on floor. At the end of this activity, the birds get up and vigorously shake the litter particles off their feathers. Dust bathing is triggered by internal and external (heat, and light) factors (Duncan, 1998; Vestergaard, 1980; Vestergaard, 1982). Dust bathing is important for feather grooming and the removal of external parasites. Research has found that dust bathing is important to balance lipid levels in the feathers (Olsson and Keeling, 2005; Shields, et al., 2004; Van Liere and Bokma, 1987). Since dust bathing is partly due to internal factors, birds reared in the battery cage system and even featherless chickens also dust bath (Vestergaard et al., 1999). Furthermore, it had been reported that dust bathing is not a need but is an activity that birds derive pleasure from (Widowski and Duncan, 2000). The inability for birds to express dust bath behavior results in stressful (Duncan, 1981; Nicol, 1987; Tanaka and Hurnik, 1992; Appleby 2004).

Aggressive Behaviors

In birds, aggressive behaviors include pecking, fighting, and cannibalism. Birds express aggressive behaviors in frustrating situations. Factors that initiate aggressive behaviors include uncomfortable environment, poor management, and genetics (Blokhuis, 1989; Hughes and Duncan, 1972; Newberry, 2004; Tauson et al., 1999). High corticosterone levels increases feather pecking. In addition, limited housing conditions without foraging materials may also encourage feather pecking (El-Lethey et al., 2000).

Cannibalism

Cannibalism in poultry is the act by which birds vigorously peck as well as feed on the flesh of their counterpart. Cannibalism is described as a behavior that is learnt from one bird to another (Cloutier et al., 2002). Cannibalism is expressed by birds regardless of the housing system (Newberry, 2004), and is difficult to eradicate when there is an outbreak. The potential for the behavior to spread may be increased in large flocks (Scientific Panel on Animal Health and Welfare, 2005). Consequently, birds that refuse to learn cannibalism often time become victims (Newberry, 2004). The occurrence of cannibalism is higher in deep litter housing system compared to the conventional laying cages since birds' movements are restricted (Appleby, 1991). Subsequently, when cannibalism occurs in conventional laying cages, attacked birds are not able to escape.

Furthermore, if certain behavioral expressions are to be taken into consideration, then environmental enrichment strategies which address such need would be desirable. Some means of assessing animal welfare from the behavioral (Dawkins, 2004) perspective include (1) rearing birds in environments with different forms of enrichment placed at different locations and no enrichment in other locations, (2) observing the birds as they express preferences for choice of enrichment, and (3) identifying the behaviors expressed in the course of utilizing the preferred enrichment. In poultry, some behaviors are expressed individually, such as wing flapping, preening, and feeding, while others are expressed to their counterpart such as mating and aggression. When birds are reared on deep litter systems, or more spacious housing systems, the occurrence of wing flapping was increased (Duncan, 1998). However, when reared in conventional laying cages, wing flapping was almost absent. Pereira (2007) reported that at normal environmental temperatures, birds expressed more preening, feather ruffling, and foraging behaviors. The amount of time spent by birds to preen is reduced when birds are in ‘frustrating situations’ (Duncan, 1998; Duncan and Wood-Gush, 1972). Hence, preening and wing flapping are some of the examples of comfort behaviors because their occurrences are more frequent when birds are in optimum conditions. Dust bathing in poultry is a social behavior because it is expressed by birds in groups. Hence, a bird can initiate the whole process in the flock (Olssen et al., 2002). Poultry translate their psychological state into the behaviors they express. When birds experience conditions such as boredom, illness, aggression, pain, frustration, and fear (Jones et al., 1996; Jones and Roper, 1997; Mills and Faure, 1990), they express it. These behaviors provide clues about the state of welfare of the birds in question. Birds naturally display other behaviors such as nesting, perching, and dust bathing when left alone in the wild or reared on a free range housing system. When these behaviors are absent in confined birds, psychologists would describe the welfare of such birds as poor.

Physiology (Corticosterone and heterophil to lymphocyte ratio)

Welfare definition can be coined from the physiological response standpoint in that an increase in stress hormones indicates poor welfare (Bareham, 1972). Moberg (2000) explained that animals can become stressed without substantial physiological responses, while some activities that appear to be exciting may also stimulate physiological stress responses. In chickens, excessive production of corticosterone (Beuving and Vonder, 1978) and an increase in the amount of circulating heterophil to lymphocyte ratio (Gross and Siegel, 1983) are positive indicators of stress. In poultry, corticosterone is the glucocorticoid of interest and its effects are mediated by blood-borne carrying proteins known as corticosteroid-binding globulins (CBG) and two different receptors (Type I and Type II). There is a positive correlation between corticosterone level and heterophil to lymphocyte ratio. Hence, when there is an increase in plasma corticosterone levels, there is also an increase in blood heterophil to lymphocyte ratio in chickens (Gross, et al., 1980). Corticosterone is usually measured in the blood plasma and can also be measured in the feces in some other poultry species (Dehnhard, et al., 2003). Circulating corticosterone can be transferred from broiler breeders (Janczak, et al., 2007), quails (Hayward and Wingfield, 2004), and laying hens (Rettenbacher, et al., 2006), into the yolks of their eggs in stressful conditions. The continuous exposure of birds to frequent handling does not necessarily increase plasma cortcosterone level (Jones and Faure, 1981). Interestingly, Guesdon et al., (2004) also did not find any significant difference in plasma corticosterone levels between birds reared in standard and enriched cages. Beuving and Vonder (1978) injected groups of young and old laying hens with ACTH and found abrupt increases in corticosterone levels in the younger birds compared to the older hen. Furthermore, elevated corticosterone levels in the older hens persisted for a longer period compared to the younger ones.

Genetics also influence the amount of corticosterone secretion in laying hens in that the corticosterone content in the egg yolk of white laying hens is higher than that of brown laying hens when exposed to similar stressful conditions (Navara and Pinson, 2010). In chickens, the exposure of the embryo to corticosterone alters offspring development thereby causing retarded growth and fearful behaviors in chicks (Janczak, et al., 2006; Janczak, et al., 2007). Conditions that can result in elevated corticosterone levels include extreme hot or cold temperatures as well as management practices (Lin, et al., 2006).

Excess production of white blood cells (WBCs) is an indication that the body is challenged by micro-organisms. Of the component of the WBCs, the increase in the amount of circulating heterophil to lymphocyte (H/L) ratio is an indication of stress condition in poultry (Gross and Siegel, 1983). Avian heterophils are produced in the bone marrow, which is synonymous to neutrophils in humans, and both are similar in function. Lymphocytes originate in the spleen. There is a correlation between the immune system and stress in that there is higher incidence of diseases when animals are stressed (Moberg and Mench, 2000). Heterophils migrate to the site of infection especially in large numbers and ingest microorganisms through phagocytic process (Montali, 1988). Therefore, in stressful conditions, the amount of heterophils increases. Studies show that feed restriction in birds (De Jong, et al., 2003; Hocking, et al., 1996), and heat stress in broiler chicks (McFarlene and Curtis, 1989) are some of the conditions that increase H/L ratios.

Materials and Methods

Broiler Breeder Management

Prior to the commencement of this experiment, the research protocol was assessed and certified to be in accordance with the Institutional Animal Care and Use Committee (IACUC) requirement of the University of Tennessee. This experiment was carried out at the Johnson Animal Research and Teaching Unit (JARTU) of the East Tennessee Research and Education Center. The experiment was repeated in order to avoid room bias in the results obtained. Each experiment lasted for 20 weeks, and a total number of 336 (48 roosters and 288 hens) 21-week-old broiler breeder birds (Cobb 500, Pilgrim's Pride, Flat Rock, AL) were used in each experiment.

Upon arrival, birds were assigned to their respective treatments in two temperature controlled rooms (Figures 1 and 2), each with separate automatic timer to turn the lights on at 0700, and off at 2100, thereby providing a 16h: 8h light: dark schedule. One room was maintained at 23°C to mimic a thermoneutral setting while the other room cycled between 23°C and 30°C to mimic a heat stressed environment. Each room had four treatments, and each treatment was replicated three times to make a total of 12 pens. The individual pen housed 12 female and 2 male broiler breeders for a female: male ratio of 6:1. In both thermoneutral and heat stress rooms, the birds in each pen had limited auditory and visual contact with their counterparts housed in other pens. The birds were raised in accordance with the Cobb 500 broiler breeder management manual. One male and one female from every pen were weighed at the end of every week to monitor weight gain and make sure that their weights corresponded with the Cobb 500 broiler breeder management standards target values.

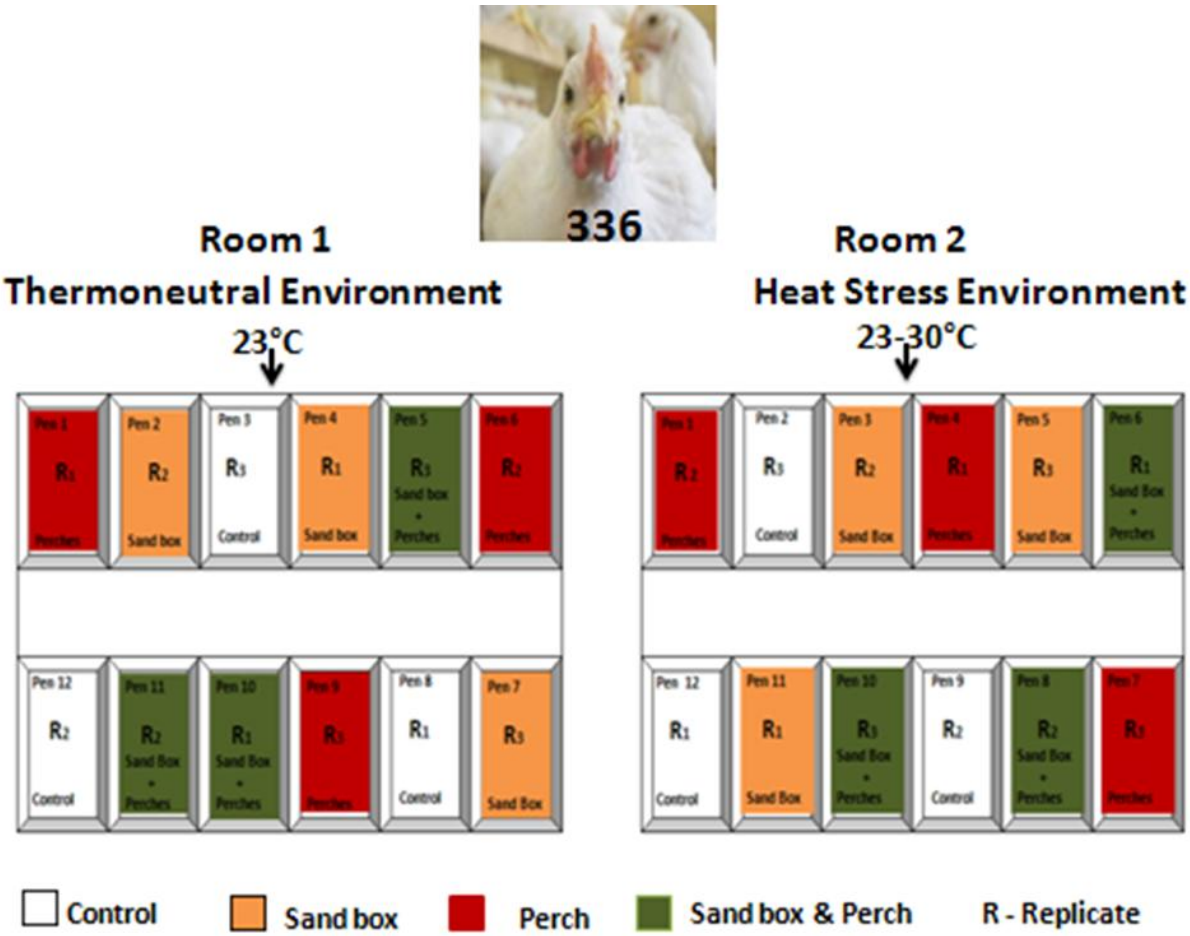


Figure 1. The distribution of broiler breeder birds into experimental units

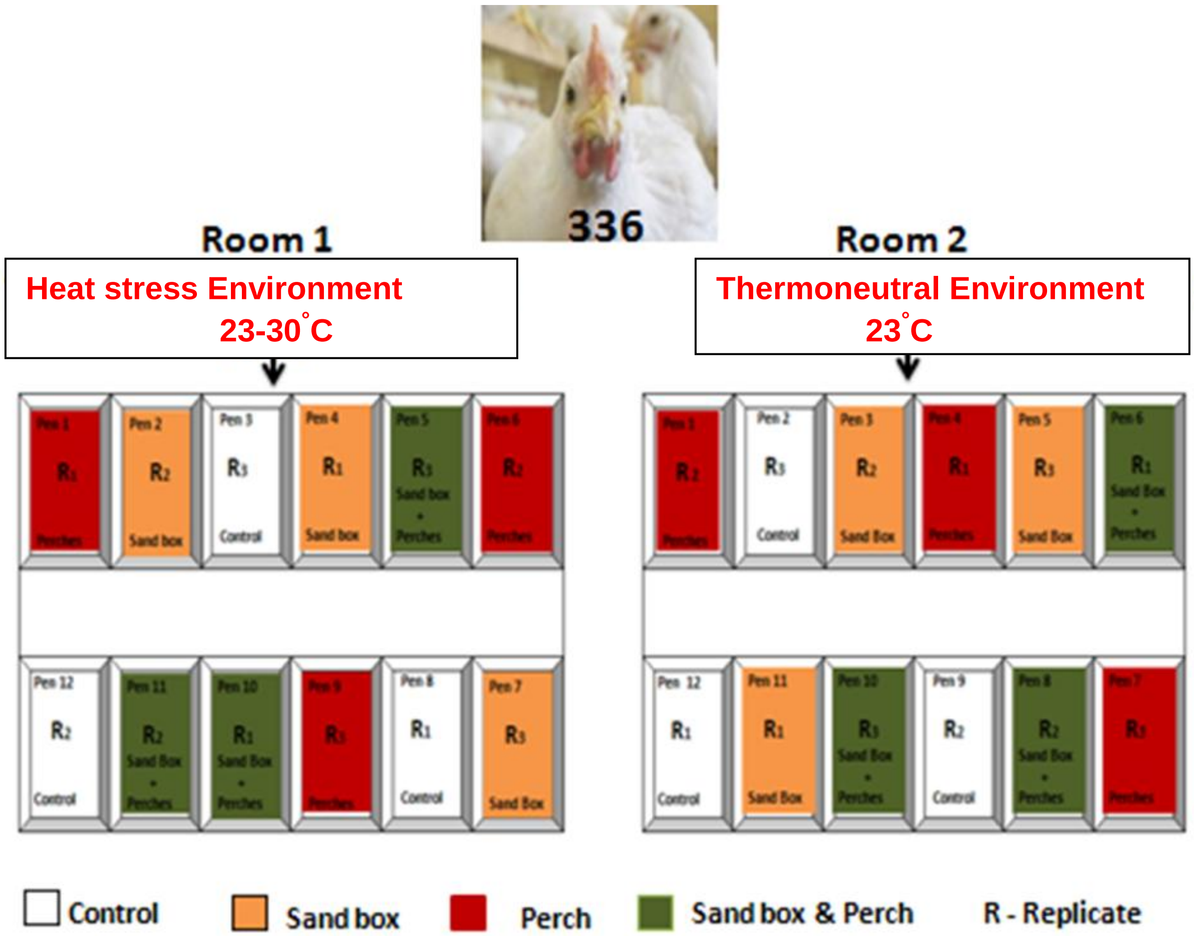


Figure 2. The distribution of broiler breeder birds into experimental units for the repeat experiments

Figure 3. The experimental unit design (pen) containing the white colored perch and the black colored sand box

The results obtained from the weekly weight gains along with the recommendations from the manual were used to regulate the quantity of feed provided for the birds.

The pen design (Figure 3) was similar throughout the experiment. The pen had an elevated slatted portion and a floor section with shredded cardboard as litter material. Included on the slatted portions of every pen were nest boxes, female feeders, and drinking water cups. The floor section accommodated the male feeders and the different forms of enrichment structure(s). The dimensions of the slatted portions were 121.92 x 192.024 cm and the litter areas were 192.024 x 192.024 cm. The slatted areas were raised 40.64cm inches above the litter and the pens were 228.092 cm high. The male feeders were red plastic containers, which were continually raised above the ground level as the male birds grew, to prevent the hens from eating therein (averagely 52.07 cm high). The female feeding troughs were long metal containers, enclosed on both sides, with vertical bars spaced 5.08 cm apart and a barrier on top to prevent the roosters from gaining access to the feed. The feed was formulated to meet the nutritional requirements (NRC, 1998) for broiler breeder at the laying stage of growth and manufactured specifically for this project by Tennessee Farmers' Cooperative, Lavergne, TN.

Water was provided for *ad-libitum* consumption through plastic cup drinkers (three cups per pen) fixed 35.56 cm above the slat and regulated automatically. The nest boxes in each pen (KUHL Corporation Flemington, N.J), had 6 compartments, cushioned with nest pads for the bird to lay. All birds had two separate identification tags affixed on different body parts namely a metal numbered identification tag on the shank of the birds, and a colored paper tag (Heartland Tag LLC, Fair Play, MO.) affixed to the wing web. The birds in separate pens had different wing tag colors and numbers for easy identification.

Enrichment

Enrichment structures (Figure 3) were placed on the floor section of the designated pens to attract the hens to the floor since hens typically spend more time on the slated portion (Jones and Prescott, 2000). The forms of environmental enrichment that were used in this research included sand placed in a four cornered plastic box, and a perch structure. The four treatments in each room were (1) no enrichment to serve as the control, (2) sand, (3) perch and, (4) a combination of sand & perch. For the first experiment, the plastic boxes with dimension of (79.248 x 74.295 x 5.08 cm) were used to hold the sand. In the second experiment, slightly deeper plastic boxes with dimension of (69.85 x 50.165 x 15.24 cm) were used to hold the sand. Premium play sand (sharp sand) was used for this research. The sand in the sand box was topped up daily to ensure that there was enough sand for birds to dust bath. A white, four cornered, mobile perch with a dimension of 66.04 x 49.53 x 25.4 cm made from Polyvinyl chloride (PVC) pipe material was used for this experiment.

Parameters

The parameters measured were in three categories (1) production (egg production, egg weight, floor eggs, fertility, and hatchability), (2) behavior (aggressive fighting, aggressive pecking, attempted mating, complete mating, preening, wing flapping, frequency and duration of sand use, frequency and duration of perch use, (3) physiology (corticosterone and heterophil to lymphocyte ratio).

Production

The total numbers of eggs laid by the birds in various pens (those laid in the nests and those laid on the floors) were recorded daily throughout the experiment, and were used to calculate the percentage hen-day egg production. Twelve eggs per pen were randomly selected, weighed and set into the incubator on weeks 26, 28, 32, 36 and 40. These weeks sums up to a total of 5 cycles of eggs set throughout each experiment. A Jamesway single -stage incubator (Jamesway incubator company Inc., Cambridge, Ontario, Canada) was used to hatch the eggs throughout the study. Eggs to be set were briefly stored in a cooler at 12°C. A day prior to each cycle (set time), eggs were removed from the cooler, cleaned of any dirt with the use of emery paper, and candled for good internal qualities such as a correctly positioned air cell and the absence of meat spot. The eggs were further examined for other external qualities such as good shape, non-cracks and ideal sized egg. Eggs deemed appropriate to set in the incubator were numbered for identification, and left on the plastic egg crate in an enclosed room overnight to assume room temperature. The setter portion of the incubation system was turned on 24 hours prior to egg set for uniform temperature at 99.7°C. The following morning, the eggs were transferred on to the white plastic incubator egg flats and placed in the setter for 18 days. The egg fertility was determined by candling on day 10 of each cycle. However, the infertile eggs remained with the fertile eggs in the incubator until day 21. On day 19, eggs were transferred to the hatching trays in the hatcher, which was set at 98.5°C. After the eggs hatched on day 21 of each cycle, the un-hatched eggs were broken out to identify the causes for non-hatched. The number of chicks that hatched were recorded and used to determine percent hatchability.

% Egg production= (total # of eggs laid/number of hens)*100

% egg fertility = (total # of fertile eggs/total # of eggs set) *100

% egg hatchability = (total # of chicks hatched/total # of egg set) *100

% floor egg = (total # of eggs laid outside the nest boxes/total number of hen)*100

Behavior

A total of four Noldus MPEG recorder cameras (two cameras per room) were mounted on the walls, with each camera set to record the behaviors exhibited by birds in two separate pens/ treatment. Hence, all four treatments' behaviors were recorded at the same time in both thermoneutral and heat stress rooms. The cameras were connected to a computer such that the recordings captured by the four cameras could be previewed at the same time on a computer which was located in a separate room. The recordings were saved on external hard drives in such a way that the behavioral recordings of the selected pens could be analyzed over time with the aid of Noldus Observer XT 8.0 program. Some of the behaviors monitored by the cameras includes frequencies of mating (attempted, completed, and total), aggression (fighting, pecking), individual behavior (preening, wing flapping), frequency and duration of the use of enrichment structures (perch and sand). Attempted mating included inability for a male bird to completely mount a female bird and achieve cloaca contact. Attempted mating was recorded when the effort made by the male bird to mount the female bird was not achieved. This is characterized by the male seizing the back of the head or the comb of the female. Factors responsible for attempted mating include inability of the male bird to maintain stability on top of the female bird, mating interrupted by other males, and the loss of mating interest by the male birds (Moyle, et al., 2010). Completed mating was recorded when a female crouched on the floor for the male to appropriately mount and achieve cloaca contact. Total mating was the number of times the birds displayed both attempted and completed mating. Aggressive fighting was identified when birds violently attacked other bird. Pecking was considered as bird using its beak to aggressively strike another bird. Preening behavior was characterized as a bird using its beak to rearrange its feather. Wing flapping behavior was noted when the bird stretch out both feathers and energetically swing in an up and down movement.

