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E-mail: editorijps@gmail.com

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Influence of Atmospheric Ammonia on Serum Corticosterone, Estradiol-17β and Progesterone in Laying Hens

Frank W. Edens
Prestage Department of Poultry Science, North Carolina State University,
Box 7635, Raleigh, NC 27695-7635, USA

Abstract: Hormonal profiles of hens in curtain side-walled high-rise deep pit and flush-waste layer houses were initiated at placement of hens in cages in August and continued through a 50 weeks long laying trial. In mid-January, a cold weather-related incident caused the rupture of water lines and flooding of the manure pits in the deep pit layer house, allowing atmospheric ammonia to rise to a high of 135±29 ppm and remain high through mid-February when the pits were finally emptied. After removal of wet manure from the deep pit, atmospheric ammonia in the deep pit house decreased to 25±9 ppm compared to 21±4 ppm in the flush-waste house. Elevated atmospheric ammonia in the deep pit house caused the hens, compared to hens in the flush-waste house, to increase significantly ($p \leq 0.05$) their serum corticosterone from mid-January through February followed by a decrease until June when it increased again in response to high summer temperatures. Serum progesterone and estradiol-17β in those hens in the deep pit house decreased significantly ($p \leq 0.05$) in response to elevated atmospheric ammonia. Egg production of hens in the deep pit house was decreased from mid-January through February ($p \leq 0.05$) followed by a slight rebound in March. Overall egg production remained lower in the deep pit house compared to production in the flush-waste house through July. Elevated atmospheric ammonia is a strong stressor, which will induce increased serum corticosterone that leads to loss of egg production associated with decreased serum concentrations of serum estradiol-17β and progesterone.

Key words: Ammonia, layers, corticosterone, estradiol-17β, progesterone

INTRODUCTION

Prolonged exposure to high concentrations of atmospheric ammonia in poultry houses can cause a variety of disorders ranging from irritated mucous membranes in the respiratory tract, which includes ciliostasis in the trachea that can then lead to increased susceptibility of bacterial infection through the respiratory system. It is not uncommon to observe conjunctivitis and corneal lesions of the eyes, which are both ammonia-concentration and -exposure time dependent. Exposure of caged laying hens to high concentrations of atmospheric ammonia (NH_3) in poultry houses has been long-recognized as a potential problem affecting their welfare and productivity (Charles and Payne, 1966a,b). After one week of exposure to the high concentration of atmospheric ammonia at 100 ppm or greater, laying hens showed decreased body weight and feed consumption. Egg production was also decreased after seven weeks exposure to 105 ppm atmospheric ammonia, but provision of a diet containing greater levels of protein, vitamins and minerals prevented the negative effects of exposure to 104 ppm atmospheric ammonia (Charles and Payne, 1966b). Hens on a low protein diet exposed to 104 ppm ammonia showed a rapid decrease in egg production (Charles and Payne, 1966b). Exposure of growing pullets to an atmospheric ammonia concentration of 200 ppm for a period of 17 d before housing in laying cages caused the ammonia-exposed hens to have a significantly reduced hen-day egg production (Deaton *et al*., 1984). An earlier study by Deaton and colleagues (Deaton *et al*., 1982) showed that laying hens exposed to an atmospheric ammonia concentration of 100 ppm for a period of 28 days did not decrease significantly their hen-day egg production (2.9% lower than control), but their body weight decreased significantly along with an 8 g per day decrease in feed intake (Deaton *et al*., 1982). The performance results from these earlier studies suggested that atmospheric ammonia in high concentrations, even for short periods of time, while tolerated, can be considered a significant stressor for the exposed hens.

It has been reported that 25 ppm of atmospheric ammonia is aversive to caged laying hens (Kristensen *et al*., 2000). In the United States and in the United Kingdom, maximum levels of ammonia in poultry houses have been set at 25 ppm by the National Institute of Occupational Safety and Health (NIOSH) (CDC, 2005) and the United Egg Producers (UEP, 2008) and 50 ppm by the Occupational Safety and Health Administration (OSHA, 2006). These levels have been established based on human safety and represent the limits for 8 hours of exposure. OSHA considers 50 ppm to be the lowest level to cause irritation to the eyes, nose and throat of the most sensitive individuals (UEP, 2008). However, it is not uncommon for atmospheric ammonia

concentrations in cage layer facilities to exceed the 25 to 50 ppm safety limits for humans, especially during winter when producers reduce ventilation to conserve energy (Liang *et al*., 2003; UEP, 2005).

