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Effects of Diets With Vermiculite on Performance, Meat Morphological Parameters of Broiler Chickens

Tolkyn Abdigaliyeva,
Kazakh National Agrarian University, 050010, Kazakhstan, Almaty, Abay, 26

Nurzhan Sarsembayeva,
Kazakh National Agrarian University, 050010, Kazakhstan, Almaty, Abay, 8

Bozena Lozowicka,
Institute of Plant Protection – National Research Institute, 15195, Poland, Bialystok, Chełmońskiego, 22

Altay Ussenbayev
Kazakh National Agrarian University, 050010, Kazakhstan, Almaty, Abay, 26

Ainur Dzhangabulova
Kazakh National Agrarian University, 050010, Kazakhstan, Almaty, Abay, 26

Abstract

This is the first study of broilers fed with vermiculite (V) mineral feed additive from Kazakhstan. The objectives of this study were to determine the effect of V and fish meal (FM) supplementation on histological profile of broiler chicken meat. Additionally, the blood parameters, growth performance, meat quality were assessed. One hundred, 1-d-old Arbor Aycres breed broiler chickens were divided into five groups. They were used in a 42-d experiment and fed either an unsupplemented basal diet or similar diets supplemented with 3.0, 5.0%V, 3.0%-mix of V and FM (1%V+2%FM) and 5.0%-mix of V and FM (1.5%V+3.5%FM). In both groups, where (V+FM) were used, the blood hemoglobin content of chickens increased slightly. It was established that supplementation of feed additives increased significantly body weight gain, feed intake and feed conversion ratio ($p<0.05$). The higher levels of these indices were obtained when the 5%V+FM was used. Morphological characteristics of meat were not changed in experimental and control groups of chickens.

Keywords: feed additive; vermiculite; fish meal; growth performance; broiler chickens; histological properties.

1. INTRODUCTION

Chicken has become one of the most popular meats consumed in the world. Modern, intensive poultry production has achieved phenomenal gains in the efficient and economical production of high quality and safe chicken meat, eggs and poultry bioproducts. At the same time, the industry is focused on maximizing the health and well-being of the birds and minimizing the impact of the industry on the environment [16].

According to the Kazakhstan Statistics Agency, broiler meat production in Kazakhstan has expanded, the number of birds has increased from 65 thousand tons in 2006 to expected 160 thousand tons in 2016 and broiler production has been expected twice higher, reaching 246 thousand tons up to 2020. The Kazakh government has made a priority to support domestic poultry production for the construction of new and modernization of existing poultry farms [15].

Consequently, chicken nutrition and feeding is an important part of effective production in developing countries [3; 6; 20]. The use of feed additives has been an important part of poultry production [12]. Common feed additives used in poultry diets include antimicrobials, antioxidants, emulsifiers, binders, pH control agents and enzymes [5; 8; 11]. In particular, it has been suggested that additives play an essential role in maintaining the health of poultry [1]. The efficiency of poultry production, as was mentioned, depends on a complete and balanced mineral diet of birds.

The problem of mineral nutrition of broiler chickens is solved by the use the natural minerals, which have a wide range of properties [10]. The natural minerals are found to be effective as non-toxic, cheap, ecologically advantageous and affordable materials based on their high-sorption capacity and ion exchanges properties. So, they are widely used in many fields of industry, agriculture, environment protection, sanitation, veterinary medicine, and animal nutrition.

One of the most promising natural minerals, suitable for use in agriculture, is vermiculite [19]. V is a silty mineral that is a product of weathering or hydrothermal decomposition of biotite, flogopite, some chlorites and other silicates rich in magnesium [4]. The basic deposits of V are concentrated in the USA, the South African republic, Russia and other countries such as the Republic of Kazakhstan [14]. V has been widely added to animal feed for poultry feeding to improve growth performance and health, to reduce toxic residues and to minimize costs of production. However, the data on the effect of V supplementation as a feed additive on poultry growth and productivity are limited. To the best of our knowledge, the influence of V on quality especially on the level of protein content during storage and FM has been not published.

Studies on applying of V as a feed additive for broiler chickens are limited. Therefore, future studies are required to recognize the effects of the feed additive on health status and meat quality of poultry.

2. MATERIALS AND METHODS

2.1. Feeding of chickens.

The material of the study was V of *LLP Kulantau*, Kazakhstan, brand M-150, fraction 0.5-3.0mm. Diets and experimental design were as follows: 100 *Arbor Acres* broiler chickens were randomly blocked and allotted to one of five dietary treatments.

