KnE Life Sciences

DonAgro
International research conference on Challenges and Advances in Farming, Food Manufacturing, Agricultural Research and Education
Volume 2021

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Conference Paper

# The Effect of Complex Feed Supplements on the Growth and Development of Long Bones in Broilers

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## Abstract

Poultry farming as one of the most intensive branches of animal husbandry, and it uses a large amount of various feed supplements in order to obtain the maximum production volume. This article presents the results of a study on the effect of feed supplements used for feeding broiler chickens. Until the age of 42 days, the chickens received a phytase-containing enzyme supplement, mineral supplements Mn and Ca in a citrate form and a vitamin supplement of 1,25-dioxycholecalciferol (vitamin D3, calcitriol). The live weight of the chickens was measured at the age of 1, 4, 7, 14, 21, 28, 35 and 42 days. The femur development of the chickens was studied through morphological methods (morphometry and histological examination). The influence of various supplements (from the combined to the mutually absorbing ones) on the growth and development of the long bones of broilers was identified.

**Keywords:** poultry, broilers, feed supplements, vitamin D, mineral supplements, enzyme supplements, histology, bones.

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Published: 5 April 2021

Publishing services provided by Knowledge E



Selection and Peer-review under the responsibility of the DonAgro Conference Committee.



## 1. Introduction

The broiler poultry industry is one of the important suppliers of animal protein for human nutrition. Chicken meat is a highly valuable dietary product having characterized by high digestibility, assimilability, and ease of processing. These factors, as well as the relatively low cost of products, make broiler meat popular, and broiler poultry farming highly profitable [1].

Correct feeding is the key to the health and high productivity of farm animals. At present, both practitioners and theorists recognize that the reserves for increasing the productivity of broiler chickens have been exhausted. Despite all the measures taken, there is an imbalance between the growth and development of bone and muscle systems. Further changes are possible in two directions: the use of genetic engineering

achievements and attempts to adjust physiological and biochemical processes in the body. The latter has the direct goal of improving the digestibility of feed, simplifying the use of consumed substances for building the body, and reducing the level of a developmental imbalance [2].

For this purpose, a variety of feed supplements have been developed. In intensive meat (broiler) poultry farming, numerous feed supplements are use. When testing these supplements, the result of their use is subjectively evaluated, and the effect of simultaneous administration of supplements is not determined at all.

The ambiguous results of feeding with several supplements suggest the interaction of supplements at different levels - in the gastrointestinal tract cavity or in the internal environment of the body [3, 4]. Supplements are used for a specific purpose - their effect on a specific organ or tissue is assumed [5]. However, as a rule, biologically active substances have a wider spectrum of actions on the body, which are not taken into account. Thus, vitamin D, which is widely used to strengthen bones [6] can change the connective tissue in various parts and organs of the body by affecting the cells of fibroblasts, and muscle tissue. Enzyme supplements are used to improve the digestibility / absorption of feed nutrients. The study of compatibility of dietary supplements is necessary to optimize the use of feed supplements in animal husbandry.

We studied the effect of different feed supplements - vitamins, minerals and enzymes - on the development of long bones of the pelvic limb of broilers. The development of the tubular bone is a multi-stage process, which includes several diverse, simultaneously occurring processes. The process involves osteoblasts and osteocytes, which produce the intercellular substance of the bone, as well as osteoclasts that can destroy it. The work shows complex relationships in the animal body, which must be taken into account when using multiple feed supplements.

## 2. Materials and Methods

### 2.1. Materials

The material was Smena-4 cross broiler chickens divided into six groups:

group No. 1 - control, basic diet (BD);

group No. 2 - BD + multienzyme complex (IFC - 350 mg / kg, a complex supplement containing phytase and enzymes that break down non-starchy polysaccharides);

group No. 3 - BD + supplement containing 1,25-dioxycholecalciferol (active form of vitamin D3) - 0.069 mg / kg.

group No. 4 - BD + IFC (350 mg / kg) + vitamin D (0.069 mg / kg),

group No. 5 - BD + MFK + vit. D + Mn citrate;

group 6 - BD + IFC + vit. D + Mn citrate + Ca citrate.

### 2.2. Methods

The poultry was removed from the experiment at the age of 1 day, 3 days (since the fourth day, the chickens began to receive supplements), 7, 14, and 28 days, and when the marketable weight was reached, at the age of 42 days. The average live weight was calculated at each time point for each group (the number of birds in the group was 50 at the beginning of the experiment and 20 - at the end).

For the morphological study, birds with a live weight close to the average value were selected. The left femur was isolated, measured and weighed.

For the study, femur samples were taken in the middle of the diaphysis (the narrowest section of the bone). A section of the bone of 10 mm in length was cut.

