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ORIGINAL PAPER

EFFECTS OF SUPPLEMENTING LAYING HENS' DIETS WITH VERMICULITE ON MORPHOMETRIC PARAMETERS, CHEMICAL COMPOSITION, FATTY ACID PROFILE AND EGG PRODUCTION*

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Abstract

This experiment was designed to study the effects of supplementing laying hens' diets with vermiculite (V) and vermiculite mixed with fishmeal (FM) on productivity, morphometric parameters, chemical composition and fatty acid profile of eggs as well as on some blood plasma indicators of laying hens. Hajseks White birds were randomly divided into 5 groups (20 birds per group) and 24-weeks-old hens were fed for 2 months with: A-base diet (BD) (control group), B and C-base diet with 3% or 5% vermiculite and D and E-base diet and 1% vermiculite + 2% fish meal or 1.5% vermiculite + 3.5% fish meal. In two tested groups of hens fed with V+FM, the productivity of eggs was higher than in the control group. Hens in groups B and C laid 100 eggs more than the control group and group D and E produced about 200 eggs more after feeding with V+FM. A higher content of protein, carbohydrates and energy value of eggs were observed. The average egg weight was the highest in group E, where also the weights of protein, white and yolk of eggs were the highest. Eggs of group C hens had the highest parameters of weight, thickness and density of the shell. The results showed that supplementation of V+F significantly affected the concentration of fatty acids (FA) and the protein content in the yolk and white. Feeding a diet supplemened with minerals and proteins led to an increase in the total fatty acid content and dietary mono- (MUFA) and polyunsaturated fatty acids (PUFA). The level of *n*-6 FA (especially linoleic and linolenic acids) was significantly increased in eggs of hens from groups D

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and E. The content of protein, WBC, HGT, calcium in blood of hens group fed with combination of V+FM was higher. The observed high mineral nutritional value implicates that a vermiculite additive could be used in the production of high quality poultry products. For the future of the poultry industry, especially in developing countries, this novel knowledge of vermiculite as a feed additive is very important.

Keywords: mineral, additives, feed, Hajseks White, egg quality, blood parameters.

INTRODUCTION

A balanced diet is necessary for optimal poultry production. The use of mineral supplementation has been an important part of the feed industry (Wenguang 1998, Langhout 2000, Yildiz et al. 2014), and minerals are typically classified as macro- or micro-minerals, depending on the levels needed in a diet.

Unconventional mineral feed additives, e.g. zeolites, vermiculites and bentonite, would increase the efficiency of feed utilization and strengthen the feed resources. In diets containing fishmeal, meat and bone meal, supplementation with inorganic vermiculite improves the growth performance and health of birds, reduces toxic residues and lowers production costs (Patkowska et al. 2008). The chemical formula of vermiculite is $(Mg,Fe^{+2},Fe^{+3})_3[(Al,Si)_4O_{10}](OH)_2 \cdot 4H_2O$ and, depending on the of origin of deposit, the elements: K, Na, Ca, Ti and Cr may be present in small amounts. The Republic of Kazakhstan is one of the biggest producers of vermiculite in the world, with an output of ten thousand tons annually (Syrmanova et al. 2012).

Eggs have always been an important component of human nutrition. They contain several nutrients which are essential for life and they are enclosed in a high-calcium shell (Miranda et al. 2015). The egg white is mostly high-protein, low-fat food, having hardly any cholesterol and the vitamin it contains is riboflavin (vitamin B_2). Uncooked egg whites consist of avidin, an antinutrient which binds biotin a B complex vitamin. The egg yolk is high in fat, cholesterol and protein; it is also an excellent source of vitamin A, D, E and K (Mazalli et al. 2004).