This report describes an accidental and prolonged elevation in atmospheric ammonia concentrations in a high-rise deep pit cage laying house (Pit) compared with a flush-waste cage laying house (FW) adjacent to the high-rise house. The flocks had been subjected to several managerial problems including bouts of enteritis before and immediately after housing in the laying facilities and development of cage layer fatigue (osteomalacia) during the first two months in the laying cages, which was due to inappropriately manufactured feed with low dicalcium phosphate, low phosphorus and no supplemental vitamin D_3. Before housing in the cage laying houses, pullets were bled and on a monthly basis laying hens were bled for determination of serum corticosterone, estradiol-17β and progesterone to ascertain housing influence on stress responses and reproductive steroids. As a result of a mid-January freeze-induced rupture in water lines during an especially cold period, the manure pits in Pit were flooded and atmospheric ammonia increased to very high concentrations with a transitory decrease in egg production and changes in the serum steroids.

MATERIALS AND METHODS

Animals and husbandry: The hens in this study were a part of the 23rd North Carolina Random Sample Egg Laying Test (Martin, 1983) and standard husbandry practices established for the egg laying test were applied to these hens. Two laying houses located in Rowan County, NC, USA were involved with one being an insulated curtain sidewall flush-waste house (FW) and the second being an insulated curtain sidewall high-rise deep pit house (Pit).

The pullets were hatched from eggs obtained from primary breeders or their distributors and were brooded and reared in flat-deck (1.22 x 1.02 m) brood-grow cages in an insulated curtain sidewall house until placement in the Pit and FW houses at 21 weeks of age into laying cages with three hens per cage, which provided 387 cm^2 per hen. At the time of placement into the Pit and FW houses, lighting, via incandescent lamps in the ceiling, was stepped up to 16 h light and 8 h darkness per day and was maintained on the hens throughout the 50 week laying period.

At hatch, all chicks were vaccinated against Marek's Disease with a cell-associated live turkey herpes virus vaccine. Additional vaccinations included Newcastle (B1) at one week of age, Newcastle La Sota strain at four and 16 weeks of age, bronchitis via water at one and 16 weeks of age, wing web fowl pox at 12 weeks of age and avian encephalomyelitis via wing web at 14 weeks of age.

At one week of age, all chicks were subjected to precision beak trimming. At 12 weeks of age, the pullets' beaks were inspected and all, that needed further trimming, were subjected to the trimming procedure.

Feeds: Common corn-soya mash diets, purchased from a commercial feed manufacturer, were provided *ad libitum* to the growing pullets and to caged layers. Pullets were reared on low energy step-down protein starter-grower diets (Anderson *et al*., 1995, 2013) and caged layers were subjected to a phase feeding program with step-down protein in which feeds provided a minimum of total sulfur amino acids (TSAA) at 0.7 g and 0.65 g for brown and white egg strains, respectively to 40 weeks of age or to hen-day (HD) production of 88% or less (Martin, 1983). From 88% HD production to 75% HD production, the diets provided 0.65 g and 0.6 g TSAA for brown and white egg strains, respectively and at HD production under 75%, the diets provided 0.6 g and 0.58 g TSAA, for brown and white egg strains, respectively.

Blood sampling: At 17 weeks of age, selected pullets, which were reared on-site of the North Carolina Agricultural Research Station in Rowan County, NC and designated for placement into either the Pit or FW cage laying facilities were bled initially for determination of serum steroids (corticosterone, estradiol-17β and progesterone). Blood samples were collected at each sampling period during the afternoon. A single pullet and later a single hen was removed randomly from their respective cages and bled from the ulnaris vein in the wing. No other bird was removed from a pen where a hen had been sampled. Before blood collection, the birds were palpated to determine if an egg was present in the shell gland and if an egg was present, the bird was not subjected to blood sampling. If the bird struggled violently, it was not bled in an effort to prevent elevated serum corticosterone related to handling stress (Beuving and Vonder, 1977, 1978, 1981). No effort was made to take a second bird from the cage where a struggling hen had been taken and then returned. Blood was collected in a volume of 3 mL in syringes and was then expressed into Becton-Dickenson Vacutainer® serum separation tubes (Fisher Scientific, Raleigh, NC 27604), which contained a coagulant. The coagulated blood samples were held over night at 4°C before they were centrifuged at 800 x g at 4°C for 30 min and serum was decanted from each sample into vials, which were capped and frozen at -20°C until processed for steroid hormone concentrations. A total of 48 blood samples per house per sampling time were taken from August until the following July. A total of 1152 serum samples were assayed by radioimmunoassay for each of the three steroids.

