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Effects of environmental enrichment on health and bone characteristics of fast growing broiler chickens

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ABSTRACT Providing environmental enrichment for broilers is a potential strategy to increase welfare, activity, and health. The aim of this study was to evaluate the effect of environmental enrichment on health and leg bone characteristics of broilers. One control and 8 types of enrichment were included: 2 distances between food and water (7 and 3.5 m), roughage, vertical panels, straw bales, 2 platforms (30 and 5 cm), and a lowered stocking density (34 kg/m^2) . Birds were kept according to conventional Danish guidelines. The study included 58 pens with approximately 500 birds each. On day 35 of age, 25 birds per pen were killed and included in a postmortem analysis of wooden breast, body condition scores, pathological conditions (femoral head necrosis, arthritis, tenosynovitis, fractures, tibial dyschondroplasia, and twisted tibiotarsus), muscle width of the lower leg, and tibiotarsus properties (bone strength, weight, length, and proximal diameter, middle diameter, and distal diameter). It was predicted that environmental enrichment would have a positive effect on pathology with the exceptions that environmental enrichment that increased activity would pose a risk factor for wooden breast development, and straw bales would be a risk factor for bacterial infections (arthritis, tenosynovitis, and femoral head necrosis). Furthermore, it was hypothesized that enriched groups would have increased muscle width, bone strength, and dimensions of the tibiotarsus. Broilers with 7 m between food and water had a longer distal diameter of the tibiotarsus than those with straw bales (P = 0.04). The birds provided with vertical panels had wider leg muscle than the treatments with roughage (P = 0.045), 3.5 m distance (P = 0.049), and straw bales (P = 0.044). No effects were found for the remaining outcomes. These results suggest that provision of vertical panels and increased distance between resources can result in larger muscle and bone dimension, possibly having a positive effect on leg health. Furthermore, the provision of environmental enrichment does not appear to be a risk factor for wooden breast or bacterial infection.

Key words: broiler chicken, environmental enrichment, welfare, leg health, pathology

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INTRODUCTION

Modern broilers are bred for fast growth, and studies have found that they spent approximately 80% of their time resting (Weeks et al., 2000; Zuidhof et al., 2014). The combination of fast growth and inactivity poses a risk factor for the development of lameness and pathologies related to the locomotor system (Bradshaw et al., 2002) and is likely to result in reduced welfare for the birds, because of pain, and in economic losses for the producer. In a recent investigation of walking ability (gait scores) in conventional broilers in Denmark, 77.4% of birds had an abnormal gait, and 5.5% had severe gait defects (gait score > 2). In addition, 4.7% of broilers had tibial dyschondroplasia, and 6.5% had inflammation of the hock joint tendon sheath (tenosynovitis), and both of these conditions were correlated to lameness (Tahamtani et al., 2018a). Providing environmental enrichment is a potential strategy to increase activity and has been shown to increase activity in studies which also found positive effects on lameness (Reiter and Bessei, 2009; Blatchford et al., 2012) and foot pad dermatitis (Ohara et al., 2015). Furthermore, a reduction in the mortality because of leg disorders has been achieved by adding enrichment in the form of reduced

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stocking density (Hall, 2001) and increased distance between food and water (Reiter and Bessei 2009), and applying an intermittent light schedule has been found to decrease the prevalence of tibial dyschondroplasia (Soerensen et al., 1999; Sanotra et al., 2002). However, there are also possible disadvantages of enrichment. The provision of straw bales or chopped straw has resulted in increased severity of foot pad dermatitis (Bilgili et al., 2009; Tahamtani et al., 2019), and Thoefner et al. (2019) have shown a correlation between foot pad lesions and bacterial infections. Thus, the provision of straw bales may pose a risk factor for conditions with a bacterial etiology such as arthritis, tenosynovitis, or femoral head necrosis (Bradshaw et al., 2002).

In addition to leg problems, physiological measures of the leg such as muscle and bone dimensions have been shown to be affected by enrichment (Castellini et al., 2002). Examples of bone measures affected by enrichment include weight, length, diameter, cortical thickness, and strength of tibiotarsus (Reiter and Bessei, 2009; Yildiz et al., 2009; Buijs et al., 2012; van der Pol et al., 2015). Generally, enrichment resulted in larger and stronger bones, with the exception of Buijs et al. (2012) who found that tibiotarsus length and breaking strength decreased at lowered stocking densities. Thus, the effect of enrichment on bone measures and the importance in relation to leg problems is a subject that is not yet fully understood. Here, we hypothesize that increased activity in birds kept in enriched environments is associated with an increase in muscle and bone dimensions and bone strength.

