Age determination of poultry: a challenge for customs

Leeftijdsbepaling bij kippen: een uitdaging voor de douane

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ABSTRACT

The age-related degree of ossification of the sternum and the long leg bones (femur, tibiotarsus and tarsometatarsus) of chickens was determined both macroscopically and radiographically in ten broilers ranging from 7 to 14 weeks in age, and in four laying hens of different ages and breeds. The caudal half of the sternal crest was still cartilaginous in 14-week-old broilers. This relatively slow ossification rate of the sternum interfered with accurate age determination. The length of the long bones of the shank varied with age but was also breed dependent. In contrast, the diaphyseal diameter of the long leg bones and the thickness of the articular cartilage covering the femoral head, femoral condyles and proximal tibiotarsal surface varied little in 7 to 14-week-old broilers. The thickness of the femoral and proximal tibiotarsal articular cartilage was more than 3 mm in broilers and less than 1 mm in the older laying hens. The closure of the growth plates in the tibiotarsus and the tarsometatarsus was the most reliable criterion for age determination in broilers.

SAMENVATTING

Om de verbening van het borstbeen en van de lange pootbeenderen (femur, tibiotarsus en tarsometatarsus) te evalueren, werden 10 mestkippen van verschillende leeftijd en 4 volwassen leghennen zowel macroscopisch als radiografisch onderzocht. Bij mestkippen van 14 weken oud was de caudale helft van het sternum nog steeds kraakbenig. De trage verbening van het borstbeen interfereert met een nauwkeurige leeftijdsbepaling. De lengte van de lange pootbeenderen is leeftijdsafhankelijk maar hierbij moet rekening gehouden worden met grote rasverschillen. De diameter van de schacht en de dikte van het gewrichtskraakbeen van de lange pootbeenderen variëren weinig bij mestkippen van 7 tot 14 weken oud en kunnen bijgevolg niet als betrouwbare leeftijdsindicators gebruikt worden. De dikte van het femorale en proximale tibiotarsale gewrichtskraakbeen bedroeg meer dan 3 mm bij mestkippen en minder dan 1 mm bij oudere leghennen. Het sluiten van de groeiplaten in de tibiotarsus en de tarsometatarsus vormt het meest betrouwbare criterium voor de ouderdomsbepaling van mestkippen.

INTRODUCTION

To safeguard participation in international trade, the European Community has made provisions for financial restitution when exporting poultry meat to third countries (Council Regulation (EC) No 2777/75 and 2779/75). Customs tariffs are based on the age of the exported animals as determined by the state of ossification of the sternum and the long bones of the legs (Council Regulation (EC) No 2091/2005). Restitution is disbursed when the sternal tip, femur and tibiotarsus are not completely ossified.

These regulations require a precise knowledge of the ossification process in birds, but literature data on this subject are scarce. Similar to the situation in fetal mammals, primary ossification centers arise prenatally in the middle part of the diaphyseal cartilage of the avian long bones (Fell, 1925). However, unlike in mammals, no secondary ossification centers are formed in the epiphyses of the appendicular skeleton of birds. Epiphyseal growth plates are therefore lacking in avian bones, except at the distal end of the tibiotarsus and the proximal end of the tarsometatarsal bone, both of which have a growth plate formed by the fusion lines between tarsal bones and the tibia or the metatarsal bones, respectively (Church and Johnson,

Table 1. Overview of the animals used in this study.

Breed	Sex	Age (weeks)	Weight (kg)
broiler	male	7	2.8
broiler	male	7	2.8
broiler	male	8	3.5
broiler	male	8	3.5
broiler	male	10	4.25
broiler	male	10	4.9
broiler	male	12	5.2
broiler	male	12	6
broiler	male	14	5.4
broiler	male	14	6.6
Rhode Island Red	female	22	1.4
Rhode Island Red	female	22	1.4
Orpinghton	female	>104	1.4
laying hen	female	>104	-

1964; Hogg, 1980; Naldo *et al.*, 1998). Additionally, a secondary ossification centre is situated in the craniodorsal part of the tibia. It corresponds to the tibial crest center of mammals (tuberositas tibiae), to which the patellar ligament is attached (Church and Johnson, 1964; Hogg, 1980; Naldo *et al.*, 1998).