Hormone analyses: A double antibody ^{3}H-labeled radioimmunoassay was used to quantify serum

concentrations of corticosterone (Cambridge Medical Diagnostics, Billerica, MA 01865) and double antibody ^{125}I-labeled radioimmunoassays, following the manufacturer's instructions, were used to quantify concentrations of estradiol-17β and progesterone (Radioassay Systems Laboratories, Inc., Carson CA 90746).

To extract corticosterone, each serum sample of 100 µL in a 13 x 100 mm polypropylene tube was extracted twice with 1 mL of a 1:100 mixture of ethyl acetate:isooctane to remove progesterone and other progestins. The samples were frozen in an acetone-dry ice bath followed by centrifugation and decanting of the organic phase. The remaining aqueous phase was extracted twice with 3 x 1 mL methylene chloride followed by centrifugation and freezing in an acetone-dry ice bath. The organic phase was decanted into 16 x 100 mm glass tubes and the pellet was discarded. The methylene chloride fraction was dried under nitrogen followed by reconstitution in 500 µL the assay buffer (phosphate buffered saline gel mixture). A volume of 200 µL of this reconstituted mixture was then used for the ^{3}H-labeled double antibody radioimmunoassay following the recommendations of the kit manufacturer (Cambridge Medical Diagnostics, Billerica, MA 01865). Samples were run in duplicates. The extraction efficiency for corticosterone was 94% and the intra-assay and inter-assay coefficients of variation were 6.7 and 11.9%, respectively. Counting of the radioactivity was accomplished in a Beckman LS100C using the ^{3}H window (Beckman Instruments, Fullerton, CA 92835).

Progesterone was measured using a commercial double antibody ^{125}I-labeled radioimmunoassay method with an assay sensitivity of 0.1 ng/mL (Radioassay Systems Laboratories, Inc., Carson CA 90746). Estradiol-17β was also measured using a commercial double antibody ^{125}I-labeled radioimmunoassay method with a sensitivity of 2.9 pg/mL (Radioassay Systems Laboratories, Inc., Carson CA 90746). All serum samples of 600 µL volume were extracted with a mixture of ethyl acetate-hexane (3:2 by volume) and shaken for 1 min. The organic phase was decanted into 13 x 100 mm glass tubes and dried under nitrogen. The sample residue was reconstituted with 2.5 mL of the assay buffer and incubated at 25°C in a water bath for 60 min. Following the manufacturer's protocol, ^{125}I-labeled progesterone or estradiol-17β were added to respective sample tubes and allowed to rest at room temperature for 90 minutes. After this incubation step, a volume of 100 µL of the precipitating second antibody was added to all assay tubes, which were then allowed to rest at room temperature for at least 60 min followed by centrifugation of the assay tubes for 15 min at 1000 x g at 4°C. The supernatant was then decanted with drying followed by counting of the pellet in a Tracor Analytic Model 1185 gamma counter (Tracor Analytic, Elk Grove Village, IL 60007). Extraction efficiency was 93.3% for progesterone and 96% for estradiol-17β. The intra-assay and inter-assay coefficients of variation for progesterone were 6.9 and 10.2% and the intra-assay and inter-assay coefficients of variation for estradiol-17β were 6.2 and 11.1%.

Atmospheric ammonia concentration: Atmospheric ammonia was measured in the Pit and FW houses commencing within one week after the freezing and rupture of the water lines in the Pit house. Atmospheric ammonia concentrations were measured with a Bendix/Gastec Precision Gas Detecting System model 400 (Fisher Scientific, Raleigh, NC 27604) using Gastec Ammonia Specific Colorimetric Stain Detector Tubes (Fisher Scientific, Raleigh, NC 27604) with detection ranges between 5 and 70 ppm and 2.5 and 200 ppm (±15%). One time per day, when animal caretakers first entered each house in the morning, multiple measurements at cage level in the Pit and FW houses were made and averaged to obtain the daily atmospheric ammonia measurement for each house. Mean atmospheric ammonia was calculated for monthly reference for each house. Atmospheric ammonia measurements were obtained from January through July for each house.

