# Effect of a Dietary Herbal Mixture Supplement on the Growth Performance, Egg Production and Health Status in Chickens

# Влияние на билкова комбинация върху растежа, яйчната продуктивност и здравния статус при кокошки

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## Abstract

The experiment was carried out with dual-purpose fowls developed for rural backyard farming – indigenous Katunitsa chicken (line "AN"), in a free range management system from hatching to 52 weeks of age. The purpose of the investigation was to establish the influence of a blend of herbs as a dietary supplement on some serum biochemical parameters (alanine aminotransferase, aspartate aminotransferase, total cholesterol, gamma-glutamyl transferase, triglycerides and creatinine), growth performance, egg production, egg fatty acid composition, and health status. The composition of this blend of herbs included: 0.05% garlic powder (*Allium sativum*), 0.3% cinnamon powder (*Cinnamomum verum)* and 0.03% of each of the following dried herbs: yarrow (*Achillea millefolium),* rosemary (*Rosmarinus officinalis)*, thyme (*Thymus serpyllum)*, basil (*Ocimum basilicum)* and oregano (*Origanum vulgare).*

The birds were divided into two groups – control (n=60) without supplemental mixture to the diet and experimental (n=700) with supplemental mixture to the diet from the first day of age to the end of the experiment.

The results showed that the herbal mixture supplement decreased significantly the blood serum cholesterol and triglyceride levels – at 7 (P<0.001) and at 52 weeks (P<0.05) of age.

The content of phospholipids, triglycerides and linoleic acid in the egg yolk was higher in the experimental group compared to the control group (P<0.05). There was no significant difference in the egg yolk cholesterol levels between both groups (P>0.05).

Over the entire investigation period the chickens from the experimental group had a better feed conversion ratio (with 3.37%) and a higher egg laying capacity (with 1.79%).
At the end of experiment (52 weeks of age), hens from the control group exhibited generalised fatty degeneration of liver parenchyma, while these in experimental group, the major part of hepatocytes had no fat droplets in their cytoplasm.
The death rate from 0 to 7, from 8 to 21 and from 22 to 52 weeks of age was 18.33%, 0% and 8.57% in the control group and 1.00%, 0.26% and 2.62% in the experimental group, respectively.
Therefore in conclusion, it can be affirmed that the use of this blend of herbs had a beneficial effect in the treated fowls and improved their egg productivity, vitality and health condition.

**Keywords:** blood biochemical parameters, chickens, dried herbs, egg production, garlic, growth performance, mortality

## Резюме

Проучването е проведено с кокошки от общоползувателно направление, предназначени за свободно отглеждане във фамилни ферми – Катунска кокошка, (линия "AN") от излюпване на пилетата до 52- седмична възраст. Целта е да се установи влиянието на билкова смес използувана като добавка в комбинирания фураж върху някои серумни биохимични показатели (аланин аминотрансфераза, аспартат аминотрансфераза, общ холестерол, гамаглутамилтрансфераза, триглицериди и креатинин), растежа, носливостта, мастнокиселинния състав на яйчния жълтък и здравния статус. Съставът на билкова смес е: 0,05% чесън на прах, 0.3% канела *(Cinnamomum verum)* и по 0,03% от следните изсушени билки: бял равнец *(Achillea Millefolium),* розмарин *(Rosmarinus Officinalis),* мащерка *(Thymus serpyllum)*, босилек *(Ocimum Basilicum),* риган *(Origanum vulgare).*
Бяха сформирани две групи – контролна (n=60) без добавка на билковата смес и експериментална (n=700) с добавка на билковата смес към комбинирания фураж от еднодневна възраст до края на опита.
Резултатите показаха, че билковата добавка намалява значително нивата на холестерола и на триглицеридите в кръвния серум на птиците – на 7 ($P<0.001$ ) и на 52 седмична възраст ($P<0.05$).
Яйчният жълтък е с по-високо съдържание на фосфолипиди, триглицериди и линолова мастна киселина при птиците от втора спрямо тези от първа група ($P<0.05$). Не се установи доказана разлика в нивата на холестерола на яйчния жълтък между двете групи ($P>0.05$).
За целия период на проучването конверсията на фураж беше по-добра с 3,37 % и носливостта беше по-висока с 1.79 % при птиците от втора група.
В края на експеримента (52-седмична възраст) при кокошките от контролната група се установи обща мастна дегенерация на чернодробния паренхим, докато при тези в експерименталната липсва.
Смъртността от 0 до 7, от 8 до 21 и от 22 до 52 седмична възраст беше 18.33%, 0% и 8,57 % в първа група и 1%, 0,26 % и 2,62 % във втора група.