The proteins of eggs have an appropriate concentration of all essential amino acids, and are in general 99% digestible, thus serving as the standard to which all other proteins are usually compared (Raes et al. 2004). The benefit of omega-3 and omega-6 polyunsaturated fatty acids (PUFA) for health is unquestionable. These PUFA are essential because they cannot be synthesized by the body and must be obtained from a diet. Omega-3 have been shown to reduce inflammation and help prevent risk factors associated with chronic diseases such as heart disease, cancer and arthritis; they also appear to be important for the proper cerebral and behavioral functions (Ansenberger et al. 2010). Epidemiological studies indicate that populations that consume high amounts of omega-3 fatty acids have lower incidence of breast, prostate and colon cancers than those consuming less omega-3 fatty acids (Hanf, Gonder 2005). The amount of saturated or monounsaturated fatty acids in eggs is influenced by the quantities of lipids in the feed. In contrast, the PUFA content and profile in the egg can be modified through dietary supplementation (Fraeye et al. 2012).

There is scarcity of data on the effects of dietary inclusion of vermiculite from Kazakhstan's deposits on the quality performance and chemical composition of poultry eggs (Fendri et al. 2012).

Thus, the present study was conducted to evaluate the effects of four dietary levels of vermiculite, including two variants with fish meal, on egg production, morphometric parameters, chemical composition and the and fatty acid profile of eggs as well as some blood plasma indicators of laying hens.

MATERIAL AND METHODS

Laying hens and diets

The experiment was carried out at a poultry farm in the region of Almaty and involved laying hens of the race Hajseks White. A flock of medium weight (1990 g) laying hens in the first half of their production cycle (24 week of age) was randomly allocated to five-tier battery cages holding 20 laying hens each, and maintained under conventional conditions of ventilation, temperature (17-19°C) and lighting (16 h light d^{-1}). All birds had free access to diets and water and were fed with a standard commercial diet. The protocol for this study was approved by the local Animal Experimentation Ethics Committee. Feed mixtures contained the same components, the only difference being that the mixtures designed for the experimental groups were supplemented with vermiculite: (A experimental group) basal diet (BD) without vermiculite (V), (B) 97% of BD supplemented 3% V, (C) BD supplemented with 5% V, (D) 97% BD + 1% V + 2% fish meal (FM) and (E) 95% BD and 1.5% V + 3.5% FM (Table 1). The feeds where the additives consisted of vermiculite and fishmeal showed good storability.

The feeding, weight control and maintenance procedures were according to the management guide designed for Hajseks White hens. The vermiculite used in this study was provided by the company LLP «Kulantau» (Kazakhstan). Its brand name is M-150, and it contained the fraction sized 0.5-3.0 mm. Treatments were assigned randomly and consisted of the incorporation of vermiculite into commercial ingredients of diets. Vermiculite is characterized by a high content of Fe_2O_3 (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. Approximate dimensions of the crystals used for acquiring diffraction data were 0.4×0.2×0.2 mm.

Table 1
Composition of basal diets for laying hens and supplementation with vermiculite and vermiculite with fishmeal

Composition (%)	Groups				
	A	B	C	D	E
Wheat	54	51	49	51	49
Corn	22	22	22	22	22
Soybean meal	1	1	1	1	1
Sunflower meal	11.5	11.5	11.5	11.5	11.5
Prelac	1	1	1	1	1
Fodder yeast	5	5	5	5	5
Calcium	1	1	1	1	1
Meatbone meal	3.5	3.5	3.5	3.5	3.5
Premixes	1	1	1	1	1
Vermiculite	0	3	5	1	1.5
Fishmeal	0	0	0	2	3.5

METHODS

Egg production and morphometric parameters of eggs

The intensity of eggs production by laying hens, which was determined as a ratio of the number of eggs laid in the experimental period and expressed as a percentage, was monitored for 8 weeks. Morphometric parameters of eggs were investigated at 3^{th}, 6^{th} and 9^{th} week of the experiment. The eggs were weighed and the average eggshell thickness was measured. The density was measured with a hydrometer. Next, eggs were broken to assess albumen quality using an egg analyzer. The sampling method and organoleptic analysis were performed according to the GOST national procedures.