Despite the expected positive consequences of environmental enrichment on leg health, negative effects on muscles may also be seen. Environmental enrichment throughout the rearing period for conventional broilers may affect development of the muscle abnormality described as wooden breast (Sihvo et al., 2014; Petracci et al., 2015) as this phenomenon has been shown to be initiated already at 2 to 3 wk of age (Radaelli et al., 2017; Papah et al., 2018). Wooden breast is characterized by pale and bulging hard areas (Sihvo et al., 2014) and has several implications for the quality of the fresh and processed products (Bowker et al., 2018). Increased movement and muscle activity encouraged by enriching the environment may affect wooden breast development as oxidative stress as well as hypoxia indicators and pathways for muscle fiber repair have been identified in wooden breast-affected tissue (Mutryn et al., 2015). Furthermore, exercise overload in the enriched environment increases the risk of inflammation, which also characterizes wooden breast tissue (Sihvo et al., 2014; Velleman and Clark 2015).

Newberry et al. (1995) defined the effect of environmental enrichment as "An improvement in the biological functioning of captive animals resulting from modifications to their environment". Using this definition, environmental enrichment can be any modification that improves biological functioning and does not necessarily include the addition of something new to the production environment. Some examples of enrichment for broilers

are the provision of straw bales, perches or platforms, changes to the light schedule, or a reduction in stocking density (Pedersen and Forkman 2019). Many studies that evaluate the effect of enrichment compare one type of enrichment to a non-enriched control. While a meta-analysis may yield important information (EFSA, 2010), it can be difficult to compare values for a specific measure because there is often variation between studies in a number of important aspects (Pedersen and Forkman 2019). To give an example, 2 studies investigated the effect of enrichment on tibial dyschondroplasia (Sorensen et al., 2000; Birgul et al., 2012). The investigated types of enrichment were provision of perches (Birgul et al., 2012) and a reduced stocking density (Sorensen et al., 2000). However, the 2 experiments differed in regards to choice of broiler (Ross 208 and Ross 308) and used different scoring methods to assess tibial dyschondroplasia. This challenge with variation between individual studies can be solved by combining a number of different enrichments in the same study making comparison and evaluation of several enrichment alternatives possible.

In the present study, our aim was to compare the effect on health and leg measures in broiler chicken of 8 types of environmental enrichment and a nonenriched control. The types of enrichment tested were 2 increased distances between food and water (7 m and 3.5 m), maize roughage, vertical panels, straw bales, 2 types of platforms (30 cm and 5 cm), and a lowered stocking density (34 kg/m^2) . The included outcomes were postmortem observations including body condition score, wooden breast assessment, various pathological conditions, and bone and muscle measures of the leg. The hypothesis was that the provision of environmental enrichment could pose a risk factor for the development of some pathological conditions such as wooden breast and bacterial infections, while possibly having a positive effect on others such as tibial dyschondroplasia. Furthermore, it was hypothesized that increased activity in enriched treatment groups would result in increased leg muscle mass, as well as increased bone strength and dimensions of the tibiotarsus. The present study was part of a larger experiment at Aarhus University, Denmark, that evaluated the effect of environmental enrichment on broiler welfare (Tahamtani et al., 2019), learning and fear (Tahamtani et al., 2018b), activity budget (Bach et al., 2019), and production parameters (unpublished data).