В заключение може да се твърди, че използваната комбинация от чесън и билки имаше добро влияние върху третираните птици и подобрява тяхната яйчна продуктивност, жизненост и здравословно състояние.

**Ключови думи:** билки, биохимични параметри на кръвта, кокошки, носливост, смъртност, чесън

## Introduction

One of the primary priorities of poultry science and practice is the quest for alternative nutritional supplements aimed at ensuring optimal health, amount and quality of produce. Since ancient times, herbs and a number of spices containing biologically active substances are used under the form of drug.

The use of herbs and herbal extracts as poultry feed ingredients is continuously increasing after the ban of nutritive antibiotics in the European Union (Regulation 1831/2003/EC). Their positive effect is demonstrated mainly on growth and egg productive performance, quality egg traits, differencial blood count and immunity response (Dorhoi et al., 2006; Canogullari et al., 2009, 2010; Ghasemi et al., 2010; Mohebbifar and Torki, 2010; Navid et al. 2014). There are many evidence to suggest that they may have beneficial influence on useful gut microflora altogether with inhibitory effects on pathogenic and conditionally pathogenic bacteria – clostridia, colibacteria, salmonellae etc. (Dorman and Deans, 2000;  Griggs and Jacob, 2005; Bölükbaşi and. Erhan, 2007; Windisch et al., 2008; Frankič et al., 2009; Khan et al., 2012).

The purpose of the present study was to investigate the effect of the combination of 0.05% garlic powder, 0.03% cinnamon powder and five dried herbs − yarrow, rosemary, thyme, basil and oregano in an equal proportion (0.03% for each herb) on the growth performance, egg laying capacity, serum biochemical parameters, fatty acids in the egg yolk and the viability of chickens reared in free range management system.

## Materials and Methods

### Birds, housing and feeding

The experiment was conducted in a poultry farm located in the village of Katunitsa near Plovdiv, with dual-purpose fowls with colour plumage developed for rural backyard farming – indigenous Katunitsa chicken breed (line “AN”) from hatching to 52 weeks of age (11 June 2008 – 11 June 2009) – Figure 1.

The birds were reared in a free range management system on deep litter under natural conditions of light and temperature, in a poultry shed with three sides, with an option for closing during the cold period of the year. The shed was supplied with a walking yard divided into sections, allowing the birds to exhibit their natural behaviour – cleaning, digging, dust bathing. The rearing conditions for the experimental hens were fully compliant with minimum requirements for humane treatment of breeder hens (Regulation 25/2006; Regulation 44/2006).

Except for the routine vaccinations, birds did not receive any medications used in conventional poultry farming for either prevention or treatment.

For the purposes of the experiment, two groups were formed: first group (control) with 60 one-day-old chickens and second group (experimental) – with 700 one-day-old chickens. The only difference between both groups was that the diet for the experimental group included a dietary phyto supplement containing 0.05% garlic powder, 0.03% cinnamon (*Cinnamomum verum)* and dried herbs: yarrow (*Achillea millefolium)*, rosemary (*Rosmarinus officinalis)*, thyme (*Thymus serpyllum)*, basil (*Ocimum basilicum)* and oregano (*Origanum vulgare L.)* in an equal proportion (0.03% of each herb) from the one day of age to the end of the experiment. The diets were calculated by licensed software Komfu 2000 of Exbit® Ltd and prepared at the farm (Table 1). Until 42 weeks of age, the birds were fed *ad libitum,* and then the daily ration was limited to 150 g in order to restrict the increase in body weight. Feed consumption was measured at the day of weighing of birds.