The broiler chickens were held in isolated sections of cages on deep litter with a partial mesh floors. The average body weight of the chicks was 46.18±1.75g. The chicks were reared for 42 days under controlled management (34°C at day one, reduced by 1°C per day until a temperature of 22°C was reached, with humidity of 60–70%).

Birds had free access to feed and tap water which, along with their excrements, were inspected microbiologically. Birds were fed a starter diet containing all the necessary nutrients until day 21 and a growth diet from day 22, providing 2.950 and 3.100 J/kg of ME (metabolisable energy, which indicates the nutritional value). V (characterized by high Fe_2O_3 content - 20.59%; SiO_2 - 17.8%; K_2O - 8.18%; Al_2O_3 - 7.22%; MgO - 6.4%; TiO_2 - 2.27%; CaO - 1.79%, and FeO - 0.56%, data unpublished) was used as feed additives.

Control chicks (A) were fed a basal standard diet of the *LLP Saru Bulak* (BD) without V. There were offered four different diets based on optimized ratio of FM to V: B) 97% BD + 3% V; C) 95% BD + 5% V; D) 97% BD + 1% V + 2% (FM) and E) 95% BD + 1.5% V + 3.5% FM.

2.2. Hematological and biochemical blood parameters of chickens.

Blood samples for hematological and biochemical examinations were taken from the *vena basilica* of the left wing; these were collected using syringe-needle assemblies flushed with heparin. The heparinized blood was immediately centrifuged at 837 rmp×g at 4°C for 10 minutes, and plasma samples were stored at −80°C in Eppendorf test tubes until the analyses were performed. The samples for hematological examination were collected in tubes with EDTA and analyzed immediately. Selected plasma biochemical parameters (total protein, calcium, and phosphorus, HGB, HCT, RBC and WBC) were measured. Hematological studies were performed on automatic hematology analyzer Swelab Alfa Basic 4/3 (Sweden).

The chickens were weighed individually on 1, 14, 28 and 42 d of the experiment.

2.3. Tasting assessment of meat

Tasting assessment of meat and broth was provided by 10-point scaleat samples from randomly selected 5 chickens' carcasses of control group A and experimental groupE with the highest gain performance [17].

2.4. Methods of histological examination of meat

Histological examination was carried out in accordance with GOST19496-93 "Meat. The method ofhistological examination", GOST R 51604-2000 "Meat and meat products. Histological method for identifying the composition". Meat samples for morphology research were collected as in section 2.3. Moreover, each assay was done in triplicate to assess reproducibility.

2.4.1.Preparing a mixture of egg white with glycerol and processing microscope slides.

Fresh egg white, egg yolk without admixture was whisked to foam, poured on to a large filter (filter paper), pre-soaked with distilled water and filtered over night. Into the filtered protein, glycerol was added at a ratio of2:1, stirred, and 0.1g of camphor was added to prevent rotting. The resultant mixture was applied to degreased glass slides triturated using a gauze swab, and dried over a flame burner.

2.4.2 Preparing of eosin solution.

1 g ofwater-solubleeosin was dissolved in100cm^3 of distilled water.

2.4.3. Preparation of Ehrlich hematoxylin.

Ehrlich's hematoxylin was prepared by mixing20 cm^3 of a 10% alcoholic solution of, 80 cm^3 of 96% alcohol, 100$cm^3$of glycerol, 100$cm^3$of distilled water, 10$cm^3$of glacial acetic acid and 3g of potassium alum. The resulting solution was poured into a wide-mouth jar, tied with gauze and left in the light to mature for four weeks. The ripened solution was filtered. For fixing, the samples were placed in a 10% neutral formalin aqueous solution and were sealed tightly. The fixed samples were then placed in flask and inserted through the glass funnel, which was washed with cold running water for 15 minutes. Prepared sample pieces were collected and sealed in gelatin. Well-washed slices were washed in a 12.5% aqueous gelatin solution for six hours and then in a 25% aqueous gelatin solution for 24 hours in an oven at a temperature of 37°C. The pieces were then laid out in a Petri dish filled with a fresh blocked 25% aqueous gelatin solution and rapidly cooled in the refrigerator. After cooling, the excised blocks were added to a 20% formalin solution for 12 hours. Before cutting with the microtome the blocks were washed. From fixed specimens were excised 15x15x4mm size pieces as well as the underlying layers, to a depth of 15 mm. Slices were placed in a microtome, and frozen sections were prepared in thicknesses from 10 to 30 microns. With a microtome knife finebrushslices were transferred to a Petri dish containingtap water. Under in tact slices a glass slidetreated with egg white and glycerin was quickly introduced. A slice was removed from the water to the middle of the glass by holding it in the position with a dissecting needle. Then the slice was covered with dry filter paper and by pressing he paper by hand, stuck onto a glass slide.