The chemical composition of eggs

The chemical composition of eggs was determined. Moisture was assessed by drying at 105°C, the fat content was determined with the Soxhlet method, while total protein was tested according to a modified method by Kjeldahl. The ash content was measured in a muffle furnace by heating at 550°C for eight hours.

Sample preparation of yolk and determination of fatty acids (FA)

The eggs were manually broken and separated into egg white and yolk. Yolk lipids were extracted using a standard procedure according to Folch et al. (1957), i.e. applying chloroform and methanol (2:1 v/v). The FA composition of the yolk lipids was determined by the saponification/methylation procedure (EN ISO 12966-1:2014/AC:2015). To the extracted yolk lipids (100 mg), placed in a screw capped glass tube, 4 ml of 2 M NaOH was added and heated on a heating block. After 10 min, 5 ml boron trifluoride-methanol complex was added and samples continued to be heated. Next, 3 ml isooctane was added to the boiling mixture and heated for 1 min. After removing the flask from the heat source, without allowing the flask to cool, 20 ml 1% NaCl solution was added. Then, 2 ml of the upper isooctane layer was transferred into a vial to which a small amount of anhydrous sodium sulfate was added. Chromatographic analysis was performed using a Hewlett-Packard-6890 gas chromatograph with a flame-ionization detector (FID) and Supelcowax 10 capillary column (100 m×0.25 mm). The conditions of the separation: carrier gas-helium; flow rate – 1.5 ml min^{-1}; detector temperature 250°C; column temperature 60°C increase of 5°C min^{-1} to 180°C. Methyl esters of acids were identified by retention times and compared with a mixture of methyl esters of fatty acids (Supelco 37 Component FAME Mix, 10 mg ml^{-1} in methylene chloride (Folch et al. 1957).

Haematological and biochemical blood parameters of laying hens

Blood samples for haematological and biochemical examinations were taken from the vena basilica of the left wing; they were collected with a syringe and needle that had been flushed with heparin. The samples were collected within 1 min of the capture to ensure that the levels of the monitored parameters were not affected by any stress induced by presampling handling (Chloupek et al. 2009). The heparinised blood samples were immediately centrifuged at 837 g^{-1} at 4°C for 10 min, and plasma samples were stored in Eppendorf test tubes at -80°C until the analyses were performed. The samples for haematological examination were collected in tubes with EDTA and analysed immediately. Selected plasma biochemical parameters (total protein, calcium, and phosphorus, HGB, HCT, RBC and WBC) were measured. Haematological studies were performed on an automatic hematology analyzer Swelab Alfa Basic 4/3 (Sweden).

RESULTS

Egg production and morphometric parameters

Morphometric measures of eggs, which can vary, are the main economically important parameters of poultry production. The age of hens and their diet have a significant effect on the whole egg, white, yolk solids and on the yolk: white ratio of eggs (Ahn et al. 1997, Drażbo et al. 2014). In this study, hens' diets containing added vermiculite caused a change in the morphometric parameters of eggs (Table 2). The intensity of egg production by laying

Table 2

Productivity, morphometric and quality parameters of eggs of lying hens fed different levels of vermiculite

Parameter	Groups				
	A (Control)	B (3% V)	C (5% V)	D (3% V+FM)	E (5% V+FM)
Number of eggs	820	1034	1030	1266	1284
Number of eggs per hen	41	46.35	46.25	52.15	52.6
Hen house eggs production (%)	68.3	77.25	77.08	86.92	87.67
Defective eggs	18	6	3	11	7
Egg weight (g)	58.07±0.32	58.71±0.13	59.13±0.42	61.25±0.2^{b}	63.31±0.30^{c}
Albumen (g)	35.31±0.14^{a}	35.58±0.23	35.68±0.14^{c}	37.81±0.31	38.45±0.32^{a}
Yolk (g)	17.32±0.17^{a}	17.51±0.21^{b}	17.48±0.08	17.96±0.09^{a}	19.33±0.06^{a}
Shell (g)	5.44±0.18^{a}	5.62±0.16^{a}	5.97±0.23^{a}	5.48±0.22	5.53±0.16^{b}
Shell thickness (mm)	0.381±0.002^{c}	0.423±0.006	0.453±0.004^{c}	0.392±0.003	0.405±0.004
The density of eggs (g cm^{-3})	2.14±0.30^{a}	2.31±0.30	2.37±0.20^{a}	2.21±0.10^{b}	2.25±0.40^{b}