Data analysis: All the data were subjected to statistical analyses using the general linear models procedure of the Statistical Analysis System (SAS, 2004). When significantly different ($p \leq 0.05$), means were separated by Duncan's multiple range test (SAS, 2004).

RESULTS

Before the extreme cold weather (-23°C) associated accidental water line breakage in the Pit house, atmospheric ammonia was not an issue of concern in either the Pit or FW houses, but after the accident, atmospheric ammonia in the Pit house rose to 135±29 ppm versus 44±9 ppm in the FW house (Table 1). Atmospheric ammonia remained high in the Pit house (107±35 ppm) through February compared to 32±9 ppm in the FW house. After the wet material in the deep pit was cleaned, atmospheric ammonia in the Pit house returned to concentrations similar to that found in the FW house (Table 1).

The serum corticosterone profiles of hens in the Pit and FW houses are presented in Fig. 1. After placement in laying cages, the hens in both houses experienced bouts of enteritis and cage layer fatigue, which were associated with a mild elevation in serum corticosterone followed by a period (October to December) in which serum corticosterone decreased. Following the water line breakage in January, serum corticosterone in hens in the Pit house rose significantly and remained high through February. When the deep pit was cleaned,

Table 1: Atmospheric ammonia (ppm±SEM), measured in the morning using a manual gas detector system, in the high-rise deep pit (Pit) house and in the flush-waste (Flush) house from mid-January through mid-July

House	Jan	Feb	Mar	Apr	May	Jun	Jul
Pit	135±29[a]	107±35[a]	25±9[a]	29±9[a]	32±7[a]	29±4[a]	27±5[a]
Flush	44±9[b]	32±9[b]	23±4[a]	24±4[a]	22±5[a]	20±2[a]	19±3[a]

[a,b]In a column, means (±SEM) with unlike superscripts differ significantly ($p \leq 0.05$)

serum corticosterone decreased in the hens in the Pit house to a concentration similar to the serum corticosterone in the hens in the FW house. Comparable serum corticosterone concentrations were evident in the hens in both houses through July when the laying trial was terminated. A secondary rise in serum corticosterone was measured in hens in both houses during June and July period, which was associated with increased summer ambient temperatures (Fig. 1).

Serum estradiol-17β profiles of hens in the Pit and FW houses are shown in Fig. 2. Generally, there was a transitory elevation in serum estradiol-17β after hen placement into laying cages, which was followed by a general decline in serum concentrations in hens in both houses. However, after atmospheric ammonia was dramatically elevated (January through February) in the Pit house, serum estradiol-17β concentrations declined significantly in the hens in that house and remained low until the atmospheric ammonia returned to normal concentration in March. Serum estradiol-17β concentrations in the hens in the Pit house returned to concentrations similar but somewhat lower than serum estradiol-17β concentrations in hens in the FW house through the end of the laying trial in July. In June and July serum estradiol-17β concentrations in hens in both houses declined in response to summer temperatures (Fig. 2).

Serum progesterone profiles of hen in both the Pit and FW houses are presented in Fig. 3. Following hen placement into laying cages in August, serum progesterone rose significantly and remained high through May. After peak serum progesterone concentrations were achieved in November, there was a small but steady decrease in its concentration through May, which was followed by a high temperature related decrease in June and July (Fig. 3). When atmospheric ammonia was elevated in the Pit house, there was an approximately 50% decrease (January through February) in serum progesterone in the hens residing therein in response to elevated atmospheric ammonia. After atmospheric ammonia was returned to normal concentrations following pit cleaning, serum progesterone in the hens in the Pit house returned to concentrations similar to that in hens in the FW house (Fig. 3). Serum progesterone in hens in both Pit and FW houses decreased in response to summer temperatures during June and July (Fig. 3).

Hen-day egg production of hens in both the Pit and FW houses is presented in Fig. 4. Egg production increased significantly shortly after the hens were placed into laying

