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Mathieu Rutten, Christine Leterrier, Paul Constantin, K. Reiter, Werner Bessei

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Original article

Bone development and activity in chickens in response to reduced weight-load on legs

Mark RUTTEN[a], Christine LETERRIER[b]*, Paul CONSTANTIN[b], Klaus REITER[a], Werner BESSEI[a]

[a]University of Stuttgart Hohenheim, Garbenstr. 17, 70593 Stuttgart, Germany
[b]Station de Recherches Avicoles, INRA, Centre de Tours, 37380 Nouzilly, France

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Abstract — In order to find out whether a reduction in load-bearing on chicken legs would modify the activity and leg bone tissue, an original suspension device was developed in order to alleviate half of the weight-load on the legs. Thirty-six 5 d-old male meat-type chicks were assigned to 12 groups, each of 3 birds: a control bird (C), a bird equipped with a harness (H) and one equipped with a harness and suspended from a balance (S). The counterweights of the balance reduced the load on the legs by 50%. Behaviour in the birds was recorded twice for a 22-hour period. The birds were slaughtered at 19 d of age. Their legs were then weighed and the humeri, tibiotarsi and femurs were used for computation of length, biomechanical stiffness and composition. Tibiotarsi were also used for histomorphometry. Body weight at 19 d of age was significantly higher in the C birds (642 g vs. 586 g in the H and S groups, $P < 0.05$). The distance travelled as measured in the second week of the experiment was greater in S birds (338 m per 22 h vs. 246 in the C group and 252 in the H group, $P < 0.05$). The length of the tibiotarsi and femora was greater in the S birds when corrected for body weight ($P < 0.05$). Bone stiffness, composition and histological parameters were not significantly different in the 3 groups. We thus concluded that a reduction in load-bearing on the legs of young chicks enhances locomotor activity and longitudinal growth of leg bones. Bone quality was not affected, probably due to the contradictory effects of increased exercise and reduced weight.

chicken / locomotion / exercise / bone / growth

Résumé — **Développement osseux et activité chez le poulet de chair lors d'une réduction du poids appliqué sur les pattes.** Chez le poulet de chair, on suppose que le poids corporel élevé des animaux est à l'origine d'une faible activité physique, et secondairement d'une médiocre qualité du tissu osseux. Les modifications induites sur l'activité et le tissu osseux lors d'une réduction des forces appliquées au membre inférieur sont étudiées au moyen d'un dispositif permettant une réduction de 50 % du poids appliqué sur les pattes. Trente-six poussins sont répartis dans 12 groupes de 3 animaux : un animal

* Correspondence and reprints
Tel.: 33 (0)247 427 945; fax: 33 (0)247 427 778; e-mail: Christine.Leterrier@tours.inra.fr

témoin (C), un animal harnaché (H) et un animal équipé d'un harnais et d'un système de suspension (S). Le comportement est enregistré deux fois pendant une période de 22 heures. Les animaux sont abattus à 19 jours et une analyse anatomique, biomécanique et histologique est menée sur les os des ailes et des pattes. Le poids du corps est significativement plus élevé chez les poussins C. La distance parcourue est significativement augmentée dans le lot S, ainsi que la longueur du fémur et du tibiotarse corrigée pour le poids vif. Les paramètres biomécaniques et histologiques ne sont pas différents dans les 3 lots. En conclusion, la réduction artificielle du poids appliqué aux membres inférieurs augmente l'activité locomotrice et la croissance en longueur des os des pattes. La qualité osseuse n'est pas modifiée, probablement à cause des effets adverses de l'augmentation d'exercice et de la réduction des forces induites par le poids.

poulet de chair / locomotion / exercice / os / croissance

1. INTRODUCTION

Leg weakness is one of the major welfare problems in rapid growing meat-type chickens [2, 7]. The causal interrelationships between the incidence of leg weakness and locomotor activity are not fully understood. It has been found that increasing activity in meat-type chickens either on a treadmill or by increasing the distance between feeder and drinker improved the walking ability and bone development [21–23, 26, 29]. However, concurrently with an increase in growth rate, the amount of time spent sitting increases and the locomotor activity of meat-type chickens decreases [3]. This reduction in activity seems to be related to an increase in body weight. Therefore, it was of interest to discover whether a reduction in load-bearing on the legs would modify the activity and leg bone tissue. For this purpose, an original suspension device was developed which alleviated a certain amount of the weight load on the legs. The first results using this device are reported here.