2.4.4. Staining of slices.

Sections were first stained with Ehrlich's alum hematoxylin for three minutes and after further two minutes were washed in water. To remove excess hematoxylin, slices were added to a 1% solution of hydrochloric acid until a pink color was attained, then ammonia water until a blue color was attained, and then washed with water for

two minutes. Slices were stained with 1% aqueous in for one minute and rinsed with water. Thereafter, slices were placed undera cover slip. Prepared histological preparations were examined under a biological microscope BIOLAMM-1. Ready preparations were examined and photographed using a microscope LEICADM4000B.

2.4.5. Statistical analysis.

Analysis of experimental birds' weight was provided with Duncan's multiple range tests in SAS 9.3 (SAS, 2012). Statistical analysis of hematological blood parameters was performed by using Turkey'smultiple range tests in Statistics 7 (Analytical Software, 2000). The statistical significance was defined as $p<0.05$.

Animal experiments were carried out following the protocol approved by the local Ethical Committee in agreement with international principles for biomedical research.

3.RESULTS

3.1 The influence of vermiculite as a feed additive on the hematological and biochemical blood parameters of broilers.

This study was focused on hematological parameters during 42 days testing the growth of broilers, which is considered a significant factor. The hematocrit values reached the highest level (39.8%) in the group treated with 5%V+FM and the lowest levelof this parameter was in the group treated with 3%V+FM (Table 1). Hemoglobin showed mean value ranging from a minimum of 119.2 g/l for group treated without feed additive (group A) to a maximum of 129.0 g/l (for the group E treated with 5%V + FM), with statistically significant differences. The number of red blood cells (RBC) was higher in the experimental groups B, D, E of broilers than in the control group of birds. The mean values ranged from $4.20 \cdot 10^{12}$/l (5%V) to $4.73 \cdot 10^{12}$/l (5%V+FM). Data referring to the leukocyte (WBC) parameters development, as presented in Table 1, showed very significant statistical oscillations of the total leukocyte number, $6.6 \cdot 10^{9}$/l (3%V+FM) to $8.5 \cdot 10^{9}$/l (5%V+FM).

The values of the total protein content in most birds were within the range of 37–42g/l [2; 18]. The broilers fed with feed additive 5%V+FM (group E) had higher protein concentration 39.1g/l than the control (group A, 35.3g/l). In comparison with control group the increased Ca amount was observed in the all experimental groups of chicks. This was associated with the highest ion-exchange activity of vermiculite. The highest increase of the concentration of calcium compared to control group (1.4mmol/l) showed the broilers fed with combination of 3%V+FM (1.83 mmol/l). The amount of phosphorusin the serum for 3%V+FM (1.56 mg/l) with respect to the control group (1.3) has small increase.

3.2 Growth of broiler chickens.

Several indices are used to evaluate the performance of a flock of broilers – growth rate, days to market, mortality, and feed efficiency [5]. Results of this research indicated that an average weight of birds in the all experimental groups fed with V feed additives was higher than this parameter of broilers of the control group (Table 2).

Table 1.Hematological and biochemical profile of broilers

Indicators	Groups				
	A(BD)	B (3% V)	C (5% V)	D (3% V+FM)	E (5% V+FM)
HGB, g/l	119.2±0.4	124.0±0.2	124.0±1.2 [a]	128.1±0.4 [c]	129.0±0.4
HCT,%	36.8±1.81 [d]	39.0±1.23 [b]	39.0±0.45 [d]	37.7±0.60 [c]	39.8±0.12
RBC,$\times 10^{12}$/l	4.47±0.15 [a]	4.8±1.21 [b]	4.2±0.62[b]	4.64±0.44 [c]	4.73±0.61 [d]
WBC,$\times 10^{9}$/l	8.0±0.21 [c]	6.9±0.46 [d]	7.7±0.15 [a]	6.6±0.14	8.5±0.16 [d]
Calcium,mmol/l	1.4±0.62 [a]	1.6±1.13	1.6±0.14 [d]	1.83±1.62	1.78±0.64 [a]
Phosphorus,mg/l	1.3±0.28 [b]	1.43±0.51	1.6±0.14 [a]	1.56±1.17	1.38±0.31
Protein, g/l	35.3±0.51 [a]	37.3±2.02 [d]	37.8±1.23[d]	38.2±1.44	39.1±0.14 [d]