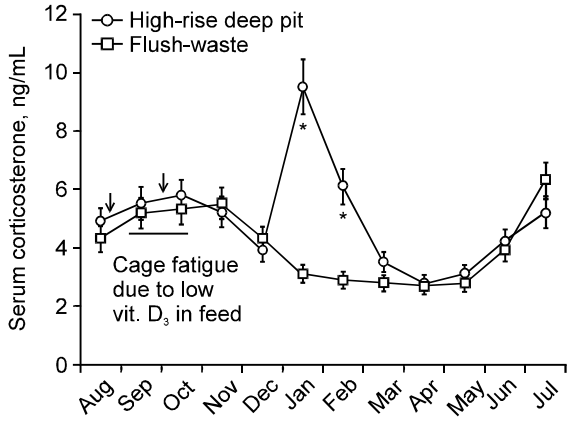

Fig. 1: Influence of accidentally elevated atmospheric ammonia on serum corticosterone in caged laying hens in high-rise deep pit and flush-waste curtain sidewall houses. *Indicates a greater difference ($p \leq 0.05$) in serum corticosterone concentration in hens in the high-rise deep pit house over time compare to changes in hens in the flush-waste house. Arrow indicates development of enteritis in the flocks

cages in both the Pit and FW houses, but due to bouts of enteritis and cage layer fatigue due to dietary deficiencies in Vitamin D_3, phosphorus and dicalciumphosphate, egg production was interrupted during the September to November time period. Peak egg production was noted in November and remained in a normal profile for hens in the FW house through the termination of the 50 weeks long laying trial in July. A similar egg production profile was noted for the hens in the Pit house until atmospheric ammonia was increased, which caused a 15% decrease in hen-day egg production in January (Fig. 4). Decreased egg production was found in hens in the Pit house through March when it rebounded to approach the egg production rate of the hens in the FW house. Nevertheless, overall Pit house hen-day egg production remained lower than the production of hens in the FW house. The normal decrease associated with the end of lay egg production was exacerbated by the summer heat of June and July (Fig. 4).

DISCUSSION

In this investigation, there was a unique opportunity to examine the influence of significantly elevated atmospheric ammonia on stress and reproductive

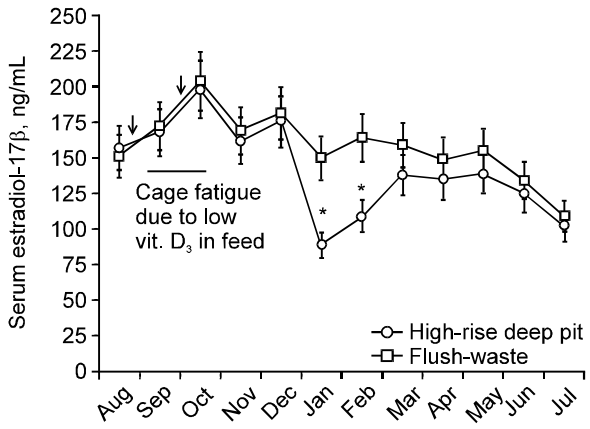

Fig. 2: Influence of accidentally elevated atmospheric ammonia on serum estradiol-17β in caged laying hens in high-rise deep pit and flush-waste curtain sidewall houses. *Indicates a greater difference ($p \leq 0.05$) in serum estradiol-17β concentration in hens in the high-rise deep pit house over time compared to changes in hens in the flush-waste house. Arrow indicates development of enteritis in the flocks

steroids in layers caged in FW and Pit houses. The results of this field study clearly indicated that atmospheric ammonia is a potent stressor to laying hens, which caused reproductive dysfunction associated with decreased hen-day egg production. Subjective assessment of atmospheric ammonia in the Pit and FW houses was not unusual before mid-January and was not recorded. However, on an exceptionally cold night in mid-January, the water lines in the Pit house froze and ruptured flooding the deep pits under the cage lines. Within a few days after this accident, atmospheric ammonia concentrations increased in the Pit house and when first measured, the ammonia concentration was in excess of 135±29 ppm (Table 1). Atmospheric ammonia remained in high concentration until the pits were cleared during February and returned to acceptable concentrations in March (Table 1). During January and February, slightly elevated atmospheric ammonia (44±9 ppm and 32±9 ppm, respectively) in the FW house were within currently acceptable levels of atmospheric ammonia (25-50 ppm) (UEP, 2005, 2008; OSHA, 2006) and likely were trending upward due to a managerial effort to conserve house temperature via reduced ventilation. With the advent of warmer seasonal temperatures commencing in March, morning atmospheric ammonia levels in both the Pit and FW houses were significantly lower than the ammonia concentrations found in January and February. In March and later times, the lower atmospheric ammonia concentrations in both houses was attributed to more ventilation and to clearance of wet manure from the PIT house. Yet, in the Pit house atmospheric ammonia tended to be slightly higher than in the FW house and this likely was due to accumulation of more droppings in its pit area, which allowed for increased emission of ammonia into the atmosphere. Regular removal of the manure from the FW house resulted in more acceptable atmospheric ammonia concentrations.