**Growth performance**

Live weight (g) of chickens was measured on electronic balance Taurus to the nearest 1 g on a random sample - 50% of control and 8% of experimental groups, on a weekly basis up to 8 weeks of age and at 2-week intervals from 8 to 24 weeks of age, as well as at 52 weeks of age. From 4 weeks of age, the live weight was presented separately for each gender. Slaughter analysis was performed on 6 chickens from each of groups at the end of the experiment.

**Egg productivity**

The eggs were gathered daily and weighed on electronic balance Ohaus-2000 with a precision of ±0.01 g once weekly.

**Determination of lipid content, fatty acid composition and cholesterol in egg yolk**

The lipid content analysis was performed in the Institute of Biophysics and Biomedical Engineering, BAS - Sofia. Total yolk lipids were extracted as per Bligh and Dyer (1959), and total phospholipids – by the method of Kahovkova and Odavic (1969).

The fatty acid compositions of phospholipids and triacylglycerols as well as cholesterol quantitation were conducted on a gas chromatograph Perichrom (France). As internal standard for quantitative determination of fatty acids, heptadecanoic acid (C17:0), and for cholesterol – cholestan, were used.

**Blood biochemical examination**

Blood for analysis (5 ml) was sampled from v. *Subcutanea ulnaris* of six birds from each group by the 7$^{th}$ and 52$^{nd}$ weeks of age in accordance with the Regulation No 15/2006.

The blood was allowed to clot for one hour at room temperature (25 °C) and the samples were centrifuged at 2000 g for 10 min.

Blood serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), gamma-glutamyl transferase (GGT), triglycerides (TG), total and HDL cholesterol, and creatinine – were determined with an automated biochemical analyzer Cobas mira at an accredited biochemical lab in the Diagnostic and Consultation Medical Centre “St. George”- Plovdiv.

**Histological examination**

The material for the histological study – pieces of about 1 $cm^3$ in size was obtained from different parts of the intestines - ileum (from the middle part) and caeca (from the middle of the left caeca body) and the liver immediately after the slaughtering of six hens from each group in accordance with the Regulation No 15 (2006). The samples were immediately placed into 10% neutral formalin (Fluca, code No 47630). After fixation, the samples were washed with running water, dehydrated in an alcohol series (Valerus, code No 1065), cleared in xylene (Aldrich, code No X104-0), and embedded in paraffin (Fluka, code No 33509). The formed blocks were cut on a paraffin microtome Reichert. The 6-µm cuts were stained with haematoxylin and eosin. The observation, micromorphometric examination and photographing were done on a Hund microscope. The metric study of the preparations was performed with a standardized eyepiece-micrometer.

**Statistical analyses**

Results are expressed as a mean and standard error. Data were subjected to one-way analysis of variance (ANOVA) using GraphPad InStat 3.06 software to determine the level of significance among mean values.

Figure 1. Fowls during the egg-laying period (original)

Table 1. Composition of diets (in %)