One replicate pen of each treatment was randomly selected for video recording. The birds housed in the 8 recorded treatments (pens) were individually marked with the use of twist-up livestock markers (blue for the 2 males, pink, orange, and green each per 4 hens) at different body parts (head, back, tail, and both head & tail) for identification and analysis (Prima Tech, Kenansville, NC). The behaviors of 4 females and 2 males per treatment were monitored and analyzed. Only behaviors that were captured by the cameras were analyzed while behaviors expressed behind barriers such as in the nest boxes or other obstructed areas were not considered since they were out of focus. The term "use of sand box" refers to birds in close contact with the sand box. Examples include birds staying within the sand box, or standing outside the sand box with their body parts (e.g. beak, leg, wing, and other body parts) on the sand or the box that held the sand. The term ‘use of perch’ represent birds that were in close proximity with the perch in the form of standing, seating or staying within the four corners of the perch.

The behaviors of the birds were monitored for 50 minutes in the morning and 50 min in the afternoon on weeks 22, 23, and 24, which corresponded with bird acquisition and the start of laying. After the start of lay, birds’ behaviors were video monitored, for 30 min during three time periods: (1) prior to eating morning, 0730 - 0800; (2) after eating (1330 - 14002); and (3) evening (1930 -2000). These observations were carried out for a one-day period during weeks 26, 28, 32, 36 and 40. Sexual behavior was observed for 2 hours from 1900 - 2100 on weeks 26, 28, 32 and 40. Particular attention was paid to this 2-hour time period because studies had shown that 70% of mating activity takes place during the last four hours of light (Bilcik and Estevez, 2005). At week 26, the morning and afternoon video recording experienced minor technical difficulty and the files could not be accessed. Thus only one video recording (2hours in the evening) was accessed and analyzed. In experiments 1 and 2, the data from the 30 minutes behavioral recordings on weeks 26, 28, 32, 36, and 40 were multiplied by 5/3 to put them on a 50 minutes equivalent basis. This was done for accurate comparisons of the 50 minutes birds’ behaviors that were recorded at weeks 22, 23, and 24.

Physiology

Blood samples (between 3-5mls) were drawn from the brachial veins of four randomly selected females and two males from each pen on weeks 24, 32, and 40. The birds that were bled were gently taken out of their pens, placed into a four cornered mobile cage, and moved out of the thermoneutral and heat stress rooms. Outside the rooms, the birds were bled out of earshot and visual contact from their counterparts. For bleeding, each bird was placed on a table such that it laid on its side and one of its wing stretched out. The vein was identified and the needle was used to aspirate blood. Heparinized 6ml Kendall syringes with 23 gauge 1 inch needles (Becton Dickinson and company Franklin Lakes NJ) were used to draw blood from the veins of the selected birds. The blood samples were transferred into labeled heparinized vacutainer tubes (BD, Franklin Lakes, NJ) and mixed by gentle inversion, and immediately placed on ice. Blood from one of the 6ml tubes was centrifuged at 6 x 1000*rpm* for 20 minutes and the plasma was saved in the two 2 mls microcentrifuge tubes with the aid of a transfer pipette (Samco Scientific, Mexico). The cap of the two 2mls microcentrifuge tubes and stored at -20°C until it was analyzed for corticosterone (CORT) concentration. Blood smears were made from whole blood sampled from the second tube for analysis of heterophil and lymphocyte cell numbers.

Radioimmunoassays

Radioimmunoassays (RIAs) were conducted to measure the corticosterone level in the plasma samples. Prior to corticosterone analysis, the plasma was thawed, vortexed and centrifuged using a microcentrifuge. Procedure was in accordance with the kit instruction with plasma to diluent samples at a ratio of 1: 5, respectively. Automatic gamma counter (PerkinElmer, Hebron, KY, USA) was used to determine the corticosterone content of each tube. The intra-assay CVs for the high and low were 8.91 (10.3ng/mL) and 23.06% (5.00ng/mL) respectively for corticosterone levels.

Cell Counting

The slides were stained using PROTOCOL Hema 3 staining system (THERMO Fisher Scientific., Kalamazoo, MI) and staining procedures were in accordance with the Wright-Giemsa staining method. Stained slides were left to dry after which the heterophils and lymphocytes cells were counted by light microscopy at 100x. On every slide, one hundred white blood cells were counted with the use of a laboratory counter (Clay Adams, Division of Becton, Dickinson and company Parsippany, N.J). The white blood cells counted were the granular (heterophils, eosinophils, and basophils) and aggranular (lymphocytes and monocytes) cells. The number of the different cells per slides were recorded and the heterophil: lymphocyte (H:L) ratios were calculated for each sample. Heterophils appeared granular and their nucleus had 3-5 lobes connected by chromatin threads and their cytoplasm contained fine granules. Lymphocytes are mononuclear and their cytoplasm stains pale blue.

Statistical Analysis

The production parameters were analyzed using a completely randomized design (CRD) with repeated measures while the physiology and behavior parameters were analyzed using CRD split plot with sampling and a factorial in the whole plot. Pen was the experimental unit and the response variables were analyzed with mixed model analysis of variance (SAS 9.2, Cary, NC, USA). The between subjects model were used to test treatment effects using pen variation as the experimental error. The within subjects model tested the changes across weeks and interactions with treatments, using an autocorrelation covariance structure as needed. Least squares means were compared using Fisher's protected LSD. Data were examined for normality and equality of variances. Data that had normality problems were transformed logarithmically and ranked where necessary and reanalyzed. However, the values shown in this paper were the non-transformed values. Correlation and regression analyses were used to study relationships among the response variables. Principal component analysis assessed the degree of commonality among the variables with significance level of $p < 0.05$.

Results

Experiment 1

Egg production

In experiments 1, egg production significantly increased (P<0.0001) each week from weeks 24 until 28, when the increase was gradual (Figure 4). Immediately after peaking, there were few declines in some weeks but the overall egg production gradually declined towards the end of the study at week 40. The highest and lowest egg productions in experiment 1 were obtained at weeks 29 and 24. The numbers of egg laid were higher (P=0.049) in treatment SP of the HS room compared to the same treatment in the TN room (Figure 5). When the least square means of each treatment were summed up regardless of the room temperatures, there was a trend (P=0.08) for increased egg production in treatment S than the control (Figure 6). Additionally, when the egg production data were reanalyzed using statistical contrast, the birds that had sand (either alone or in combination with perch) laid more (P=0.01) eggs.

Egg Weight

Egg weight significantly increased (P<0.0001) with age from weeks 26 to 40 (Table 1) regardless of room temperatures and enrichment (treatments). There were no differences in the egg weights between the HS and TN rooms (Table 2). Enrichment did not affect egg weight in neither the TN nor HS rooms.

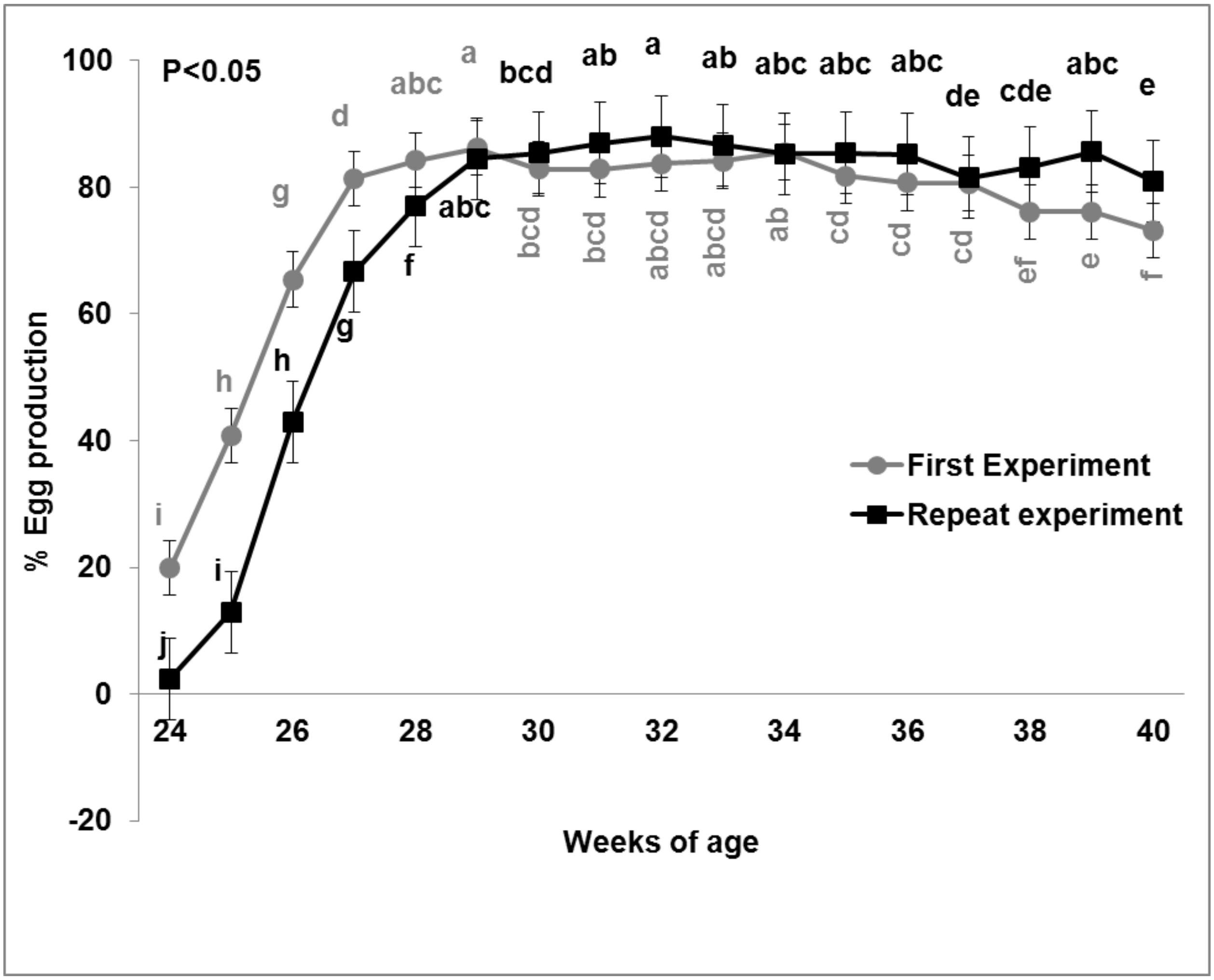
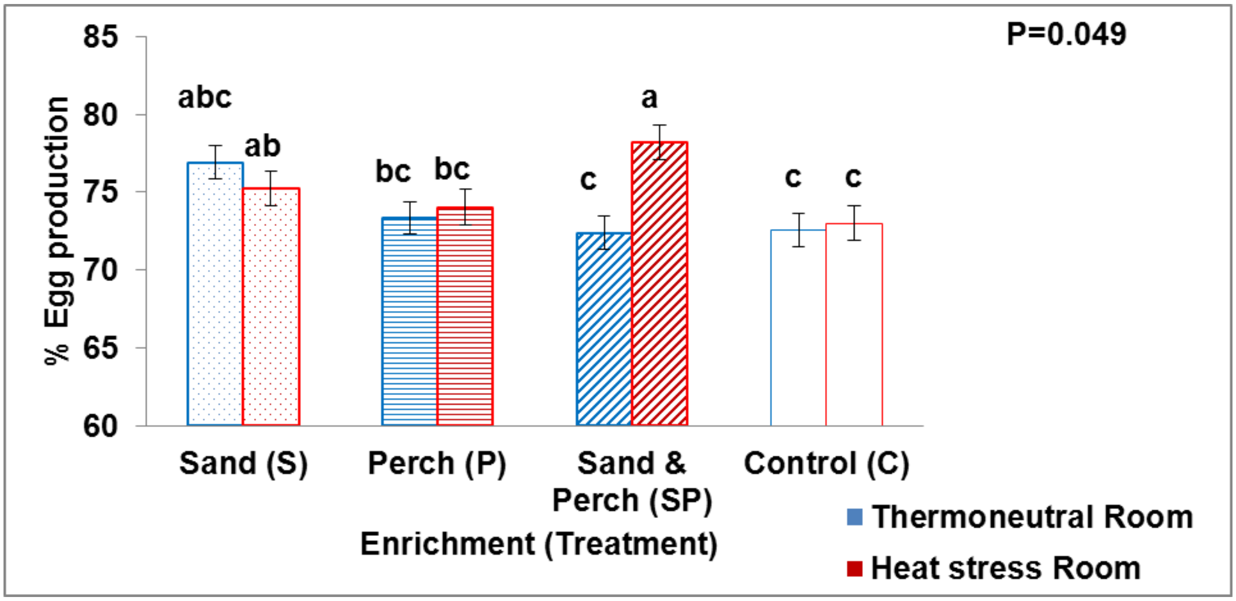
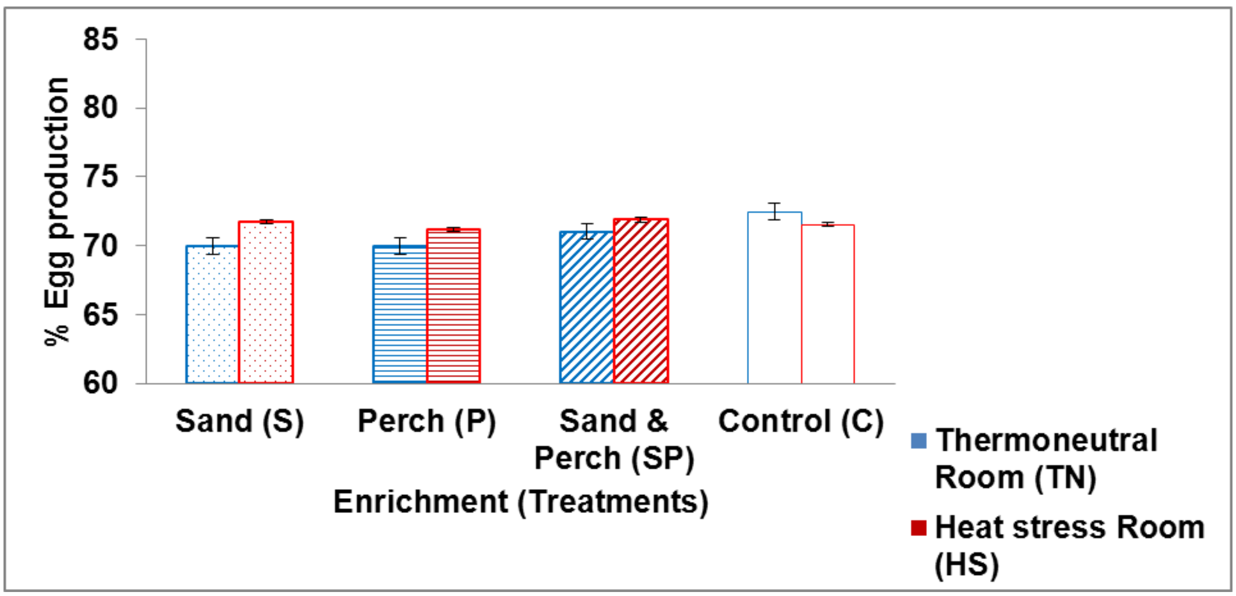


Figure 4. Effects of age on egg production in experiments 1 and 2.

Values are mean ± SE. Egg production means within experiments, that do not share similar superscript are different (P<0.05).



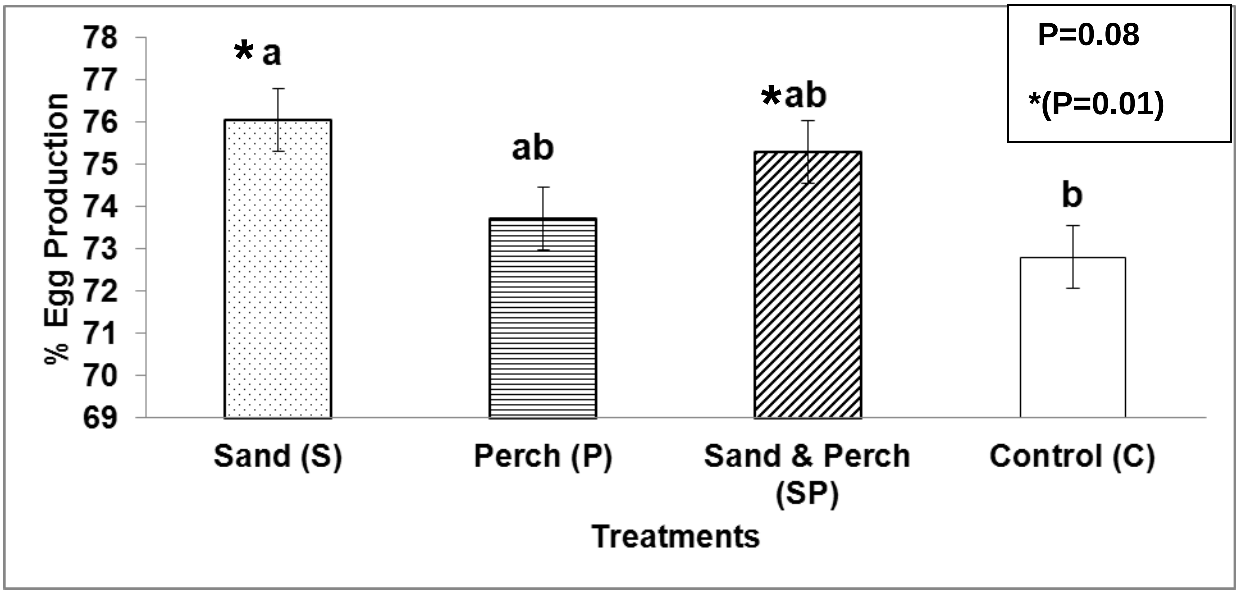
Experiment 1



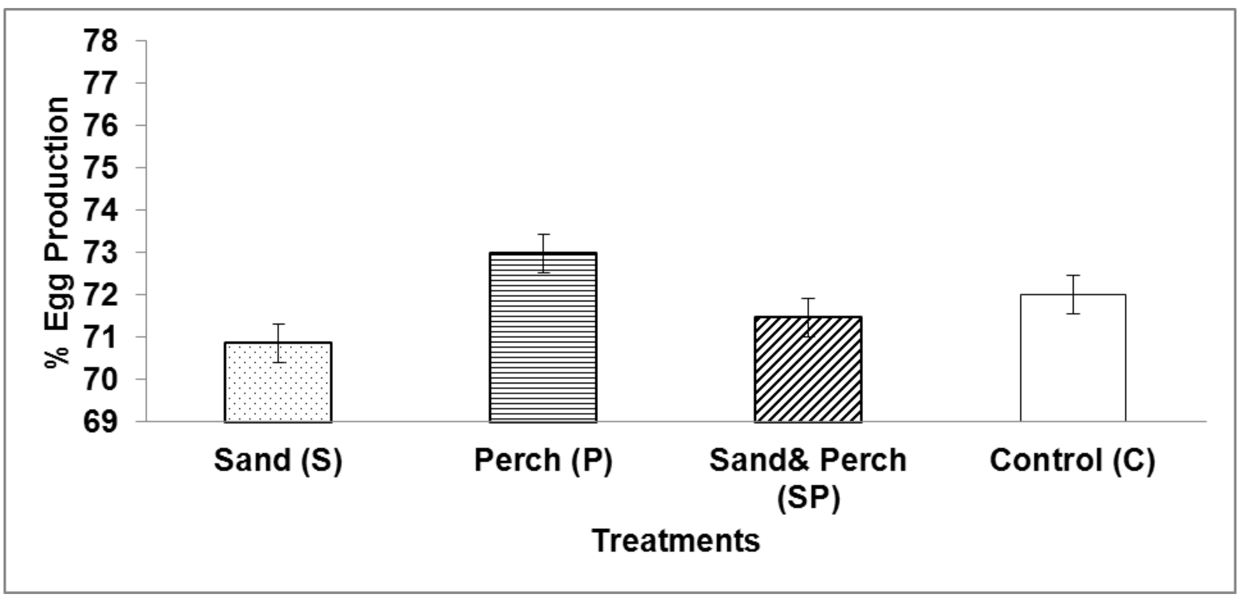
Experiment 2

Figure 5. Effects of enrichment and heat stress on egg production in experiments 1 and 2.

Values are expressed as mean ± SE. Means within experiments, that do not share similar superscript are different (P<0.05)



Experiment 1.



Experiment 2.

Figure 6. Effects of enrichment on overall egg production in experiments 1 and 2.

Values are expressed as mean ± SE. Means within experiments that do not share similar superscript tend to differ at P<0.10. (*) denote enrichment differ at P<0.05.

Table 1. Least squares means for weekly egg weights (grams) for experiments 1 and 2 as birds age.

Weeks	Experiment 1	Experiment 2
26	52.35± 0.24[e]	48.34±0.19[e]
28	54.44± 0.12[d]	54.89±0.12[d]
32	59.70± 0.14[c]	59.26±0.13[c]
36	63.10± 0.13[b]	61.36±0.11[b]
40	65.27± 0.17[a]	63.27±0.18[a]

Values are expressed as mean ± SE. Means within the same column that do not share similar superscript are different at P<0.05.

Table 2. Least squares means of egg weights (grams) for thermoneutral (TN) and heat stress (HS) rooms regardless of treatments.

Experiment 1.