The Belgian Customs authorities commissioned a preliminary study of skeletal ageing changes in poultry. This study was carried out in 10 broilers and 4 laying hens. The broilers were kept until 14 weeks old in order to determine the exact age of closure of growth plates in the leg bones. Once the growth plates are closed, the bones are completely ossified and no restitution will be paid to the exporter. Because the customs authorities are also confronted with the export of frozen poultry meat, this preliminary study was not restricted to the macroscopic examination of osseous changes in poultry meat, but radiographic data were also collected because this non-invasive imaging technique can provide a useful method for fast and reliable age determination of frozen samples.

MATERIALS AND METHODS

Ten male *Ross* broilers, which were kept on a commercial diet ad libitum and had unlimited access to water, were euthanized in pairs at the age of 7, 8, 10, 12 and 14 weeks, respectively. Two 22-week-old *Rhode Island Red* hens, a 26-month-old *Orpinghton* hen and a more than 2-year-old laying hen of unknown breed were also included in this study (Table 1). The broilers and the laying hens were all permitted to roam freely.

After death, the sternum and the two femoral and two tibiotarsal bones from each animal were carefully exposed by dissecting the surrounding soft

Figure 1. Sternum and surrounding bones of a 10-week-old broiler (left lateral view): 1. total length of the sternal crest, 2. length of the cartilaginous sternal crest, 3. ribs, 4. coracoid, 5. clavicula.

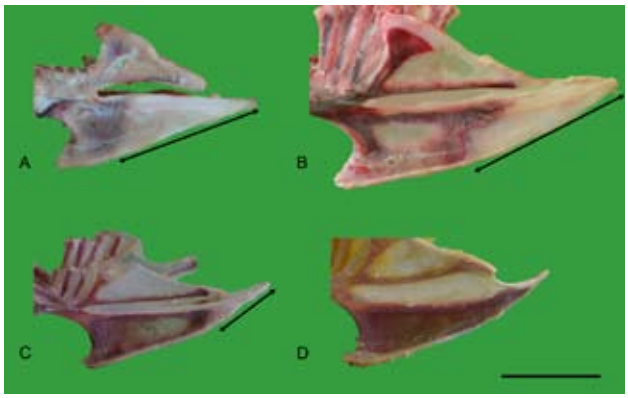

Figure 2. Left lateral view of the sternum (scale bar 5 cm): A. broiler 7 weeks, B. broiler 14 weeks, C. laying hen 22 weeks, D. laying hen 2 years. Notice the decrease of cartilage with advancing age

Table 2. Mean values (± SD) of the femoral, tibiotarsal and tarsometatarsal dimensions of broilers and laying hens at different ages (* broilers of 8 weeks old).

		Broiler of 7 weeks	Broiler of 14 weeks	Laying hens
Femur	**length (cm)**	**8.75 ± 0.19**	**11.6 ± 0.15**	**8.7 ± 0.34**
	width (cm)	**1.05 ± 0.06**	**1.2 ± 0.06**	**0.8 ± 0.08**
Tibiotarsus	**length (cm)**	**11.5 ± 0.84**	**16.05 ± 0.29**	**12.1 ± 0.35**
	width (cm)	**0.85 ± 0**	**1.19 ± 0.14**	**0.7 ± 0.16**
Tarsometatarsus	**length (cm)**	**8.85* ± 0.07**	**10.95 ± 0.21**	**8.1 ± 0.36**
	width (cm)	**1.15* ± 0.07**	**1.35 ± 0.04**	**0.8 ± 0.09**