$^{a\text{-}d}$ Means in the same raw with different superscripts are significantly different at $p < 0.05$.

hens in experimental group (C) made up 77.25%, and in the experimental group (E) – 87.67% against 68.30% in the control group. In the end, D and E groups produced by 3 and 5 units more than the control; productivity per hen in the E test group was 52.6 eggs in two months. This was 22% higher than in the control group. In all the tested groups of hens (B-E) fed with vermiculite plus fishmeal, productivity of eggs was higher than in the control group. Hens fed the vermiculite additive (groups B, C) laid 100 eggs more than the control group and about 200 eggs more after being fed with vermiculite plus fishmeal (groups D, E). The solids content of a whole egg, white and yolk varied within a narrow range among egg sizes. The average egg weight was the highest in group E (63.3 g). The content of albumen and yolk of egg was also the highest in B-D groups. Eggs of hens fed with 5% V (group C) had the highest weight of the shell, shell thickness and density. Eggshell breaking strength is a key indicator of egg quality. This research has shown that the eggshell breaking strength increased slightly in hens fed with V + FM.

The chemical composition and nutritional value of eggs

Determination of the chemical composition and nutritional value of eggs is one of the important steps in the veterinary sanitary examination. The chemical composition influenced the nutritional value of eggs and defined their physiological role as a source of biologically active substances (AJUYAH et al. 2003, VALSTA et al. 2005, DÍAZ et al. 2010, LONERGAN et al. 2014, KUMAR, RANI 2014, KICZOROWSKA et al. 2015). By modifying appropriately broiler chicken diets, it is possible to modify the fatty acid profile in poultry products and increase their nutritional value. The chemical composition of eggs, which is not constant, is characterized above all by the content of water, nitrogenous substances, lipids, minerals, carbohydrates and vitamins. Essentially it depends on a species, habitat and type of feed, rearing and other factors.

Table 3 shows data on the concentrations of protein, carbohydrates, fat and ash, separately in yolk and white of eggs of the control and experimental groups.

While evaluating the quality of eggs in this experiments, it was found that the protein content in yolk and white of eggs laid by hens receiving the V+FM diet was higher than in the control group by about 2-3% (groups D and E). The amount of protein in eggs of the experimental groups showed a significant increase of 0.5-1.0 % in groups B, C and D, and the highest differences were observed between the control and group E (2-3%, yolk and white). The lipid percentage in yolk was observed to be 31.1% in the control group and 30.3%, 29.8%, 34.5 and 35.8% in groups B, C, D and E, respectively, meaning that it and increased by 3.4% and 4.7% in groups D and E, respectively, compared to the control. The moisture content was significantly lower in all the experimental groups than the moisture content of the control yolk and white. Conversely, the carbohydrates in yolk and white of hens from groups D and E were the highest. The dry matter in yolk from the experimental groups was practically on the same level (45.2% in the control group and 45.5%w in the first test group).

Thus, the analysis of the chemical composition and nutritional value of eggs from hens supplemented with mineral and fish meal additives in diets showed the positive influence on some eggs quality indicators. A higher content of protein, more carbohydrates and a higher energy value of eggs were observed. This allowed us to conclude that the observed high nutritional value sugests that the tested feed additive could be used in the production of high quality poultry products.