MATERIALS AND METHODS

Animals and Housing

Day-old mixed-sex Ross 308 broilers were kept in 2 identical houses with 5 pens per house. A control group and 8 treatments (types of environmental enrichment) were included. Each pen measured 9.6×3.1 m and contained 497 birds/pen resulting in a stocking density of 40 kg/m². In pens were the environmental enrichment occupied floor space, the number of birds was reduced

to maintain a stocking density of 40 kg/m². All birds received commercial feed and water ad libitum. The intensity of light was approximately 20 lux. A commercial light schedule was applied consisting of 23L:1D on day 1 of age with a gradual increase in the dark period to reach 18L:6D from day 6 of age and throughout the remaining experiment. The litter material was a 4-cm deep layer of wood shavings. The indoor climate was managed using an automatic ventilation system (Skov A/S) that monitored and controlled temperature and humidity. The average air humidity was 61.8%, and the ambient temperature was kept at 34°C from day-old and gradually decreased to reach 20°C from day 28 of age and for the rest of the experiment. The experiment consisted of 6 consecutive blocks each lasting 35 D. The treatments were randomly distributed over the pens and blocks by drawing of lots and subsequently balanced between the 2 houses, resulting in 6 to 8 replicates per treatment (Table 1). The data from 2 pens were excluded from block 1 because of water damage in the pens.

Treatments

All treatments consisted of different types of environmental enrichment each illustrated in a schematic design in Figure 1. In treatment A and B, the distance between food and water was increased to 7 m and 3.5 m, respectively, as opposed to a distance of 1.5 m in the remaining treatments. In treatment A, the distance was reduced to 1.5 m from day 22 of age to ensure the food and water intake of the birds, whereas the increased distance was maintained throughout the experiment in treatment B. Treatment C consisted of a feed supplement in the form of maize roughage in addition to the ad libitum commercial feed. The roughage was given daily on 3 circular pans (0.4 m in diameter) on the floor of the pen. In treatment D, 5 opaque vertical panels ($60 \text{ cm} \times 60 \text{ cm}$) were evenly distributed in the pen. For treatment E, 3 straw bales (42 cm \times 48 cm \times 122 cm, height \times width \times length) were evenly distributed in the pen. In treatment F and G, platforms of a height of 30 cm and 5 cm, respectively, were placed in the pen. The platforms were rectangular $(5.4 \text{ m} \times 0.6 \text{ m})$ and made from perforated plastic slats, and the area under the platforms was fenced off. Two access ramps with a 14.5° incline were attached to the 30 cm high platforms. For treatment H, the stocking density was lowered to 34 kg/m^2 . Treatment I was the control without any form of environmental enrichment and a stocking density of 34 kg/m^2 .

Data Collection

A random sample of 25 birds per pen was selected for the postmortem examination. At 35 D of age, the birds were killed by cervical dislocation and subsequently marked with unique wing tags. The total number of birds for the random sample was 1,450, of which 21 birds were excluded from the analysis because of lost wing marks or missing or insufficient data. After scoring of wooden breast, birds were kept in a freezer and thawed at 4°C before the remaining postmortem examination was carried out by the same observer who was blinded to the treatments.

Wooden Breast Observations for wooden breast were carried out shortly after the birds had been killed on all birds from the random sample from block 2 to 6. The pectoralis major muscles were exposed and removed from the carcass. The classification, into 3 categories, of the pectoralis major was conducted by palpation performed by 2 trained assessors that were blinded to the treatment (Dalgaard et al., 2018). Muscles with no wooden breast abnormality were given a score 0, moderate abnormality was given a score 1, and severe abnormality was given a score 2.

Body Condition Score Body condition scores modified from Gregory and Robins (1998) were defined as follows: Score 0: below normal, prominent keel bone with poorly developed breast muscles; Score 1: normal, plump breast muscles which provide a smooth contour with or protrude slightly above the keel bone; Score 2: above normal, very plump breast muscles which protrude notably above the keel bone.

Muscle Width of the Lower Leg All observations for leg health were carried out on both the left and right leg. To measure the width of the muscles of the lower leg (i.e., the drumstick), the leg was distended in a 90° angle from the body. An index finger was placed on the knee joint, and the width (ventral to dorsal) was measured just below the index finger using a digital caliper. The measurements were noted in millimeters to 2 decimals.

Leg Problems The following conditions were assessed on a dichotomous scale (Yes/No): femoral head

Table 1. Overview of treatments, flock size, and replicates included in the experimental setup.