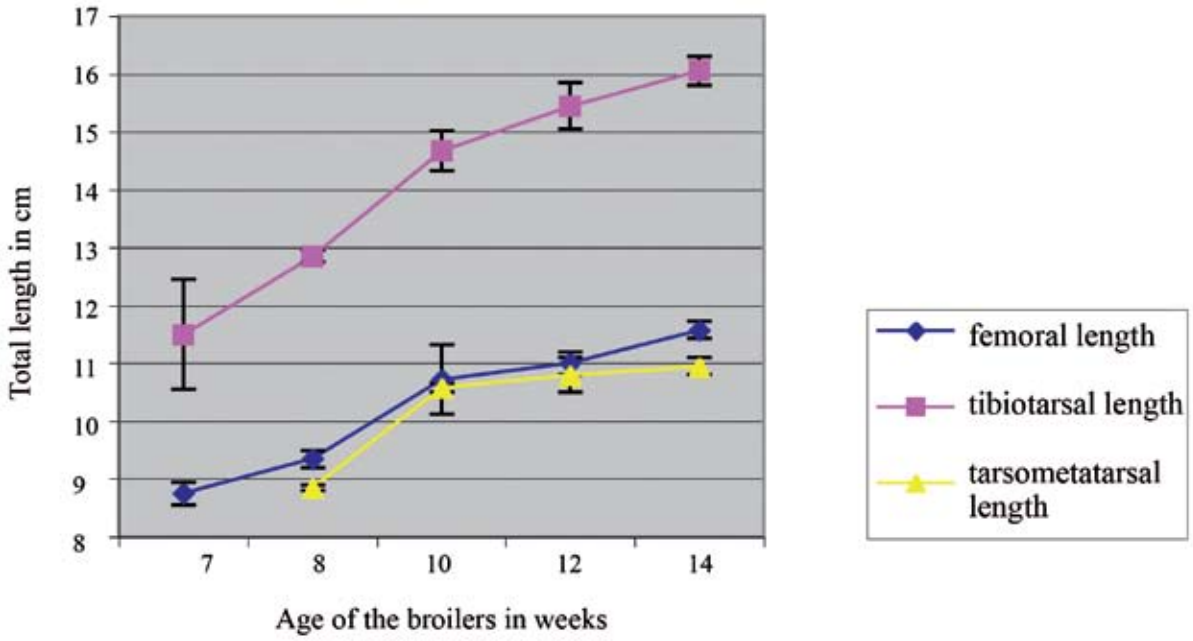

Figure 3. Line graph of the elongation of the femoral, tibiotarsal and tarsometatarsal bones of broilers.

tissues. The total length and the length of the cartilaginous part of the sternal crest were measured along the ventral border, both on the dissected specimens (Figure 1) and on the radiographs. The ossification of the sternum was determined by comparing the length of the cartilaginous part of the sternum to the total length of the carina. The total length and the width at the level of the midshaft of the two femoral bones and of the two tibiotarsal bones were measured macroscopically and radiographically. Morphometric analysis of the tarsometatarsus was performed on radiographs by measuring the length of the tarsometatarsus from the proximal epiphysis to the third articular trochlear surface, and the width was measured at the location of the first metatarsal bone. In order to measure the thickness of the articular cartilage and to visualize the growth plates, the femora and the tibiotarsi were sectioned with a band saw either sagittally or coronally. The thickness of the proximal and distal epiphyseal cartilage was measured by a ruler and the presence of growth plates was evaluated. Digital photographs of the femur and the tibiotarsus were taken both before and after sectioning.

Ventrodorsal and lateral radiographs of the sternum, craniocaudal and lateromedial radiographs of the femur and tibiotarsus, and dorsoplantar and lateromedial radiographs of the tarsometatarsus were taken using 42 kV and 49 kV for the sternal and long leg bones, respectively. All radiographs were made at 4 mA. The radiographic results were compared to the macroscopical measurements and evaluation of the growth plates.

RESULTS

The sternal crest of the 7-week-old broilers consisted of cartilage for more than 60% of its entire length. The relative length of the cartilaginous part diminished gradually with increasing age, but in the 14-week-old broilers 42-52% of the total carinal length was still cartilaginous. In the 22-week-old *Rhode Island Red* laying hens, 10.5-26% of the ster-