Fatty acid composition of egg yolk

Many authors have studied effects of different fat sources in broilerss nutrition on the concentration of fatty acid, mainly PUFA, in chicken meat (SANZ et al. 1999; CRESPO, ESTEVE-GARCIA 2001, CASTELLINI et al. 2002). However, there are no reports on the effect of a mineral diet of hens on FA levels in eggs, and this study has shown that vermiculite supplementation has an influence on the fatty acid level in egg yolk. Hens are able to deposit dietary lipids into the egg yolk and to modify the fatty acid (FA) composition of the egg (CASTELLINI et al. 2002). However, the eggs of hens provided the standard

Table 3

Composition and nutritional value of eggs of lying hens fed different levels of vermiculite

Indicators	A		B		C		D		E	
	yolk	white	yolk	white	yolk	white	yolk	white	yolk	white
Moisture (%)	54.84±0.54	87.13±0.32	54.53±0.44	87.17±0.35	53.955±0.51	86.56±0.74	54.13±0.74	86.35±0.67	54.37±0.44	86.13±0.34
Dry matter (%)	45.27±0.544	12.90±0.54	45.58±0.54	12.97±0.52	46.15±0.56	13.53±0.38	45.98±0.33	13.78±0.87	45.76±0.45	13.95±43
Protein (%)	15.21±0.32	9.94±0.43	14.86±0.44	10.14±0.26	14.95±0.65	9.95±0.77	16.82±0.43	11.25±0.56	17.28±0.65	12.13±0.66
Lipids (%)	31.15±0.54	0.26±0.26	30.38±0.55	0.27±0.09	29.88±0.54	0.13±0.11	34.55±0.23	0.36±0.10	35.87±0.34	0.38±0.08
Carbo-hydrates (%)	0.66±0.21	0.55±0.22	0.54±0.12	0.4±0.13	0.52±0.16	0.54±0.21	0.7±0.22	0.77±0.19	0.78±0.21	0.89±0.19
Energy value (kcal 100 g^{-1})	344.0	43.9	334	44.2	330.0	43.1	381.0	50.9	394.0	54.9

feed were poor in linolenic acid (LNA; 18:3 *n*-3) and did not contain eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6 *n*-3) fatty acids. Many studies on laying hens have been conducted to enrich eggs with *n*-3 polyunsaturated FA (PUFA) because of their health improving effects on humans (SANZ et al. 1999, MAZALLI et al. 2004, LAWLOR et al. 2010). Less research has been performed regarding the enrichment of broiler eggs. However, it is hypothesized that these *n*-3 PUFA can provide similar health betterment effects on the broiler offspring. Increased consumption of *n*-3 PUFA should be beneficial for the cardiac function, brain development, mental health and prevention of cancer, autoimmune diseases or diabetes (SANZ et al. 2010).

Dietary mineral plus protein sources had a significant effect on the FA profile in yolk (Table 4). Feeding with supplementing diet led to an increase in total FA content, a little changing of level of saturated fatty acids (SFA), increasing dietary mono (MUFA) and polyunsaturated fatty acids (PUFA). Regardless of V+FM inclusion levels or combination, contributed to a significant increase in the concentrations of C18:2 *n*-6 and C18:3 *n*-3 fatty acids and total PUFAs in yolk lipids.