Treatment description	Treatment group letter code	No. of replicates	Flock size/birds per replicate
Distance between food and water, 7 m	A	6	497
Distance between food and water, 3.5 m	В	6	497
Feed supplement in the form of maize roughage	С	7	497
Opaque vertical panels	D	6	497
Straw bales	E	7	482
30 cm high platform with access ramps	F	6	437
5 cm high platform without access ramps	G	7	437
Lowered stocking density, 34 kg/m^2	Н	6	422
Control	Ι	7	497



Figure 1. Schematic design of each treatment pen. Grey circles represent round feeders. Grey lines represent the water line with drinking nipples. Circles labeled "R" in treatment C represent circular pans used for the allocation of maize roughage. Lines labeled "P" in treatment D represent solid vertical panels. Rectangles labeled "S" in treatment E represent straw bales (Figure reproduced from Tahamtani et al., 2018b).

detachment, femoral head necrosis, arthritis, tenosynovitis, fractures, tibial dyschondroplasia, and twisted tibia. A bird was recorded as having the specific condition if the left, the right, or both legs were affected. The upper leg was dislocated from the acetabulum, and it was noted if the articular cartilage of the femoral head detached from the growth plate (Y/N). Femoral head necrosis was recorded as present if 1 of 2 criteria was present: (1) Lesion in the growth plate following separation of the articular cartilage (Durairaj et al., 2009) or (2) focal yellow areas of caseous exudate or lytic areas following incision on the femoral head (McNamee and Smyth, 2000). The tibiotarsus was cut free at the knee and hock joint, and the joints and the bone were assessed for abnormal findings, that is arthritis and tenosynovitis seen as swollen joints and tendon sheaths with increased exudate (Bradshaw et al., 2002) and fractures. Tibial dyschondroplasia was defined as the presence of cartilage plugs in the proximal tibiotarsus (Tablante et al., 2003). Finally, twisted tibiotarsus was defined as an inward or outward rotation of the tibiotarsus along the long axis of the bone (Bradshaw et al., 2002).



Figure 1. Continued

Tibiotarsus Weight, Length, Diameter, and **Strength** All muscle and cartilage were removed from the tibiotarsus, and subsequently the bone was weighed in grams to 2 decimals using a digital laboratory scale. Length and diameter (proximal end, middle, distal end) were measured in millimeters to 2 decimals using a digital caliper. The length was measured in a straight line from the proximal to distal end, and the diameter was measured from the posterior to the anterior surface of the bone. These bones were then frozen at -20° C and stored until all bones had been collected. Bone strength was measured after the bones were defrosted using a FTC TMS-Touch Texture Analyzer fitted with a 1,000 N intelligent load cell (Food Technology Corporation). Measurements were performed at 48 mm/min. Bone strength was assessed halfway along the length of the bone as the peak load at fracture, that is the total load, in N, required for a fracture.

Statistical Analysis

Analyses of the postmortem data were performed using software SAS and RStudio. The effect of treatment on wooden breast abnormalities was tested using the GLIMMIX procedure with pen nested in block as a random factor and treatment as a fixed factor. Body condition scores were combined to generate binominal outcomes (e.g., 0 vs. >0). The body condition score was analyzed using a generalized linear mixed-effect model to test for the effect of treatment on the outcome. Pen and block were included in the model as random factors, and pen was nested in block. With this model, a pairwise comparison of all treatments was performed. Hereafter, a Holm correction was performed to correct for multiple comparisons. The effect of treatment on the prevalence of arthritis, tenosynovitis, or general inflammation of the hocks (either arthritis or tenosynovitis) were tested using a binary GLIMMIX procedure, with random factor pen nested in block and treatment as a fixed factor. The continuous outcomes (muscle width and tibiotarsus weight, length and diameter) for the left and right foot were averaged per bird and analyzed in the same manner as the binominal outcome, except for use of a linear mixed-effect model instead of the generalized linear mixed-effect model. The effect of treatment on bone strength, femoral head necrosis, tibial dyschondroplasia, and twisted tibiotarsus was analyzed using ANOVA with pen nested in block as a random effect and treatment as a fixed effect.

Ethical Statement

All animal procedures were carried out in accordance with the Danish Ministry of Justice Law no. 20 (January 11, 2018) concerning protection of animals and Law no. 474 (May 15, 2014) concerning animal experimentation and Law no. 54 (January 1, 2017) and Act no. 1047 (August 13, 2018) concerning broiler production.

RESULTS

Wooden Breast

Results from the assessment of wooden breast are presented in Table 2. There was no effect of treatment on wooden breast scores (F_{8, 1131} = 0.38; P = 0.93). In over 50% of the birds (54.5–72.4%), no wooden breast

Table 2. Distribution of broilers (%) within the different scores ofwooden breast (WB) per treatment.

