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THE TETRACYCLINE LABELLING METHOD FOR MEASURING GROWTH OF BONES IN THE CHICKEN

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The objective of this study was to estimate the internal dynamics of leg (tibia) and wing (humerus) bones of light anf heavy types of chicken, reared in cages and on the floor. The relation of floor vs. cages area was 3:1. All chicks were fed a standard broiler mixture: starter (0-4wk) with 0.9% Ca; 0,7% P and grower (4-7 wk.) with 0,8% Ca ; 0,5% P. Each bird was given two intraperitoneal injections of 30 mg/Kg of oxytetracycline at a 2-day interval.

The results showed no significant differences between floor and cage reared birds in tibial osteon appositional rates. However, there was a significant effect of rearing system on humerus internal dynamics. Seven week old chicks of the light type, reared on the floor had humeri with higher appositional rates than those reared in the cages.

Key words: Growth, chicken, bones, tetracycline

INTRODUCTION

Cortical remodeling is the destruction of compact bone followed by the construction of a new Haversian system. The osteoblasts of the bone remodeling unit form a resorption tunnel within the bone, which is refilled centripetally by the osteoblastic apposition of concentric lamellae to form a Haversian system or osteon. At the osteon level, bone formation occurs in two stages, matrix formation and mineralization, which are separated both in time and in space (Redker, 1983). Much of our knowledge of osteoblasts and bone formation has been obtained by the use of in vivo markers, especially the tetracyclines (Frost, 1965; 1969; Tapp, 1966; Hansson et al., 1974; LaCroix, 1972; Tam et al., 1974). The antibiotic tetracycline forms a complex with calcium ions at the surfaces of newly formed apatite crystals and thus marks the site of initial mineralization which can be visualized by its fluorescence with UV light, as a golden yellow band or ring (Sandhu and Jande, 1981).

Leg weakness in broiler chichens causes a significant and increasing economic loss in the poultry industry. In order to understand the pathology or etiology of bone disease in poultry, it is necessary to have a thorough under-

standing of bone physiology. However, studies of histological differenced in bone growth between various strains are rare in the chicken (Wise, 1970; Poulos et al., 1978) and in turkeys (Leblanc et al., 1986).

This study was undertaken to compare by microscopy the bone dynamics of normal skeletal growth in two type of chickens reared on the floor and in cages. One was a light type without significant bone problems and the other a heavy type (broilers) with significant bone problems.

MATERIALS AND METHODS

The two strains of chickens: heavy — Ross-1 and light — Issa Brown, used in this study, were reared in cages and on the floor. The relation of floor vs. cage area was 3:1. All chicks were fed with a standard broiler mixture: starter (0-4 wk.) with 0.9% Ca; 0.7% P and grower (4-7 wk.) with 0.8% Ca; 0.5% P. Groups of 5 normal male chicks of each strain and group were studied at 5 and 7 weeks of age. Each bird was given two intraperitoneal injections of 30 mg/Kg of oxytetracycline at a 2 day interval. The second dose was administrated 2 days prior to sacrifice. The left tibia and humerus were used for histological studies. Transverse blocks were cut through the middle diaphysis, fixed in buffered formol saline and embedded in a plastic mixture (methylmethacrylate monomer, polymethylmethacrylate and benzoyl peroxide). Seven microns thick undecalcified sections were cut using a Reichart-Jung microtome (Model 5000, polycat). A Letz Dialux 20 microscope with a blue filter for fluorescence (D, BP 355-425 nm) was used for labeling studies. The bone appositional rate was the mean of four measurements of the distance between the oxytetracycline labels in eight osteons per section, expressed in μ/day.

RESULTS AND DISCUSSION

All birds showed double oxytetracycline labeled primary osteons in the tibial cortex (Figure 1).

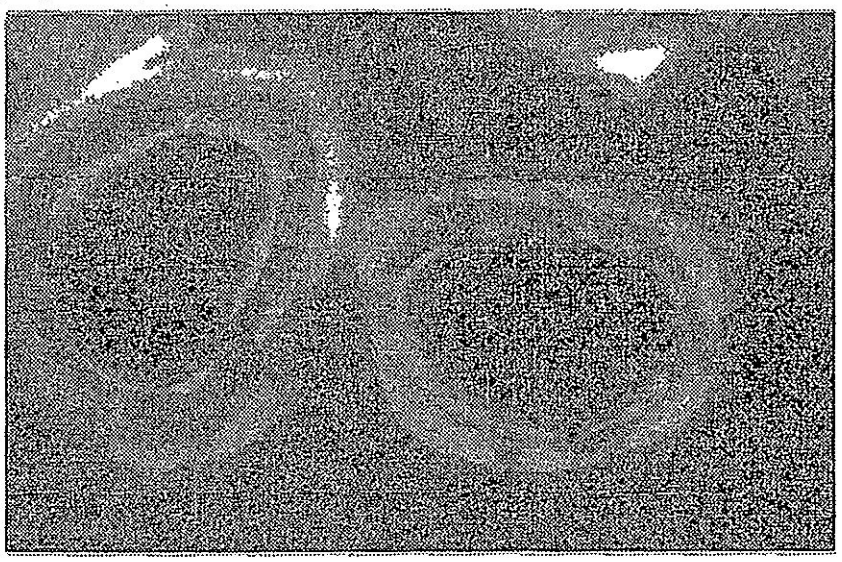

Figure 1. Two concentric fluorencent rings of tetracycline labeled osteons in a transversal section of tibial middle diaphisis, of a 5-week old heavy type of chicken reared of the floor. x 25.