The SFA: palmitic, arachidic acid decreased about 0.1-0.3% while margaric, stearic, docosanoic increased about 0.2×0.5% in group E. Addition of vermiculite increased clearly the level of myristic (C14:0) acid in yolk eggs in group B (0.54%). A slight increase of stearic acid was observed when 5% V was added. No significant difference in palmitic acid content was observed compared with control. When 3% and 5% of V+FM were added, the percentage of 18:1 oleic acid increased (near two times in groups D and E). There is no significant increase of 16:1 palmitoleic acid percentage. Similar results were reported by CRESPO and ESTEVE-GARCI´A (2001). Our data shows that the addition of a natural mineral with fish meal significantly increased in yolk eggs the level of total *n*-3 fatty acids (Table 4), with this response being primarily related to higher levels of linolenic acid. Similar results were also reported by SOUZA et al. (2008) using fed diets containing microencapsulated fish oil. Fish oil is one of the best known sources of *n*-3 PUFA, as it is rich in EPA and DHA (MACLEAN et al. 2006). Especially, it is important to note that PUFAs (linoleic acid and alpha-linolenic acid) have displayed protection against lipid per oxidation increasing the levels of several cellular antioxidants such as ascorbic acid, α-tocopherol (CACHALDORA et al. 2008). The linoleic acid significantly increased over tree and four times ore in groups C, D and E than in control and B group, and gamma-linolenic seven times higher in group E, which represented a great contribution to the sum of PUFA. Most *n*-6 polygene fatty acids were noted in the yolk eggs hens fed with the V+FM feed. However, this content was higher in all the experimental groups in comparison to the control group. The results of current study indicated that the FA profile in yolk eggs is customized by a diet with mineral and fishmeal. Feeding with different kinds of diets containing 3% and 5% ratios of vermiculite and fishmeal can be possible to apply in future which was proven

Table 4

Fatty acid composition of egg yolk (control and experimental groups, g 100 g^{-1})

Fatty acid composition (g 100 g^{-1})	Groups				
	A	B	C	D	E
Myristic acid; $C_{14:0}$	0.38±0.07	0.54±0.06	0.22±0.07	0.22±0.04	0.27±0.07
Pentadecylic acid; $C_{15:0}$	0.08 ±0.02	0.07±0.01	0.07±0.02	0.09±0.01	0.08±0.02
iso-Pentadecylic acid; $C_{15:0\ iso}$	n.d	n.d	n.d	0.01±0.00	0.01±0.00
aiso-Pentadecylic acid; $C_{15:0\ aiso}$	n.d	n.d	n.d	0.01±0.00	0.01±0.00
iso-Hexadecanoic acid; $C_{16:0\ iso}$	n.d	n.d	n.d	0.03±0.01	0.01±0.00
Palmitic acid; $C_{16:0}$	27.23±0.87	23.42±0.72	23.03±1.02	26.19±0.97	26.97±1.64
Margaric acid; $C_{17:0}$	0.29±0.04	0.28±0.05	0.28±0.03	0.30±0.09	0.34±0.05
Stearic acid; $C_{18:0}$	10.86±0.34	10.2±0.28	9.49±0.14	11.86±0.64	11.06±0.12
Arachidic acid; $C_{20:0}$	0.06±0.01	0.06±0.01	0.06±0.02	0.04±0.01	0.04±0.01
Behenic acid; $C_{22:0}$	0.05±0.01	0.03±0.01	0.04±0.01	0.10±0.02	0.10±0.03
Myristoleic acis; $C_{14:1}$ *n*-5	0.02 ± 0.00	0.01±0.00	0.01±0.00	0.09±0.02	0.04±0.01
Palmitoleic acid; $C_{16:1}$ *n*-7	0.06±0.01	0.06±0.02	0.05±0.01	0.09 ± 0.01	0.04±0.01
Heptadecenic acid; $C_{17:1}$	0.11±0.03	0.11±0.02	0.10±0.02	0.14±0.03	0.16±0.04
$C_{18:1}$ t 6,9,10,11,12	0.11±0.02	0.07±0.01	0.07±0.01	0.44±0.09	0.25±0.03
Oleic acid; $C_{18:1}$ c9	25.87±0.75	28.39±0.68	26.24±0.28	41.37±1.24	42.46±2.61
Asclepic acid; $C_{18:1}$ c11	1.89±0.23	1.89±0.15	1.90±0.11	2.38±0.21	2.39±0.21
C18:1 c13	0.14±0.07	0.12±0.04	0.11±0.03	0.13±0.05	0.14±0.04
C18:1 t 16	n.d	n.d	n.d	0.09±0.02	0.08±0.02
Eicosenoic acid; $C_{20:1}$ *n*-9	0.21±0.06	0.18±0.03	0.20±0.02	0.13±0.02	0.34±0.07
Conjugated linoleic acid; $C_{18:2}$ t13c9	0.01±0.00	0.01±0.00	0.01±0.00	0.06±0.01	0.02±0.01
Conjugated linoleic acid; $C_{18:2}$ t12c9	0.04±0.02	0.03±0.01	0.03±0.01	0.05±0.01	0.05±0.01
Conjugated linoleic acid; $C_{18:2}$ t11c15	0.01±0.01	0.01±0.00	n.d	0.03±0.01	0.02±0.01
Linoleic acid; $C_{18:2}$ *n*-6	8.65±0.65	8.76±0.36	25.69±0.79	28.40±1.78	31.55±1.54
α-linolenic acid; $C_{18:3}$ *n*-3	0.37±0.09	0.33±0.04	1.82±0.48	1.98±0.34	2.41±0.24
Conjugated linoleic acid; $C_{18:2}$ c9 t11	n.d	n.d	n.d	0.07±0.01	0.07±0.01
Eicosadienoic acid; $C_{20:2}$ *n*-6	0.11±0.03	0.33±0.07	0.10±0.02	0.33±0.06	0.46±0.07
Arachidonic acid; $C_{20:4}$ *n*-6	1.92±0.12	1.85±0.14	1.83±0.03	2.14±0.05	3.51±0.11
Docosapentaenoic acid; $C_{22:5}$ *n*-3	0.28±0.08	0.15±0.04	0.15±0.02	0.61±0.05	0.93±0.9
Docosahexanenoic acid; $C_{22:6}$ *n*-3	1.05±0.11	1.11±0.09	1.07±0.07	1.15±0.07	1.51±0.08
Total SFA	38.95	34.61	33.19	38.85	38.89
Total MUFA	28.41	30.83	28.68	44.86	45.90
Total PUFA	12.44	12.58	30.70	34.82	40.53

