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USE OF A CALCIUM PARTICULATE AND FEEDING TIME ALONE OR IN COMBINATION WITH 25-HYDROXYHOLECALCIFEROL TO REDUCE FRACTURE SUSCEPTIBILITY IN LAYING HENS

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Abstract

The aim of this article is to present the possibility of reducing sternum fractures (KBF) in laying hens by using calcium particulate 2 hours before turning off the lights (CaT), alone or in combination with 25-hydroxyholecalciferol (Hyd). For the experiment, a total of 240 birds were used that were exposed to one of four treatment combinations in a 2X2 factorial design (Factor 1: CaT+ or Control (CaT-); Factor 2: Hyd vs. Control (Hyd-). All birds were housed in a commercial aviary until 33 weeks of age when they were moved to a test barn and randomly assigned to one of the eight pens (30 hens/pen). After one week of habituation to the novel housing, all birds began to receive one of the four treatment combinations (n = 2 replicate pens/treatment combinations). The ability of the treatment combinations to reduce fracture susceptibility was tested using an ex vivo impact testing protocol. After 39 weeks of age the keels were removed and scored for damage using an established three-point severity scale. The likelihood of an experimental fracture occurring in the CaT-/Hyd- was 3.6 times more likely compared to the CaT+ /Hyd+ treatment (1.1 to 5.8).

Key words: *laying hens, keel bone damage, calcium timing (CaT).*

INTRODUCTION

The extremely high frequency and severity of keel bone damages (KBD) is one of the biggest animal welfare problems facing the industry. Most of the leading animal welfare organizations, including the United Kingdom's Animal Welfare Committee and EFSA, are sounding the alarm. The prevalence of KBD has increased dramatically since the introduction of changes imposed by EU legislation banning the use of conventional cages since January 2012. Despite good intentions to improve the welfare of chickens, the unexpected consequences are cause for serious reflection. The poultry industry is facing the unexpected challenge of significantly increasing KBD, which is compromising animal welfare and reducing farm productivity.

The problem of keel bone damage is widespread with similar but very varying levels reported in different countries such as Switzerland (Kappeli et al., 2011), United Kingdom (Wilkins et al., 2011), Netherlands, Belgium, Germany (Rodenburg et al., 2008; Heerkens et al., 2016) and Canada (Petrik et al., 2015). KBD affects different genetic lines of laying hens (Kappeli et al., 2011), as well as all types of breeding systems (Wilkins et al., 2011; Petrik et al., 2015), including organic production systems (Bestman and Wagenaar, 2014). Despite their ubiquity, the causes and influencing factors of KBD remain largely unknown to the research community, a circumstance that makes it very difficult to develop effective strategies to reduce their occurrence and severity. According to most experts working in this field, the main cause of bone damage in laying hens is the early onset of the egg-laying period. Skeletal growth in hens ends at about 16-18 weeks of age. In most industrial egg-laying hybrids, laying begins at about 17 weeks of age. Ossification of the sternum, however, is not complete until about 40 weeks of age. This fact underlies the hypothesis that due to the need for large amounts of calcium to form the eggshell during early laying, the sternum (still cartilage) receives less than the calcium needed for proper ossification. Many authors confirm that high productivity and long egg-laying periods leave bones weak and prone to breakage (Whitehead and Fleming, 2000; Whitehead, 2004). Egg production demineralises bones to provide the calcium needed for the shell. The bulk of the eggshell is formed during the dark phase of the day, when there is no intake of food and minerals in the digestive system (Thiele, 2015). The main sources of minerals during this part of the day are bones. About 30% of the required calcium is extracted from them. Increasing the amount of calcium in the feed is ineffective because absorption is a limiting factor (Newman and Leeson, 1997).

The aim of the present study was to implement an established technique (CaTiming; CaT) commonly used in laying hens to improve shell quality (Thiele, 2015) in a new way to reduce sternal fractures in hens in the early stages of laying. Our specific goal was to test the following hypotheses:

- Providing some of the daily calcium in the 2-hour period before stopping lighting (CaT) increases the resistance of the sternum to fractures and improves bone strength;
- The inclusion of (Hyd) in the diet provides an additional effect.

It was expected that the combination of the two factors (CaT) and (Hyd), which is known to increase calcium absorption (Soares et al., 1995), will further increase bone strength.