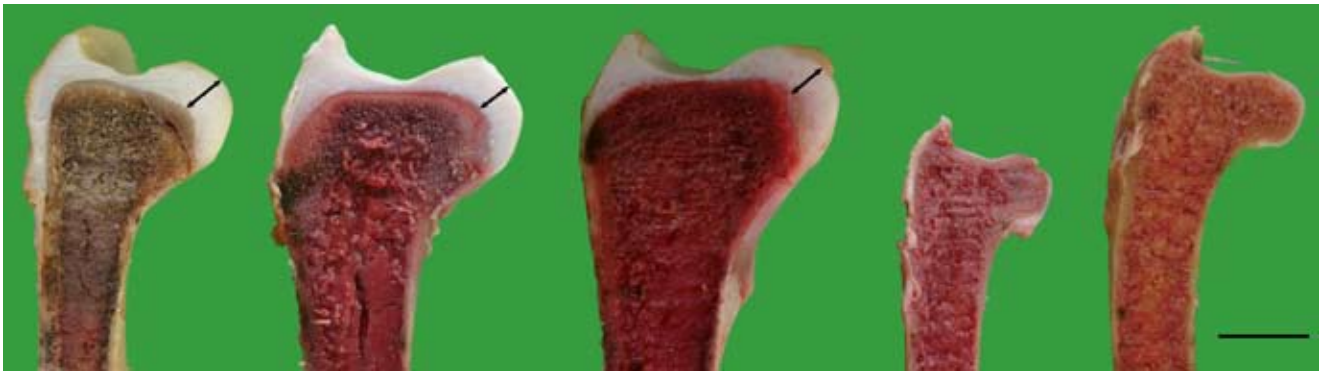

Figure 5. Longitudinal section through the femoral head (scale bar 1 cm). From left to right: broiler 8 weeks, broiler 10 weeks, broiler 14 weeks, laying hen 5 months and laying hen 2 years. Notice the progressive decrease of cartilage covering the femoral head (black arrows).

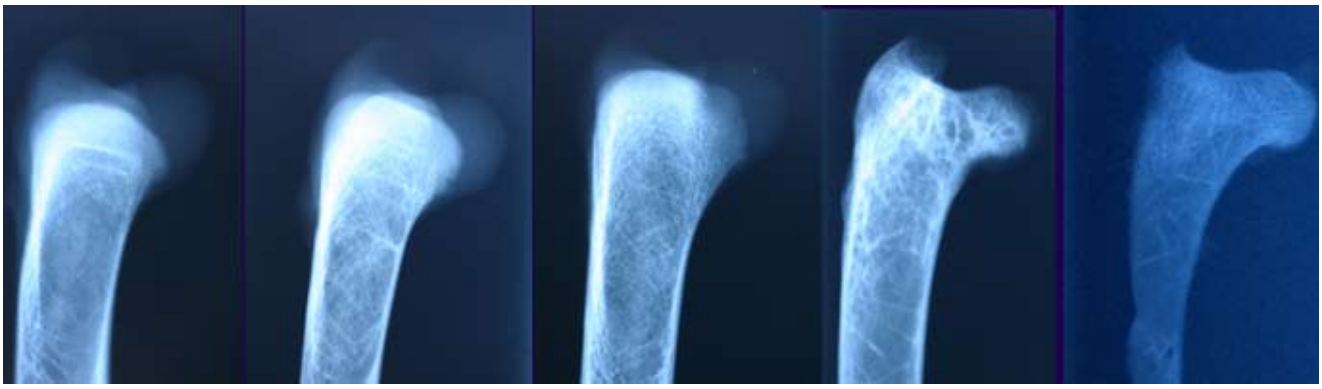

Figure 6. Radiographs of the specimens of Figure 5 (lateromedial view).