2. MATERIALS AND METHODS

A total of 36 meat-type male chickens (IJV 915, ISA Hubbard) was used. The birds were housed under commercial conditions from one to five days of age. On the 5th day, 36 chicks were assigned to 12 groups, each of three birds. Care was taken to minimise the difference in body weight within the groups of three. The groups were transferred to special compartments of 2×0.50 m (length $\times$ width).

In order to partially remove the load of the body weight a harness of elastic cohesive retention bandage (Easifix 8 cm, 10 cm, Smith+Nephew, Laboratoires Fisch Vibraye, France) was placed around the body of the chicks. The harnesses weighed between 8 and 11 g depending on the age of the chicks. The legs and wings were free so that they could move with minor disturbance. A thin braided plastic wire was attached to the harness and connected to a balance. The balance was fixed on the wheels connected to special rails of 2 m length that were fitted above the compartments (Fig. 1). Using variable counterweights on the balance, the load on the legs of the birds was reduced by 50 percent of the body weight. This device was original and had never been used in previous experiments. Individual body weight was recorded daily from 5 to 18 days of age and the harnesses and counterweights were adjusted daily. The suspended birds could walk throughout the whole compartment. The second bird in the group was equipped with a harness as described above, but was not suspended. The third bird was handled as the other birds but did not have a harness.

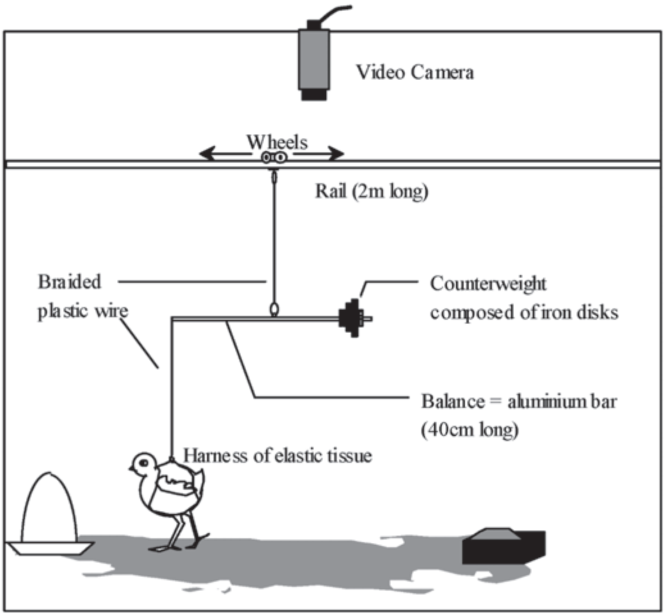

Figure 1. Suspension device developed to alleviate half of the weight loaded on the legs.

The following abbreviations will be used for the different treatments: C for no harness and no suspension, H for harness with no suspension, S for harness and suspension. Each group of three birds consisted of one C bird, one H bird and one S bird that were then reared in a compartment of 2 × 0.50 m.

An artificial lighting schedule was followed (24L:0D, 0 to 1 d; 23L:1D, 2 to 19 d). Room temperature was adjusted according to the recommendation for commercial meat-type chickens and a standard pellet diet was given ad libitum (EM = 3100 kcal; CP = 22%; Ca = 1.15%; available P = 0.42%). One feeder and one bell drinker were placed at the opposite short sides of each compartment so that the chicks had to walk about 2 m when going from the feed to the water.

The behaviour of the birds of each group was continuously video-taped for a 22 h period during the first and the second week of the experiment. Behavioural observations were made for a 20-minute period at two-hour intervals. In order to estimate walking distance, a grid of rectangular spaces of 0.25 (h) × 0.20 (w) m was marked on the side walls of the compartments. Crossings of the grid lines were counted and 0.20 m were calculated for each crossing in the straight direction along the compartment. The mean distance of the diagonal line was taken for each crossing from the left to the right side of the compartment.

The birds were slaughtered at 19 days of age and the following traits were recorded or computed: live weight, weights of the left and right legs, percentage of the leg weight as a percentage of the total body weight, length and weight of the left and the right femur and tibia and of the right humerus. The Quetelet Index was calculated for each bone by its weight (in mg) divided by the square of its length (in mm^2).

Humeri, femurs and tibiotarsi were frozen until processing. A three-point flexure test was carried out on the right bones when thawed (Instron Number 1102, High Wycombe, UK). The rate of travel of the mobile anvil was 5mm/min and the width of the bearer was 25, 25 and 40 mm, respectively, for the humeri, femurs and tibiotarsi. Stiffness was calculated as the slope of the loading curve before the bioyield point [9], i.e. the inflection point of the loading curve.