Increased concentration of serum corticosterone is an indicator of a non-specific stress response in all classes of domestic fowl (Beuving and Vonder, 1977, 1978, 1981; Edens, 1978; Edens and Siegel, 1975; Edens *et al*., 1984). Elevated atmospheric ammonia concentrations in the Pit house during January and February increased the serum corticosterone in the resident hens, but the hens in the FW house did not experience the any increase in corticosterone. In March, after the wet manure had been cleared from the pits of the Pit house, serum corticosterone of the resident hens in the Pit house decreased to a concentration less than that found in January and February and continued to decline to concentrations that were similar to that of hens in the FW house (Fig. 1). Before the January-February time period, serum corticosterone concentrations in the hens housed in the Pit and FW houses were elevated slightly due to handling stress associated with transfer from growing cages to laying cages, high summertime temperatures and bouts of enteritis from September through October. From November through December, the serum corticosterone concentrations of hens in both houses were in a decreasing profile. Serum corticosterone in the hens in the FW house continued to show a decreasing profile through the January to May time period and from April through July the serum corticosterone concentrations were similar in hens in the Pit and FW houses. With high early summertime temperatures in June and July, serum corticosterone in hens in both the Pit and FW houses increased (Fig. 1), which was consistent with a generalized stress response to heat (Edens, 1978; Edens and Siegel, 1975; Edens *et al*., 1984).

Serum estradiol-17β increased after hens were placed into cages in both the Pit and FW houses (Fig. 2) and was maximized in the September-October time period at a time when egg production should have been reaching a peak (Fig. 4). After maximal serum estradiol-17β occurred, there was a general decline in its concentration through the end of the 50 week laying trial in July. In January, coincident with the elevated atmospheric ammonia in the Pit house, serum estradiol-17β decreased significantly in the resident hens in that house whereas in hens resident in the FW house the serum estradiol-17β did not change significantly (Fig. 2). Serum estradiol-17β remained depressed in Pit house resident hens through February and increased toward the concentration of the hormone in hens resident in the FW house during the month of

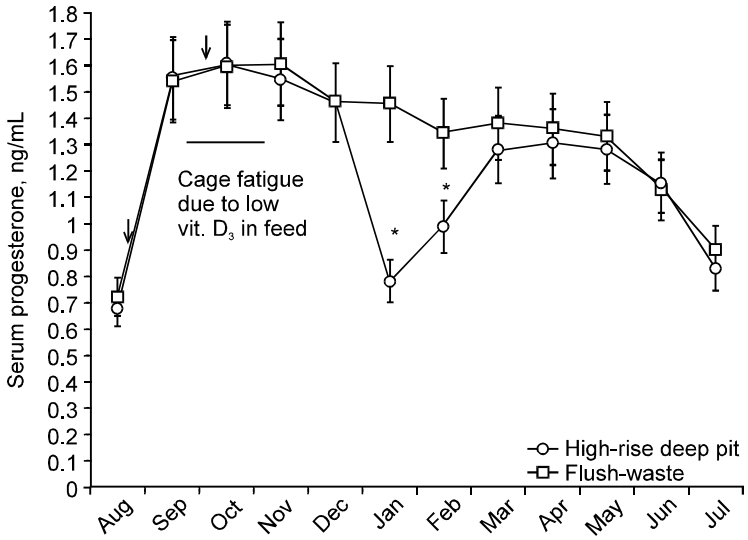

Fig. 3: Influence of accidentally elevated atmospheric ammonia on serum progesterone in caged laying hens in high-rise deep pit and flush-waste curtain sidewall houses. *Indicates a greater difference ($p \leq 0.05$) in serum progesterone concentration over time in hens in the high-rise deep pit house compared to changes in the hens in the flush-waste house. Arrow indicates development of enteritis in the flocks

March (Fig. 2). From April through July, the serum estradiol-17β in hens in the Pit and FW houses were similar. These observations suggested that the stress effect of elevated atmospheric ammonia was transitory and had not caused any chronic problems in the hens in the Pit house.