Treatment	WB 0 (normal)	WB 1 (moderate)	WB 2 (severe)
A	59.7	38.7	1.6
В	68.5	27.4	4.0
С	68.1	27.0	5.0
D	68.1	28.3	3.5
E	65.3	28.6	6.1
F	72.4	26.0	1.6
G	54.5	44.4	1.0
Н	56.1	40.7	3.3
Ι	64.5	33.9	1.6

Treatments: A—7 m distance between feed and water; B—3.5 m distance between feed and water; C—maize roughage; D—vertical panels; E—straw bales; F—30 cm elevated platform; G—5 cm elevated platform; H—low stocking density; I—control.

abnormalities (WB 0) were observed. Only a small percentage (1.0-6.1%) of broilers had severe wooden breast (WB 2); the highest percentage (6.1%) was observed in treatment E (straw bales).

Body Condition Scores

The distribution of body condition scores 0, 1, and 2 (below normal, normal, above normal) in the different treatments is presented in Figure 2. Treatment A and B (7 m and 3.5 m between food and water, respectively) had the highest percentage of birds with a body condition score 0 (below normal). However, when the correction for multiple comparisons was included, no significant effects of treatment on body condition scores were found ($\chi^2 = 9.61$; df = 8; P = 0.3).

Muscle Width of the Lower Leg

There was an effect of treatment on the width of the leg muscles ($\chi^2 = 20.8$; df = 8; P = 0.008). The mean widths of the muscles of the lower leg for each treatment are presented in Table 3. In treatment D (vertical panels), the leg muscles were larger than in treatments B (3.5 m between feed and water), C (maize silage), and E (straw bales) and had a tendency to be larger than in treatment F (30 cm platform).

Leg Problems

Inflammation of the hocks, either by arthritis or tenosynovitis, was found in 11.9% of the broilers (n = 1,429). Figures 3 and 4 show the percentage distribution per treatment of broilers with arthritis and tenosynovitis, respectively. There was no effect of treatment on frequency of arthritis ($F_{8,1371} = 0.8$; P = 0.61), tenosynovitis ($F_{8,1371} = 0.2$, P = 0.99), or both of these combined ($F_{8,1371} = 0.34$, P = 0.95). The majority of birds (96.57%) had a detachment of the articular cartilage from the growth plate of the femoral head. This high percentage was considered to be an artefact from the freezing process and was not further analyzed. The percentage of broilers with femoral head necrosis, fractures, tibial dyschondroplasia, and twisted tibia in



Figure 2. Percentage distribution of body condition scores across the treatments. Treatments: A—7 m distance between feed and water; B—3.5 m distance between feed and water; C—maize roughage; D—vertical panels; E—straw bales; F—30 cm elevated platform; G—5 cm elevated platform; H—low stocking density; I—control.

each treatment group is listed in Table 4. There was no effect of treatment on the prevalence of femoral head necrosis ($\chi^2 = 6.12$; df = 8; P = 0.64), fractures ($\chi^2 = 6.16$; df = 8; P = 0.63), tibial dyschondroplasia ($\chi^2 = 4.53$; df = 8; P = 0.81), or twisted tibia ($\chi^2 = 4.11$; df = 8; P = 0.85).

Tibiotarsus Weight, Length, Diameter, and Strength

Overall, the mean weight of tibiotarsus following slaughter on day 35 was 12.16 ± 2.02 g (n = 1,429 bone pairs). Further, the mean dimensions of the tibiotarsus were 93.61 ± 3.24 mm in length, 18.67 ± 1.36 mm in diameter at the proximal end, 7.36 ± 0.76 mm in diameter at the middle, and 13.18 ± 1.06 mm in diameter at the distal end of the bone.