Proximal ends and 10-mm-thick mid-diaphyseal cylinders were cut from the proximal half of the tibiotarsi for histomorphometry. Proximal coronal sections and diaphyseal cylinders were dehydrated in a graded series of ethanol solutions and embedded in methyl metacrylate. The blocks were then cut with a low speed diamond wheel saw to produce 120 µm sections that were then polished to 90 µm with a DAP7 polishing machine (Struers, Copenhagen, Denmark). Morphometric analysis of the growth plate was performed with a graduated reticule at low magnification in order to measure the width of the proliferating zone and of the hypertrophic zone [10]. The pre-hypertrophic zone was not distinguished from the hypertrophic zone because of the gradual process in chondrocyte differentiation. Bone volume [18] was quantified in the metaphyseal area using the Visilog 4.1 software package (Noesis, 78140 Vélizy, France). Diaphyseal cross-sections were also analysed with the Visilog software in order to quantify cortical and marrow areas and diameters. The cortex was divided into a peripheral active zone, a mature zone and a resorption zone [14, 17].

The humeri, the proximal and distal epiphyses and the diaphysis of femurs, the distal diaphysis and the distal epiphysis of the tibiotarsi were then defatted in ether for 24 h, dried (110 °C for 12 h) and weighed. The bones were ashed (550 °C for 14 h) and the ash weight was calculated relative to the tibial dry weight in order to obtain the ash percentage.

The effect of the treatments within the groups was subject to a Friedman procedure for related samples. In the case of significant treatment effects, the differences between the individual means were tested for significance by the Wilcoxon matched pair test [28]. A 5% level of significance was chosen.

3. RESULTS

The body weight of the experimental groups did not differ significantly at the beginning of the experiment (five days of age). At 19 days of age, however, it was significantly higher in the C-group as compared to the S and H groups (Tab. I). The distance travelled did not differ among groups in the first week of the experiment (day 6 to 12). In the second week (day 13 to 19) this trait was significantly higher in the S group than in both the C and the H group (Tab. I). Significant differences between all groups ($P < 0.009$) were found for the mean weight of the legs, the C group showing the highest (76.0 ± 7.5 g) and the S group the lowest weight (67.8 ±5.7 vs. 71.7 ±5.8 g in the H group, $P < 0.05$). However, the weight of the legs in percent of body weight was higher in the H group (24.5 ± 0.6% BW) than in the C and S groups (23.7 ±1.0 and 23.2 ± 0.9% BW, respectively). There was no significant effect of the treatments on the weight of the humerus and tibiotarsi (Tab. II). The weight of both femurs in the S group was significantly lower than in the other treatments. There was no significant effect between treatments for the gross length of the leg and wing bones with the exception of the left femur that was shorter in the H group than in the others (Tab. III). Table IV shows that the S group had a relatively light but long tibiotarsi and femurs

Table I. Mean values for body weight development at 19 days of age and distance travelled in response to different treatments.

Parameters	Experimental group[1]			*P*-value[2]
	C	H	S	
Body weight (g)				
at 5 days	113.8	114.4	113.9	0.910
at 19 days	642.3[a]	586.1[b]	585.6[b]	0.043
Distance travelled (m per 22 h)				
at 6–12 days	488.8	353.3	464.4	0.110
at 13–19 days	246.4 [b]	252.6[b]	338.3[a]	0.010

[1]C: no harness, not suspended; H: harness, not suspended; S: with harness, suspended.
[2]Results of the Friedman test for related samples; mean values labelled by the same letter do not differ statistically ($P < 0.05$; Wilcoxon matched pair test).

Table II. Mean values for bone weight at 19 days of age in response to different treatments.

Parameters	Experimental group[1]			*P*-value[2]
	C	H	S	
Right humerus (g)	2.8	2.7	2.7	0.460
Right femur (g)	3.8[a]	3.7[a]	3.4[b]	0.017
Left femur (g)	3.8[a]	3.6[a]	3.4[b]	0.009
Right tibia (g)	5.3	5.0	4.8	0.080
Left tibia (g)	5.4	5.0	4.8	0.080

[1]C: no harness, not suspended; H: harness, not suspended; S: with harness, suspended.
[2]Results of the Friedman test for related samples; mean values labelled by the same letter do not differ statistically ($P < 0.05$; Wilcoxon matched pair test).

Table III. Mean values for the length of different bones at 19 days of age in response to different treatments.