Note: [a-d] Means in the same raw with different superscripts are significantly different at $p<0.05$

Table 2.Effects of feed additives on growth performance and body weight changes of broiler chickens

Groups			A (BD)	B (3% V)	C(5% V)	D(3% V+FM)	E (5% V+FM)
Period (d)	IW,g	1	47.4±1.2	46.2±1.2	44.6±2.7	47.3±1.3	45.4±2.2
	BWG,g	14	786.7±25.6*	793.7±31.0*	796.0±24.4*	805.3±15.5*	812.2±14.3*
		28	1502.1±46.3	1531.1±59.2*	1529.2±16.4	1638.5±24.3*	1644.3±57.0*
		42	2007.3±65.2	2158.0±14.2*	2204.2±20.9*	2403.3±20.9*	2506.5±53.8*
Growth	RG, g		1959.9	2110.82	2156.61	2356.01	2459.1
	ADG, g		46.66	50.25	51.35	56.09	58.55
	Growth rate		42.35	45.72	46.31	50.81	52.88

Note: IW – initial weight, BWG- body weight gain,ADG - average dailygain, RG - relative growth. *Means in the same raw are significantly different.

In experimental group E, where chickens were fed with V+FM, the weight gain grown up by 19% compared to the control group's birds. Addition of V and V+FM to broiler's feed had a significant impact on absolute average daily gain (ADG) and relative growth (GR). Table 2 shows that the difference in weight of the broilers at the beginning of experiment in all groups was not more than 0.4g, after 42 days ADG of groups C, D, E was 51.35g, 56.09g, 58.55g, respectively, while in the control group the difference in weight was insignificantly. The growth rate of birds in E and D groups (V+FM) was significantly higher than in the control group.

Body weight gain in broilers for the first 14 days of age given a diet supplemented with V+FM was higher than in those given only V. The body weight gain was not affected by the dietary treatments from 28 to 42 days. This observation is in agreement with the results of [9], who observed no differences in body weight gain of broiler chickens supplemented with different natural feed additives as alternatives to antibiotic growth promoters.

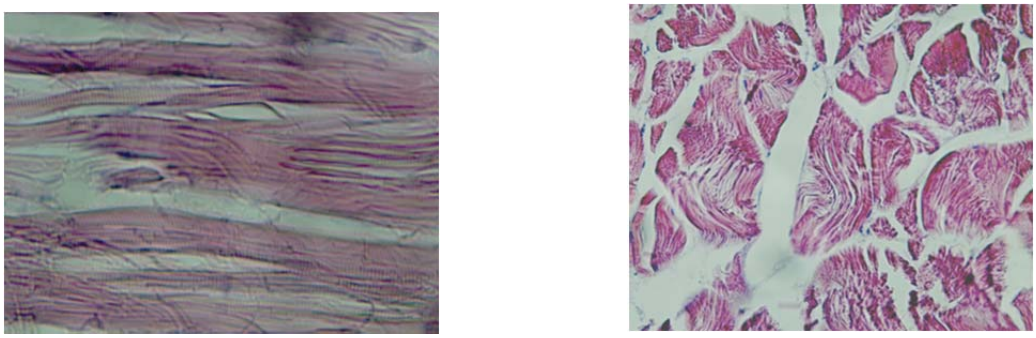
3.3 Tasting assessment of meat

The important component of meat is represented by muscles that determine nutritional values, give a specific taste, smell and color characteristic.Tasting assessment (on a 10 point scale) of meat and broth showed that the quality of the meat in the control and experimental groups has the appropriate standard indicators. Moreover, all organoleptic parameters of meat from experimental group of chickens exceeded those indices of meat from control group of broilers. The average score of meat tastewas 8.75 and 9.60; smell – 8.95 and 9.81 and colour – 8.86 and 9.75, respectively.