The appositional rate of tibia mineralization (distance between two labels / time between labels) did not differ between chickens reared in cages and on the floor (Table 1).

Table 1. Osteon appositional rate in the tibial cortex of the heavy and light types of chickens (μ/day)

Type of chickens	Age (weeks)	Rearing system	
		Floor	Cages
Heavy	5	2,38 ±0,74	2,23 ±0,62
	7	3,02 ±0,77	2,87 ±0,67
Light	5	1,81 ±0,73	1,87 ±0,62
	7	2,63 ±0,56	2,44 ±0, 65

There were no significant differences between the heavy and light types of chickens. The results obtained were similar to those from other studies of bone growth (Wise, 1970; Leblanc et al., 1986; Bain et al., 1988).

The osteon appositional rate in chicken humeri was affected by the rearing system. First, the heavy type of chicken showed that no primary osteons were double labelled (Figure 2).

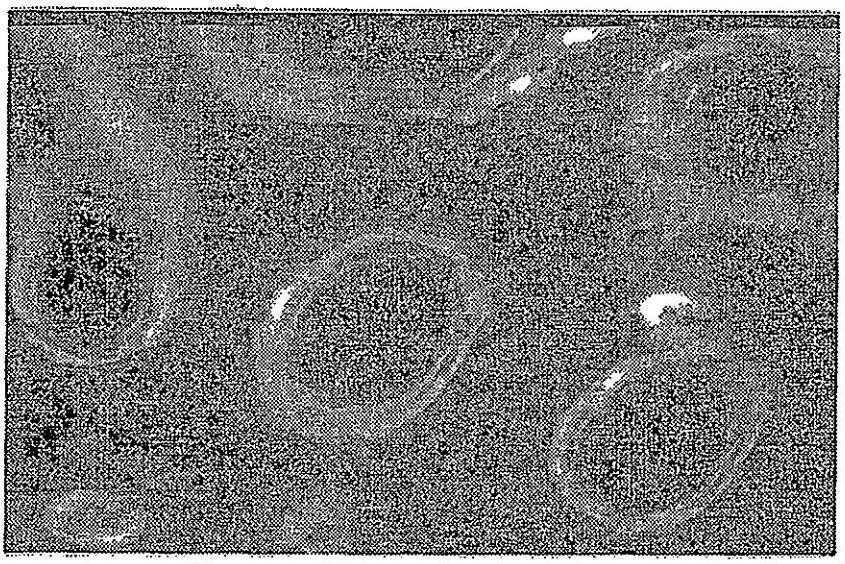

Figure 2. Abscence of second fluorescent tatracycline ring in transversal section of humerus middle diaphysis of a 5-week old haevy type of chicken reared in a cage. x 25.

These findings are not easy explain. According to Recker (1983), it can reflect a prolongation of the time interval between the onset of matrix synthesis and the onset of mineralization, or may result from an increase in the frequency or duration of the normal pauses in mineralization. Also, the interval between label administration and biopsy must be standardized. If the interval is too short, some in vitro loss of label will occur. If the interval is too long, some of the osteoid which was labeled will have completed its mineralization by the time of the biopsy, its place being taken by new osteoid which has had no chance to become labeled. Consequently, the absence of label does not neces-

sarily mean that mineralization has stopped altogether, only that its rate has been markedly reduced.

Secondly, the osteon appositional rate in the humerus of the light type of chicken differed significantly depending on the rearing system (Table 2.).

Table 2. Osteon appositional rate in the humerus cortex of the light type of chicken (μ/day)

Age (Weeks)	Rearing system	
	Floor	Cages
5	2,20 ±0,66	2,02 ±0,74
7	2,63 ±0,61 **	2,23 ±0,57

** — Statistically highly significant differences ($p<0.01$)

Thus, seven week old chicks (light type) reared on the floor had humeri with a significantly higher appositional rate than those reared in the cages.

Acknowledgement

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CONCLUSION

According to the results of this study, tetracycline is a very good marcer for in vivo measurement of osteoblasts and bone formation rates. The wing bones were more affected by the chich rearing system than the lef bones. The appositional rate in the humerus from floor reared chickens was significantly higher that in the cage reared birds.

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PRIMENA TETRACIKLINA KAO MARKERA ZA PRAĆENJE BRZINE RASTA KOSTIJU PILIĆA

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SADRŽAJ

Cilj ovoga rada bio je merenje brzine formiranja kostiju nogu (golenjača) i krila (ramena kost) pilića lakog i teškog tipa gajenih na podu i u kavezima. Eksperimentalni uslovi su bili takvi da je odnos površine poda prema površini kaveza iznosio 3:1. Za ishranu pilići su dobijali početnu smešu (0-4 nedelja) sa 0,9% Ca i 0,7% P kao i završnu smešu (4-7 nedelja) sa 0,8 Ca i 0,5 P. Svakom piletu su intraperitonealno, date dve injekcije oksitetraciklina (30 mg/kg telesne mase) u intervalu od dva dana.

Dobijeni rezultati nisu pokazali značajne razlike između podno i kavezno gajenih pilića u pogledu brzine osteonske apozicije golenjače. Međutim, u slučaju ramene kosti, ispoljio se statistički značajan uticaj načina gajenja. Sedam nedelja stari pilići, lakog tipa a gajeni na podu, imali su ramene kosti sa značajno većom brzinom osteonske apozicije u odnosu na piliće gejene u kavezima. Pokazalo se da je tetraciklin dobar marker za praćenje brzine formiranja kosti.