| Ingredients | Weeks of age | | | | |
|---|---|---|---|---|---|
| | 1-3 | 4-7 | 8-18 | 19-42 | 43-64 |
| Corn | 33.49 | 27.93 | 31.14 | 47.48 | 30.61 |
| Wheat | 35 | 40 | 35 | 20 | 45 |
| Soybean meal (44% CP) | 18 | 20 | 12 | - | - |
| Sunflower meal (34% CP) | 6 | 6 | 15 | 20 | 12 |
| Lucerne meal | 1 | 1.5 | 3 | 3 | 3 |
| L-lysine (99 % purity) | 0.26 | 0.28 | 0.27 | 0.32 | 0.29 |
| DL-methionine (99 % purity) | 0.16 | 0.17 | 0.12 | 0.04 | 0.05 |
| L- threonine | 0.11 | 0.13 | 0.1 | 0.04 | 0.04 |
| Sodium chloride | 0.3 | 0.3 | 0.25 | 0.27 | 0.27 |
| Limestone | 1.9 | 1.9 | 1.5 | 7.5 | 7.5 |
| Mono calcium phosphate | 1.13 | 1.12 | 1 | 0.75 | 0.66 |
| Ronozyme NP [(a)] | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Choline chloride 60% | 0.1 | 0.12 | 0.08 | 0.1 | 0.1 |
| Vit. E – 50% | 0.04 | 0.04 | 0.03 | 0.01 | 0.01 |
| Vit. C – 99.5% | 0.02 | 0.02 | 0.02 | - | - |
| Sel-Plex® | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 |
| Roxazyme® G2G [(b)] | 0.01 | 0.01 | 0.01 | - | - |
| Rovabio™ Excel AP [(c)] | - | - | - | 0.005 | 0.005 |
| Calculated analysis | | | | | |
| Metabolisable energy, (MJ/kg) | 12.1 | 12 | 12.0 | 11.7 | 11.8 |
| Crude protein, % | 19.1 | 18.9 | 18.3 | 14.3 | 13.1 |
| Crude fibre, % | 4.0 | 4.1 | 5.2 | 5.1 | 4.3 |
| Calcium, % | 1.07 | 1.07 | 1.00 | 2.88 | 2.84 |
| Phosphorus available, % | 0.48 | 0.48 | 0.45 | 0.36 | 0.37 |
| Lysine, % | 1.08 | 1.08 | 0.96 | 0.72 | 0.63 |
| Methionine +cystine, % | 0.79 | 0.79 | 0.78 | 0.60 | 0.55 |
| Threonine, % | 0.78 | 0.69 | 0.73 | 0.53 | 0.46 |
| Tryptophan, % | 0.22 | 0.22 | 0.21 | 0.16 | 0.14 |

Note: The same feed was fed to experimental birds, after supplementing each kilogram feed with 0.05% garlic powder and 0.03% of each of the following dried herbs: yarrow, oregano, thyme, basil, rosemary and cinnamon (total amount of the supplement 0.23 %, on the account of decreasing maize proportion by 0.23 %.

[(a)] Ronozyme® NP is a preparation of 6-phytase (EC No. 3.1.3.26) produced by a genetically modified strain of *Aspergillus oryzae* (DSM 17594) as produced by the applicant DSM Nutritional Products Ltd.

[(b)] Roxazyme® G2G contains an enzyme complex derived from *Trichoderma longibrachiatum,* whose main enzymatic activities are cellulose, xylanase and ß-glucanase.

[(c)] Rovabio™ Excel AP is a concentrated powder whose main enzymatic activities are xylanase and ß-glucanase obtained from a fermentation broth of non-genetically modified fungus *Penicillium funiculosum.*

## Results and Discussion

### Growth performance and feed consumption

The birds from both groups exhibited a high growth performance. By 12 weeks of age, they attained the appropriate slaughter live weight (Table 2). At the beginning of egg lay, the live weight of hens from both groups was over 3.00 kg, and that of roosters – over