When grouping the measures by treatment, there was some variation in the mean values across the treatments (Table 5), and the smallest and lightest bones were found in treatment E (straw bales). An effect of treatment was found only on the distal diameter of tibiotarsus ($\chi^2 = 16.27$; df = 8; P = 0.04). Broilers housed with

Table 3. Mean, standard deviation, and P-values of pairwise comparisons of muscle width of the lower leg across treatments.¹

Treatment	Width (mm)	Adjusted <i>P</i> -values ²
A B C D E F G H I	$\begin{array}{c} 41.94 \ (3.17) \\ 41.36 \ (2.91) \\ 41.45 \ (2.96) \\ 42.74 \ (2.87) \\ 41.40 \ (2.84) \\ 41.40 \ (2.99) \\ 41.46 \ (3.19) \\ 41.51 \ (3.27) \\ 41.95 \ (2.95) \end{array}$	B-D, $P = 0.049$ C-D, $P = 0.045$ D-E, $P = 0.044$ F-D, $P = 0.056$

¹Treatments: A—7 m distance between feed and water; B—3.5 m distance between feed and water; C—maize roughage; D—vertical panels; E—straw bales; F—30 cm elevated platform; G—5 cm elevated platform; H—low stocking density; I—control.

H—low stocking density; I—control. $^2\mathrm{Adjusted}$ *P*-values from pairwise comparisons of effect treatment on muscle width. Only comparisons with *P*-values below 0.07 are shown. Significant *P*-values (P < 0.05) are shown in **bold**.



Figure 3. Percentage of arthritis across treatments. Bars indicate upper and lower 95% confidence intervals. Treatments: A—7 m distance between feed and water; B—3.5 m distance between feed and water; C—maize roughage; D—vertical panels; E—straw bales; F—30 cm elevated platform; G—5 cm elevated platform; H—low stocking density; I—control.

straw bales (E) had a shorter diameter of tibiotarsus (mean \pm SD: 12.96 \pm 1.02 mm) compared with broilers housed with 7 m distance between feed and water (A; mean \pm 13.35 \pm 1.16 mm). There was no effect of treatment on the weight ($\chi^2 = 11.30$; df = 8; P = 0.19), length ($\chi^2 = 13.17$; df = 8; P = 0.11), proximal diameter ($\chi^2 = 9.45$; df = 8; P = 0.31), or middle diameter ($\chi^2 = 11.31$; df = 8; P = 0.18) of the tibiotarsus. The bone strength model estimates (mean and standard error) are displayed in Table 6. There was no effect of treatment on bone strength ($\chi^2 = 6.36$; df = 8; P = 0.61).

DISCUSSION

The current study evaluated the effect of 8 different types of environmental enrichment for broilers. The muscle width of the lower leg (the drumstick) was affected by treatment when comparing the vertical panel treatment (D) to the 3.5 m between food and water (B), roughage (C), and straw bale (E) treatments. In all cases, the birds with vertical panels had wider muscles than the other treatments. An increase in muscle width in the vertical panel treatment is likely caused by an increase in activity; the birds will cross longer distances in the more complex environment when they have to



Figure 4. Percentage of tenosynovitis across treatments. Bars indicate upper and lower 95% confidence intervals. Treatments: A—7 m distance between feed and water; B—3.5 m distance between feed and water; C—maize roughage; D—vertical panels; E—straw bales; F— 30 cm elevated platform; G—5 cm elevated platform; H—low stocking density; I—control.

navigate around the panels. Indeed, we found that broilers with access to vertical panels performed more locomotive behavior that broilers exposed to straw bales at 6 D of age (Bach et al., 2019). Likewise, Castellini et al. (2002) found that organic broilers with outdoor access had higher drumstick percentage than indoor free range broilers, likely because of increased activity. On the other hand, Chen et al. (2013) found no effect on thigh meat yield when comparing groups of slowgrowing chickens raised either indoor or with outdoor access from day 35. Even though meat yield was unaffected, the thigh meat in the birds with outdoor access did have a decreased fat content and increased quality (Chen et al. 2013). As for the panels, the straw bales also create a more complex environment and are likely to increase activity as the birds will have to navigate around the bales. However, the straw bales also serve as elevated resting places and thus do not promote increased activity in this function. Another possible explanation for slimmer muscles in the straw bale treatment may be that the birds were less inclined to walk because of foot pad dermatitis as foot pad dermatitis scores were found to be numerically highest in the straw bale treatment, among all the treatments and significantly higher than in the treatments with 5 cm and 30 cm platforms (Tahamtani et al., 2019). The roughage provides further opportunity for foraging behavior, but the behavior is restricted to the plates containing the roughage and, therefore, does not promote increased movement around the pen. Furthermore, birds with access to roughage may gain less weight and as a consequence less muscles, because of decreased intake of the ad libitum concentrate feed. Indeed, we did measure the weight of the broilers weekly (100 birds per pen) and found that birds in the roughage treatment had lowered feed consumption, lowered daily weight gain, and lowered terminal weight when compared with birds from the 30 cm platform treatment (unpublished data). Apart from these significant differences between the roughage treatment and the 30 cm platform treatment, no other effects of treatment on body weight were observed (Tahamtani et al., 2019). Regarding the treatment with 3.5 m between food and water, this setup forced the birds to move longer distances in their efforts to reach the resources. However, this treatment also had a higher percentage of below normal body condition scores. It is possible that even if the birds moved more, they did not have the sufficient energy and nutrient intake to convert this movement into build-up of muscles.