MATERIALS AND METHODS

The ability of CaT and Hyd to reduce the susceptibility of the sternum to fractures was tested using an *ex vivo* protocol (Toscano et al., 2013). The basis of this study is a direct mechanical impact with quantifiable energy loads on the sternum of killed birds, which allows assessing the likelihood of fracture in the relevant experimental group. A total of 240 Leghorn (LSL) birds were used in the study. At 18 weeks of age, all birds were housed in an industrial aviary and received a standard diet until 33 weeks of age. They were then moved to the experimental building and randomly assigned to one of the eight pens (30 hens/pen). After 1 week of habituation (34 weeks), the pens were randomly divided into four experimental groups (60 birds/dietary combination). A 2X2 factorial design was used. Group A - control receiving standard feed, Group B - calcium in the feed is reduced by 50% (18 g/kg), but 2 hours before turning off the lights birds have free access to calcium carbonate with particle sizes 3-5 mm (calcium content 38%). Group C - the diet of group B + 32.84 IE/kg Hyd and Group D - standard feed + 32.84 IE/kg Hyd.

At 39 weeks of age, hens were euthanized by sedation with Phenobarbital (600 mg/kg, IP; Esconarkon, Streuli Pharma AG, Switzerland) and subsequent cervical dislocation. After the injection, the birds were placed in a small, dark box for about 7-10 minutes, in order to provide peace of mind until their unconsciousness and avoid self-harm. Cervical dislocation was performed only when the birds were completely relaxed and did not show reflexive actions (dilation of the pupils, retraction of the nails). Prior to euthanasia, their weight was determined and recorded. Time between death and impact also were recorded and typically were within 120 s. Impact testing was performed following a protocol previously described by Michael Toscano (Toscano et al., 2013). Specially designed and manufactured test equipment (Atelier Lorraine, Bern, Switzerland) was used for the testing procedure. In order to ensure contact of the impact element with the ventral surface of the sternum, the carcasses were positioned in a supine position. The point of impact was located about 2 cm in front of the caudal end of the bone. Two different values of impact force were used: 3.248 J and 4.331 J. After mechanical impact, the sternum was removed and examined for damage using an established three-point weight scale. In addition, the presence or absence of old fractures and their severity was recorded using a rating system published by Wilkins (Wilkins et al., 2011).

The entire experimental procedure was performed at the Center for Proper Housing: Poultry and Rabbits (ZTHZ), in Bern, Switzerland, using the Commercial Genetic Line (LSL).

The experiment was approved by the Cantonal Veterinary Office and complied with Swiss regulations regarding the treatment of experimental animals.

Descriptive statistical methods were used to determine the mean and standard deviations (± SD). Fracture susceptibility was statistically analyzed using logistic regression with fractures as a binomial response, and dietary combination, impact energy, and body weight as predictors.

RESULTS AND DISCUSSIONS

The processing of the obtained results showed that there is no significant difference in the number and severity of old fractures between the experimental groups. This finding is completely expected, as up to 33 weeks of age all hens received the same diet. Almost all birds included in the study had at least one sternal fracture. About 9% of them had two fractures of different location and severity. It also confirms the view that most fractures occur in the first 40 weeks of life of the laying hens due to incomplete ossification of the sternum (Buckner al., 1947). This was not the case with fractures experimentally induced by controlled mechanical action. In experimental group D, receiving standard feed with the addition of 25-hydroxycholecalciferol, the new fractures as a result of the experimental impact were over 73% (Table 1).

Table 1. Old fractures and experimental fractures in laying hens, as a result of impact testing with different level of energy load

Groups	A (n = 56)		B (n = 56)		C (n = 56)		D (n = 56)	
Old Breaks	94.6% (53)		96.4% (54)		98.2% (55)		94.6% (53)	
Exp. Fract. in total	57.1% (32)		57.143% (32)		46.429% (26)		73.214% (41)	
Energy	L (30)	H (26)	L (35)	H (21)	L (30)	H (26)	L (30)	H (26)
	56.7% (17)	57.7% (15)	51.4% (18)	66.7% (14)	36.7% (11)	57.7% (15)	63.3% (19)	84.6% (22)