Table 2. Growth performance (in grams). Data are presented as mean±SEM

| Age in weeks | Control group | | Experimental group | |
|---|---|---|---|---|
| | ♂ | ♀ | ♂ | ♀ |
| 0 | 43±2 | | 43±2 | |
| 1 | 86±3 | | 87±3 | |
| 2 | 165±6 | | 169±5 | |
| 3 | 281±6 | | 285±5 | |
| 4 | 645±36 | 535±17 | 642±23 | 523±10 |
| 5 | 748±35 | 635±21 | 744±32 | 632±23 |
| 6 | 995±36 | 820±24 | 991±35 | 810±23 |
| 7 | 1130±58 | 991±31 | 1150±48 | 933±25 |
| 8 | 1453±60 | 1151±30 | 1472±27 | 1076±28 |
| 9-10 | 1884±58 | 1490±36 | 1914±41 | 1412±26 |
| 11-12 | 2479±37 | 1737±54 | 2486±37 | 1828±20 |
| 13-14 | 3080±31 | 2163±38 | 3143±50 | 2193±41 |
| 15-16 | 3692±35 | 2449±61 | 3770±47 | 2498±47 |
| 17-18 | 4136±39 | 2719±91 | 4141±47 | 2717±64 |
| 19-20 | 4453±27 | 3050±113 | 4418±65 | 3289±73 |
| 21-22 | 4630±7 | 3127±52 | 4778±155 | 3294±60 |
| 23-24 | 4780±72 | 3352±121 | 4806±180 | 3433±54 |
| 52 | 4667±76 | 4042±85 | 4652±69 | 4219±67 |

4.40 kg. By the end of the study (52 weeks of age), the live weight of hens from the experimental group was higher than that of controls by 4.38%, but the difference was not significant (n.s.). From 4 to 52 week of age the differences were significant ($P<0.05$) between females and males in the respective group. In the corresponding ages between the females in both groups and between the males respectively there were no significant differences.

Herbs have a specific taste and flavour which is passed to feed (Loo and Richard, 1992; Frankič et al., 2009). Previous studies with weaned pigs (Jugl-Chizzola et al., 2006) have shown that the specific flavour of garlic and herbs' combination in compound feed resulted in lower consumption. These effects were not confirmed in our experiment. At 12 weeks of age, when the chickens were fit for slaughter, the feed intake was almost equal. The birds from the experimental group consumed by 1.66% less feed and at the same time, the live weight of females was by 5.24% higher. No significant difference in the live weight of male chickens was established.

For the entire experimental period, the total compound feed intake by bird in the experimental group (44.244 kg) was with 3.36% lower than that of control group (45.787 kg). Until 42 weeks of age, when birds from both groups received feed *ad libitum*, the total feed intake of experimental birds was by 4.37% lower. In our belief, this was due to stimulation of nutrients' absorption by active compounds of herbs and their beneficial effect on gut microflora. On one side, the beneficial effect could be owed to appetite stimulating effect of cinnamon (Loo and Richard, 1992; Frankič et al., 2009), and on the other, some authors have found that oregano and its essential oil possessed a growth-stimulating effect due to improved digestion, absorption and utilisation of nutrients in the stomach and the jejunum in chickens and turkeys (Jamroz et al., 2003; Lee et al., 2003; Bampidis et al., 2005; Jamroz et al., 2006; Mikulski et al., 2008). According to Gálik et al. (2013, 2014) in laying hens nutrition, feed additives stimulate the digestive enzymes, improve feed conversion ratio, their egg production and also nutrient composition of eggs.

The garlic and herbs supplemented to compound feed had also antibacterial and antifungal activity against gastrointestinal pathogens and thus, inhibited the growth and development of enteropathogenic strains of *E. coli*, *Salmonella* spp. etc. (Pasqua et al., 2006; Windisch et al., 2008; Frankič et al. 2009). The used herbs and their active principles alter the structure of cell membranes of enteropathogenic strains, causing ion leakage and death (Windisch et al., 2008).

According to Castillo et al. (2006) the aldehyde carvacrol present in cinnamon improves the growth of lactobacilli, thus increasing the share of useful intestinal microflora. Furthermore garlic supplementation improved feed conversion ratio, body weight, carcass characteristics, as well as survivability in poultry (Javed et al., 2009; Kumar et al., 2010; Mohebbifar and Torki, 2011). All this contributes for the better nutrients utilisation and improved growth and development of chickens.

The beneficial influence of the herbal mixture on poultry growth and the better feed conversion ration could be also explained with the antioxidant activity of carvacrol, thymol, cineol and pinene – components of thyme, oregano and rosemary (Faleiro et al., 2005; Hazzit et al., 2006).