The samples were fixed in formalin, then decalcified in a 5% nitric acid solution. The decalcified samples were washed with running water and dehydrated, followed by embedding in paraffin according to the standard procedure.

The samples were cut using a microtome, and complete transverse sections of the tubular bone with a thickness of 5-9 μm were obtained. The sections were then glued onto glass slides and stained by the Masson's three-color method.

Features of the histological structure of the femur were dewsscribed and evaluated on histopreperets. Measurements and calculations were made: diameters of the bone and bone cavity, the thickness of the compact substance, maturity of the osteonic structure.

The results obtained were processed by the standard biometric methods.

## 3. Results

The multienzyme supplement and vitamin D3, both with separate and combined administration, did not have a noticeable effect on the live weight of poultry at marketable age (Table 1). The additional administration of mineral supplements - Mn and Ca citrates - increased the live weight of the bird.

The structure of the femur is a typical long tubular bone. In the middle part, the bone has an oval cross-section, large and small diameters were measured. Accordingly, the diameter of the cavity was measured twice. In the composition of the bone wall, a

compact substance consisting of osteons at different stages of maturity and a looped medullary bone were identified. On the circumference of the bone, starting from the age of 7 days, thinner and thicker sections of the wall (compact substance) were identified; two wall thicknesses were measured - the largest and the smallest.

In growing chickens, the morphology of the femur went through the following stages of age-related changes: before the bird began to receive supplements, that is, at the age of 1 to 7 days, the bone diameters increase – the large one increased from 2.4 to 2.9 mm, the smaller one increased from 1.8 to 2.2 mm. The large diameter did not change and was equal to 2 mm. Accordingly, an increase in the diameter of the bone occurs due to the wall thickness: the maximum value increased from 394 to 743 μm, and the minimum one – from 82 to 134 μm.

Table 1: Average live weight of broilers by group

| age | No of the group | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| 1 days | 44,98± 1,27 | | | | | |
| 3 days | 73,38± 0,70 | | | | | |
| 7 days | 130,84±,92 | | | | | |
| 14 days | 333,7± 4,76 | 341,7± 7,27 | 303,2± 5,79 | 316,1 ± 6,12 | 320,1 ± 6,57 | 310,5 ± 6,72 |
| 28 days | 1116,6 ± 51,77 | 1087,4± 56,14 | 1056,6 ± 27,53 | 1087,0 ± 50,41 | 1048,8 ± 64,35 | 1027,2 ± 20,43 |
| 42 days | 2063,8± 95,17 | 1913,8 ± 106,64 | 1991,4 ± 103,66 | 1908,6 ± 112,89 | 2132,8* ± 98,62 | 2206,0* ± 54,80 |

At the age of 1 day, active processes of bone formation were observed: the bone wall had a uniformly looped structure; on the outer surface, under the periosteum, there were rows of active osteoblasts. An increase in wall thickness occurs due to the appositional growth. In this case, a wide-mesh network of trabeculae or wide radially located cracks under the periosteum in the region of the thick wall developed. The structures of the medullary surface of the wall turned into osteons: the channels narrow, and the typical circular bone plates were formed (1-2 plates per osteon).

At the age of 7 to 14 days, the birds began to receive feed supplements, and their effect was immediate and persisted until the final slaughter (42 days).

During the second week (up to 14 days), the thick bone wall increased in thickness up to 1200 μm and the thin wall increased up to 200 μm. The difference in the growth rate of the wall thickness is very large: the thin wall added 200 microns in 7 days, and the thick one - 1200 microns.

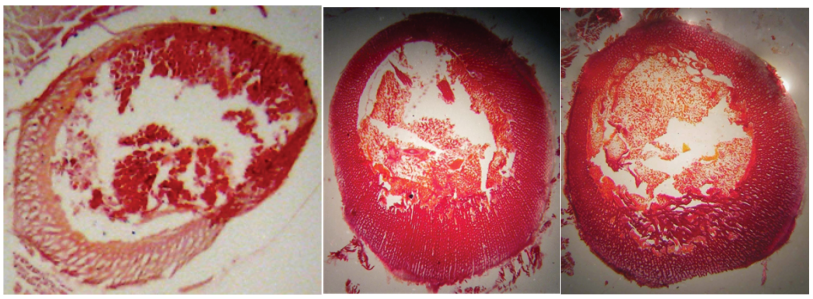


**Figure** 1: The histological structure of broiler femur: A - 3 days; B - group 1 on the 42th day; C – group 8 on the 42th day.

In the thick wall, significant structural changes were observed: its inner surface has undergone resorption and the band of mature osteons was absent. As a result of resorption, the cavity diameter increased by 0.4-0.8 mm.