This result contrasts with the findings of Sahin et al. (2009) found improved bone mineralization after the addition of 25(OH)D3 to the diet of laying quails.
This discrepancy may be due to the fact that the activity of 25(OH)D3 is highly dependent on the biological response of birds (Aburto et al., 1998). In control group A and experimental group B receiving calcium particles, this percentage was 57. In experimental group C, which received a combination of the two studied factors, the percentage of experimental fractures was the lowest - 46%. Our results support the claim of Nascimento et al. (2014) that bone strength increases as calcium levels increase.
High levels of calcium in the blood stimulate calcitonin-releasing cells, and thus reducing bone resorption leads to increased bone strength (Whitehead, 2004; Nunes et al., 2006).
A significant difference between the groups was found in both the overall percentage and the percentage of fractures resulting from the different energy impacts (Table 1).
With an energy impact of 3.248 J, the highest percentage of fractures was again in group D - 63%, followed by groups A - 57%, B - 51% and group C - 31%. As a result of the high energy impact (4.331 J) the leader was once again Group D, with a remarkable 85% of experimental fractures. In group B the result was 67%, and in experimental groups A and C - 58% (Figure 1).

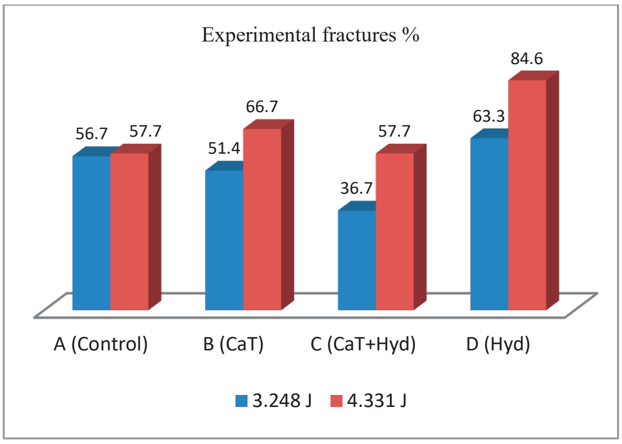

Figure 1. Percentage distribution of experimental fractures according to the force of impact

It is noteworthy that in control group (A) the difference between the two energy effects is insignificant. In experimental group B, it could be concluded that the applied dietary combination reduces the likelihood of fractures at low energy effects, but increases it dramatically at high ones.
In experimental group C the sensitivity to fractures at high energy impacts is identical to the control group, while the probability of fractures due to low energy is the lowest.

Examining birds with two old fractures, we found that in experimental group C, receiving a combination of calcium carbonate and 25-hydroxycholecalciferol, there was not a single bird with an experimental fracture (Figure 2).

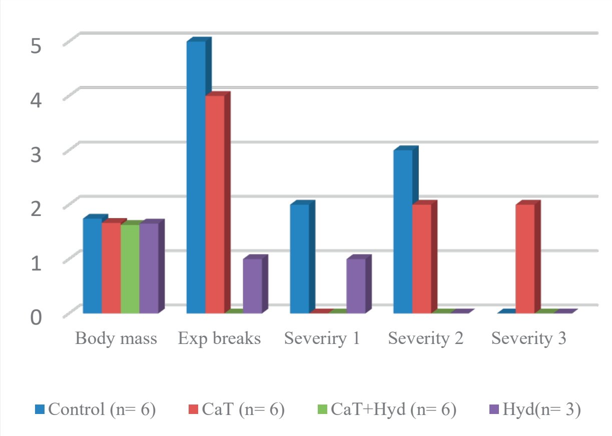

Figure 2. Experimental fractures in birds with two old fractures

In group A the result was 5 out of 6 (83%), in group B - 4 out of 6 (67%) and in group D - 1 out of 3 (~34%). This result is difficult to comment on categorically, but our assumption is that birds that have undergone multiple previous fractures have been able to achieve increased resilience thanks to the applied dietary combination of CaT and Hyd.

CONCLUSIONS

The application of CaT contributes to increasing the resilience of bones to low energy effects, but makes them more susceptible to high energy effects compared to the control group. Co-administration of CaT and Hyd enhances the positive effect at low impact energy without having a negative effect at high energy. The addition of 25-hydroxycholecalciferol alone has a negative effect on the strength of the keel bone, regardless of the strength of the impact.

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