

# Are Thirty-Five Days Enough to Observe the Stress-Reducing Effect of a Semiochemical Analogue on Chickens (*Gallus gallus domesticus*) Housed Under High Density?

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**ABSTRACT** Two similar 400-m<sup>2</sup> buildings, each housing 8,400 broilers of a commonly used industrial strain, were used to test the effectiveness of the semiochemical treatment known as mother hen uropygial secretion analogue (MHUSA). The birds in 1 building were exposed to MHUSA continuously during a 35-d growing period, whereas those in the other building received a placebo. The experiment was then repeated using precisely the same conditions but with the building treatment reversed to control for any building effect. The purpose of the trial was to evaluate the effect of MHUSA on growing performances (live weights) and stress indicators ob-

served from blood samples: heterophil-lymphocyte ratio and corticosterone level. Data analysis (ANOVA) showed that MHUSA-treated broilers had a higher mean growth rate, as shown by increased live weights at both d 17 and 35 ( $P \leq 0.001$  and  $P \leq 0.001$ , respectively). After the 35-d growing period, we observed both lower heterophil-lymphocyte ratio ( $P \leq 0.001$ ) and lower corticosterone level ( $P \leq 0.05$ ) for birds treated with MHUSA compared with placebo, further indicating that the birds were less stressed. We conclude that constant diffusion of MHUSA in buildings used to house broilers might enhance the welfare and growth of the bird by reducing stress.

**Key words:** broiler, stocking density, growth, stress, semiochemical

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## INTRODUCTION

Broiler chickens, which are used for meat, are typically housed for 35 d at a stocking density of 20 or more birds/m<sup>2</sup>. Pettit-Rilez and Estevez (2001) have shown that stocking densities of more than 15 birds/m<sup>2</sup> are stressful for broilers. At the end of the growing period, average live weight of birds is around 2,000 g (Pettracci et al., 2004), meaning that the stocking density is often more than 40 kg/m<sup>2</sup>. Martrenchar et al. (1997) observed that at densities exceeding 43 kg/m<sup>2</sup>, there were detrimental effects, including increased fighting. Added to this, most broilers are raised in buildings without outdoor access or opportunities for dust bathing. Large group size (Barnard and Burck, 1979), which makes the establishment of a dominance hierarchy impossible, and a lack of dust bathing (Vestergaard et al., 1999) tend to lead to aggressiveness (Cornetto et al., 2001). The effects of injury and stress are of concern both to producers and processing plants, because they represent a source of significant economic loss. For example, stress has been shown to lower growth

curves (Tankson et al., 2001), increase the number of downgraded broilers, and can even lead to increased mortality rate (Buitenhuis et al., 2002). Heterophil-lymphocyte ratio (HLR; Puvadolpirod and Thaxton, 2000), corticosterone (CS) level, and weight gain (Post et al., 2003) are of importance to evaluate both the stress of the birds and the economical efficiency of the production method. A study by Madec et al. (2006) has shown that the semiochemical treatment known as mother hen uropygial secretion analogue (MHUSA) reduces stress in broilers housed for 80 d at a stocking density of 11 birds/m<sup>2</sup>. The purpose of the current study is to assess the effect of MHUSA on stress and performance parameters during a shorter period of housing at a higher stocking density.

## MATERIALS AND METHODS

### *Birds and Breeding Conditions*

Birds were 1 d old when they arrived (noted d 0). The population was equally split between males and females. The strain used (Ross PM3) is a commonly studied meat-producing bird with a high standardized growth rate that is usually slaughtered from 35 to 42 d of age. The same number of birds (8,400) was kept under artificial light (18:24 pattern) in each of 2 similar 400-m<sup>2</sup> buildings with-

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out outdoor access. Birds had free access to food and fresh water. Fresh wheat straw litter was installed the day previous to the arrival of the chicks and left unchanged throughout the rearing period. Building temperature was controlled by windows and gas heaters. Because we denoted as batch a group of chicks born on the same day and housed from their arrival until slaughter, a batch is composed of 16,800 broilers (8,400 individuals per building).