Serum progesterone increased in hens after they were caged in both the FW and Pit houses (Fig. 3). In November, coincident with the approach of peak egg production by hens in both houses (Fig. 4), serum progesterone was reaching a maximum concentration and remained high in the hens in the FW house. However, in the resident hens in the Pit house, there was a significant decrease in serum progesterone during the time of elevated atmospheric ammonia in January and February. By March the serum progesterone concentrations in the hens in the two houses were similar again. Yet, serum progesterone in hens in both the Pit and FW houses was in a declining profile from March through July (Fig. 3). The greatest decline in serum progesterone, with the exception of the ammonia induced depression, occurred with the beginning of the summer season in June and July (Fig. 3). Increased ambient temperature has been reported to decrease both serum progesterone and estradiol-17β in laying hens (Mahmoud *et al*., 1996; Mashaly *et al*., 2004; Rozenboim *et al*., 2007).

Hen-day egg production peaked (84% hen-day production, which was significantly less than expected) in the November-December time period (Fig. 4). Egg production peak should have been observed between the end of September and the end of October, but due to the stress of enteritis noted for these flocks in September and osteomalacia or cage layer fatigue (Whitehead and Fleming, 2000; Randall and Duff, 1988) due to deficient dietary calcium, phosphorus and vitamin D_3 in the layer diets, hen-day egg production was depressed during this time period. With resolution of the enteritis problem and replenishment of dietary calcium, phosphorus, vitamin D_3 and supplemental oyster shell, hen-day egg production returned in November-December to a level that might be normal for the chronological age of the laying flock but never reached potential production for these two flock. In January, hen-day egg production for the Pit house decreased after the elevation in atmospheric ammonia and remained depressed significantly in February with some recovery in March whereas the hen-day egg production of hens in the FW house did not change significantly (Fig. 4). Egg production by hens in the Pit house did increase over time but not to a level that was similar to production of hens in the FW house. The normal age-dependent decline in egg production was evident from April through June, but in July, heat-related egg production rate was significantly less than the June production in both houses (Fig. 4). Many stressors can cause decreased egg production and among those diverse stressors are acute and chronic heat exposure (Bray and Gesell, 1961; Sahin *et al*., 2002), cold (Hester *et al*., 1996; Mumma *et al*., 2006; Shini *et al*., 2009) and ammonia (Charles and Payne, 1966a,b; Deaton *et al*., 1982, 1984). As reported herein, ammonia stress and eventually heat stress caused extraordinary declines in hen-day egg production (Fig. 4).

Elevated serum corticosterone in response to diverse stressors is correlated with decreased reproductive

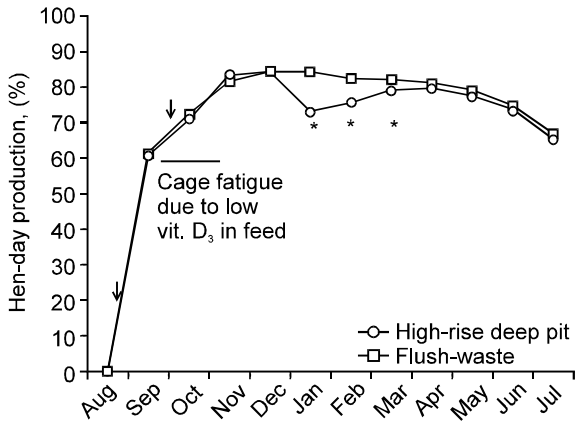

Fig. 4: Influence of accidentally elevated atmospheric ammonia on hen-day egg production of caged laying hens in high-rise deep pit and flush-waste curtain sidewall houses. *Indicates a greater difference ($p \leq 0.05$) in percent hen-day egg production over time in hens in the high-rise deep pit house compared to changes over time for the hens in the flush-waste house. Arrow indicates development of enteritis in the flocks