Resorption was more pronounced in all groups receiving vitamin D. In group 4, the combination with IFC reduced the effect of the vitamin supplement, and in groups 5 and 6, mineral supplements increased resorption. As a result, by the age of 14 days, broilers that received vitamins and mineral supplements had bones with thin walls without a zone of mature osteons, and with wide cavities in the growth zone under the periosteum.

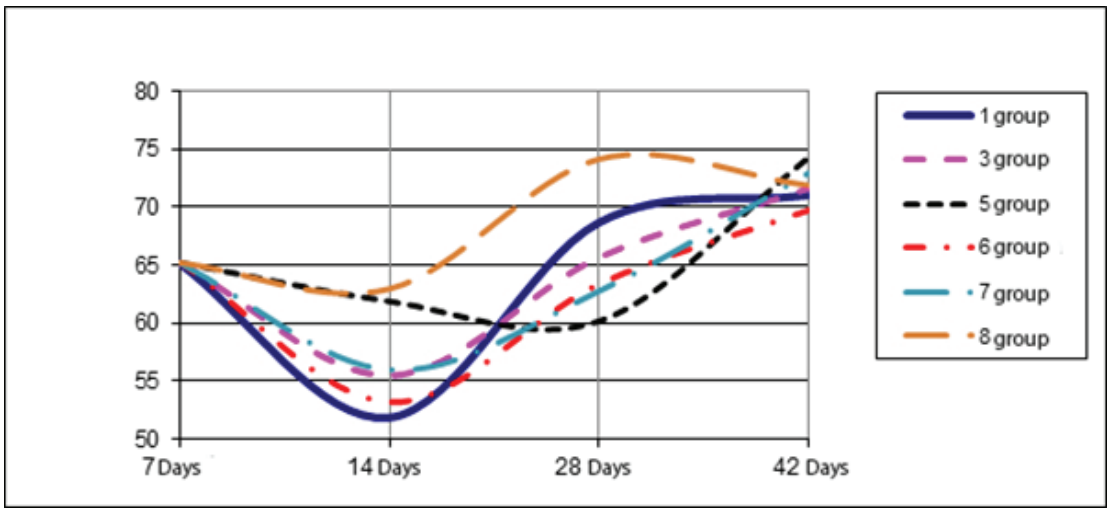


**Figure** 2: Changes in the average diameter of the bone cavity, μm

In the next 4 weeks, during the bone formation, a variable predominance of the processes of appositional growth and active resorption of the medullary surface was observed; all processes were accelerated when broilers received vitamins and mineral supplements. By the age of 28 days, with the same bone diameter in groups 1 and 6, a completely different histological picture was observed: chains of mature osteons in the

thick wall and weak resorption and growth in the control group, and strong resorption accompanied by the formation of medullary bones and significant growth in group 6 (Figure 1).

For all groups receiving vitamin D, the presence of the medullary bone in the bone cavity is characteristic.

At slaughter age (42 days), the bone retains signs of growth and rearrangement: moderate resorption of the medullary surface and moderate growth on the outside. The leading position in terms of the diameter of groups 1 and 6 is preserved. Moreover, in group 1 the animals had the thickest and most mature bone wall; in group 6, the wall had a thickness which was less than the average one. In general, by the age of 42 days, both measurements and the histological picture of the bone in all groups was more aligned.

The important indicators were the thickness of the bone wall (compact substance) in thick and thin areas and the cavity diameter. When the birds received only vitamin D, the bone thickened much more intensively, but at the same time the bone cavity also increased. Feeding with an enzyme supplement (in the absence of a positive effect on the live weight of the bird) decreased the effect of vitamin D. The indicators were equal to the ones in the control group. Inclusion of mineral supplements - Ca and Mn citrates - increased the effect of vitamin D and thickened the bone. This also decreased the thickness of the bone wall due to the size of the cavity. As a result, Group 6 had the thickest and weakest bones (see Figure 2).

## 4. Discussion

This study has confirmed the effect of different supplements on the growth and development of long bones in broilers. Insufficient strength of the pelvic limb bones leads to dislocations and fractures. This is a common cause of poultry deaths, culling and poor product quality. The effect of vitamin D on the level of calcium absorption in the intestine and bone cells was described. The results are consistent with the results of medical histological studies [7].

## 5. Conclusion

A decrease in the effect of vitamin D supplementation was found with the simultaneous introduction of a multi-enzyme supplement into the diet.

The introduction of mineral supplements led to an increase in the effect of the vitamin supplement. The unexpected result was a thickening and a noticeable weakening of the bones. The combination of supplements had a negative effect.

## Acknowledgement

The authors are grateful to their colleague for their contribution and support to the research. They are also grateful to all the reviewers who gave their valuable inputs to the manuscript and helped in completing the paper.

## Conflict of Interest

The authors have no conflict of interest to declare.

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