Five measures of size of the tibiotarsus were included in this study: weight, length and diameter at the proximal, middle, and distal part of the bone. Numerically, the birds in the straw bale treatment had the lightest and smallest bones. A significant difference was found between the distal diameter in the straw bale treatment (E) and the 7 m between food and water treatment (A). The wider bones observed in treatment A might be a result of increased exercise from walking to and from the food and water. However, other studies looking at

 Table 4. Percentage of birds per treatment with fractures, femoral head necrosis, tibial dyschondroplasia, and twisted tibia.

Treatment	Fractures	Femoral head necrosis	Tibial dyschondroplasia	Twisted tibia
A	0.67	3.36	1.34	1.34
В	6.12	4.08	2.72	2.72
С	6.90	3.45	1.72	0.00
D	3.45	2.07	2.07	0.69
Е	6.32	6.32	2.30	2.87
F	5.48	0.68	1.37	2.74
G	5.41	2.03	2.03	1.35
Η	6.00	3.33	4.00	0.67
Ι	5.61	3.57	3.57	1.53

Treatments: A—7 m distance between feed and water; B—3.5 m distance between feed and water; C—maize roughage; D—vertical panels; E—straw bales; F—30 cm elevated platform; G—5 cm elevated platform; H—low stocking density; I—control.

the effects of environmental enrichment on bone properties have found conflicting results. Stadig et al. (2017) found that broilers with outdoor access who were expected be more active had a decreased tibiotarsus length compared with broilers housed in indoor systems, but no effect was observed on bone width. Buijs et al. (2012)and Ventura et al. (2010) both recorded the effect of stocking density on tibiotarsus length in Ross 308 broilers, and while Buijs et al. (2012) found that increased stocking density resulted in shorter bones, Ventura et al. (2010) found the opposite, that increased density resulted in longer bones. Furthermore, it is not clear how tibiotarsus dimensions relate to leg problems in broiler chicken. Brickett et al. (2007) measured length and width using radiographs and found no correlation to gait scores. However, Toscano et al. (2013) showed that several tibiotarsus properties correlated to gait scores. In the current study, treatment A (7 m between food and water) which had a significantly thicker distal diameter also had the numerically lowest prevalence of arthritis and tenosynovitis. In addition, the straw bale treatment (E) which numerically had the smallest and lightest bones also had the highest prevalence of femoral head necrosis and twisted tibia, and in another part of the current experiment, it was also found that treatment E had worse footpads health compared with both treatment F and G (Tahamtani et al., 2019). Overall, the current results indicate that larger bones (longer, wider and heavier) might be associated with increased activity and possibly with better leg health, but more comprehensive knowledge on the subject is still needed. Furthermore, an analysis of the results showed no effect of treatment on bone strength. Still, further investigations are needed to yield a complete picture on the effects that these types of environmental enrichment can have on bone strength.

There was no observed effect of the treatments on the scores for wooden breast. Wooden breast is a condition with a multifactorial etiology that is not yet fully understood, but oxidative stress and hypoxia in the muscles as a result of increased activity may be a contributing factor (Mutryn et al., 2015). In another part of the current study, we investigated the percentages of broilers performing different types of active behavior and the overall activity level, and we mainly saw an increase in activity when the distance between feed and water was increased (Bach et al., 2019). The lack of effect on wooden breast suggests that the level of increased activity induced by the enrichment, in particular the increased distance between food and water, was not a risk factor for the development of wooden breast. Regarding body condition scores, treatments A and B (7 m and 3.5 m between food and water) had the highest percentage of birds with a score below normal. Although no significant effect of treatment was found, the higher percentage of below normal birds may indicate either a decreased energy uptake, the birds did not reach the food and water sufficiently, or an increased energy expenditure from walking to and from the resources. We suggest that this dilemma could be investigated further by evaluating the body composition of the birds at slaughter.