n.d – not detected

by these results including the egg's yolk level of specific fatty acid or mixture of fatty acids thought to be beneficial to human health (e.g. oleic acid).

Influence of vermiculite as a feed additive on haematological and biochemical blood parameters of laying hens

At the end of the experiment, blood samples were collected from 5 groups of hens. They were taken from randomly chosen birds, from the brachial vein, and then transferred to heparinized tubes and placed on ice. The haemoglobin concentration (HGB), red blood cells (RBC) and white blood cells (WBC) were determined. Haematological testing is one of the methods that can help detect certain changes in health, which may not be apparent from a physical examination but which can affect, for example, the condition of the birds (Lawlor et al. 2010). Haematologic studies include determination of the haematocrit value (HCT), which indicates the ratio between the volume of plasma and blood cells. The value of haematocrit in all the studied groups agreed with the physiological standards.

As shown in Table 5, the variation in the haematocrit mean value was not statistically significant, and it equalled 32.6% and 32.1% for hens given

Table 5

Blood haematological and biochemical profile of laying hens

Indicators	Groups				
	A	B	C	D	E
HGB (g l^{-1})	87.0±1.41^{a}	92.1±2.01	101.2±1.40	96.0±1.43^{d}	110.5±0.11
HCT (%)	29.7±0.82^{d}	28.6±1.15	32.1±0.16	31.7±0.61^{d}	34.7±0.21^{b}
RBC · 10^{12} l^{-1}	4.9±0.43^{b}	4.1±0.15	5.02±1.22^{d}	4.29±0.13^{c}	4.21±0.44
WBC · 10^{9} l^{-1}	6.5±1.36^{b}	12.5±1.22^{a}	7.3±0.46^{a}	6.4±0.61^{a}	5.2±0.23^{b}
Calcium (mmol l^{-1})	1.7±0.32^{a}	1.81±0.15^{a}	2.78±0.51	1.82±0.62^{a}	1.81±0.4^{b}
Phosphorus (mg l^{-1})	1.5±0.21^{b}	2.4±0.23	1.54±0.42	1.55±0.34^{b}	1.48±0.14
Protein (g l^{-1})	35.8±0.42^{c}	37.9±0.42	36.7±0.31	38.4±0.27^{a}	42.1±0.61^{d}