Weeks	**Thermoneutral (TN)**	**Heat stress (HS)**
26	53.15 ± 0.40^{ef}	51.56 ± 0.22^{f}
28	54.49 ± 0.17^{d}	54.40 ± 0.17^{de}
32	59.68 ± 0.25^{c}	59.72 ± 0.14^{c}
36	63.27 ± 0.21^{b}	62.92 ± 0.15^{b}
40	65.29 ± 0.28^{a}	65.24 ± 0.19^{a}
Mean	**58.77 ± 0.69^{a}**	**59.18 ± 0.68^{a}**

Experiment 2. * **(P<0.0798)**

Weeks	**Thermoneutral (TN)**	**Heat stress (HS)**
26	47.92± 0.20^{h}	48.76± 0.31^{h}
28	55.57± 0.15^{f}	54.21± 0.13^{g}
32	59.59± 0.17^{de}	58.93± 0.19^{e}
36	62.02± 0.16^{bc}	60.70± 0.11^{cd}
40	64.09± 0.19^{a}	62.45±0.27^{b}
Mean	***57.83± 0.76^{a}**	**57.01± 0.68^{a}**

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05)

Floor egg

The rates of floor eggs fluctuated (P=0.0071) as the birds aged but there were no difference in the numbers of floor eggs between the TN and HS rooms as the birds increased in age (Figure 7). Treatments S, P, and SP reduced (P=0.0127) the occurrence of floor egg compared to the control (Figure 8).

Fertility

Fertility increased (P=0.0141) from weeks 26 to 28 then gradually decreased until week 40, when the decline was significant (Table 3). There were no fertility differences between the TN and HS rooms (Table 3) and enrichment (Figure 9) did not affect fertility.

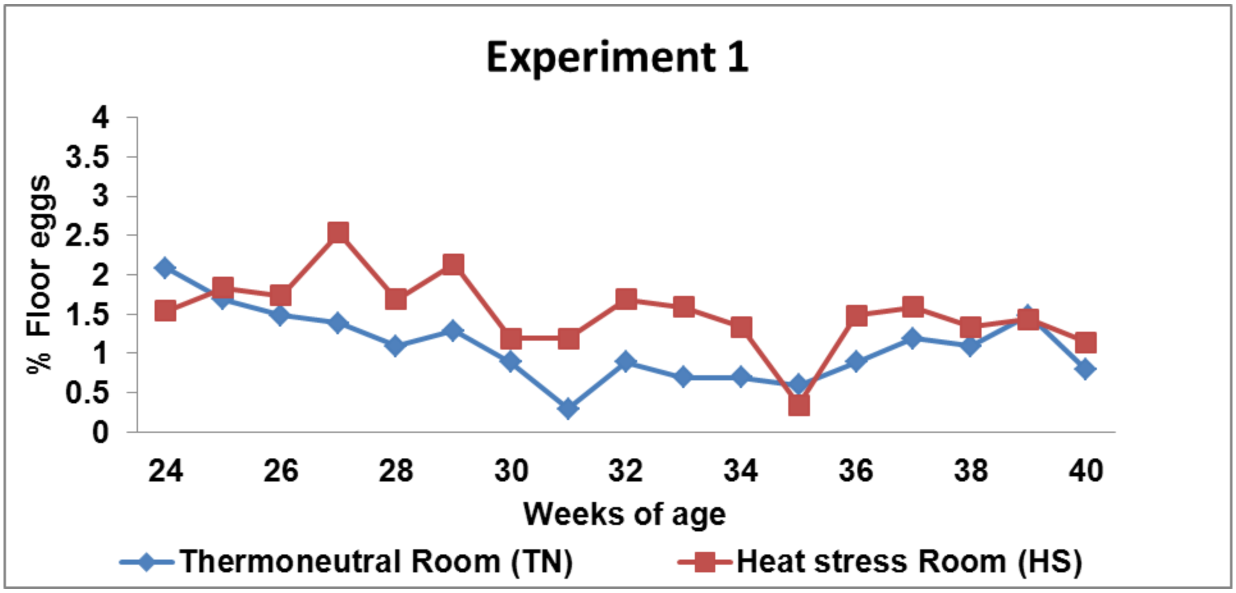
Hatchability

As shown in table 4, the mean hatchability increased (P=0.0004) from weeks 26 to 28, remained high, and significantly declined from weeks 36 to 40. There was a trend for increased hatchability mean in the HS room (P=0.10) compared to the TN room (Table 4). There were no treatment differences in hatchability (Figure 9). There was a strong positive correlation (P<0.0001, r=0.95) between fertility and hatchability.

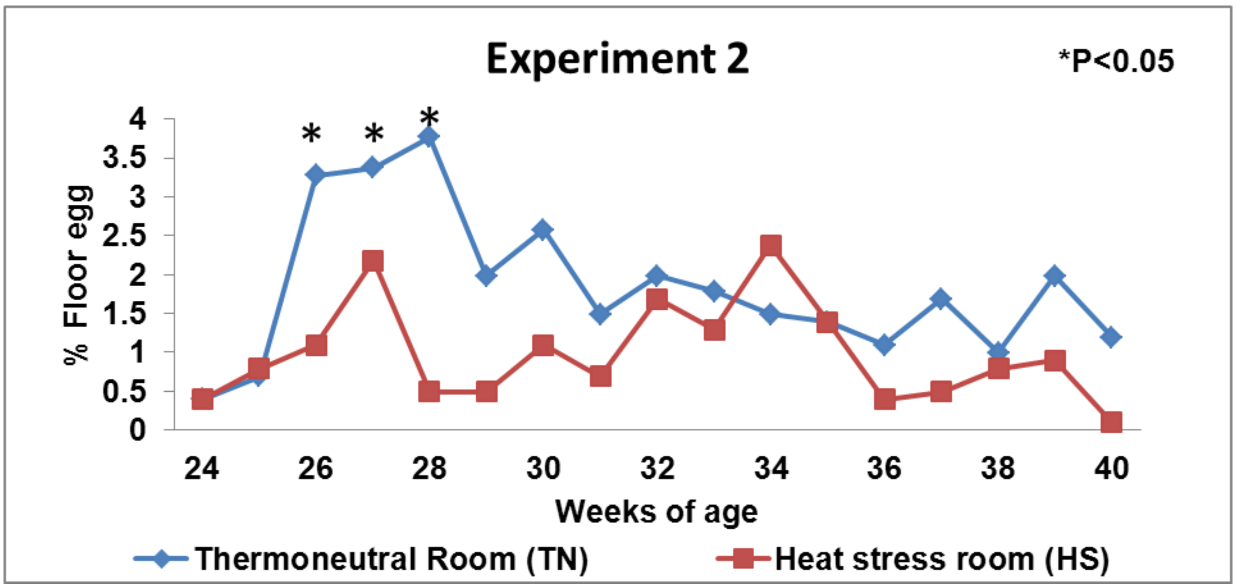
Corticosterone (CORT) **levels**

Male CORT levels

The overall mean CORT levels were lower (P=0.04) at week 32 than week 40. Although the CORT levels in the HS room were numerically higher than that of the TN room, the differences were not significant (Table 5). When data were analyzed with statistical



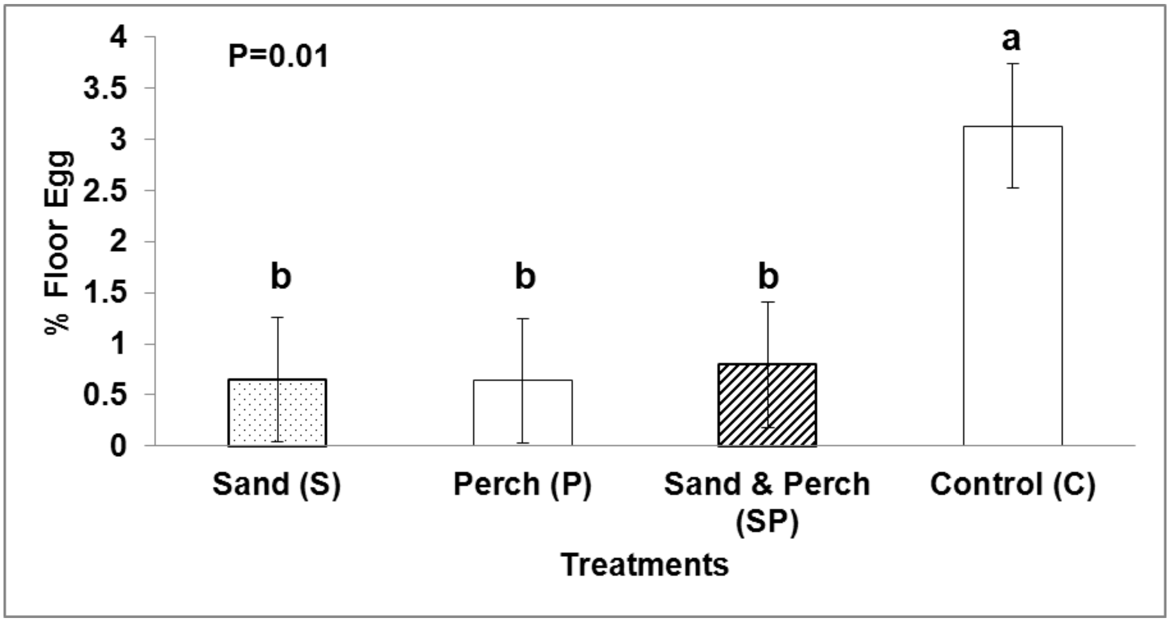
Experiment 1.



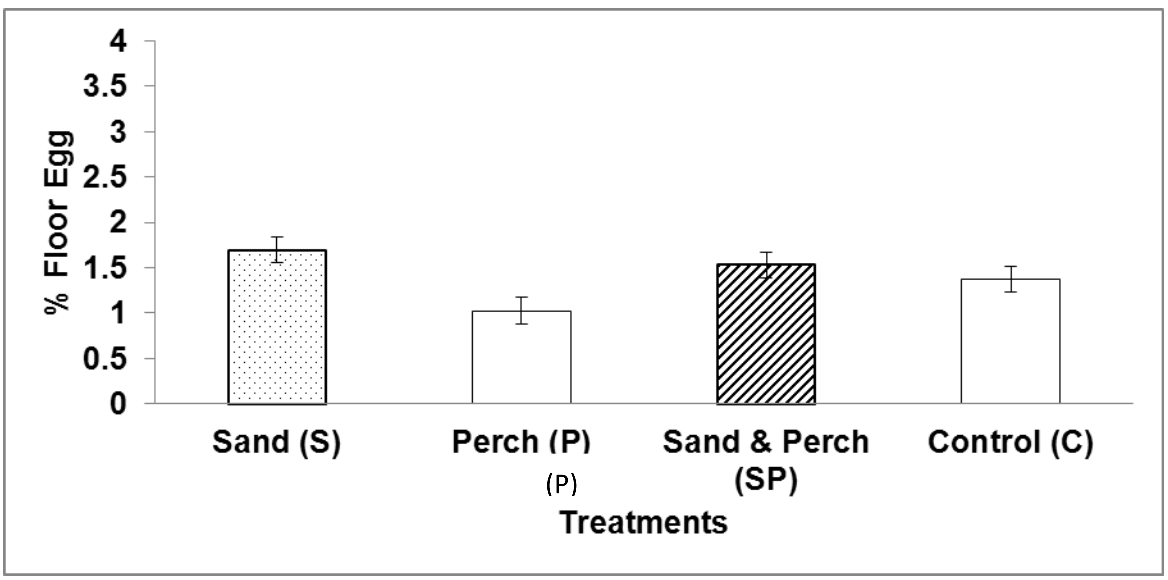
Experiment 2.

Figure 7. Effects of age and heat stress on percent floor eggs.

Least squares means of percentage floor eggs across the treatments in the thermoneutral (TN) and heat stress (HS) rooms from weeks 24-40 of experiments 1 and 2.



Experiment 1.



Experiment 2.

Figure 8. Effects of enrichment on floor eggs.

Means within experiment that do not share similar superscript are different (P<0.05)

Table 3. Least squares means for fertility in TN and HS rooms for experiments 1 and 2.

Experiment 1

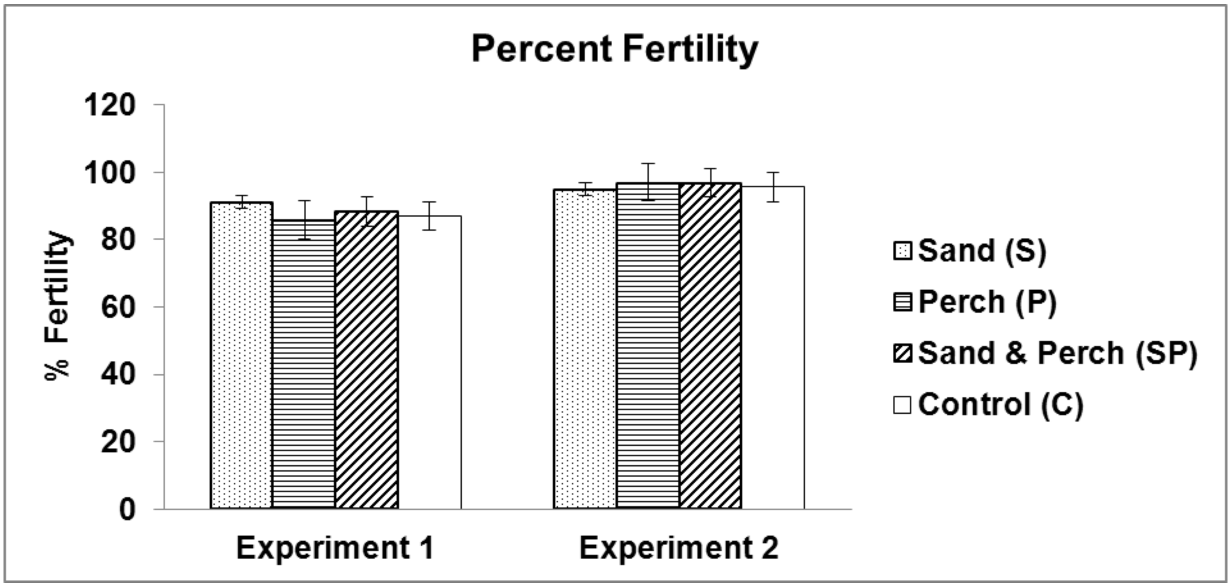
Week	Thermoneutral (TN)	Heat stress (HS)	Means
26	86.81± 1.90^{bc}	88.89± 1.35^{abc}	87.85± 1.17^{ab}
28	95.83± 0.70^{a}	90.97± 1.21^{abc}	93.40±0.73^{a}
32	88.89± 1.95^{ab}	95.14± 1.80^{ab}	92.01±1.35^{a}
36	85.42± 3.86^{ab}	90.28± 1.65^{abc}	87.85±2.10^{a}
40	74.31± 4.33^{c}	84.03± 1.90^{abc}	79.17±2.40^{b}
Means	86.25± 25 ^{a}	89.86± 1.67 ^{a}	

Experiment 2

Fertility

Week	Thermoneutral (TN)	Heat stress (HS)	Means
26	93.75± 1.26^{bc}	91.67± 0.77^{c}	92.71± 0.74^{b}
28	94.45± 0.68^{abc}	98.61± 0.60^{a}	96.53± 0.49^{a}
32	97.22± 0.51^{ab}	95.14± 0.82^{abc}	96.18± 0.49^{a}
36	97.22± 0.51^{bc}	96.53± 0.53^{ab}	96.88± 0.37^{a}
40	98.61± 0.40^{a}	96.53± 0.69^{ab}	97.57± 0.41^{a}
Means	96.25±0.78^{a}	95.70± 0.75^{a}	

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05)



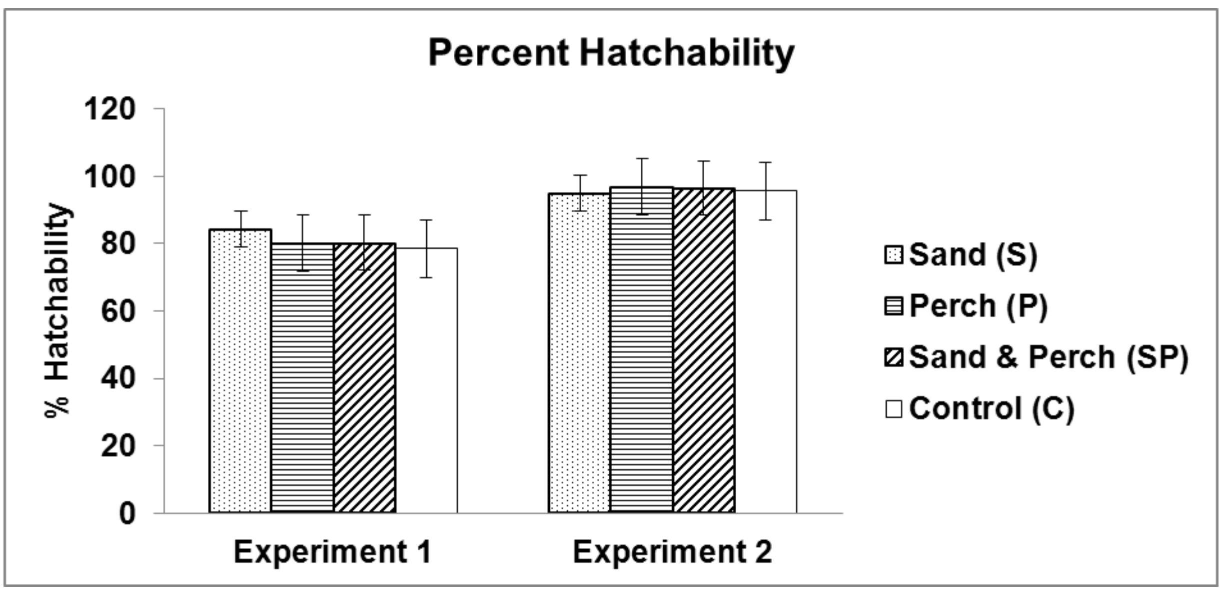


Figure 9. Effects of enrichment on fertility and hatchability in experiments 1 and 2.

Values are expressed as mean ± SE.

Table 4. Least squares means for weekly hatchability for experiments 1 and 2.

Experiment 1

Week	**Thermoneutral (TN)**	**Heat Stress (HS)**	**Means**
26	70.83±2.49^{b}	77.78±2.18^{ab}	74.31±1.68^{c}
28	88.19±1.21^{a}	81.25±1.41^{ab}	84.72±0.98^{ab}
32	80.56±1.62^{ab}	88.89±2.00^{a}	84.72±1.34^{a}
36	84.86±2.23^{ab}	85.42±1.60^{ab}	85.14±1.36^{a}
40	74.13±3.71^{ab}	76.39±2.29^{ab}	75.26±2.13^{b}
Means	**80.89±2.53 ^{a}**	***81.94±2.02 ^{a}**	

Experiment 2

Week	**Thermoneutral (TN)**	**Heat stress (HS)**	**Means**
26	83.33±1.83^{bc}	79.86± 1.36^{c}	81.60± 1.15^{b}
28	87.50±1.04^{ab}	90.28± 1.07^{ab}	88.89± 0.75^{a}
32	90.28±1.16^{ab}	90.97± 1.43^{a}	90.63± 0.92^{a}
36	90.28±1.07^{ab}	90.28± 0.87^{ab}	90.28± 0.69^{a}
40	94.45±0.81^{a}	91.67± 0.77^{a}	93.06± 0.57^{a}
Means	**89.17±1.32^{a}**	**88.61±1.27^{a}**	

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05). (*) denote a trend for higher hatchability in the HS versus TN room at P=0.10.

contrast, there was a trend (P=0.10) for perch to increase CORT levels in treatments P and SP (Figure 10).

Female CORT levels

The CORT levels (means) in the broiler breeder females across the weeks are as shown in table 5 (P<0.05). At week 32 (Table 5), the CORT levels of the females housed in the HS room were higher (P=0.0076) compared to that of the TN room. The overall mean CORT levels of the birds were similar across the weeks and the treatments did not affect the female CORT level (Figure 10).

Total CORT

The male and female CORT levels were analyzed together (Figure11). From the result, the room temperature and enrichment (Figure 12) did not affect total CORT levels. However the data were reanalyzed with statistical contrasts and as a result (Figure 20), birds' in treatments P and SP had increased (P<0.03) CORT levels (Figure12). There was a slight positive correlation between CORT and H:L ratio (r=0.12, P=0.01).

Male heterophil/lymphocyte (H:L)

The mean H:L ratio in broiler breeder males was least (P=0.0009) at week 24 compared to weeks 32 and 40 (Table 6). At week 32, H:L ratio was greater (P=0.0031) in the TN room versus the HS room. There was a trend (P=0.08) for higher H:L ratio in the control treatment of the TN room than the same treatment in the HS room.

Table 5. Least squares means for male and female corticosterone levels (ng/mL) for experiments 1 and 2.

Experiment 1

.	Week	Thermoneutral	Heat stress	Means
Male	24	2.60 ± 0.14^{ab}	3.37 ± 0.19^{a}	2.98 ± 0.12^{ab}
	32	2.50 ± 0.13^{b}	2.48 ± 0.11^{b}	2.49 ± 0.08^{b}
	40	2.98 ± 0.17^{ab}	3.45 ± 0.20^{a}	3.22 ± 0.13^{a}
Mean		$\mathbf{2.69 \pm 0.15^{a}}$	$\mathbf{3.10 \pm 0.18^{a}}$	
Female	24	3.23 ± 0.14^{bc}	3.04 ± 0.10^{c}	3.14 ± 0.08^{b}
	32	2.73 ± 0.11^{c}	3.89 ± 0.16^{ab}	3.31 ± 0.11^{b}
	40	4.31 ± 0.17^{a}	3.96 ± 0.12^{ab}	4.14 ± 0.11^{b}
Mean		$\mathbf{3.40 \pm 0.15^{a}}$	$\mathbf{3.62 \pm 0.14^{a}}$	

Experiment 2

.	Week	Thermoneutral	Heat stress	Means
Male	24	2.45 ± 0.17^{b}	$*3.71 \pm 0.22^{a}$	3.04 ± 0.15^{a}
	32	2.77 ± 0.24^{ab}	2.52 ± 0.23^{b}	2.65 ± 0.16^{a}
	40	2.23 ± 0.15^{b}	3.01 ± 0.18^{ab}	2.60 ± 0.12^{a}
Mean		$\mathbf{2.49 \pm 0.19^{b}}$	$\mathbf{3.06 \pm 0.22^{a}}$	
Female	24	2.58 ± 0.10^{a}	3.04 ± 0.10^{a}	2.81 ± 0.07^{a}
	32	2.74 ± 0.12^{a}	2.90 ± 0.11^{a}	2.82 ± 0.08^{a}
	40	2.82 ± 0.12^{a}	3.23 ± 0.12^{a}	3.02 ± 0.09^{a}
Mean	---	$\mathbf{2.71 \pm 0.11^{a}}$	$\mathbf{*3.05 \pm 0.11^{a}}$	---

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05). (*) denotes trend for higher corticosterone level at P< 0.10.