Table 5. Mean and standard deviation of tibiotarsus weight, length, and diameter within each treatment group.

Treatments	Weight (g)	Length (mm)	Proximal diameter (mm)	Middle diameter (mm)	Distal diameter (mm)
A	12.38 (2.01)	93.76 (3.36)	18.86 (1.16)	7.44(0.84)	13.35 (1.16)
В	11.92(1.87)	93.12(2.96)	18.65(1.12)	7.26 (0.72)	13.11(1.12)
С	12.13(1.97)	93.77(3.13)	18.67 (1.08)	7.39(0.75)	13.13 (1.08)
D	12.42(1.87)	93.82(2.98)	18.82(0.97)	7.39(0.73)	13.29 (0.97)
Ε	11.94(1.97)	92.98(3.19)	18.50 (1.02)	7.33 (0.77)	12.96 (1.02)
F	12.15(2.03)	93.42(3.21)	18.65(1.00)	7.38 (0.74)	13.22 (1.00)
G	12.29(2.03)	94.16(3.05)	18.71 (1.04)	7.35(0.73)	13.21 (1.04)
Н	12.26(2.25)	93.90(3.43)	18.61(1.06)	7.49 (0.86)	13.20 (1.06)
Ι	12.04(2.08)	93.60(3.59)	18.59(1.07)	7.28 (0.70)	13.16(1.07)

Treatments: A—7 m distance between feed and water; B—3.5 m distance between feed and water; C—maize roughage; D—vertical panels; E—straw bales; F—30 cm elevated platform; G—5 cm elevated platform; H—low stocking density; I—control. Numbers written in bold within a column indicate significantly different values ($P \le 0.05$).

Table 6. Peak load (mean and SE) at fracture of tibiotars us across treatments.

Treatment	Mean load (N)	Standard error
A	400.67	7.68
В	383.88	7.67
С	397.87	7.04
D	398.81	7.68
E	397.56	7.12
F	390.03	7.90
G	394.07	8.17
Н	404.28	7.59
Ι	387.46	6.53

Treatments: A—7 m distance between feed and water; B—3.5 m distance between feed and water; C—maize roughage; D—vertical panels; E—straw bales; F—30 cm elevated platform; G—5 cm elevated platform; H—low stocking density; I—control.

There was no effect of treatment on any of the leg health issues included in the experiment. Overall, the prevalence of all the different leg problems was low which was expected since the birds were young, 35 D at the time of slaughter, and not exposed to any disease-inducing treatments. It is possible that the lack of observed effects is because of the overall low prevalence of leg health issues, rendering limited room for improvement. Although few effects on health were found in the current experiment, environmental enrichment can have other positive effects relating to the natural behavior of the bird. The provision of straw bales or roughage can serve as a substrate for foraging behavior, and perches or platforms provide areas for elevated resting which is an antipredator behavior (Newberry et al., 2001). Finally, for the leg health issues with a possible bacterial etiology that is femoral head necrosis, arthritis, and tenosynovitis, the provision of straw bales was expected to be a risk factor as opposed to having a positive effect (Bradshaw et al., 2002; Thoefner et al., 2019). Likewise, for wooden breast, environmental enrichment that increases activity could be a risk factor (Mutryn et al., 2015). Thus, the finding that neither of these conditions were affected in the current experiment is positive. However, "no evidence of effect" as we see in the current experiment is not equivalent to "evidence of no effect", and a different experimental setup is needed to conclude that enrichment is not a risk factor for wooden breast or bacterial infections (Ranganathan et al., 2015).

In conclusion, the results from the study showed that provision of vertical panels or the increase of distance between resources can result in larger muscles and bone dimensions, possibly having a positive effect on leg health. Furthermore, we found that the provision of enrichment does not appear to be a risk factor for the development of wooden breast or bacterial infection. Finally, leg measures and health are merely some of many factors that environmental enrichment can affect. Therefore, a weighing of positive and negative effects on leg health as well as other parameters is necessary when deciding which types of treatments to apply or further investigate.

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