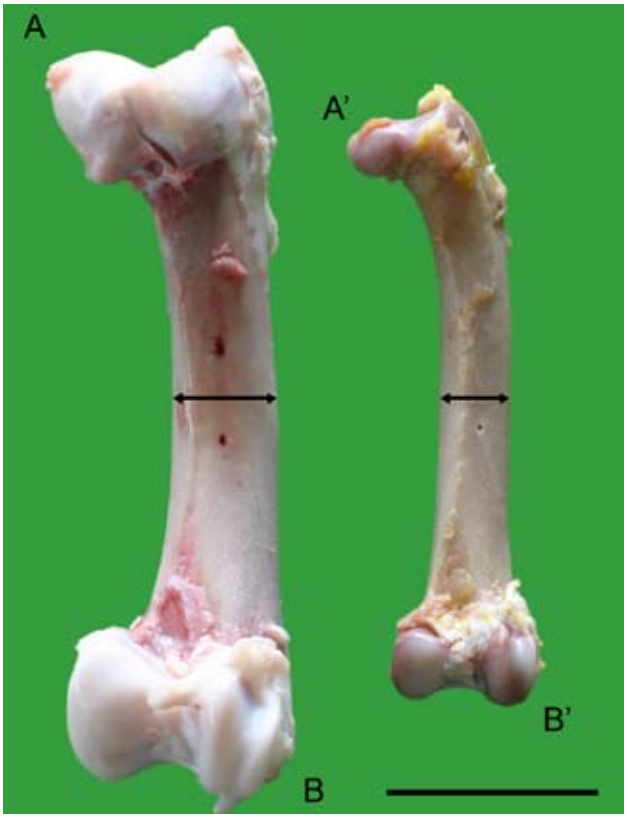

Figure 4. Difference in length and width (arrow) of the femur (scale bar 3 cm): Left: broiler of 10 weeks; Right: laying hen of 2 years. Notice the difference in the cartilage covering the femoral head (A, A') and the femoral condyles (B, B') in the broiler compared to that of the laying hen.

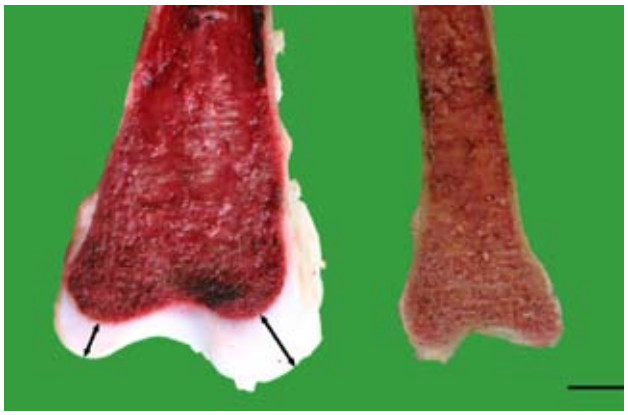

Figure 7. Longitudinal section through the femoral condyles (scale bar 1 cm). Left: broiler of 14 weeks with prominent thickness of the condylar cartilage (black arrows); Right: laying hen of 2 years with reduced condylar cartilage (less than 1 mm thick).

num consisted of cartilage, while the sternum of the 2-year-old *Orpinghton* hen and the older laying hen was completely ossified. Figure 2 shows the differences in sternal size and the progressive ossification of sternal cartilage at different ages.

The dimensions of the femoral, tibiotarsal and tarsometatarsal bones of broilers and laying hens at different ages are listed in Table 2; the elongation of the long bones of the broilers is illustrated in Figure 3.

The femoral length and width increased manifestly in broilers between 7 and 14 weeks of age (Figure 4). The cartilage covering the femoral head and the femoral condyles of the broilers was more than 3

Figure 8. Cranial view of the tibiotarsus (scale bar 5 cm). Notice the difference in length between a broiler of 8 weeks (left), a broiler of 14 weeks (middle) and a laying hen of 2 years (right).

mm thick in all age categories, but was 1 mm or less in laying hens older than 22 weeks (Figures 5 - 7). In the femora examined, no growth plates could be observed, either proximally or distally.

The morphologic changes of the tibiotarsus of broilers from 7 weeks to 14 weeks are illustrated in Figure 8. The thickness of the proximal tibiotarsal cartilage was approximately 4 mm in all age categories of broilers, while it was only 1 mm or less in laying hens older than 22 weeks. The distal tibiotarsal cartilage was clearly thinner than the proximal tibiotarsal cartilage, and it was less than 1 mm thick in 10-week-old broilers. The apophyseal growth plate located between the processus cnemialis and the tibial shaft was still visible in broilers at the age of 14 weeks (Figure 9a). In contrast, the distal growth plate formed by the fusion of the tibia and the tarsal bones disappeared in broilers at 12-14 weeks (Figure 10a). On the radiographs the proximal growth plate was clearly visible in all age categories of broilers (Figure 9b), while the distal growth plate became blurred in 12- to 14-week-old broilers (Figure 10b). In the laying hens, neither of the growth plates could any longer be observed, either macroscopically or radiographically.