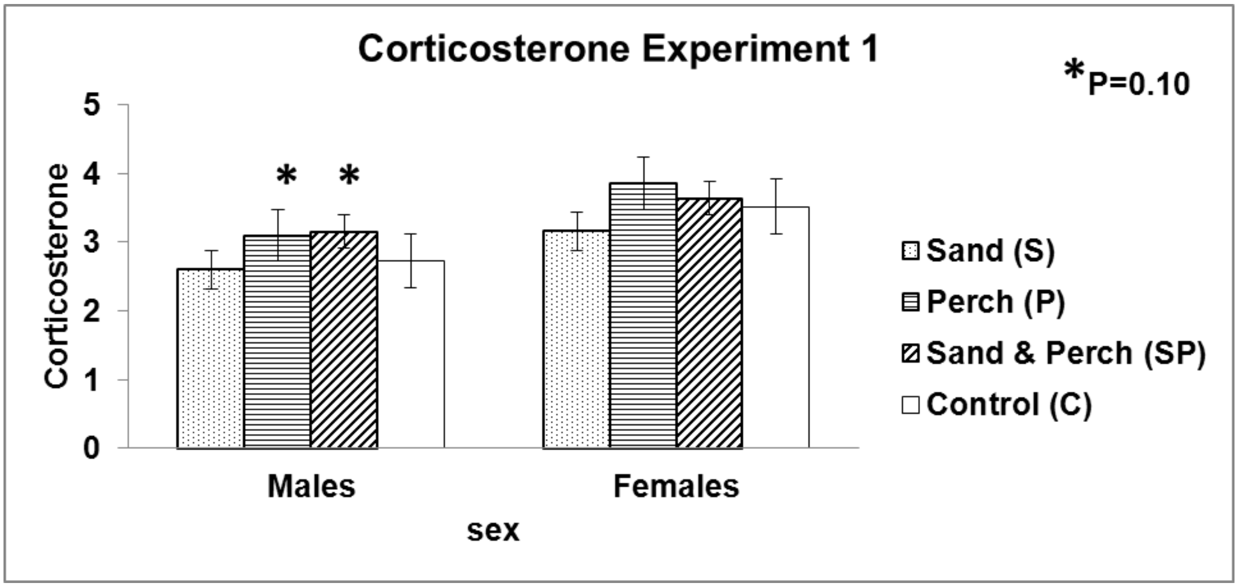
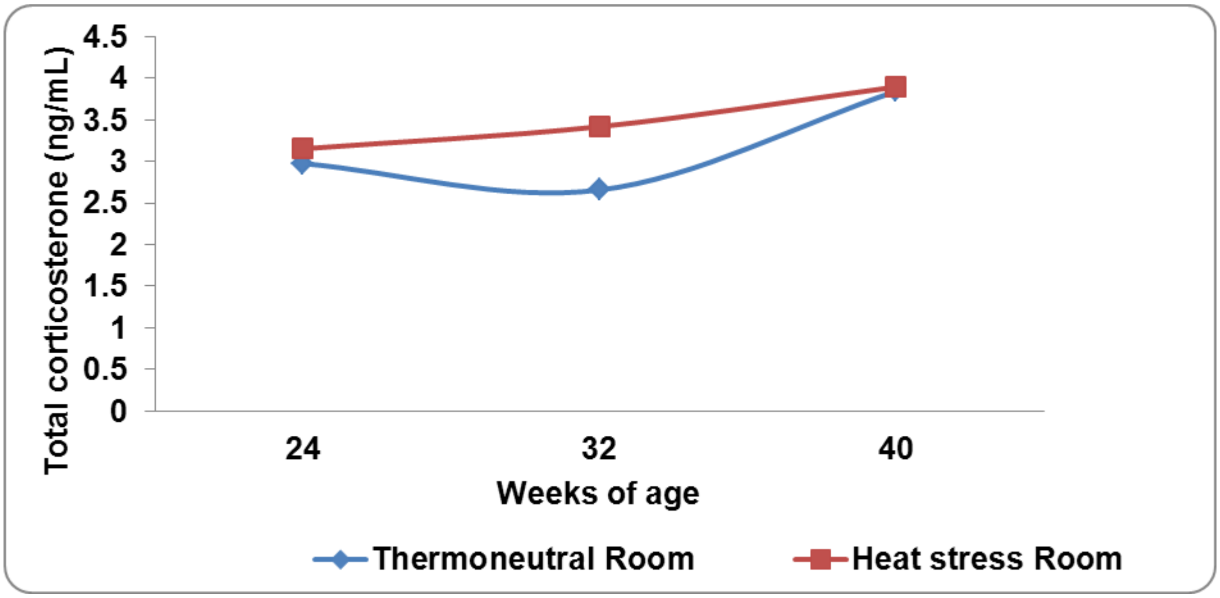
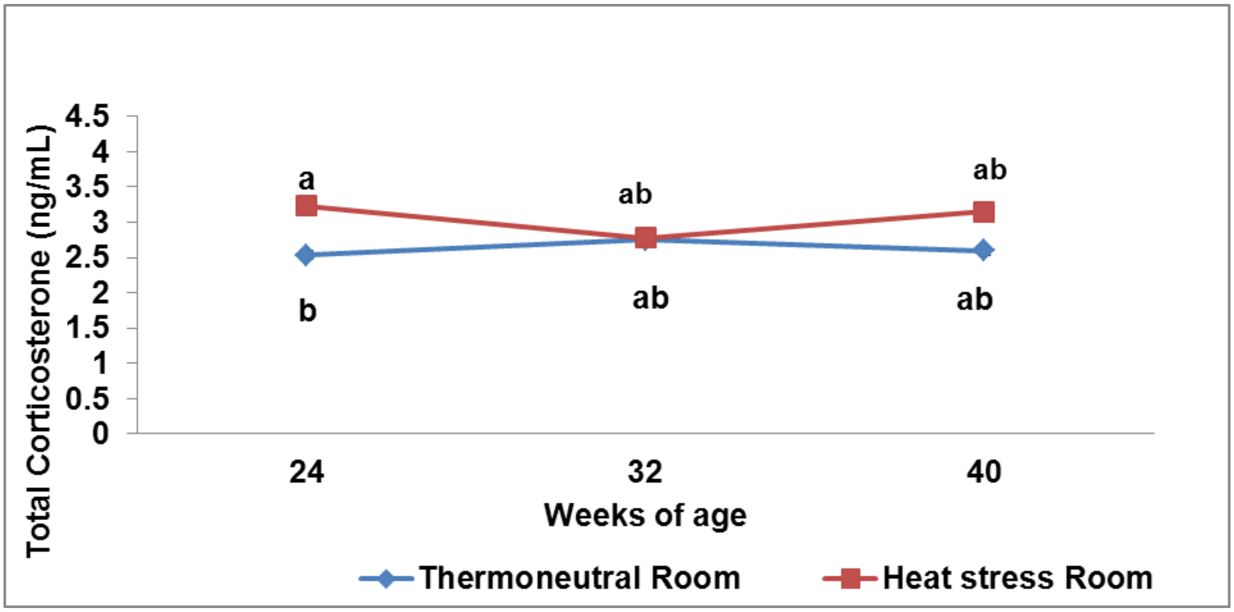


Figure 10. Effects of enrichment on male and female corticosterone levels in experiment 1.

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05). (*) denote comparison between enrichment that had perch versus non-perch



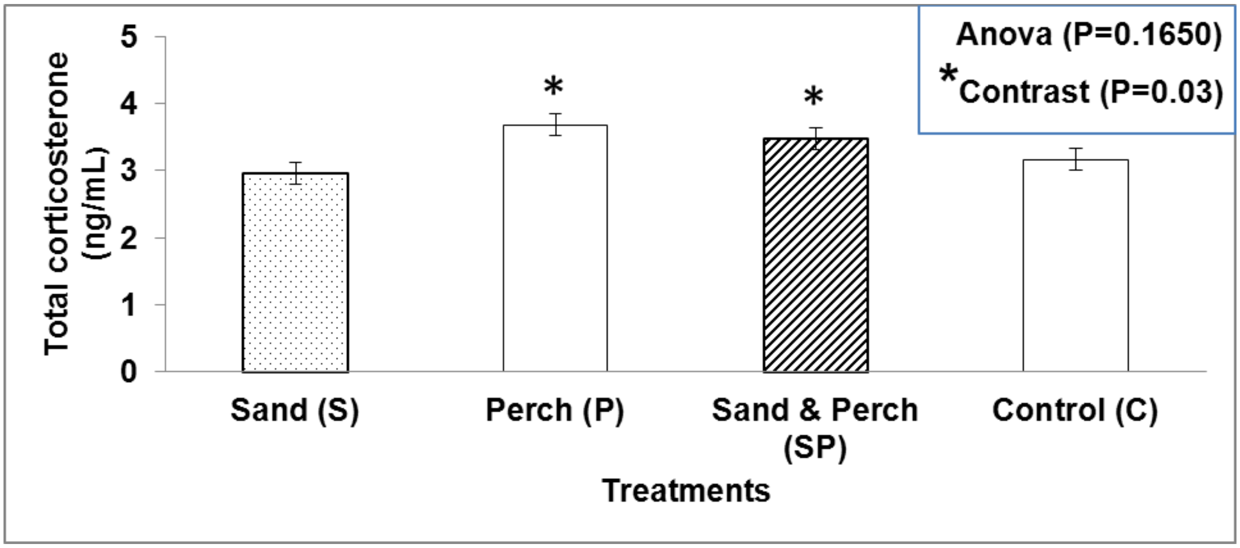
Experiment 1.



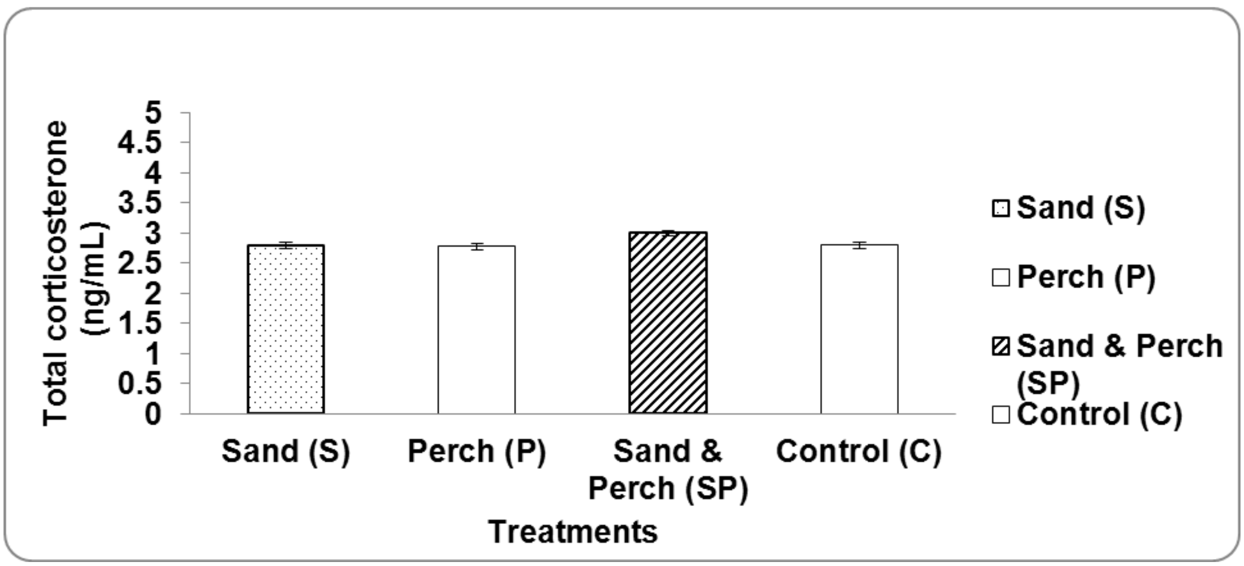
Experiment 2.

Figure 11. Effects of age and temperature on total corticosterone levels (ng/mL) in experiments 1 and 2.

Values are expressed as mean ± SE. Means that do not share similar superscript are different (P<0.05).



Experiment 1



Experiment 2.

Figure 12. Effects of enrichment (treatments) on total corticosterone levels (ng/mL) in experiments 1 and 2.

(*) denotes a trend for higher corticosterone level (P<0.10). Values are expressed as mean ± SE.

Table 6. Least squares means for male and female H:L ratios for experiments 1 and 2.

Experiment 1	Week	Thermoneutral	Heat stress	Means
Male	24	0.37± 0.02^{c}	0.42± 0.02^{c}	0.39± 0.01^{b}
	32	0.63± 0.03^{a}	0.46± 0.02^{bc}	0.54± 0.02^{a}
	40	0.46± 0.02^{bc}	0.57± 0.03^{ab}	0.51± 0.02^{a}
Mean	**--**	**0.48± 0.03^{a}**	**0.48± 0.03^{a}**	---
Female	24	0.30± 0.01^{c}	0.37± 0.01^{bc}	0.33± 0.01^{c}
	32	0.53± 0.02^{a}	0.52± 0.01^{a}	0.53± 0.01^{a}
	40	0.40± 0.01^{b}	0.50± 0.02^{a}	0.45± 0.01^{b}
Mean	**--**	**0.41± 0.02^{b}**	**0.46± 0.02^{a}**	...

Experiment 2	Week	Thermoneutral	Heat stress	Means
Male	24	0.30± 0.01^{c}	0.63± 0.05^{a}	0.47± 0.03^{b}
	32	0.66± 0.04^{a}	0.41± 0.02^{bc}	0.53± 0.02^{ab}
	40	0.73± 0.05^{a}	0.56± 0.04^{ab}	0.64± 0.03^{a}
Mean		**0.56± 0.04^{a}**	**0.53± 0.04^{a}**	
Female	24	0.32± 0.01^{b}	0.37± 0.02^{b}	0.35± 0.01^{b}
	32	0.52± 0.02^{a}	0.45± 0.01^{a}	0.48± 0.01^{a}
	40	0.55± 0.02^{a}	0.45± 0.02^{a}	0.50± 0.01^{a}
Mean	---	**0.46± 0.02^{a}**	**0.43±0.01^{a}**	---

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05)

Female Heterophil/Lymphocyte ratio

As shown in table 6, the means for female H:L ratio fluctuated ($P<0.0001$) across the weeks while the overall means were higher ($P=0.04$) in the HS room. At week 24, there was a trend ($P=0.07$) for H:L ratio of treatment S to be lower than that of SP. Similarly at week 32, treatment P has a trend for higher H:L ratio than treatments S and SP (Table 8).

Total heterophil/lymphocyte (H/L) Ratio

The male and female H:L levels were analyzed together as shown in figure 13. The circulating H:L ratio was significantly ($P<0.0001$) lower at week 24 than at weeks 32 and 40 in both the TN and HS rooms. Furthermore at week 40, H:L ratio was significantly higher in the HS room than the TN room. There was a trend ($P=0.06$) for reduced circulating H:L ratio in the treatment S compared to treatments P, SP, and C (Figure 14).

Behaviors

Preening

In figure 15-16, the birds exposed to the HS environment preened more ($P<0.05$) as they age and there was a trend ($P=0.08$) for higher preening (overall mean) frequency in the HS versus the TN room in experiment 1.

Table 7. Least squares means for female corticosterone levels (ng/mL) for experiment 2.

	Female trt*week (P=0.04)		
Treatments	**Wk. 24**	**Wk. 32**	**Wk. 40**
Sand (S)	2.79^{b}	2.88^{b}	2.58^{b}
Perch (P)	3.13^{ab}	3.11^{ab}	2.48^{b}
Sand & Perch (SP)	2.57^{b}	2.38^{b}	3.95^{a}
Control (C)	2.75^{b}	2.84^{b}	3.07^{ab}

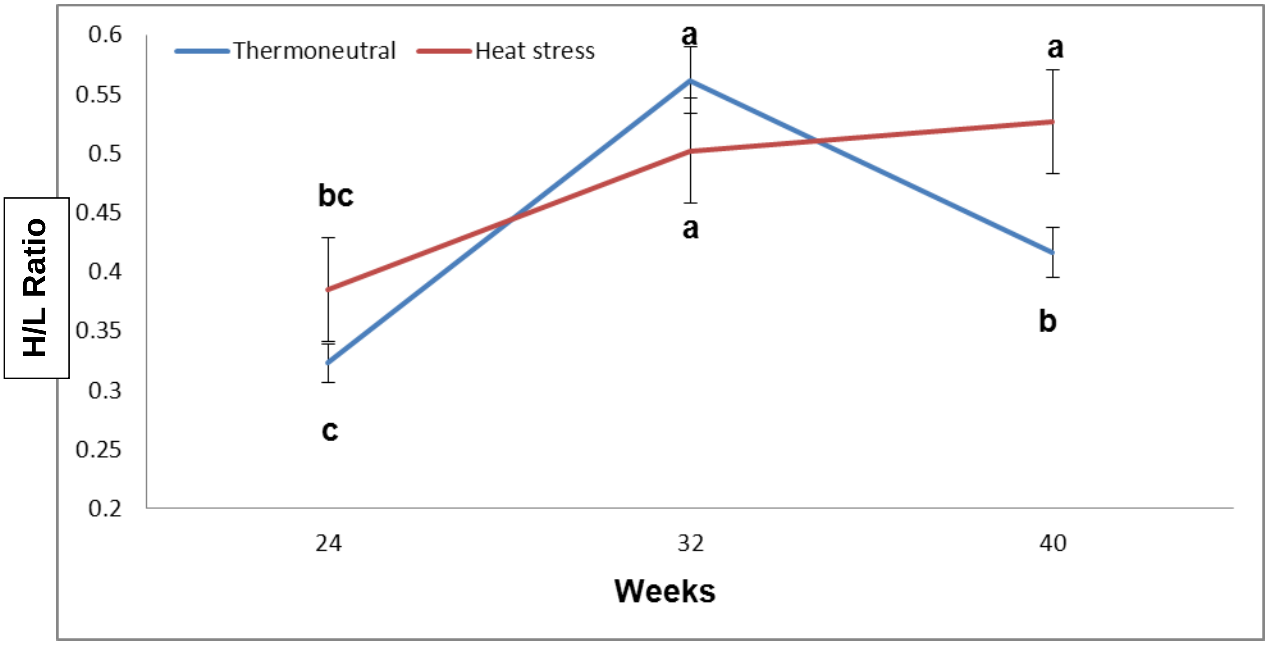
Values are expressed as mean ± SE. Within experiment, means that do not share similar superscript within the same column and rows differ (P<0.05)

Table 8. Least squares means for female H:L ratios for experiment 1

P=0.07

Weeks	**Week 24**	**Week 32**	**Week 40**
Sand (S)	0.27^{e}	0.44^{bcd}	0.45^{bc}
Perch (P)	0.31^{de}	0.63^{a}	0.43^{bcd}
Sand & Perch (SP)	$0.42b^{cd}$	0.52^{bcd}	0.4^{ab}
Control (C)	0.33^{cde}	0.52^{ab}	0.51^{ab}

Effects of age and enrichment on heterophil: lymphocyte ratios in broiler breeder females in experiment 1. Values are expressed as mean ± SE. The means that do not share similar superscript within the same column tended to differ (P<0.10).



Experiment 2.