Most probably, the better feed conversion could result from improved enzyme activity in the alimentary tract, stimulation of useful and inhibition of pathogenic microflora. All these events result in improved absorption and utilisation of nutrients (Pasqua et al., 2006; Castillo et al., 2006; Windisch et al., 2008; Frankič et al., 2009).

The slaughter analysis performed at 52 weeks of age showed no differences between both groups (Table 3).

Table 3. Slaughter characteristic at 52 weeks of age (n=6 per group)

| Traits | Control group | Experimental group |
|---|---|---|
| Body weight, g | 3819±96 | 3943±109 |
| Carcass yield, g | 2809±90 | 2903±112 |
| % | 73.52±0.63 | 73.66±0.91 |
| Edible internal organs, g | 141.23±6,80 | 148.92.±9.95 |
| - heart | 12,50±0,72 | 12.33±1.90 |
| - spleen, g | 3,02±0,22 | 3.47±0.18 |
| - liver, g | 74.81±3.02 | 73,95±6.95 |
| - gizzard, g | 50.88±4.65 | 59,17±1,73 |

**Egg production**

The beginning of lay in control chickens occurred by 21 weeks of age (2 November 2008), whereas in experimental birds – in the beginning of the $20^{th}$ week of life (24 October 2008) i.e. the difference between groups was over one week. For the entire egg production period (32 weeks) the average egg production per experimental hen was 85.5 eggs with mean weight of 61.00±0.37 g or 5.22 kg egg mass per bird. The respective values for control birds were 84 eggs laid, with average weight 60.30±0.55 g and 5.065 kg egg mass, showing that the supplementation of the feed with the herbal combination has not influenced significantly the egg productivity.

After the beginning of lay, egg production increased slowly but at a steady rate regardless of the decrease in daily average temperatures from 13-14 ºC to values below zero by the 12th week of lay which was during the last third of January (Figure 2). The peak egg production was attained by 31-32 weeks of laying period, i.e. by the end of the experiment. The analysis showed that sharp temperature deviations in both directions had a particularly adverse effect. The average egg weight increased gradually in both groups from 42-43 g and by the end of the experiment attained 66-67 g. Morphometric studies for eggs' shape index showed values from 75.07 to 79.39%, except for the first three weeks in experimental chickens, i.e. the birds from the studied local population laid more rounded eggs. The results showed clearly that in the free range management system with free access to walking yards without maintenance of optimum microclimatic parameters except for lighting duration, ambient temperature was a very powerful environmental factor.