$^{a-d}$ Means in the same raw with different superscripts are significantly different at $p < 0.05$.

diets with 3% and 5% V, and 31.7% and 34.7% for groups D and E, respectively. Haematocrit (HCT) of laying hens fed with various doses of minerals was significantly higher in all the experimental groups than in the control (29.7%), except for group B (28.6%). The highest increase in HCT, i.e. by 5% and 3%, was observed in group E. The amount of haemoglobin (HGB) is an indirect indicator of the body's iron saturation. Low haemoglobin was determind in the control group, where hens received only standard feed. Haemoglobin showed oscillations in the mean value ranging from a minimum of 92.1 g l^{-1} for the laying hens in group B to a maximum of 110.5 g l^{-1} for group E hens, with statistically significant differences. The highest concentration increase was demonstrated in group E hens (110.5 g l^{-1}). The count of red blood cells (WBC) in groups B, D and E was lower than in the control. The mean values ranged from $4.12 \cdot 10^{12}$ l^{-1} (B) to $5.02 \cdot 10^{12}$ l^{-1} (C). Data referring to the leukocyte (WBC) parameters, as presented in Table 4, showed statistically very significant oscillations of the total leukocyte counts in laying hens, in the range of $6.4 \cdot 10^{9}$ l^{-1} (D) to $12.5 \cdot 10^{9}$ l^{-1} (3% V), while in the control group it equalled $6.5 \cdot 10^{9}$ l^{-1}. When using natural feed additives or their combination with fishmeal in all the experimental groups, the amount of protein increased comparing to the control. This was associated with more rapid metabolism, which was also confirmed by the higher productivity of hens. The values of the total protein content in most of the birds were within the range of 35-42 g l^{-1} (Nagao et al. 2005). The hens from group E had a higher protein concentration (42 g l^{-1}) than the control (35 g l^{-1}). The increase in calcium was observed in the all the experimental groups of hens, in general 40% higher than in the control. This was associated with the highest ion-exchange activity of vermiculite. The highest increase in the concentration of calcium relative to control group (1.75 mmol l^{-1}) was shown by group C hens (2.78 mmol l^{-1}). The amount of phosphorus in the serum of eggs produced by laying hens from group B (2.37 mg l^{-1}) was slightly higher than in the control (1.52 mg l^{-1}), with the exception of the group of hens fed 5% V (1.48 mg l^{-1}). The content of protein, WBC, HGB and the amount of calcium were higher in the hens from the group fed with the combination of V+FM.

CONCLUSIONS

The study of the chemical composition and nutritional value of eggs from hens fed diets supplemented with mineral and fishmeal additives has shown the positive influence on egg quality indicators and morphometric parameters. A higher content of protein, carbohydrates and energy value of eggs was observed. The dietary supplementation had a significant effect on the fatty acid profile of amino acids in the egg yolk. Feeding hens with a diet supplemented with minerals and proteins led to an increase in the content of total fatty acids and dietary mono (MUFA) and polyunsaturated fatty acids (PUFA). Vermiculite contributed to a significant increase in the concentrations of C18:2 *n*-6 and C18:3 *n*-3 fatty acids and total PUFAs in yolk lipids. The observed high mineral nutritional value substantiates the conclusion that vermiculite could be used as a feed additive in the production of high quality poultry products. For the future of the poultry industry, especially in developing countries, this novel knowledge on vermiculite as a feed additive is very important for better understanding how to improve properties and quality of eggs and ensure consumers' better health and safety.

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