### **Treatment**

To construct the MHUSA, samples of the natural secretions were obtained from hens by squeezing their uropygial glands once a day for 14 d after the hatching of their chicks. When the pattern was analyzed, we observed a stabilization of the uropygial secretion from 12 d post-hatching. Thus, samples from 12 d posthatching were analyzed using gas chromatography-mass spectrometry (Turbo Mass, Perkin-Elmer, Courtaboeuf, France). A synthetic analogue was then created (MHUSA, Pageat, 2002). This consisted of a synthetic reconstruction of a fraction of the natural secretion, composed of 12 to 18 carbon-length fatty acid methyl esters. The treatment was incorporated into a commercially available gelatin matrix block (Nicols S.A., Bertry, France) weighing 150 g and composed of water (135 g), nonionic surfactant (7 g), and a gelling gum (5g), plus either 3 g of water (placebo) or 3 g of MHUSA. The gelatin matrix block (placebo or MHUSA) was held in a specially manufactured perforated plastic container suspended 120 cm above the ground, out of reach of the birds. The components of MHUSA are heavier than air, and this delivery arrangement allowed the treatment to diffuse into the air around the birds. Finally, treatment was blinded, because it was impossible to tell the difference between MHUSA and placebo matrixes (their odor and appearance were identical), and the persons involved in the trial were unaware of which group was given which treatment.

### **Experimental Design**

Two buildings and 2 batches were used in the study (buildings denoted A and B). To avoid cross-contamination between the 2 treatment groups, treatments were used in separate buildings. For the first batch, birds in building A acted as the control (i.e., received placebo blocks), and birds in building B received the semiochemical (i.e., MHUSA blocks). After this first experiment, the birds were removed and the buildings were completely emptied and thoroughly cleaned. There was a 14-d wash-out before introducing the next batch of chicks. The whole experiment was then repeated precisely as before, but with the building treatments reversed to control against building effect. Eight treatment blocks were installed in each building on the day before the arrival of the chicks (1 block/50 m<sup>2</sup>). These blocks were replaced with fresh ones every 15 d, meaning that 24 blocks were used per building and per batch.

### **Studied Indicators**

At both 17 (d 17) and 35 d of age (d 35, two days before slaughter), live weight (LW) was measured (Bird Weighing System-1050, Weltech International, Cambridgeshire, UK) for 400 individuals (100 males and 100 females from each treatment group). A further 50 males and 50 females were taken at random from each building for wing-vein blood sampling for CS and HLR measurement. The HLR was estimated from blood film smears using May-Grunwald and Giemsa stain (Lucas and Jamroz, 1961). A 3-mL portion of each sample was kept in dry tubes at +4°C before centrifugation (7,000 × g, 10 min) to collect serum. This was then conserved at -18°C for CS analysis by the RIA method by a specialized laboratory (LDH, ENV Nantes, La Chantrerie, Nantes, France). These samples were analyzed following a procedure described by De Jong et al. (2001).

### **Statistical Analysis**

Pooled data were examined by 2-way ANOVA using Statistica 5.0 software (Statsoft, Maison-Alfort, France). Comparisons were made between placebo and MHUSA treatments after replication. Data analysis was then performed for LW (at d 35), HLR, and CS. For LW at d 17, data were analyzed using the same software but by a Student *t*-test, because sexual dimorphism is not sufficiently well developed to tell the difference between male and female birds at that age (Mignon-Grasteau et al., 1999). For each indicator, we looked for both treatment and sex effect as well as for a sex × treatment interaction. Significance was accepted for values of  $P \leq 0.05$ .

## **RESULTS**

As presented in Table 1, broilers who received treatment with MHUSA were heavier at both weighing time points ( $P \leq 0.001$  and  $P \leq 0.001$  at d 17 and 35, respectively). At d 35, we observed a sex effect, because males were significantly heavier compared with females ( $P \leq 0.001$ ). Results for physiological indicators are shown in Table 2. We observed a treatment effect on both HLR and CS, with the means of both indicators being significantly lower in the MHUSA treatment group ( $P \leq 0.001$  and  $P \leq 0.05$  for HLR and CS, respectively). There was a significant sex effect on HLR (females > males;  $P \leq 0.05$ ) and a close to significant sex effect on CS (males > females;  $P = 0.07$ ). The only sex × treatment interaction found was for CS ( $P \leq 0.01$ ).

## **DISCUSSION**

According to our results, MHUSA treatment reduces the effect of stress. Birds reared in an environment continuously perfused with MHUSA showed significantly better mean growth rate, as assessed from LW values. It is known that stress leads to lower LW compared with unstressed or less-stressed chickens (Siegel, 1995). In

**Table 1.** Live weight depending on age of birds<sup>1</sup>

Day <sup>3</sup>	Placebo		MHUSA <sup>2</sup>		Significance ( <i>P</i> )		
	Males	Females	Males	Females	Treatment	Sex	Treatment × sex
17	466.0 ± 72.4		491.3 ± 63.9		***	NC <sup>4</sup>	NC
35	1,939.5 ± 201.1	1,676.5 ± 179.6	2,034.5 ± 256.6	1,747.8 ± 205.8	***	***	NS

<sup>1</sup>Means are indicated ± SE.