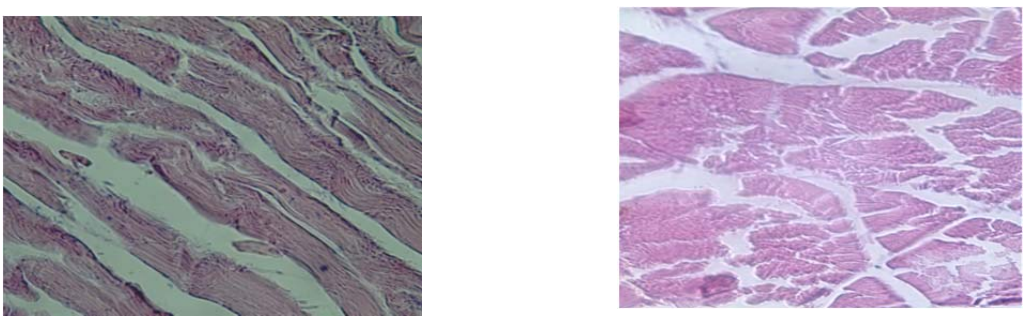
3.4. Morphological changes of valuable species of chicken`s meat.

Figure 1 shows the transverse and longitudinal sections of the broiler chickens`s meat tissue of experimental and control groups. Smaller in diameter muscle fibers in the tibia of the chicken-broilers of the experimental group give the meat a tender consistency. Broiler meat is classified as a dietary product, with a high level of digestibility [13].

Skeletal muscle of chicken`s meat is presented with striated muscle tissue. On longitudinal sections of the muscle fibers were visible shell fibers having the form of the contour line. Under the sarcolemma at the periphery of the nucleus fibers were elongated with small clumps of chromatin. The central part of muscle tissue was occupied by myofibril fibers which had longitudinal striations, stand-out differently in different fibers. The spaces between the striated muscle fibers were filled with layers of loose connective tissue called endomysium.



A) Experimental group



B) Control group

Figure 1. The striatedmuscle tissue of broiler chickens. Stained with hematoxylin-eosin. 10x40

When studying the histological sections on the muscles of the experimental and control groups, it was established that the pattern of muscle bands was clearly expressed. In the fields of vision the single hypertrophied muscle fibbers were observed. In the interstitial connective tissue fatty vacuoles were single, their number was insignificant. So muscle tissue of broiler chickens in both control and experimental groups had the same histological and morphological properties.

4. DISCUSSION

Hematological testing is one of the methods that can help detect certain changes in health that may not be apparent from physical examination, but which affect, for example, the condition of the birds [7]. Hematologic studies include hematocrit value (HCT) which indicates the ratio between the volume of plasma and blood cells. The value of hematocrit in all studying groups matches the physiological standards. The HCT of broilers fed with various treatments of minerals was significantly higher in all experimental groups than in the control group. The amount of hemoglobin (HGB) is an indirect indicator of the body's iron saturation. There was established a lower hemoglobin content at the control group, where broilers received only standard feed. The natural mineral – V has a high biological activity and cation exchange capacity. More developments that are relevant were found in case of hemoglobin, with very significant differences. When using different concentrations of the natural feed additive or combinations of them with FM in all experimental groups the amount of protein increased compared to the control group. It was associated with faster metabolism, which was confirmed by higher productivity of birds.

In this report, the results demonstrated that broilers fed with the 5%V+FM diets had significantly greater body weight, better average daily gain, relative growth gains and growth rate than broilers fed a control diet during the experimental period. Based on results during the study we can confidently state the biological and economic effectiveness of the new feed additive based on V for broiler chickens. The introduction of V with FM into the diet has a positive effect on the morphological state of the muscles of broiler chickens.

5. CONCLUSION

This research provides the first comprehensive demonstration of vermiculite from Kazakhstan as feed additive on broilers performance and meat quality. V was not investigated so far as a feed additive and had no toxicity and beneficially influenced on FM. The presence of macro and microelements in V composition in a sufficiently large amount distinguishes it from other natural minerals.

Results of research suggested that mineral supplementation guaranteed a high-quality of meat. Dietary supplementation with V had an effect on the performance of growing chickens.V showed positive action on the hematological and biochemical blood parameters of broiler chickens. Broilers fed with the 5% V+FM had significantly greater body weight, better average daily gain, relative growth gains and growth rate than the control group. Introduction into the diet of broiler chickens fodder feed additive based on V by weight of the diet did not cause pathological changes in the muscle of experimental chicks.

For future poultry industry, especially in developing countries, this new knowledge on V as a feed additive is very important for better understanding how to improve meat properties and quality ensuring consumers' health and safety.

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