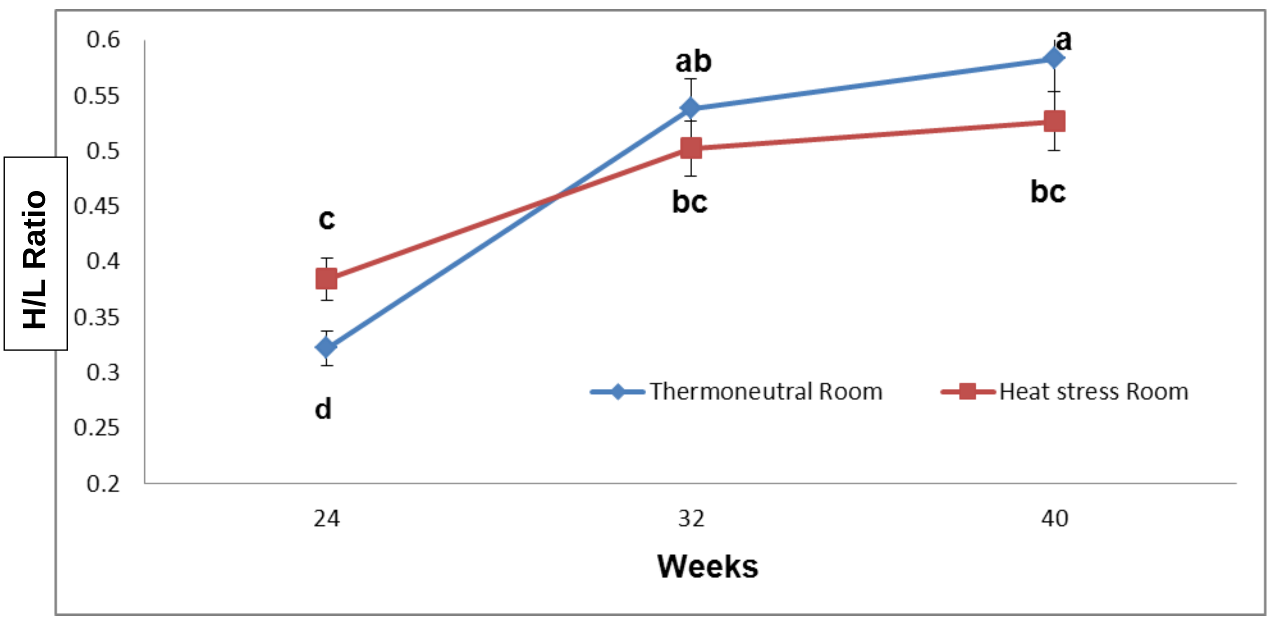
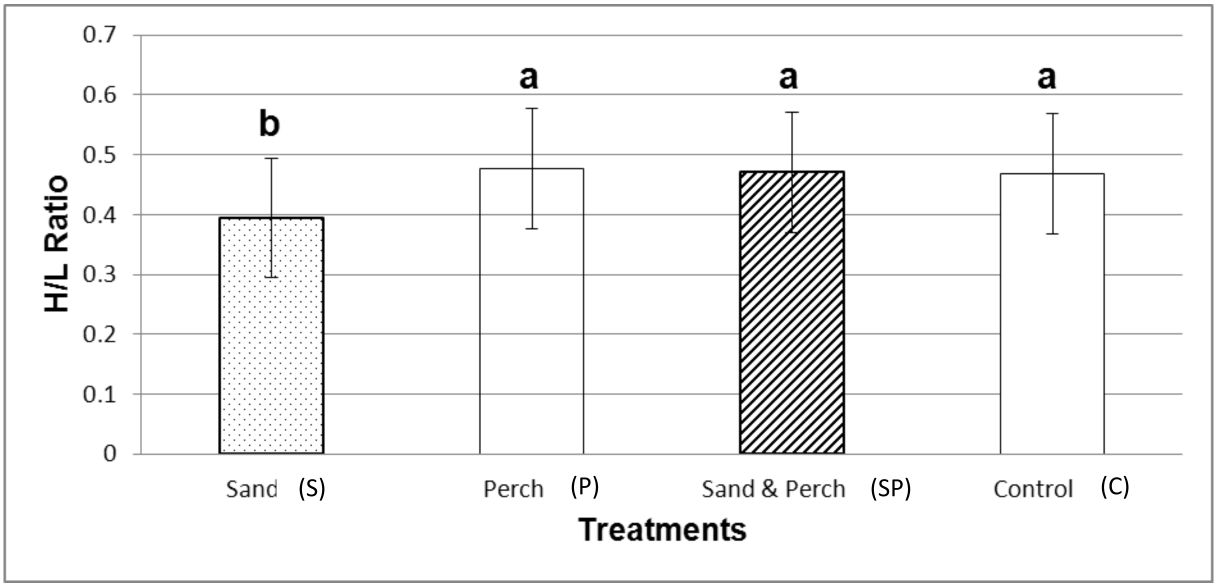
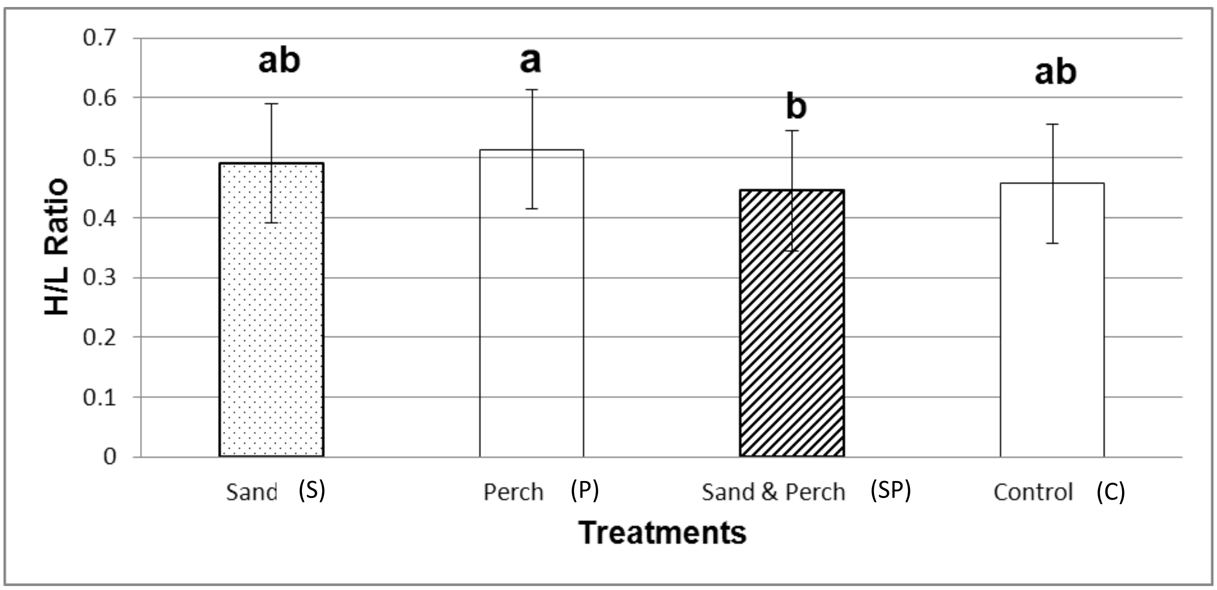


Figure 13. Effects of heat stress on total H:L ratios in experiments 1 and 2. Means that do not share similar superscripts are different (P<0.05).

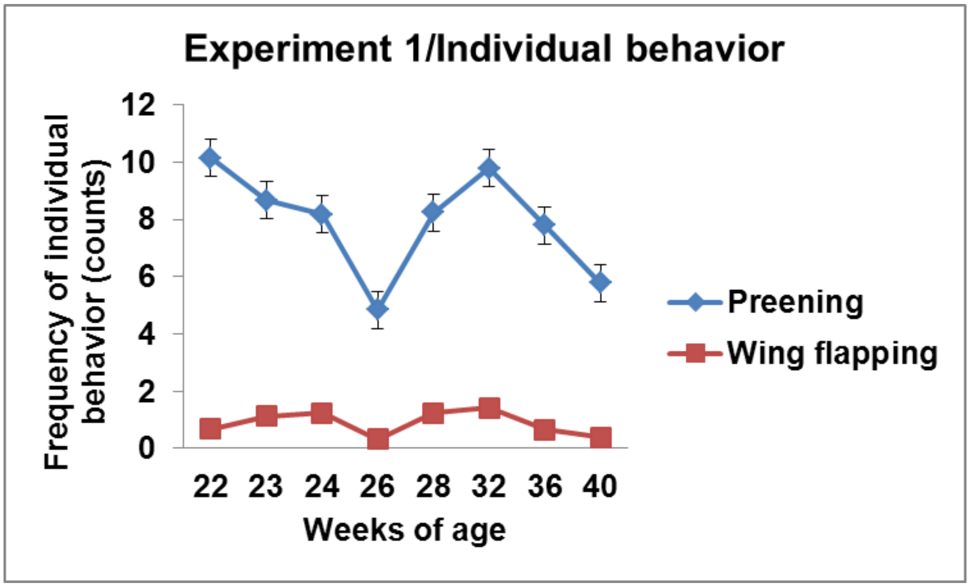


Experiment 1.

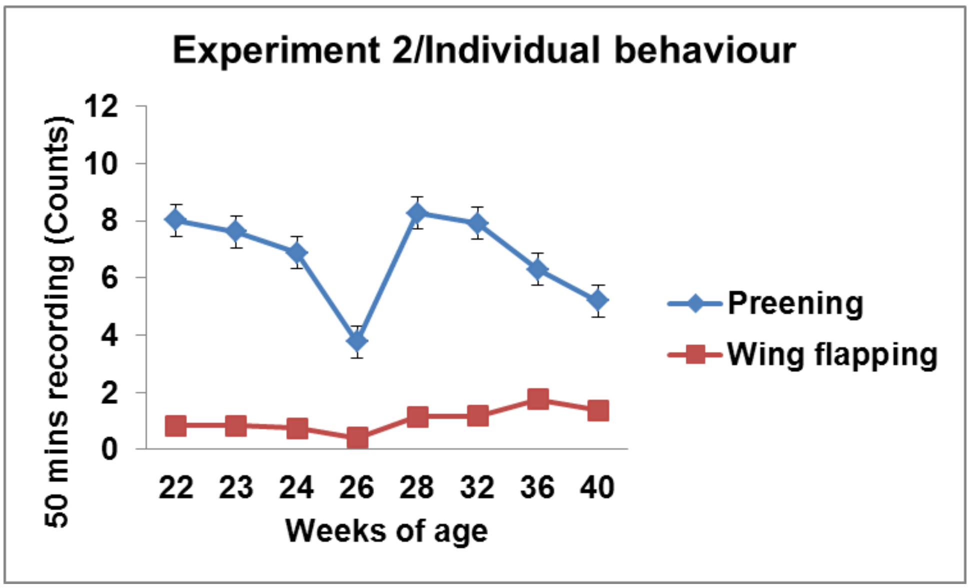


Experiment 2.

Figure 14. Effects of enrichment (treatments) on H:L ratio in experiments 1 and 2 Means that do not share similar superscripts within experiments tended to differ (P<0.10)



Experiment 1



Experiment 2

Figure 15. Effects of age and heat stress on individual behavior (preening and wing flapping) in experiments 1 and 2.

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ ($P<0.05$)

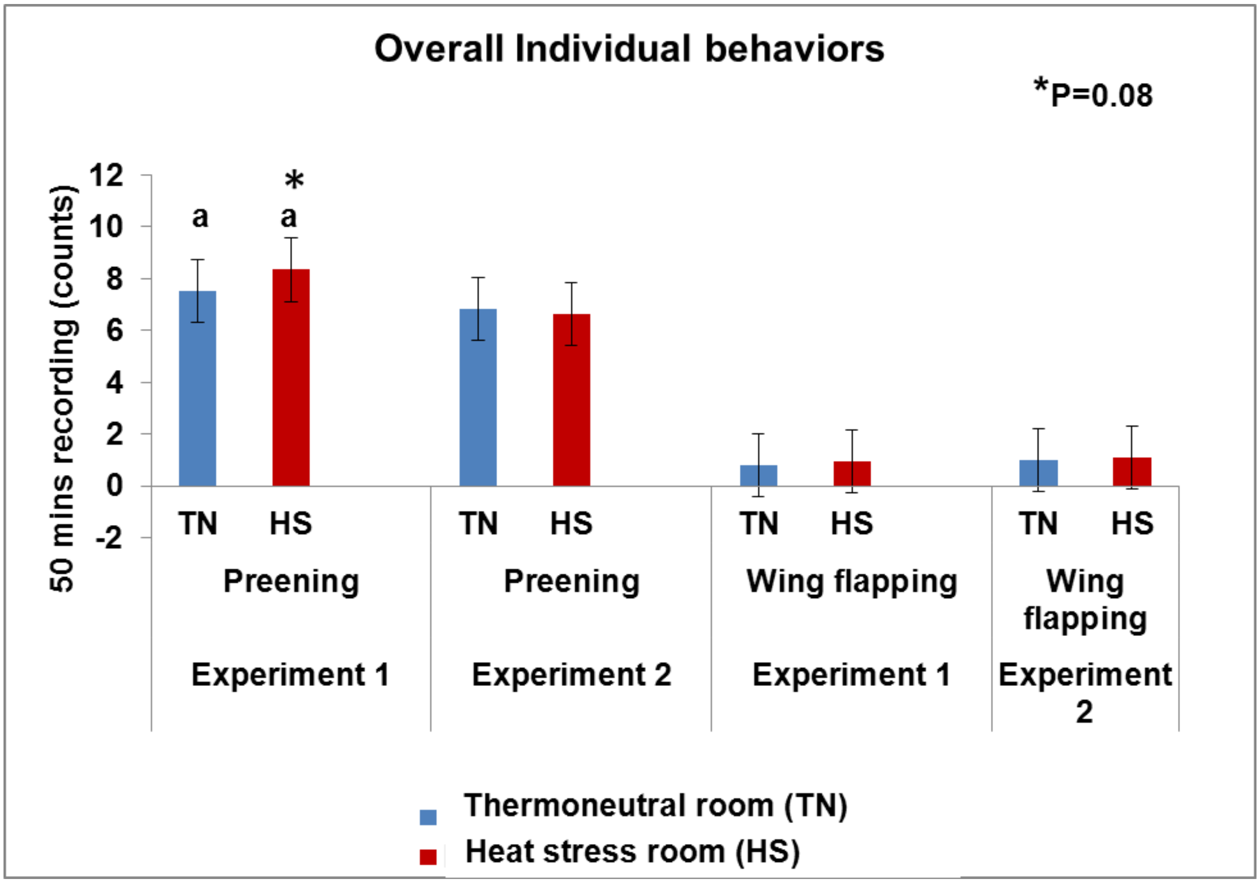


Figure 16.

Figure 16. Effects of heat stress on individual behaviors (preening and wing flapping) in experiments 1 and 2.

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05). (*) denotes comparison between the TN and HS rooms of the same experiment at P<0.10

Similarly (Figure 17), there was a trend (P=0.07) for higher preening frequency in treatment P than S. The room temperatures affected preening (Figure 16) of the birds in each treatments separately (P=0.0005). Thus, the birds in the S and C treatments of the HS room preened more than their counterparts in the TN room. Also, the birds housed in treatment P of the TN room preened more than their counterpart in the HS room.

Wing flapping

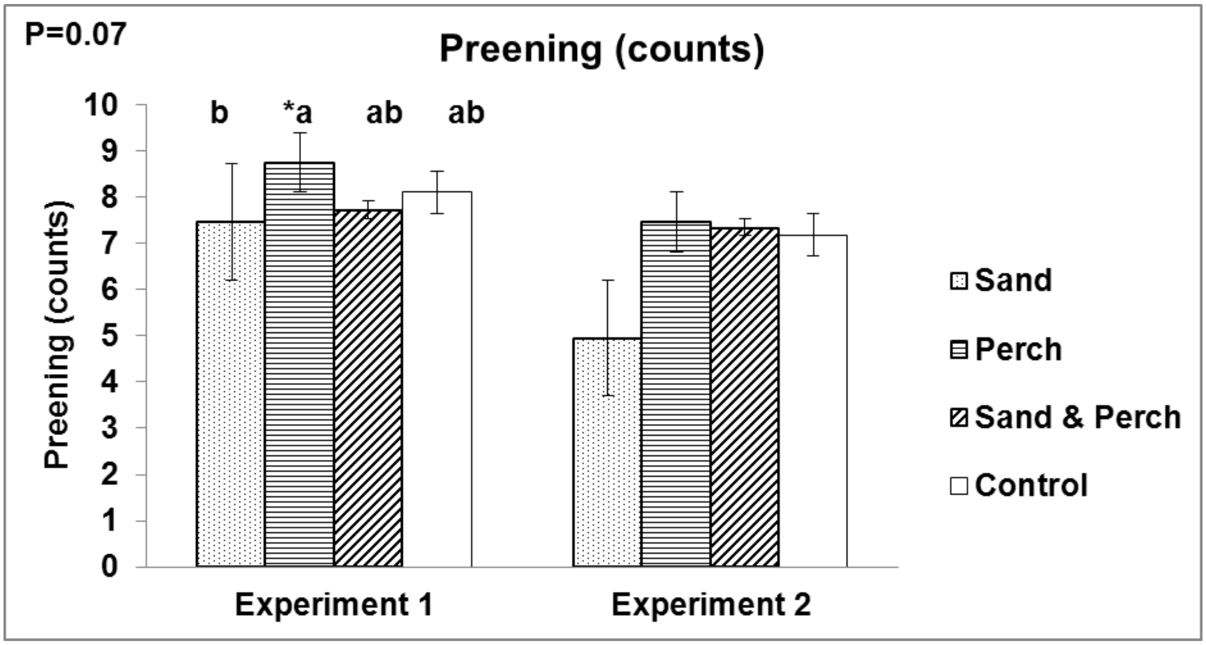
The frequency of wing flapping as the birds' age is shown in figure 15. Room temperature did not affect the overall wing flapping behavior (Figure 16). The birds in the control treatment tended to flap their wings more frequently (P=0.05) compared to those in the perch treatment (Figure 17).

Frequency and duration of sand use

The frequency and duration of sand use between treatments S and SP are as shown in figures 19-20. The frequencies of sand use were highest (P=0.0015) at week 23 and lowest at week 28 (Figure 15). Room temperatures and the treatments S and SP did not influence the frequency and duration of sand use (Figure 21).

Frequency and duration of perch use

The frequency and duration of perch use as birds' age in treatments P and SP are as shown in figures 19-20. The frequency of perch use was higher (P=0.00201) in treatment SP of the HS room at week 28 compared to the same treatment in the TN room. There was a trend (P=0.07) for increased frequency (overall mean) of perch use in treatment SP than P (Figure 22).



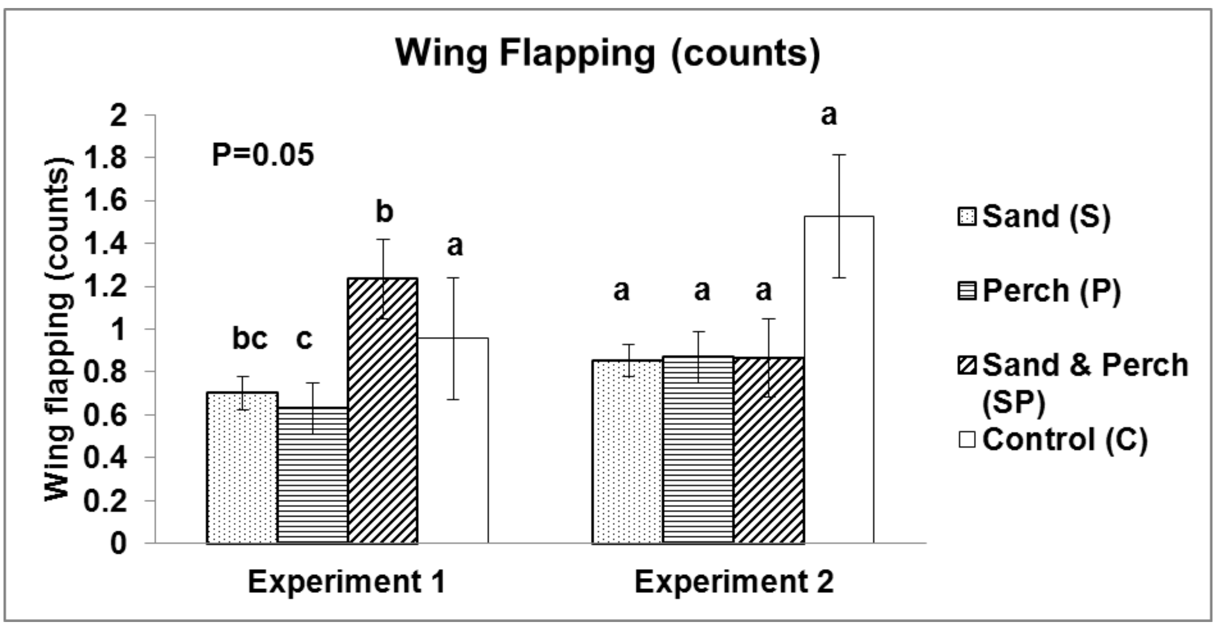
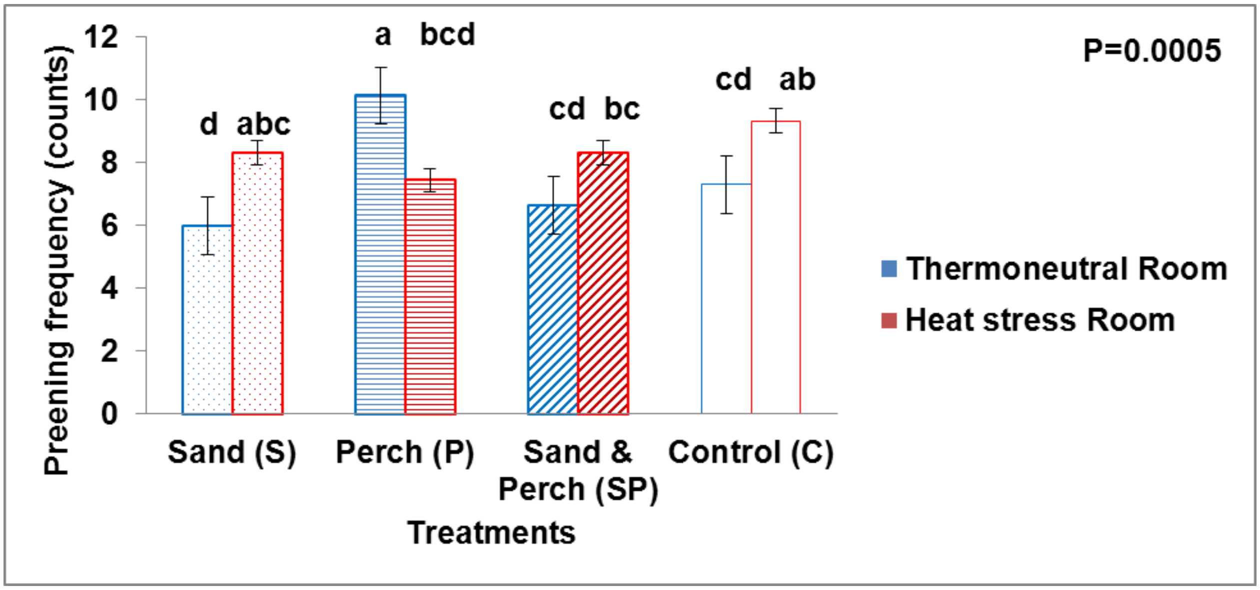
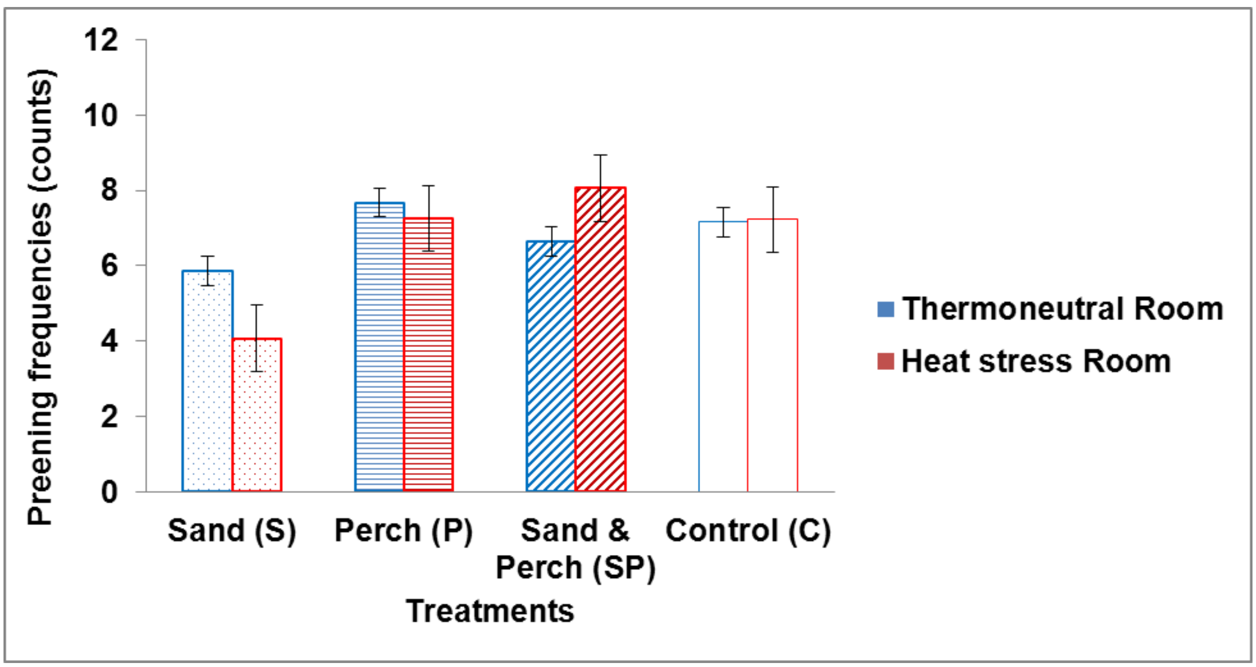


Figure 17. Effects of enrichment (treatments) on individual behavior in experiments 1 and 2.