Parameters	Experimental group[1]			*P*-value[2]
	C	H	S	
Right humerus (mm)	46.3	45.9	46.3	0.780
Right femur (mm)	48.0	47.0	48.5	0.120
Left femur (mm)	48.1[a]	47.0[b]	48.7[a]	0.039
Right tibia (mm)	67.1	65.8	67.7	0.080
Left tibia (mm)	67.2	65.9	67.5	0.170

[1] C: no harness, not suspended; H: harness, not suspended; S: with harness, suspended.
[2] Result of the Friedman test for related samples; mean values labelled by the same letter do not differ statistically ($P < 0.05$; Wilcoxon matched pair test).

Table IV. Mean values for the Quetelet Index of different bones at 19 days of age in response to different treatments.

Parameters	Experimental group[1]			P-value[2]
	C	H	S	
Right humerus ($mg \cdot mm^{-2}$)	1.29	1.28	1.25	0.780
Right femur ($mg \cdot mm^{-2}$)	1.66[a]	1.66[a]	1.43[b]	0.001
Left femur ($mg \cdot mm^{-2}$)	1.65[a]	1.65[a]	1.42[b]	0.001
Right tibia ($mg \cdot mm^{-2}$)	1.18[a]	1.15[a]	1.05[b]	0.017
Left tibia ($mg \cdot mm^{-2}$)	1.19[a]	1.15[a]	1.05[b]	0.009

[1]C: no harness, not suspended; H: harness, not suspended; S: with harness, suspended.
[2]Result of the Friedman test for related samples; mean values labelled by the same letter do not differ statistically ($P < 0.05$; Wilcoxon matched pair test).

Table V. Mean values for stiffness in the humerus, femur and tibiotarsus at 19 days of age in response to different treatments.

Parameters	Experimental group[1]			P-value[2]
	C	H	S	
Humerus ($N \cdot mm^{-1}$)	73.1	75.2	80.8	NS
Femur ($N \cdot mm^{-1}$)	80.0	74.5	78.9	NS
Tibia ($N \cdot mm^{-1}$)	55.0	51.3	52.2	NS

[1]C: no harness, not suspended; H: harness, not suspended; S: with harness, suspended.
[2]Results of the Friedman test for related samples; mean values labelled by the same letter do not differ statistically ($P < 0.05$; Wilcoxon matched pair test).

(low Quetelet Index) and the H and C groups had relatively heavy but short tibiotarsi and femurs (high Quetelet Index). This difference was significant.

The stiffness of the humeri, femurs and tibiotarsi was not significantly different between the 3 groups (Tab. V). The ash percentage was reduced in C birds only in the tibial diaphysis (Tab. VI). There was no significant group effect on the total width of the growth plate, nor on the thickness of the proliferating and hypertrophic zones (Tab. VII). Cross-section area, cortical and medullary areas and external diameters were not significantly different between the groups (Tab. VIII).

4. DISCUSSION

It was the intention of the experiment to produce birds of approximately the same weight in all treatments. In contrast to our expectation the harnessed birds, whether suspended or not, showed a significant delay in growth as compared to the C birds (Tab. I). This could be explained by the insulating effect of the harnesses. In order to minimise the pressure of the harness on the skin, an elastic tissue was used which covered a large part of the breast and the abdomen, and thus may have impaired the dissipation of metabolic heat. Since the ambient temperature was adjusted to normal

Table VI. Mean values for ash/dry matter percentage of different bones at 19 days of age in response to different treatments.

Parameters	Experimental group[1]			P-value[2]
	C	H	S	
Total humerus (%)	42.0	42.4	43.2	0.10
Proximal femur (%)	35.0	34.8	35.9	0.78
Diaphyseal femur (%)	58.0	58.8	57.9	0.56
Distal femur (%)	34.4	34.3	34.6	0.72
Diaphyseal tibia (%)	61.2[b]	63.1[a]	63.0[a]	0.05
Distal tibia (%)	36.5	37.1	35.8	0.34

[1]C : no harness, not suspended; H : harness, not suspended; S : with harness, suspended.
[2]Results of the Friedman test for related samples; mean values labelled by the same letter do not differ statistically ($P < 0.05$; Wilcoxon matched pair test).

Table VII. Mean values for the thickness of the tibial growth plate and for tibial metaphyseal bone volume at 19 days of age in response to different treatments.