The length and width of the tarsometatarsal bones are documented in Table 2 and Figure 3. The fusion line between the tarsal and the metatarsal bones became blurred in broilers at the age of 14 weeks, was hardly visible in the *Rhode Island Red* laying hens at the age of 22 weeks, and was completely absent in the older laying hens (Figure 11).

Figure 9a. Longitudinal section through the proximal growth plate (arrows) of the tibiotarsus. From left to right: broiler 8 weeks, broiler 10 weeks, broiler 14 weeks, laying hen 5 months, and laying hen 2 years. Notice the progressive closing of the growth plate with increasing age.

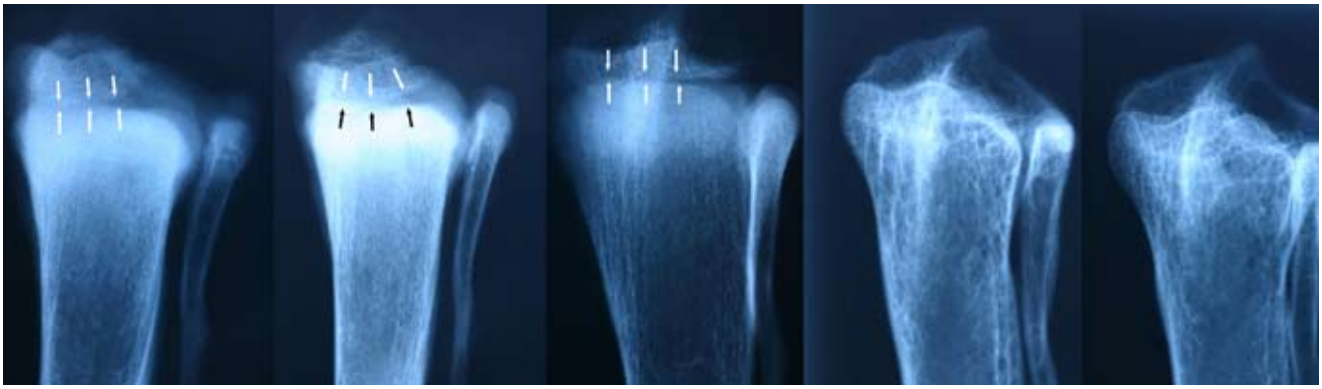

Figure 9b. Radiographs of the specimens of Figure 9a (dorsoventral view).

Figure 10a. Longitudinal section through the distal growth plate (arrows) of the tibiotarsus. From left to right: broiler 8 weeks, broiler 10 weeks, broiler 14 weeks, laying hen 5 months, and laying hen 2 years. Notice the closing of the growth plate with increasing age.

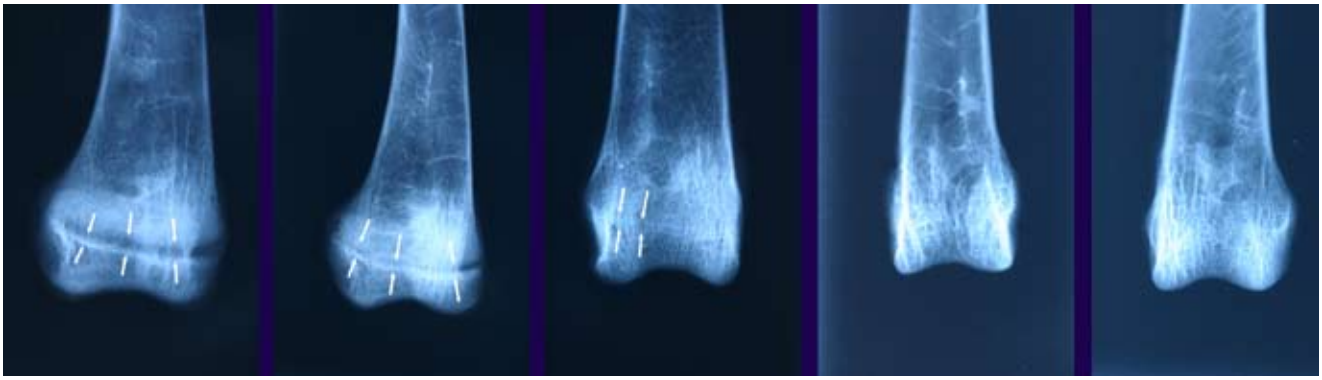

Figure 10b. Radiographs of the specimens of Figure 10a (dorsoventral view).