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript differ (p<0.05). (*) denotes means that do not share similar superscript differ within the experiment tended to differ at p<0.10.



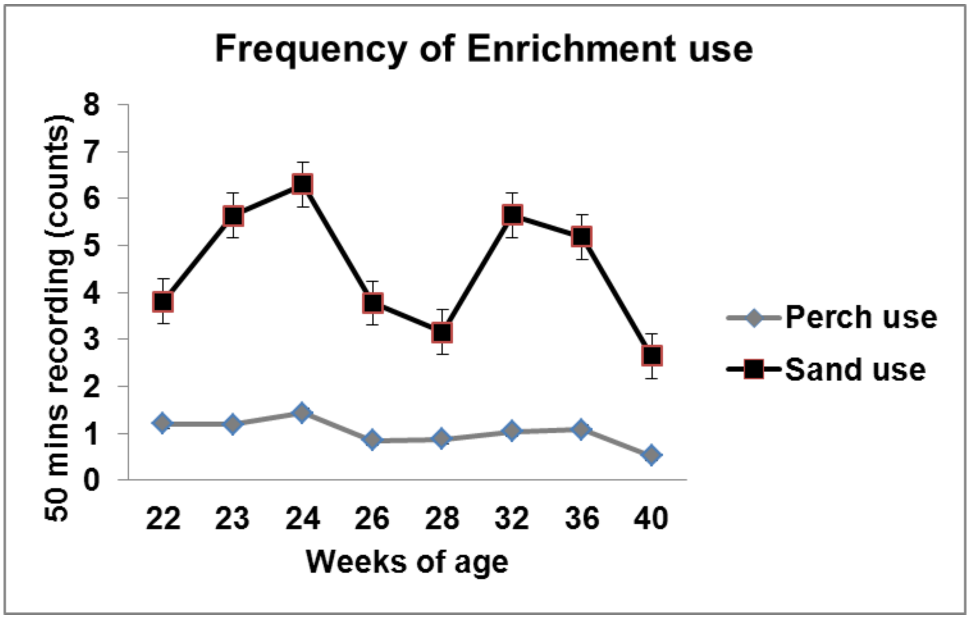
Experiment 1



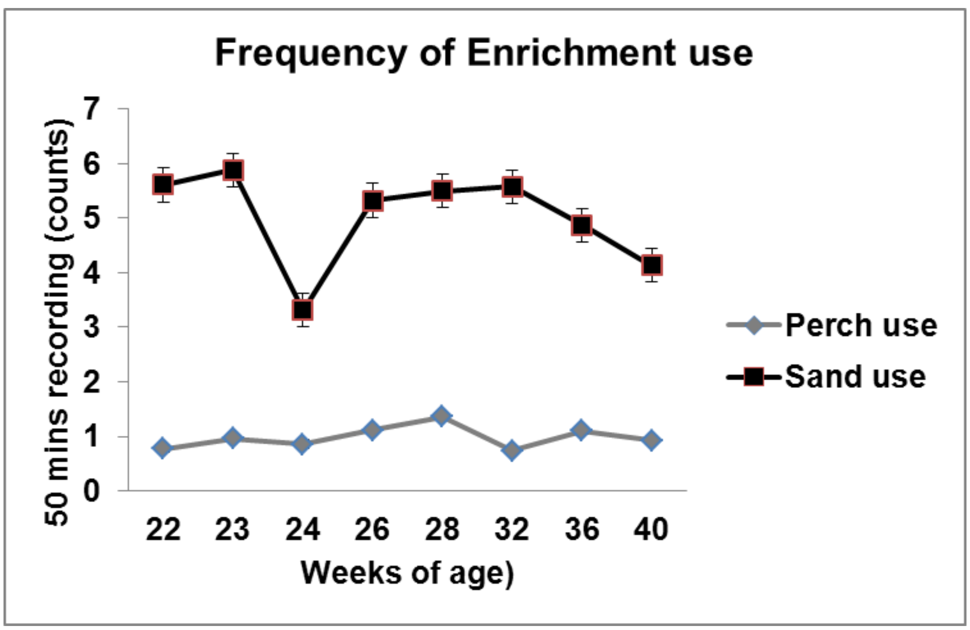
Experiment 2

Figure 18. Effects of enrichment (treatments) and heat stress on experiments 1 and 2.

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript (P<0.05)



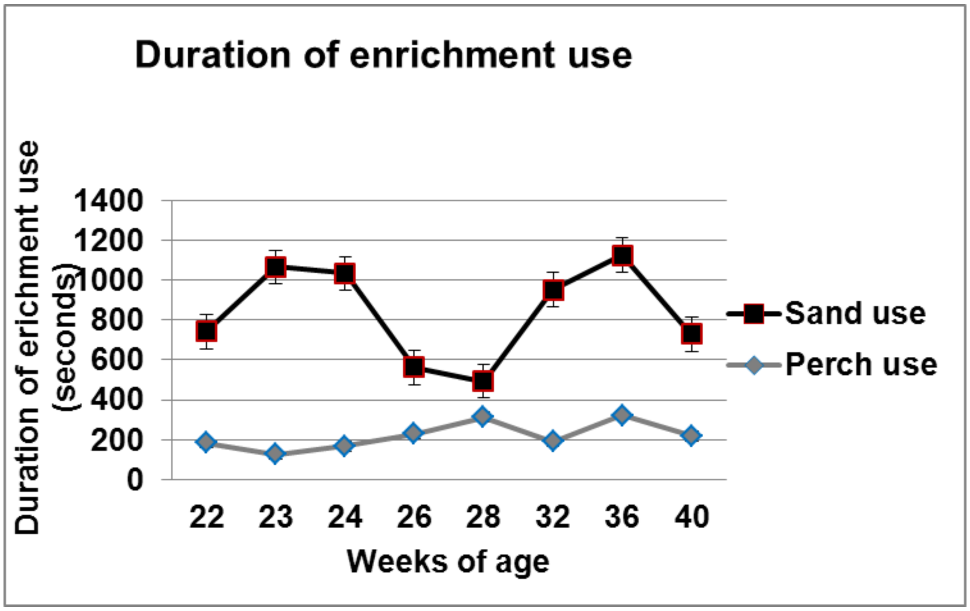
Experiment 1



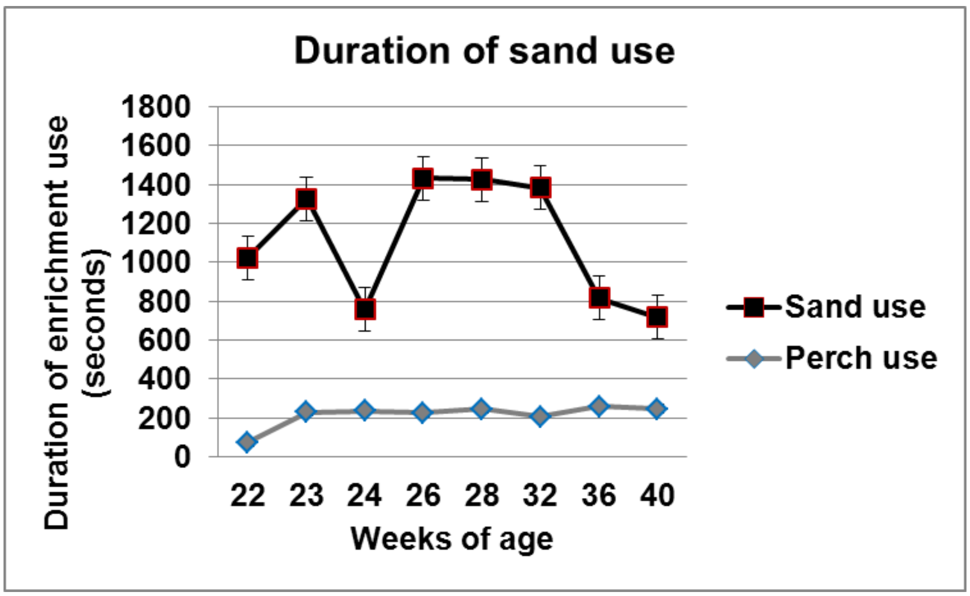
Experiment 2

Figure 19. Effects of age on frequency of enrichment use

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05)



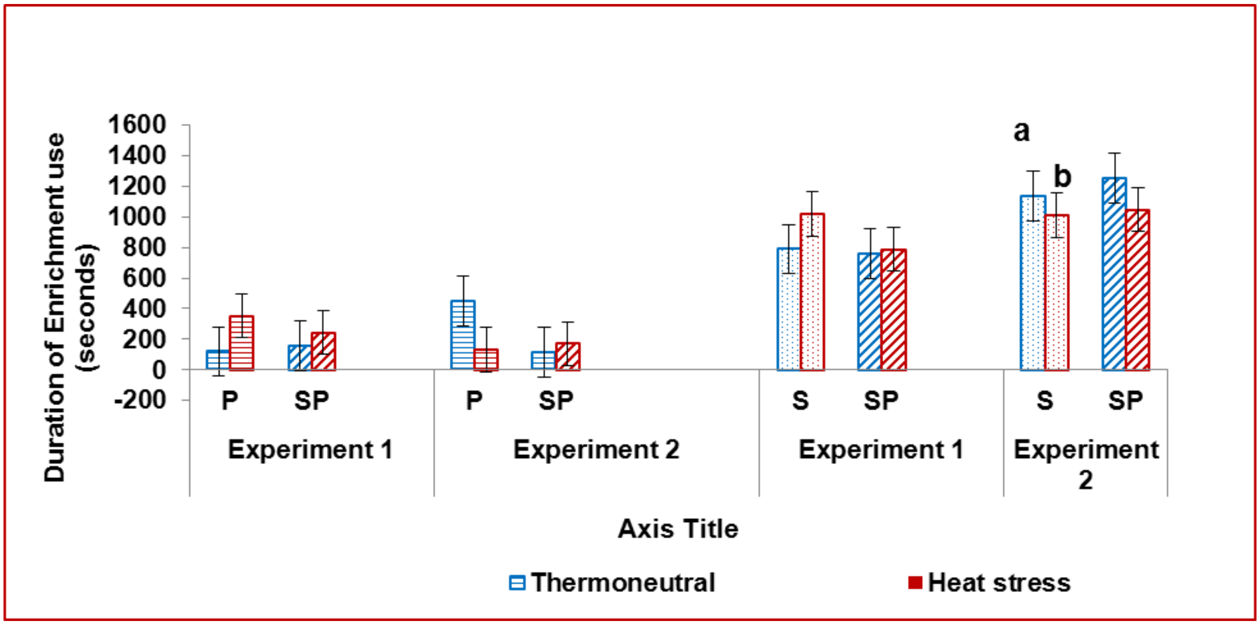
Experiment 1



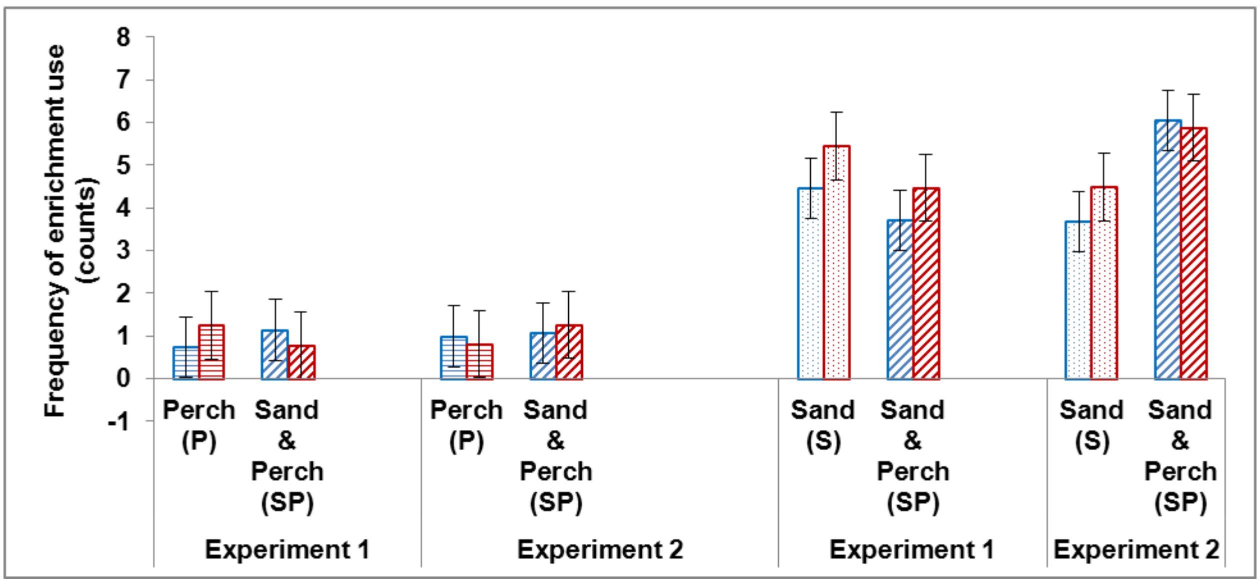
Experiment 2

Figure 20. Effects of age on duration of enrichment use.

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05)



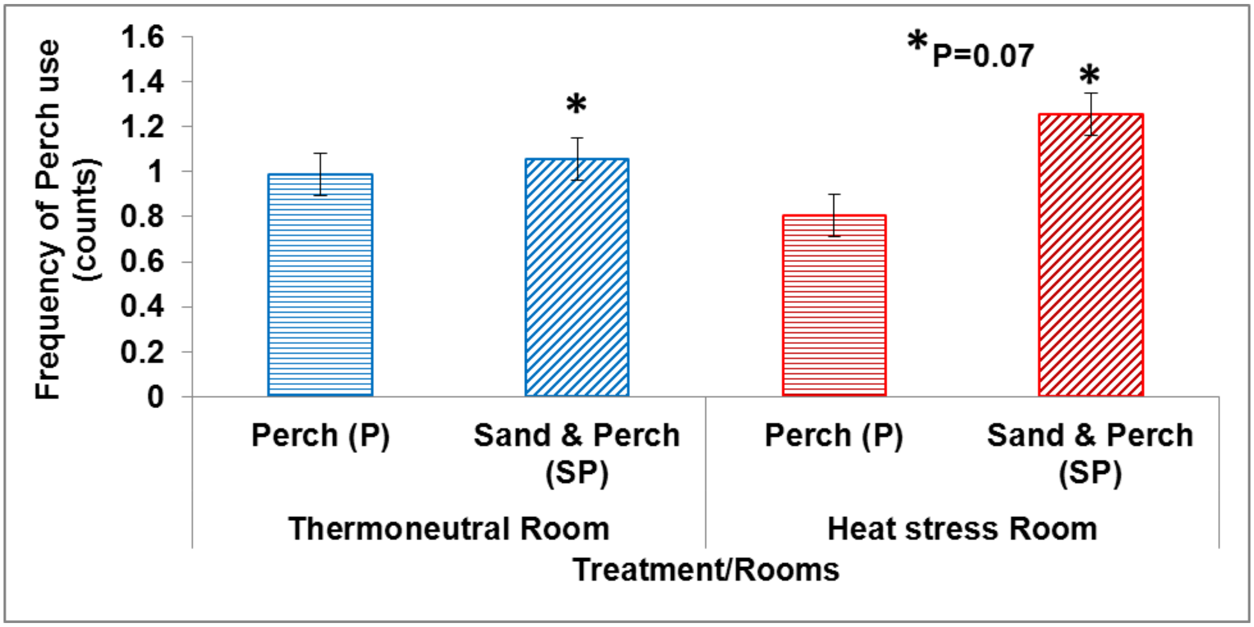
Experiment 1



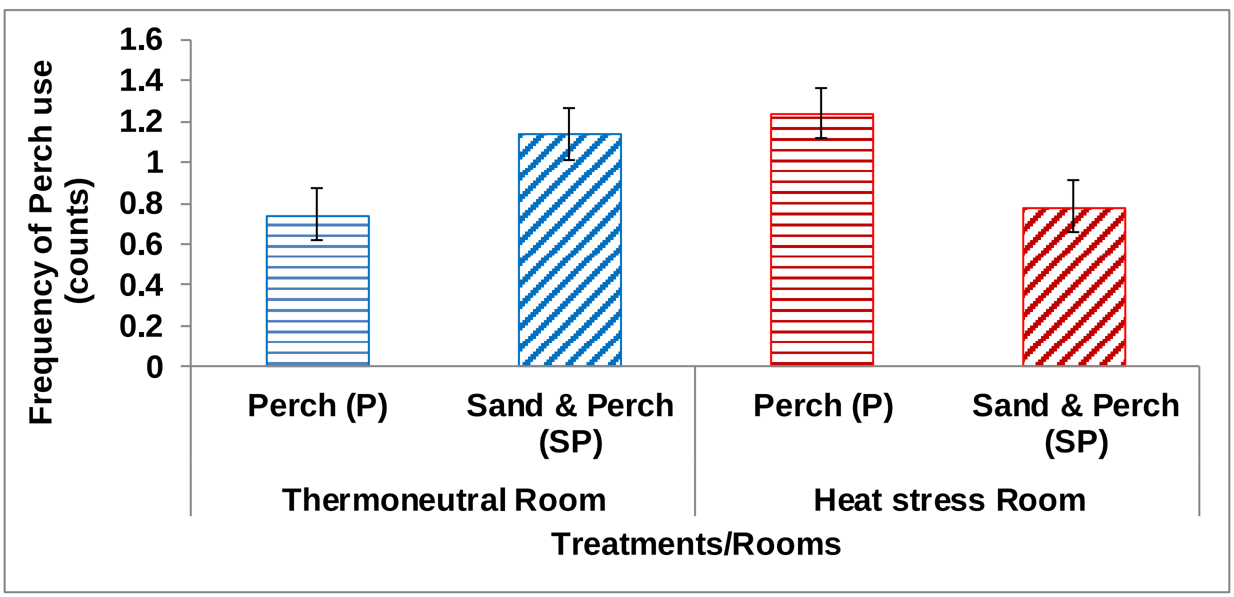
Experiment 2

Figure 21. Effects of treatments and heat stress on the frequency and duration of enrichment use.

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05)



Experiment 1



Experiment 2

Figure 22. Effects of perch use in treatments P and SP.

Effect of perch use in treatments S and SP of the TN and HS rooms. Values are expressed as mean ± SE. (*) denotes that treatment within the same room tend to differ at P<0.1

The frequency of perch use tended to be higher (P=0.05) in treatment SP of the HS room versus those of the TN room as birds age.

Attempted Mating

There were differences (P<0.0001) in the occurrence of attempted mating as shown in figure 23. There was a higher (P=0.02) frequency of attempted mating at week 26 of in the TN room compared to the HS room. The overall frequency of attempted mating was greater (P<0.01) in the TN room compared to the HS room (Figure 24). The perch treatment had the least (P=0.03) occurrence of attempted mating (Figure 25).

Completed Mating

The incidence of completed mating increased (P=0.0067) towards the latter part of the experiment as shown in figure 23. There was a higher frequency of completed mating in the TN room at week 26 (P=0.0082). Regardless of the treatments (Figure 24), birds in the TN room tended to performed more (P=0.05) completed (overall mean) mating compared to the HS room. As shown in figure 26, birds in treatment S of the TN room performed increased (P=0.0029) rate of completed mating at week 26. Likewise, birds in treatment SP of the TN room performed more completed mating than their counterpart in the HS room at week 26. Birds in treatment C of the TN room performed more completed mating compared to those in the HS room.