Parameters	Experimental group[1]			P-value[2]
	C	H	S	
Total width (mm)	4.92	4.81	4.98	0.70
Hypertrophic zone[3] (mm)	3.51	3.57	3.69	0.31
Proliferating zone (mm)	1.41	1.27	1.23	0.10
Metaphyseal bone volume (%)	20.7	22.7	23.8	0.72

[1]C: no harness, not suspended; H: harness, not suspended; S: with harness suspended.
[2]Result of the Friedman test for related samples.
[3]The thickness of the pre-hypertrophic zone is included in the thickness of the hypertrophic zone.

conditions, the harnessed chicks may have been exposed to a mild heat stress. It is well documented that meat-type chickens under these conditions reduce their food intake and growth rate is lowered accordingly [11]. Wearing a harness was also maybe stressful for the chicks and this stress may have impaired growth [13].

The locomotor activity of all groups fell from the first to the second week of the experiment carried out during the second and third week of age. Similar results have been reported by various other studies [3, 24]. The reduction in activity of the S birds was less than in both other groups. This led to the significant difference between the S birds in comparison to the H- and C birds. The similar activity of the H and C birds shows that the suspension procedure obviously did not impair the movement of the chicks. Since the H birds had the same body weight as the S birds but an activity similar to the heavier C birds, the higher activity of the suspended chicks cannot be directly attributed to reduced body weight. It may be speculated that alleviation of 50 percent of the body weight may have reduced potential pain in the bones and joints [6], and thus encouraged more locomotion.

The relative weight of the legs, at 11 to 12% of the body weight, is in the normal range for meat-type chickens [8]. Experimentally

Table VIII. Mean values for characteristics of the cortex of tibial diaphysis in 19-day old meat-type chickens in response to different treatments.

Parameters	Experimental groups[1]			*P*-value[2]
	C	H	S	
Cross-section area (mm^2)	23.1	22.2	22.4	0.92
Cortical area (mm^2)	18.9	18.3	18.1	0.56
Medullary area (mm^2)	4.2	3.9	4.3	0.34
Latero-medial diameter (mm)	5.8	5.8	5.8	0.72
Antero-posterior diameter (mm)	5.1	4.9	5.0	0.76
Active zone[3] (%)	22.3	22.0	25.9	0.26
Mature zone[3] (%)	71.7	70.1	69.3	0.56
Endosteal zone[3] (%)	6.0	7.9	4.8	0.49

[1]C: no harness, not suspended; H: harness, not suspended; S: with harness, suspended.
[2]Results of the Friedman test for related samples.
[3]Thickness of each zone is given as a percentage of the total cortex thickness ($mm \cdot mm^{-1}$).

induced extra exercise via a ramp in between the feeder and drinker led to a significant increase of the relative leg weight [16]. In this experiment increased locomotor activity had no effect on leg development, probably due first to the fact that modifications in muscle growth appear only after several weeks of exercise [27], and secondly because muscle growth was not stimulated by load-bearing strength. The reduced strength applied to the legs in the S birds might explain why leg and femur weights were reduced in this group. In the C group, growth was faster which is known to promote breast muscle growth and so reduce leg percentage [1]: the higher relative leg weight of the H group, which showed low body weight and was submitted to the anabolic load-bearing effect, could then be explained. The significantly lower weight of the femurs in the S vs. C and H chicks can be explained by the reduced weight load in the S group. The higher longitudinal growth of the leg bones in S birds is in agreement with previous demonstrations [5, 19] that compression on the growth plate reduces longitudinal bone growth. In contrast to leg bones, the treatment had no effect on the longitudinal growth of the humerus, suggesting that there were no endocrine effects of local growth factors on growth plates in the upper limb.

The device had no significant effect on bone quality. Improvement of bone density seems to be related to exercise level [26] which could explain why no effect on bone was observed with the suspension device since differences in locomotor activity appeared in the S birds during the second week of the experiment only. Many studies have demonstrated that reducing load on the limb reduces bone mineral density whereas increased activity level has a well-known effect on bone density and structure [12]. However, this effect is still poorly documented in meat-type chickens [4, 26, 30] whereas turkeys have been extensively used over a long period of time to study the relationship between bone strain and bone apposition [25]. Moreover, bone response to strain is lower and different in meat-type chickens compared to egg-type crossbreeds [20]. Ash percentage in tibial diaphysis was reduced in C birds only, suggesting that the high growth rate in the C birds could be related to poor mineralisation of the diaphysis. However, whether growth is negatively related to bone mineralisation is much debated [15] and the result found on the tibiotarsi was not corroborated by data on femurs.

In conclusion, the suspension device was efficient in reducing load-bearing on legs without impairing the birds displacement. Reducing load-bearing on the legs of meat-type chickens with this device even enhanced locomotor activity and longitudinal growth of leg bones. Slight modifications of the harness in order to reduce heat accumulation should allow further investigation of the consequences of reducing load-bearing on the legs of meat-type chickens on their activity and bone quality.

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