performance in layers (Petitte and Etches, 1989, 1991; Mumma *et al*., 2006). The primary characteristic of a non-specific stress response in poultry species is elevated serum corticosterone (Edens, 1978; Edens and Siegel, 1975; Edens *et al*., 1983; Harvey *et al*., 1984). Among the various morphological effects associated with stress-induced elevations in corticosterone is female reproductive tract collapse (Petitte and Etches, 1991). Petitte and Etches (1991) infused corticosterone continuously into laying hens and determined that the high level of corticosterone caused cessation of laying and regression of the ovary, which has been confirmed by other research (Petitte and Etches, 1988, 1989; Tilly *et al*., 1991; Mumma *et al*., 2006; Shini *et al*., 2009). It has been suggested that corticosterone directly affects the hypothalamic-hypophyseal-gonadal axis (Petitte and Etches, 1988, 1989, 1991; Tilly *et al*., 1991). Additional evidence for the negative effect of stress-induced elevation in serum corticosterone on the ovary can be seen in the decrease in serum concentrations of progesterone and estradiol-17β in this report. Porter *et al*. (1989) and Tilly *et al*. (1991) noted that progesterone and estradiol-17β are secreted by granulosa cells in large ovarian follicles and theca cells in small ovarian follicles, respectively, under the influence of luteinizing hormone (LH) and follicle stimulating hormone (FSH). It has been observed that circulating corticosterone and LH are inversely related in laying hens (Wilson and Cunningham, 1980), suggesting central mechanisms governing the relationship between the generalized stress response and reproduction. The concept of central mediation of stress-related decreased egg production in hens assumes that a stressor causes input into the hypothalamus to initiate the activation of the hypothalamic-pituitary-adrenal axis (HPA). In this process, corticotropin releasing hormone (CRH) from the hypothalamus stimulates adrenocorticotropin (ACTH) release from the anterior pituitary, which then stimulates synthesis and release of corticosterone from the adrenal cortex. With activation of the HPA, elevated CRH can have a direct inhibitory influence on release of gonadotropin releasing hormone from the hypothalamus, which results in decreased release of LH and FSH from the pituitary (Rivier and Rivest, 1991). Reduced output of LH and FSH subsequently would cause reduced output of progesterone and estradiol-17β from the granulosa and theca cells, respectively, leading to reduced ovulations and egg production.

It has been suggested that corticosterone also can have a direct effect on ovarian function causing decreased secretion of both progesterone and estradiol-17β (Rozenboim *et al*., 2007; Henriksen *et al*., 2011), but these observations do not abrogate the possibility that a central influence, mediated by the generalized stress response, can decrease reproductive performance of animals, including laying hens (Petitte and Etches, 1988, 1991; Rozenboim *et al*., 2007; Henriksen *et al*., 2011). Furthermore, there is evidence suggesting that the inhibitory activity exerted by activation of the HPA in mammals and the domestic fowl involves more than CRH inhibition of GnRH secretion (Tsutsui *et al*., 2000). A novel hypothalamic peptide, gonadotropin inhibitory peptide (GnIH) has been demonstrated to inhibit release of gonadotropin from anterior pituitary in both quail and chickens, causing depression of LH secretion from the anterior pituitary (Tsutsui *et al*., 2000) and in both mammals and birds, GnIH is stress-inducible (Kirby *et al*., 2009). The modulating role played by GnIH on reproduction in the adult hen remains equivocal (Ciccone *et al*., 2004). *In vitro*, GnIH inhibited release of both FSH and LH from pituitaries from cockerels, but its effect in laying hens was limited to inhibition of LH release from the pituitary (Ciccone *et al*., 2004). Another centrally-mediated mechanism involves an elevation in triiodothyronine, which has been demonstrated to cause cessation of ovarian steroidogenesis and inhibition of egg production (Sechman, 2013). The involvement of an elevated thyroid status likely could have influenced decreased secretion of ovarian steroids in this study since the increased atmospheric ammonia in the Pit house occurred during the coldest time of the year. Elevated plasma concentrations of corticosterone are known to increase plasma triiodothyronine and this is mediated via CRH, the stimulator of ACTH release, which is a potent inducer of TSH secretion that stimulates thyroid hormone synthesis and release (Liu *et al*., 2012; De Groef *et al*., 2005).

The results of this field investigation show that laying hens are sensitive to the reproduction inhibitory properties of stress-related elevations in circulating corticosterone, which is a keystone non-specific stress response in all poultry species. Elevated concentrations of atmospheric ammonia over a one month period caused a prolonged increase in serum corticosterone that was inversely related to decreased serum progesterone, estradiol-17β and egg production in that time period and beyond. The mechanism for the diminished reproductive status, while not elucidated directly, appears to be centrally mediated through the negative impact of the activated hypothalamic-pituitary-adrenal axis acting on the function of the hypothalamic-pituitary-gonadal axis. Atmospheric ammonia is tolerated in concentrations less than 100 ppm by most varieties of poultry, but atmospheric ammonia in concentrations above 100 ppm should be considered as a potent stressor, which can negatively influence reproductive status of laying hens. It must be kept in mind that ammonia also has negative effects in broilers and breeders alike. When atmospheric ammonia begins to increase in layer and breeder houses, efforts to reduce its concentration should be exercised immediately in attempts to keep it under 25-50 ppm concentration as required by governmental agencies and animal welfare audit tools.

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