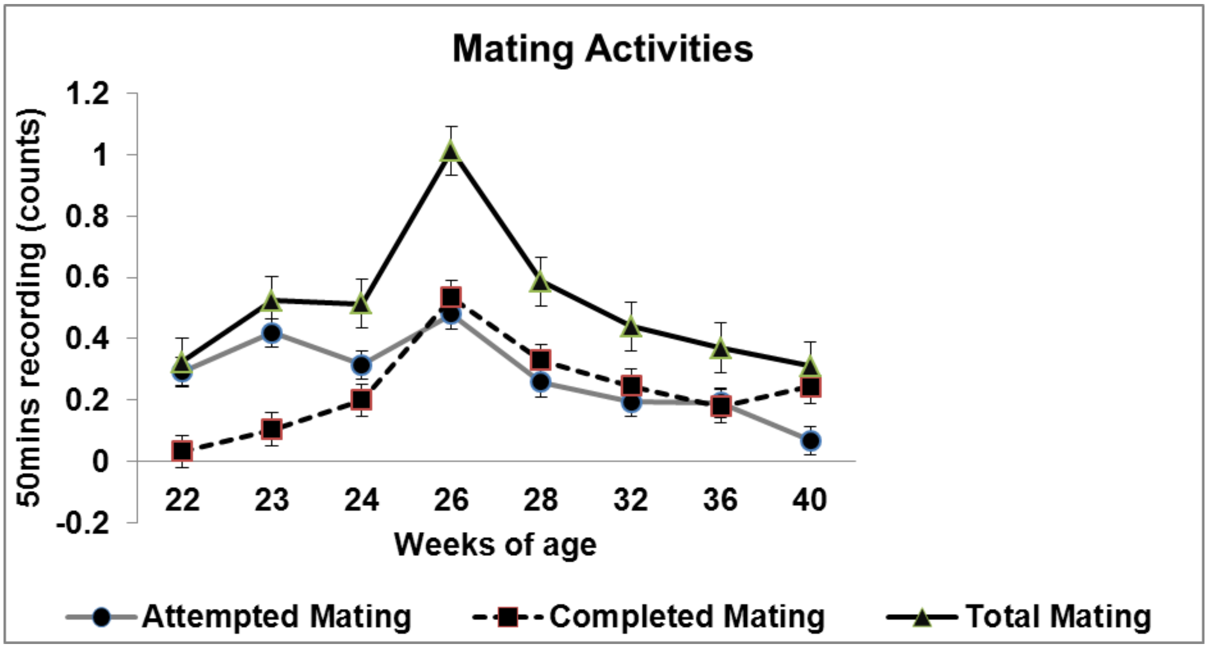
Total Mating

Both attempted and completed mating was examined together (Figure 23). There was a trend (P=0.08) for increased overall mating in TN room compared to the HS room as the birds age. The different treatments in the TN and HS rooms tended to differ (P=0.05) across the weeks. At week 26, the birds in the sand treatment of the TN room tended to perform more mating activities compared to those in the HS room. The total mating in birds that had perch in their housing was similar across the weeks. The birds in the SP treatment tended to perform more mating in the TN room than the HS room at weeks 22, 26, and 40. In experiment 1, the overall mating activities were higher (P=0.0013) in the birds reared in the TN room compared to the HS room (Figure 19).

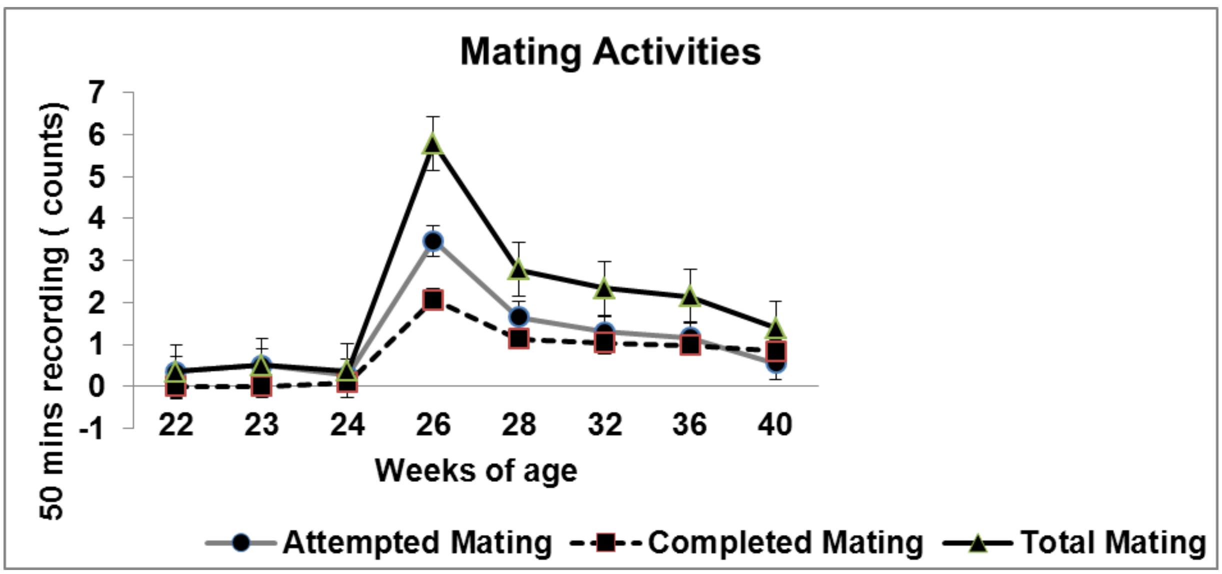
Aggressive Behavior (fighting and pecking)

The occurrence of aggressive fighting and pecking were very low in the first experiment such that the data were analyzed as all or none. Hence, regardless of the number of times the aggressive fighting and pecking occurred, it was scored 100 and when absent, aggressive behavior was scored zero.

Temperature and treatment did not affect the rate of aggressive fighting (Figure 27).



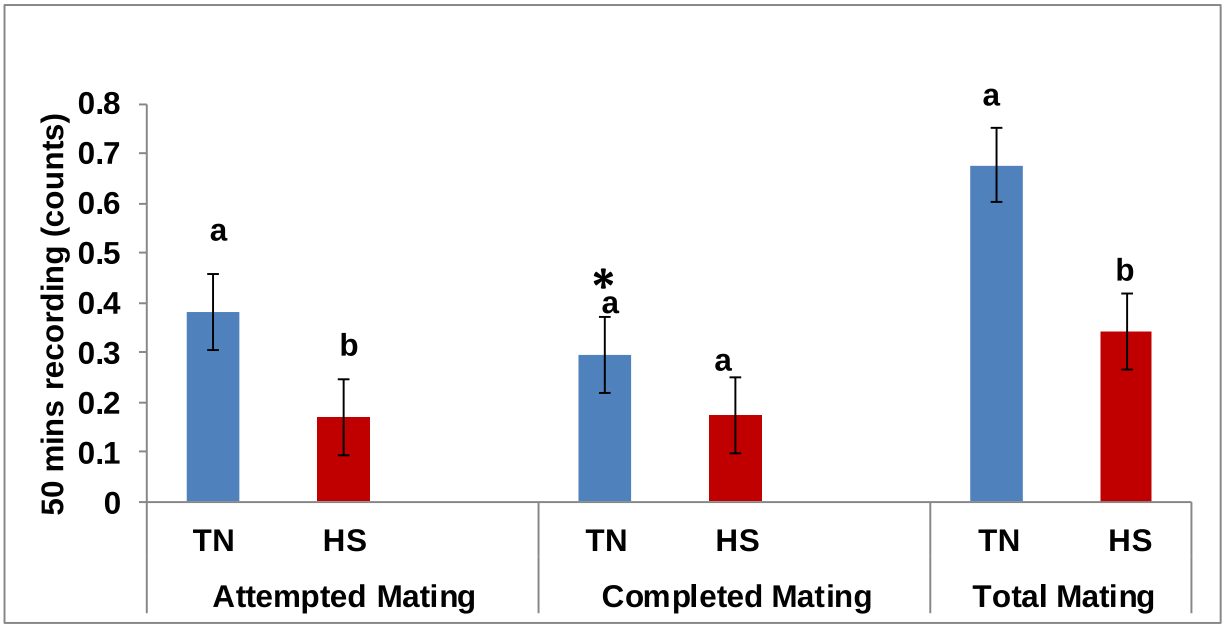
Experiment 1



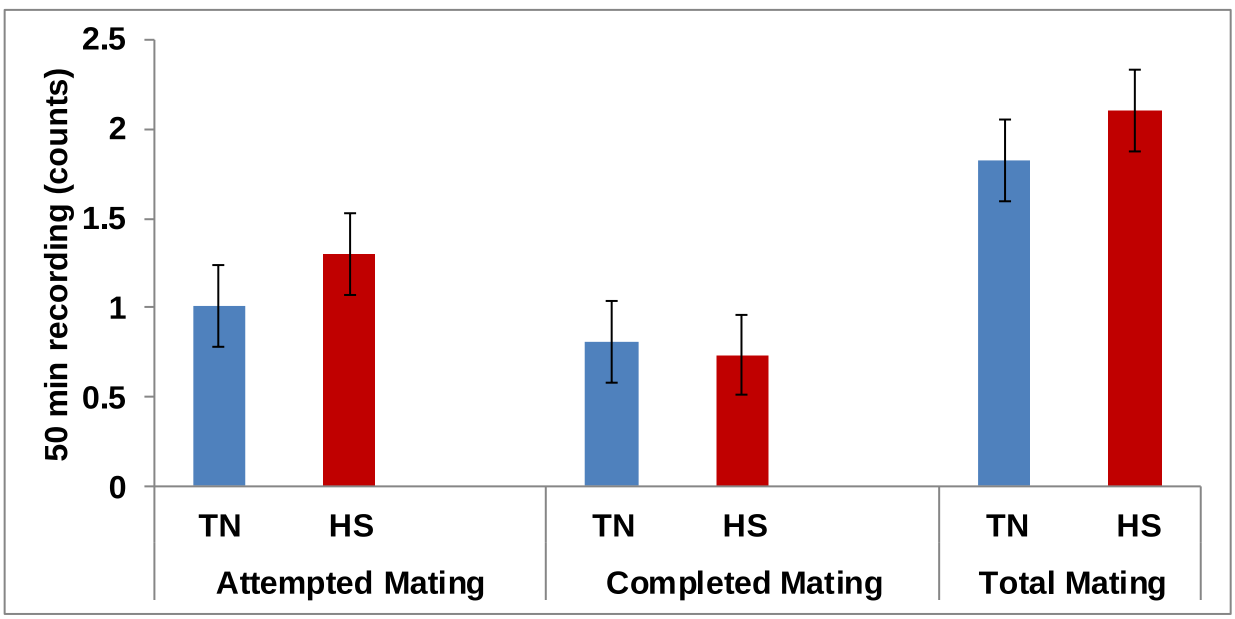
Experiment 2

Figure 23. Effects of age on mating activities (attempted, completed, and total) in both experiments 1 and 2

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05)



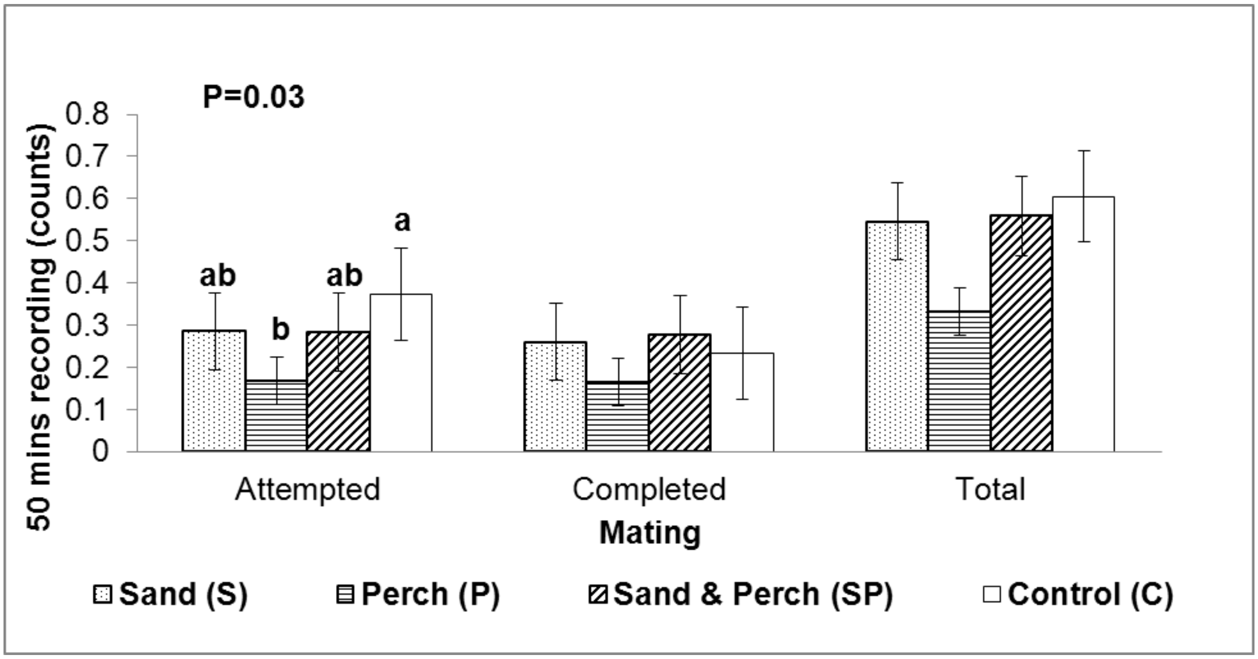
Experiment 1



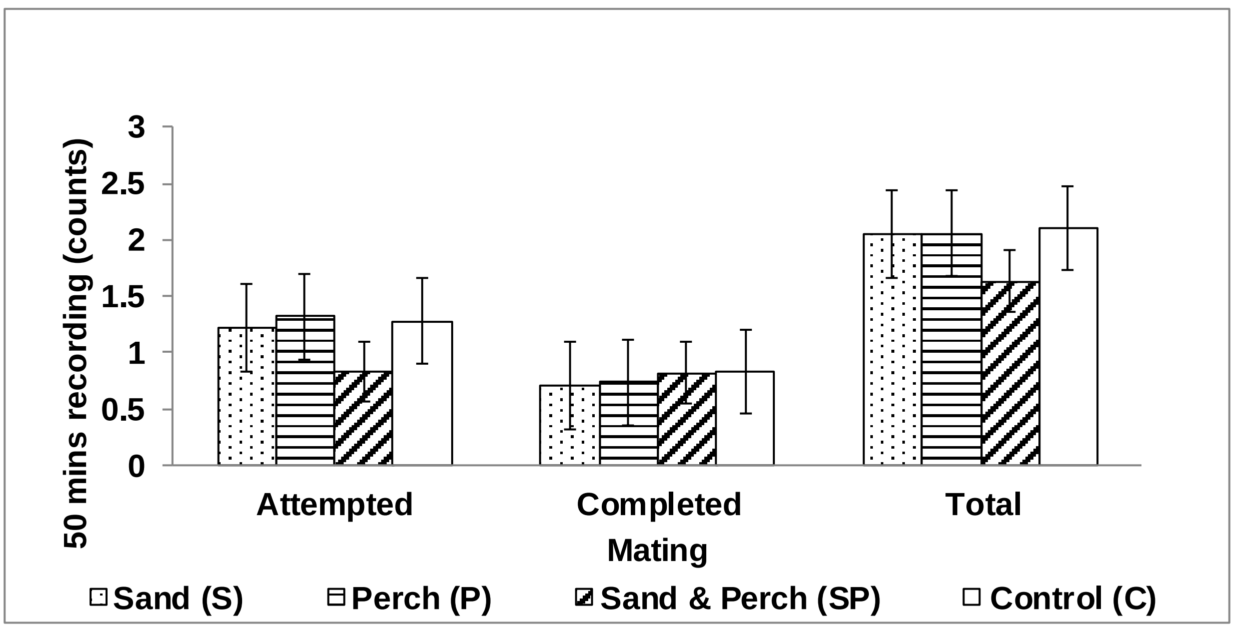
Experiment 2

Figure 24. Effects of heat stress on mating activities in experiments 1 and 2.

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same parameter differ (P<0.05). (*) denotes trend for subject to differ



Experiment 1



Experiment 2

Figure 25. Effects of enrichment on mating activities regardless of the rooms (thermoneutral and heat stress)

Values are expressed as mean ± SE. Within experiment, the means that do not share similar superscript within the same column and rows differ (P<0.05)

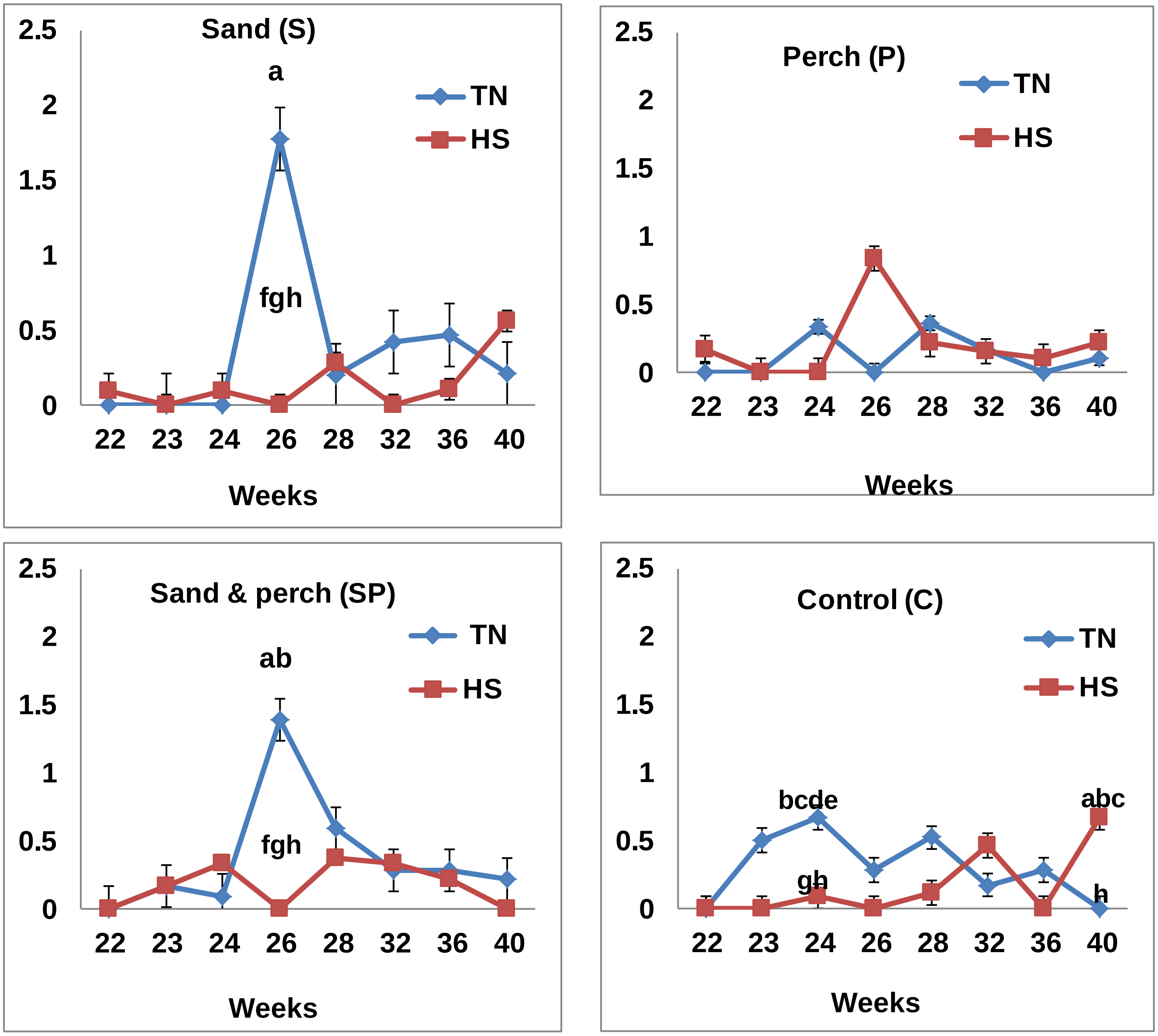
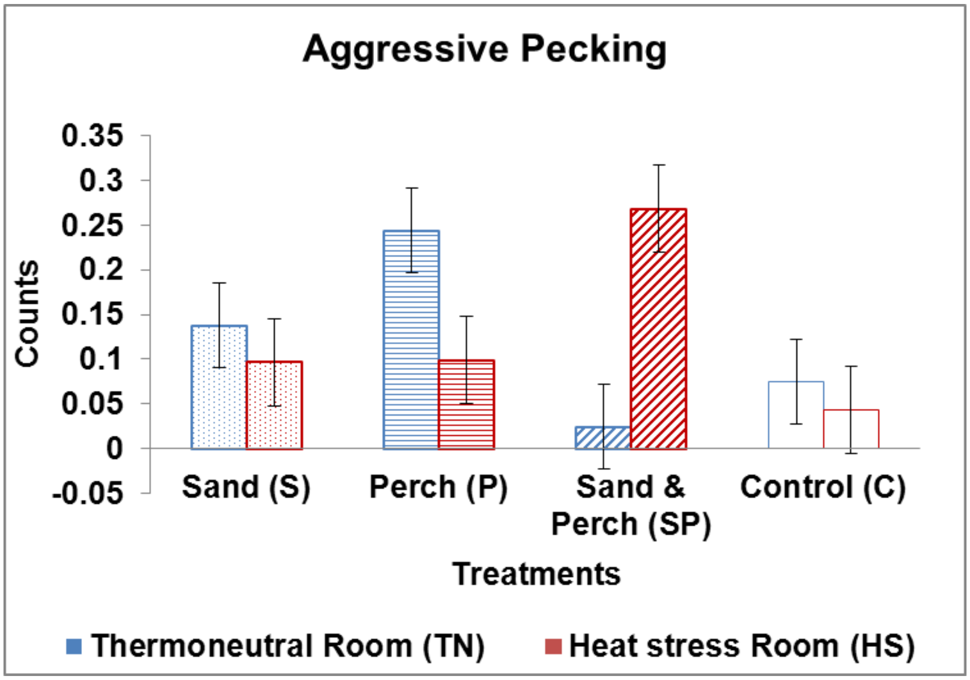
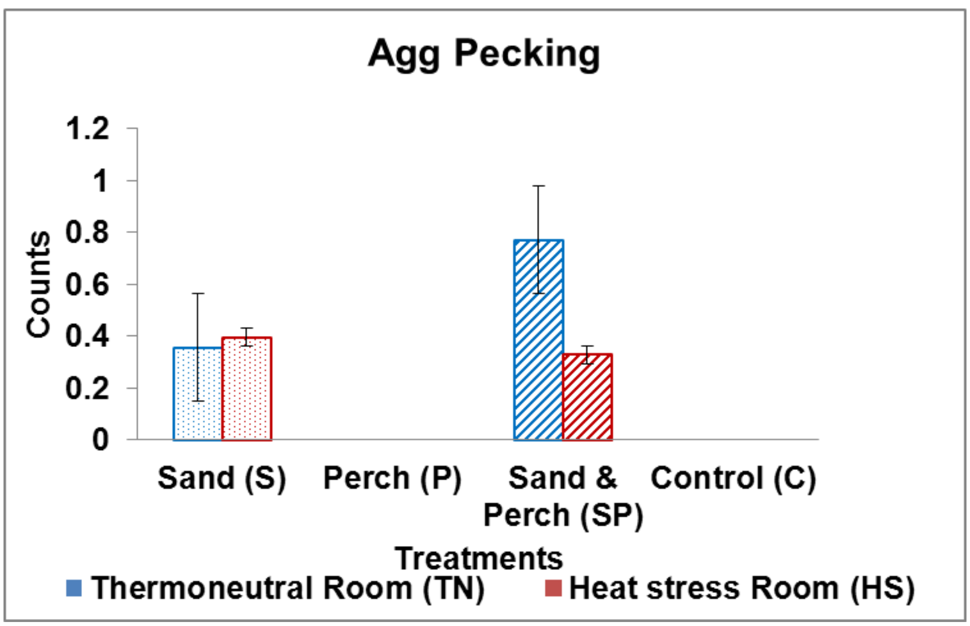


Figure 26. Effects of age and treatments on completed matings in experiment 1

Complete mating in the four treatments in both thermoneutral (TN) and heat stress (HS) rooms in Experiment 1



Experiment 1



Experiment 2

Figure 27. Effects of enrichment on aggressive pecking.