<sup>2</sup>MHUSA = mother hen uropygial secretion analogue.

<sup>3</sup>17 = pooled (males + females) live weights at 17 d (g); 35 = live weights at 35 d (g).

<sup>4</sup>NC = not calculated.

\**P* ≤ 0.05; \*\*\**P* ≤ 0.001.

other species, maternal secretions have been shown to have a positive effect on growth rate of their young. For example, McGlone and Anderson (2002) showed that exposure to a synthetic analogue of a secretion from the teats of lactating sows improved the weight gain of piglets. These findings are comparable to the effects of MHUSA on the growth rate and LW of broilers in the present study. Additionally, in the study by McGlone and Anderson (2002), the treated piglets engaged in significantly less agonistic behavior, compared with placebo, indicating a lower level of stress. Numerous references show a positive correlation between stress and LW in poultry (Beuving et al., 1988; Puvadolpirod and Thaxton, 2000; Campo et al., 2005). Our findings appear to be in accordance with the literature, in particular with McGlone and Anderson (2002). Our results, showing that the effect of stress on broilers is reduced under MHUSA treatment, are also broadly in accordance with findings by Madec et al. (2006) on slow-growing broilers. However, our results showed lower HLR in the MHUSA group, as opposed to results by Madec et al. (2006), in which no difference in HLR was observed between treatments. In that study, broilers were reared for a longer period (80 d) housed in lower-density housing (11 heads/m<sup>2</sup>) with outdoor access. Thus, according to Campo et al. (2005), the HLR results for the present study may be linked to chronic stress experienced by the broilers due to higher stocking density than was seen in the study by Madec et al. (2006).

In the same way that HLR and LW are known to be strongly linked (Puvadolpirod and Thaxton, 2000), there is also a correlation between CS and LW. For example, in the case of CS-induced stress, chickens showed lower LW (Puvadolpirod and Thaxton, 2000). In the present study, we observed lower CS level and higher LW in the

MHUSA group than in the placebo group. Time taken to carry out sampling can have a significant effect on measured CS, because CS rises 30 min after a stress event (Yoa et al., 2004). This did not influence our findings, because each bird was blood-sampled in less than 2 min.

It appears that typical industrial housing conditions for broiler chickens are less than optimal; indeed, we found that it was possible to reduce stress for these birds and that this reduction in stress had significant effects on growth rate. In poultry, access to water and dust baths, appropriate stocking density, and group size are needed to stabilize dominance (Jones and Faure, 1981; Zuidhof, 2005). In the flock studied in the present investigation, these needs were not met. This creates environmental stress (Zuidhof, 2005) as well as social stress (Vestergaard et al., 1999), which may explain the observed treatment effect on HLR and CS levels. The observed sex effect for these physiological indicators is similar to that found by Madec et al. (2006). Results from other studies indicate that a sex effect on weight should be anticipated (Woelfel et al., 2002).

Our results show that MHUSA increases growth and decreases stress. This suggests that chicks, and growing birds, possess olfactory systems that are able to detect and respond to MHUSA in the air, without actual contact with it. This mechanism is the same as would be expected in a natural response to uropygial secretions. Porter et al. (2002) have shown that chicks do have olfactory abilities, which supports the idea that they may be able to detect MHUSA. Nevertheless, this has not yet been ascertained. It would be interesting to know more about the precise effects of MHUSA, such as its ability to attract chicks. In their study, McGlone and Anderson (2002) stated that olfactory signals can modulate adaptation to the environ-

**Table 2.** Heterophil-lymphocyte ratio and corticosterone level from blood samples at d 35<sup>1</sup>

Item <sup>3</sup>	Placebo		MHUSA <sup>2</sup>		Significance ( <i>P</i> )		
	Males	Females	Males	Females	Treatment	Sex	Treatment × sex
HLR	0.214 ± 0.110	0.270 ± 0.008	0.181 ± 0.081	0.213 ± 0.085	***	*	NS
CS	6.23 ± 6.01	7.22 ± 5.49	7.04 ± 5.09	2.63 ± 3.68	*	<i>P</i> = 0.07	**

<sup>1</sup>Means are indicated ± SE.

<sup>2</sup>MHUSA = mother hen uropygial secretion analogue.

<sup>3</sup>HLR = heterophil-lymphocyte ratio; CS = corticosterone (ng/mL).

\**P* ≤ 0.05; \*\**P* ≤ 0.01; \*\*\**P* ≤ 0.001.

ment in ways that may improve performance and welfare. Thus, using MHUSA may not only improve poultry welfare in poultry but also produce economic benefits. This is a strong argument for the use of MHUSA as a routine part of poultry husbandry.

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