Figure 11. Radiographs of the proximal segment of the tarsometatarsus, including the proximal growth plate (arrows). From left to right: broiler 8 weeks, broiler 10 weeks, broiler 14 weeks, laying hen 5 months, and laying hen 2 years. Notice the closing of the growth plate with increasing age.

DISCUSSION

Restitution for chicken meat export is only disbursed when the sternum, femur and tibia of the exported products are not completely ossified. Because of the incomplete ossification of the sternal tip, broilers of 14 weeks and laying hens of 5 months are still regarded as young poultry and thus restitution is paid to the exporters. The femur shows no growth plates and therefore only the thickness of the cartilage covering the femoral head and condyles can give an indication of the age of the animal. In contrast, the tibiotarsus contains growth plates whose closure provides a criterion for age determination. However, the chronology of the ossification process in chickens is poorly documented in the literature.

The present study demonstrated that ossification of chicken bones is not completed in over-aged 14-week-old broilers.

Ossification of the sternum proceeds at a slow rate, and a large part of the sternal crest is still cartilaginous in 14-week-old broilers and in 5-month-old laying hens. This thwarts the evaluation of chicken breast parcels in which the sternum is the only major bone present. Further studies are necessary to determine the exact age of complete sternal ossification in male and female chickens of different breeds.

In recent decades there has been much selection for fast growth and meat production in poultry (Kirkwood *et al.*, 1989). The femoral length of the broilers in the present study was around 11.6 cm at 14 weeks of age, whereas previous studies on White Leghorns

(Latimer, 1927) and New Hampshire x Barred Rock crossbreeds (Church and Johnson, 1964) showed a maximum length of about 9 cm at 20 weeks. Similarly, the tibiotarsus of the broilers in this study was larger (16.05 cm at 14 weeks) than that of the male *White Leghorns* (14.51 cm at 20 weeks; Latimer, 1927) and crossbred poultry (13.8 cm at 24 weeks; Church and Johnson, 1964). In a recent comparative study, Williams *et al.* (2000) found that at the age of 5.5 weeks the tibiotarsus reached a length of 9.4 cm in a slowly growing poultry strain, and 11.6 cm in a fast growing strain. This finding is comparable to the mean tibiotarsal length of 11.5 cm in the 7-week-old broilers of the present study. Kirkwood *et al.* (1989) demonstrated that in adult poultry the length of the tarsometatarsal bone is correlated to the body weight, but breed differences in growth rate and final length of long bones have frequently been described (Wise, 1970; Naldo *et al.*, 2000; Williams *et al.*, 2000). In the present study the length and diameter of the leg bones were larger in the fast growing broilers than in the laying type chickens. The maximum length of the long bones of broilers exceeds that of non-broilers, but at any given body weight the long bones of broilers are always shorter than those of laying hens (Williams *et al.*, 2000).

The length of the long leg bones of the laying hens in the present study was similar to that of female *White Leghorns* as described by Latimer (1927), who introduced formulas for calculating the length of the long bones based on the age and the body weight. Application of these formulas to the laying hens of the present study resulted in calculated values of bone length that were very similar to the osteometric findings of the femoral, tibiotarsal and tarsometatarsal bones. Therefore, it can be stated that the morphometric parameters of the *Rhode Island Red* and the *Orpinghton* laying hens in the present study are very similar to those of the *White Leghorns* described 80 years ago by Latimer (1927). To evaluate recent developments in the *White Leghorn* laying hens, a new study would have to be performed.

Chicken bone length is subject to manifest gender differences, the males always having longer bones than the females. This sex-related difference appears around 16 weeks of age in *White Leghorns* (Latimer, 1927) and is possibly due to the fact that the ossification is completed earlier in females (Naldo *et al.*, 1998).