Experiment 2

Egg Production

As shown in figure 4, the highest egg production was at week 32 and the lowest at week 24, when egg production commenced. Enrichment (treatments) did not affect egg production in either the TN or HS rooms (Figure 5).

Egg Weight

Enrichment (treatments) did not affect egg weight in both TN and HS rooms. Regardless of rooms and treatments, egg weight significantly increased ($P<0.0001$) with age from weeks 26 to 40 (Table 1). There was a trend ($P=0.0798$) for higher overall mean egg weight in the TN room compared to the HS room (Table 2). At weeks 28 and 40 egg weights were higher ($P=0.0143$) in the TN room.

Floor egg

The numbers of floor eggs significantly ($P<0.0001$) differ with age. The incidence of floor eggs were significantly higher ($P=0.01$) for the TN room as birds age especially at weeks 26, 27, and 28 than those in the HS room (figure 7). The incidences of overall percent floor eggs in the TN room tended ($P=0.09$) to be higher than that of the HS room. Enrichment did not significantly affect the occurrence of floor eggs (Figure 8)

Fertility

In table 3, fertility increased ($P=0.03$) from weeks 26 to 28 and was maintained until the experiment was terminated at week 40 (Table 3). At 26 weeks of age (Table 9), hens housed in treatment P had a trend ($P=0.10$) for reduced fertility compared to the

Table 9. Least squares means for percent fertility for experiment 2.

Week	Sand	Perch	Sand & Perch	Control
26	91.67^{cd}	88.89^{d}	95.83^{abc}	94.45^{abcd}
28	95.84^{abc}	100.00^{a}	93.06^{bcd}	97.22^{abc}
32	93.06^{bcd}	100.00^{a}	95.84^{abc}	95.83^{abc}
36	98.62^{ab}	97.22^{abc}	98.61^{ab}	93.06^{bcd}
40	95.84^{abc}	98.61^{ab}	98.61^{ab}	97.22^{abc}

Experiment 2.

Values are expressed as mean ± SE. Means that do not share similar superscript within the same colum and rows tended to differ (P<0.10).

treatment SP. Similarly, there was also a trend for higher fertility in the perch treatment at week 28 and 32 compared to treatments SP and S, respectively. Room temperatures did not influence the overall fertility (mean).

Hatchability

Hatchability increased ($P<0.01$) from weeks 26 to 28 and remained high until the experiment was terminated at week 40 (Table 4). Enrichment did not affect hatchability (Figure 9). There was a strong positive correlation ($r=0.95$, $P<0.0001$) between fertility and hatchability in experiment 2.

Corticosterone (CORT) **levels**

Male CORT levels

At week 24, there was a trend ($P=0.09$) for elevated CORT levels in the HS room compared to those in the TN (Table 5). The overall CORT levels were higher ($P=0.0435$) in the birds reared in the HS room compared to the TN room (Table 5).

Female CORT levels

There was a trend for higher ($P=0.07$) overall CORT level in the HS room (Table 7). At week 40, the birds in the SP treatment had a higher ($P=0.04$) CORT levels compared to those in treatments S and P (Table 8).

Total CORT

The total CORT levels were higher at week 24 in the HS environment (Figure 11). Enrichment did not affect total CORT levels (Figure 12).

Male heterophil/lymphocyte

In table 6, H:L ratio was lower (P<0.05) at week 24 compared to week 40 in the TN room. At week 24, H:L ratio was higher in the HS room than TN room. Similarly at week 32, H:L ratio was higher in the TN room. Treatments S of the HS room and P of the TN room had trends for higher H:L ratio than their counterparts in the TN and HS rooms respectively (P=0.08).

Female Heterophil/Lymphocyte ratio

The H:L ratio was least (P<0.05) at week 24 compared to weeks 32 and 40 but there was no treatment effect.

Total heterophil/lymphocyte (H/L) Ratio

The H:L ratio in the TN room was significantly (P<0.0001) lower at week 24 compared to weeks 32 and 40 (Figure 13). At week 40, H:L ratio was higher in the TN than the HS room. The male and female H:L ratios were analyzed together. From the results (Figure 14), there was a trend (P<0.08) for lower H:L ratio in the SP treatment.

Behaviors

Preening

As shown in figure 15, preening differed (P<0.05) throughout the study as the birds age. Preening frequency was similar in both the TN and HS room (Figure 16). Numerically, the value of preening in treatment S was lower than treatments P, SP, and C but the difference was not significant (figure 14).

Wing flapping

In figure 15, the frequency of wing flapping differed ($P<0.05$) as birds age. Temperature (Figure 16) and treatments did not (Figure 17) affect wing flapping.

Attempted Mating

In figure 23, the occurrence of attempted mating fluctuated ($P<0.0001$) as the birds age. Temperature (Figure 24) and enrichment (figure 25) did not affect attempted mating behavior.

Completed Mating

In figure 23, completed mating increased ($P<0.0001$) towards the latter part of the experiment.

Total Mating

Both attempted and completed mating was examined together (Figure 23). At week 26, the birds in the sand treatment of the TN room tended ($P<0.10$) to perform more mating activities compared to those in the HS room. Enrichment did not influence total mating (Figure 25).

Frequency and duration of sand use

The frequency and duration of sand use was compared between the treatments S and SP. The frequency and duration ($P=0.05$) of sand use as birds age are shown in figure 19-20.The duration of sand use was higher ($P=0.0031$) in the TN room compared to the HS room (Figure 21). The treatments did not influence the frequency and duration of sand use (Figure 21).

Frequency and duration of perch use

The frequency and duration of perch use was compared between the treatments P and SP. Enrichment and heat stress did not influence the frequency and duration of perch use (Figure 21).

Aggressive Behavior

The occurrence of aggressive fighting and pecking was very low experiment 2 such that the data were analyzed as all or none. Hence, regardless of the number of times the aggressive fighting and pecking occurred, it was scored 100 and when absent, aggressive behavior was scored zero. Temperature and treatment did not affect the rate of aggressive fighting.

Aggressive Pecking

Aggressive pecking occurred only in treatments S and SP (Figure 27) and its occurrence is as shown in Table 10 ($P<0.0001$). The occurrence of aggressive pecking was higher ($P=0.0081$) in the TN room at week 23.

Table 10. Least squares means for weekly aggressive pecking behavior in experiments 2.

Weeks	Heat stress Room (HS)	Thermoneutral Room (TN)	Mean
22	0.4583^{bc}	0.3333^{c}	0.3958^{b}
23	0.4167^{bc}	1.3750^{a}	0.8958^{a}
24	0.3750^{bc}	0.5000^{bc}	0.4375^{b}
26	0.2083^{bc}	1.1111^{ab}	0.6597^{ab}
28	0.2778^{c}	0.04630^{c}	0.1620^{b}
32	0.1852^{c}	0.4456^{bc}	0.3154^{b}
36	0.5093^{bc}	0.2025^{c}	0.3559^{b}
40	0.4626^{bc}	0.4902^{bc}	0.4764^{b}

Values are expressed as mean ± SE. Means of aggressive pecking behavior that have different letters differ (P<.05).

Discussions

This research was carried out two times to validate the results of the parameters under study. In order to avoid room bias in the results, both experiments were designed to be replicas of each other. However, some conditions were not constant and might be responsible for the slight differences in the results especially in the first few weeks of experiments. Lighting pattern and quantity of feed consumption were examples of some of the factors that were different in both studies. The quantity of feed consumed by the birds was determined by their body weight relative to Cobb 500 broiler breeder management standards target values The birds that were used for the study in experiment 1 were immediately exposed to 16:8 hours of light: dark lighting schedule upon arrival. On the other hand in experiment 2, the lighting (14:10 hours) was gradually increased until the same hour of light was achieved (like the first experiment). This change in lighting was based on the time of the year and the recommendation from the broiler breeder company from which the birds were obtained. Experiment 1 was carried out in the summer while experiment 2 was carried out in the winter.

Lighting and feeding in poultry are very important and greatly influences the health and production of birds especially when the birds are at the point of laying (Renema, et al., 2007; Zuidhof, et al., 2007). Excess feed and light influence the production of double yolk eggs and the more the double yolk eggs laid by birds, the less the overall number of eggs laid (Robinson and Wilson, 1996). Exposure to higher light intensities makes birds reach reproductive maturity faster.

In the first few weeks into egg laying, the percent egg production increased significantly in both experiments but the increase was faster in experiment 1 than 2. Sand increased egg production in experiment 1 but had no effect on egg production in experiment 2. Previous studies by Pohle and Cheng (2009) reported that birds reared in enriched cages that had perch, nest box, and sand as dust bathing material, laid significantly increased numbers of eggs compared to the control. Van Dar Meulen et al. (2008) found that adding varying levels of sand into the feed of laying hens had no significant effect on egg production in a 4 - week experiment. On the other hand, Zhao et al. (2009) reported that including 4 - 6% levels of health sand into the diet of laying hens (Wenchang chicken) increased their rate of egg production. Sand was included as a form of enrichment in the setup of this current study, thus the amount of sand intake by the birds was not recorded. Furthermore, sand was not intended as a substitute for feed ingredients but either way, the birds consumed sand. Sand increased egg production and this increase may be associated with the percent of sand consumed. In the study, birds in the SP treatment of the HS room laid more eggs than their counterparts in the TN room. Laying of more double yolked eggs by birds in the TN room might be a possible reason for the result. The more the double yolk, the less the total number of whole eggs laid (Robinson and Wilson, 1996).

Including enrichment in the form of sand and perch did not influence egg weight when compared to the control at the end of 40 weeks. Pohle and Cheng (2009) reported that there were no differences in egg weight between hens raised in enriched cages (perch and dust bath with sand as dust bath material) versus the battery cages. In experiment 2, there was a trend for higher egg weight in the TN room than the HS room but in experiment 1, heat stress did not affect egg weight. Balnave and Muheereza, (1997) acclimatized some laying hens to continuous lightening and later increased their environmental temperatures from 25-30°C. Results from Balnave's study found that there were slight decreases in egg weights of the laying hens in response to heat stress. Marshaly et al. (2004) reported that high temperature (23.9 -35°C) significantly decreased egg weight in laying hens. In experiments 1 and 2, the cycling heat stress rooms were maintained between 23°C to 30°C which were the maximum levels approved by IACUC for this study (about 5°C lower than that of Marshaly et al 2004 study). As expected, egg weight significantly increased as birds' aged regardless of the treatment (enrichment). Shanawany (1984) also reported increased egg weight in broiler breeders with age (between weeks 28 to 44).

In experiment 1, fertility gradually declined immediately after peak egg production but in experiment 2, fertility remained high until the end of the experiment at 40 weeks. Wilson (2002) reported the peak of fertility to be around 25 to 27 weeks of age, but Hocking (1990) found the peak of fertility to be between 32 to 37 weeks of age. This may be attributable to different strains of birds. Nonetheless, fertility usually decreases with increase in body weight and age of birds (Duncan et al., 1990). Likely reasons for the differences in fertility results in addition to lighting were the mortality rate and the male social status. In this current study, some extra birds were brought in with the experimental birds that replaced dead experimental birds in the course of the study (experiments 1 and 2). A study by Chung et al., (2012) found that when new males replace existing males in a flock (spiking and double interspiking), fertility increases. Mortality rate was higher in experiment 2 than experiment 1. Furthermore, each pen had two males and twelve females but from personal observations, some of the males quickly formed dominances and subordinates. The subordinate males were allowed limited access to feed by the dominant males. At some point the dominant males were heavier than the subordinate males. The condition of weight loss in the males was more pronounced in experiment 1 than 2. (McGary, et al., 2002) et al. reported that the heavier the rooster the lower the expression of reproductive behavior while Duncan et al. (1990) found that the heavier the rooster, the higher the occurrence of attempted mating and sexual behaviors. In this study, there was a strong positive correlation between fertility and hatchability in both experiments 1and 2. However, Hocking and Bernard (2000) reported that there was no correlation between fertility and hatchability. In experiments 1 and 2, the addition of sand and perch to broiler breeder housing had no significant effect on fertility and hatchability compared to the control.

In experiment 1, enrichment (treatments S, P, and SP) reduced the incidence of floor eggs. Previous studies by Appleby et al. (1983) and Brake (1987) found that including perch structures into the poultry environment reduced the incidence of floor eggs. In experiment 2 there was a trend for more floor eggs in the TN room compared to the HS room. Birds preferred to lay their eggs in cool and isolated locations in the pen and this is a possible reason for the temperature related incidences of floor eggs (Petherick and Rushen, 1997). Cooper and Appleby (1996) reported that the occurrence of floor eggs in birds decreased with age. Other factors that increases the incidences of floor eggs apart from age are management, period of darkness, and the time of nest box introduction into the flock (Sherwin and Nicol, 1993). Eggs that are laid on the floor are usually in contact with the litter (that is most times soiled with feces) material in the poultry house. For birds to lay their eggs in the nest box rather than the floor, the nest boxes must be enclosed, easily accessible, and have loose substrate as cushion (Struelens, et al., 2005). When the floors of the poultry house have similar conditions to a nest box, birds might possibly lay their eggs on the floor. Micro-organisms such as bacteria easily penetrate and contaminate floor eggs to reduce hatchability of the egg (Messens, et al., 2005).

The corticosterone levels of the birds in the HS room were slightly higher compared to those of the TN room. Perch alone or in combination with sand had a trend of increased corticosterone levels in broiler breeders. (Heckert, et al., 2002) reported that perch increased stress level in broilers and the increase can be associated to the limited space available to the birds after the addition of perch into the poultry house. De Jong et al. (2001) also reported that plasma corticosterone levels of birds were higher when the lights were turned on than off.

Elevated temperature has been shown to increase H/L ratio (Zulkifli et al., 2003), while Spinu et al. (2003) reported that there were no differences in H:L ratios in birds that were reared either in the winter (~18°C) or the summer (~ 33°C) seasons . (Bedanova, et al., 2007) exposed broilers to stress (shackled the birds) and found that H:L ratio of the birds increased (about 0.88-1.05) relative to the control (0.17). The increase in corticosterone levels and heterophil: lymphocyte ratios are stress indicators in poultry. However, corticosterone is a better indicator for acute stress, while H:L ratio is a better indicator of chronic stress (Hocking, et al., 1996; Maxwell, 1993). The H:L ratio usually ranges around 0.4.

There were few mating activities at the early part of the experiment but these increased between weeks 24 to 26 and declined afterwards. Hocking and Bernard (2000) reported that mating was higher in broiler breeder males between weeks 27 to 29. At younger ages, the occurrence of mating in male birds is usually lower compared to that of mature males, since the younger breeder males spend time to learn how to mate (Wilson, 2002).

In experiment 1, the frequencies of preening differed throughout the experiment but were higher in the HS room. However, in experiment 2, preening in broiler breeders was similar throughout the experiment regardless of the temperature of the room. Duncan and Wood –Gush (1972) found that the frequency of preening was not different in birds regardless of the stress condition but the duration differs. Spinu et al. (2003) reported that the rate of preening was higher in broiler breeder females of similar breed that were reared during the winter (~ 18°C) season than those reared during the summer (~ 33°C). Preening increased prior to feeding and tended to decrease with age (Savory and Maros, 1993). Similarly, preening decreased as environmental temperature increased (Pereira, 2007). Shimmura et al. (2007) reared 57 week-old laying hens for 10 weeks and also found that preening increased in laying hens between 57-67 weeks of age. Furthermore, broiler breeders that were fed restricted feed were more active and preened more than those fed ad-libitum (Savory, 1992). During spray application in day-old-chicks, increased light intensity increased preening (Caldwell et al. 2001). Furthermore, there was a possibility for higher preening frequencies in the birds reared on perch (P) compared to those reared on sand (S). A probable reason is because the major activity that birds demonstrate on perch is preening. However, birds express more fun-filled behaviors in the presence of sand. Such behaviors include dust bathing, sitting, and foraging, as well as eating the sand (Arnould, et al., 2004; Toghyani, et al., 2010; Vestergaard, et al., 1997). The numerous activities done on sand could be responsible for the trend for reduced preening in treatment S than P.

In experiment 1 and 2, the frequency of wing flapping differ as the birds age but in experiment 1 the birds in treatment C flapped their wings more than those in treatment P. Davis (2010) reported that wing flapping tended to decrease with age especially in birds reared in cages while wing flapping was similar across the weeks in birds reared on the floor. The frequent occurrences of wing flapping in the control treatment than the perch treatment may be due to the available space that result from the absence of enrichment structures in broiler breeder environment that was compensated for with wing flapping (Nicole and Guilford, 1991).

In this study, the uses of enrichment differ as birds' age and the frequencies as well as the duration of enrichment use (sand and perch) were higher for sand compared to perch. LeVan et al. (2000) reported that the use of perch increased with age but decreased in heat stress environment. The sand box was topped up with sand daily or when necessary but the perch structure remained the same. The birds in treatments S and SP utilized the sand as long as it was available in the poultry house, especially immediately after the sand was topped up. Therefore the numerous addition of fresh sand into the sand box as well as social stimulation can be responsible for preference and increase use of the sand box. Some poultry behaviors are socially stimulated and example includes dust bathing. Dust bathing behavior in a flock can be stimulated by the expression of the behavior by a bird in the flock (Duncan, et al., 1998). Another factor that determines enrichment use birds by the location (Newberry and Shackleton, 1997). Barnett et al. 2009 found that when dust bath material (hard wood saw dust) was placed above the nest box, it was difficult for birds to make use of the dust bath material without perch. Thus, laying hens utilized the dust bath more in the presence of perch structures. Other factors that affect dust bath activities are the type of dust bath material and the lipid content of the material (Van Liere et al., 1991). The frequency of perch use was higher in broiler breeders reared in the TN room compared to the HS room. There was a trend for higher perch use in birds reared in treatment SP compared to perch P. The presence of sand may stimulate perch use. Pereira et al. (2007) found a positive correlation between temperature and dust bathing behavior.

Aggressive behavior was minimal in this study. In experiment 1, there was one occurrence of aggressive fighting while in experiment 2, fighting occurred six times. Aggressive fighting in experiment 2 occurred mostly in the control treatments of both the TN and HS environments. The few events of aggressive pecking by the broiler breeder females occurred when new females were introduced into the pen to replace dead hens. On the other hand the event of aggressive fighting in male occurred because the dominant male tried to prevent the subordinate male from mating the hens.

In summary, enrichment in the form of sand increased egg production in both the thermoneutral and cycling heat stress (23 - 30°C) environments. As a result of the inconsistency in experiments 1 and 2 as well as the reports from several authors, it is important to determine to certainty the actual quantity of sand inclusion level that would increase egg production. Economically, sand cost more to purchase in commercial broiler breeder farms. Therefore, the actual amount of sand that increases egg production would determine if sand would be more economical to use as an enrichment or not. Enrichment (sand and or perch) reduced the incidences of floor eggs but had no significant effect on egg weight, fertility, and hatchability. There was a trend for reduced occurrence of floor egg at higher environmental temperature. Circulating heterophil/lymphocyte ratios in broiler breeder birds tended to be reduced by adding sand to their environments. Broiler breeders reared in the HS environment utilized the enrichment structures more than those in the thermoneutral environment. Very few aggressive behaviors were notice in this study. Perhaps the birds were attracted to the enrichment structures and spent more time in its use. Birds use the perch as a form of refuge when attacked by counterparts. After spending time in the use of the enrichment structures, broiler breeders express themselves in the form of wing flapping, preening, and all these behaviors indicate content. The behavioral result was similar in both thermoneutral (23°C) and cycling heat stress (23-30°C) environment.

In conclusion this study established that (1) enriched environments will positively impact production of meat type birds parent stock under stress condition and (2), enriched environments (sand) tended to reduce as well as increase (perch) stress level of meat type bird parent stock subjected to heat stress.

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