The width of the long bones of the shank changes only little with the increasing age of the broilers. Therefore this parameter is hardly useful for precise age determination. However, there is a manifest breed difference between poultry of meat and laying types, as the width of the long bones of broilers is much larger than that of laying hens.

The articular cartilage covering the femoral head, the femoral condyles and the proximal tibiotarsus were more than 3 mm thick in the broilers of this study and presented minimal variability between 7 and 14 weeks of age. In contrast, the corresponding cartilages in laying hens older than 22 weeks were less than 1 mm thick. The distal tibiotarsal cartilage was 1 mm or less at the age of 10 weeks in both the broilers and laying hens of this study. However, we would not consider the thickness of the distal tibiotarsal articular cartilage as a good parameter for age determination because its measurement is difficult to standardize and very dependent on the orientation of the saw cut.

The disappearance of the growth plates in the long leg bones is subject to breed and gender variation, but is still useful for age determination. The proximal growth plate of the tibiotarsal bone closed around 14 weeks in the broilers studied, which is some 4 weeks earlier than in *New Hampshire x Barred Rock* crossbreeds (Church and Johnson, 1964) and manifestly earlier than in male *White Leghorns*, in which closure occurs at 27.8 weeks (Latimer, 1927). The distal growth plate of the tibiotarsal bone closed around 14 weeks in the broilers of this study and in the abovementioned cross breed (Church and Johnson, 1964), while it closed only at the age of 22.5 weeks in male *White Leghorns* (Latimer, 1927). Similarly, closure of the tarsometatarsal growth plate occurred around 14-16 weeks in the broilers in the present study and the cross breed chickens (Church and Johnson, 1964), whereas it only disappeared at 28 weeks in male *White Leghorns* (Latimer, 1927). All these differences can be explained by the persistent selection for muscularity in meat type chickens, which has led to a higher growth rate and precocity of broilers. This contrasts sharply with the situation in laying hens. In female *White Leghorns* the proximal and distal tibiotarsal growth plates close at 23 weeks and 16 weeks, respectively, while the tarsometatarsal growth plate disappears around the age of 20 weeks (Latimer, 1927). This is in line with our observations that in the 22-week-old *Rhode Island Red* laying hens both tibiotarsal growth plates were absent, while the tarsometatarsal growth plate was closing.

CONCLUSION

In conclusion, two osteologic criteria are proposed for determining the age of chickens in order to grant restitution for poultry meat export. A femoral and tarsometatarsal length of $\geq$ 10 cm and a tibiotarsal length of $>$ 14 cm indicate that the animals are at least 10 weeks old. The anatomical and radiographic disappearance of the tibiotarsal and tarsometatarsal growth plates suggests that the broilers were slaughtered at the age of 14 weeks or older. These guidelines are based on limited numbers of poultry studied and on incomplete literature data. Therefore it is suggested that the influence of genetics and of housing, activity, nutrition and other environmental factors be evaluated in future studies in order to substantiate the present findings.

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Uit het verleden

BOEREN KWEEKTEN VEE, HEREN ATEN VLEES

Een stukje vlees dagelijks voorgeschoteld krijgen, dat was gedurende het zoveel honderduizenden jaren bestaan van Homo sapiens allesbehalve evident. Het is eigenlijk een zeer recent fenomeen. Enkel voor de toplaag was dat al langer 'dagelijkse kost'. Dit zien we sprekend geïllustreerd in de Engelse taal. Na de machtsovername door de Normandiërs onder Willem de Veroveraar in 1066 onderging de Germaanse taal van de Angelen en de Saksen een sterke invloed van het Frans dat nog lang na de verovering de taal van het hof en van andere hoge wereldlijke en kerkelijke machthebbers bleef. Zo komt het dat volgende in oorsprong Franse woorden voor vlees nu nog steeds algemeen gebruikelijk zijn, terwijl de namen van de levende dieren Germaans bleven:

Beef (Frans: boeuf) tegenover cow, ox, steer.
Veal (Frans: veau) tegenover calf.
Mutton (Frans: mouton) tegenover sheep.
Porc (Frans : idem) tegenover swine, pig.

Luc Devriese