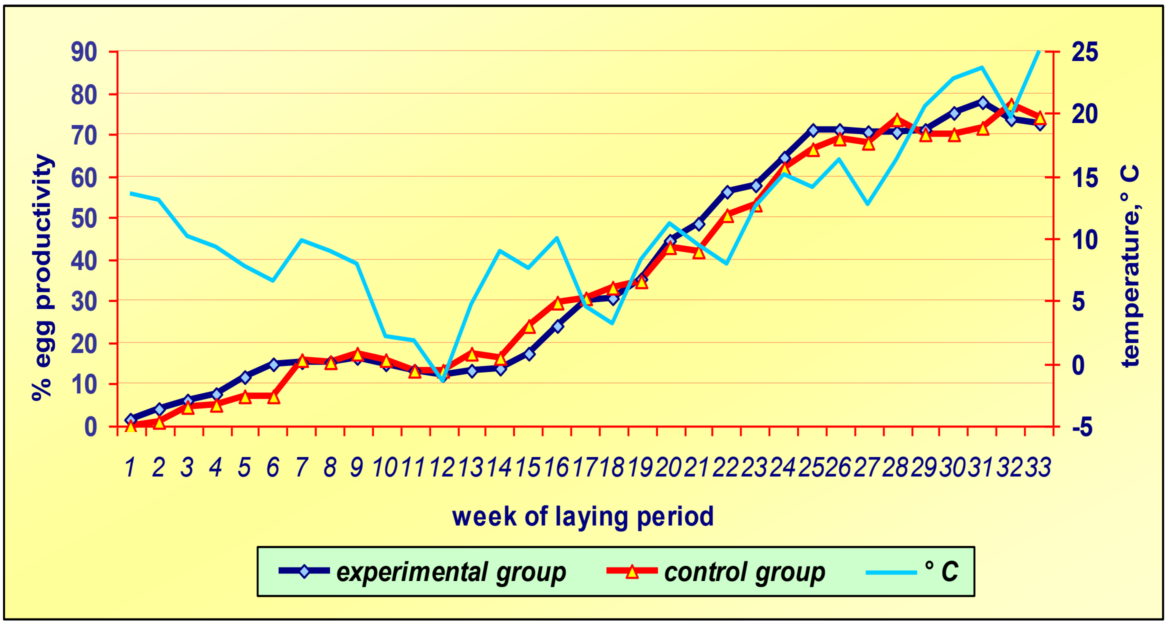



Figure 2. Dynamics of ambient temperature and egg production

**Lipid and fatty acid content of egg yolk**

The analysis of egg yolk lipid and fatty acid content showed that eggs produced by experimental hens had a higher phospholipid and triacylglycerol content – $P<0.05$ (Table 4). There were not substantial differences with regard to the total cholesterol content of egg yolk regardless of the findings of other researchers demonstrating that the serum and the egg yolk cholesterol concentrations decreased ($P<0.05$) with increasing levels of dietary garlic (Chowdhury et al., 2002, Khan et al. 2008; Połtowicz and Węśyk, 2006; Canogulari et al., 2010). Our results agreed with those of Mahmoud et al. (2006), who observed that the addition of garlic juice at 0.25, 0.50 and 1% did not change ($P>0.05$) yolk cholesterol concentration.

Table 4. Lipid content of egg yolk (n=6 per group)

| Lipids | Control group | Experimental group |
|---|---|---|
| Phospholipids, mg/g | 30.56±2.55 | 36.77±2.55 |
| Triacylglycerols, mmol/g | 142.34±12.15 | 170.11±15.71 |
| Total cholesterol, mg/g | 43.57±3.62 | 43.17±4.48 |

In our opinion, this could be attributed to the relatively constant amount of cholesterol in egg yolk, needed for the normal embryonic development, i.e. birds have physiological mechanisms for maintenance of egg yolk cholesterol. Despite the existing views about the adverse effect of foods with higher cholesterol content, this substance fulfills extremely important biological functions.

The fatty acid analysis of phospholipids and triacylglycerols showed no statistically significant differences with respect to ratio of saturated: monounsaturated: polyunsaturated fatty acids (Table 5). The more detailed analysis of fatty acid content showed that eggs laid by laying hens from experimental group contained more linolenic and docosahexaenoic fatty acids in phospholipids, more dihomo gamma-linolenic and arachidonic, as well as less docosamonoenic acid in triacylglycerols. All fatty acids, except for arachidonic, belong to the group of Ω 3-fatty acids.

Table 5. Fatty acid content in egg yolk, (in %)

| Fatty acid | | Phospholipids | | Triacylglycerols | |
|---|---|---|---|---|---|
| | | Control group | Experimental group | Control group | Experimental group |
| Myristic | C14:0 | 0.43±0.04 | 0.52±0.04 | 0.49±0.07 | 0.52±0.04 |
| Myristoleic | C14:1 | 0.09±0.02 | 0.09±0.01 | 0.11±0.03 | 0.10±0.01 |
| Palmitic | C16:0 | 28.03±0.98 | 28.81±0.54 | 28.02±1.53 | 27.65±0.63 |
| Palmitoleic | C16:1 | 4.58±0.33 | 4.53±0.22 | 5.44±0.38 | 5.07±0.27 |
| Stearic | C18:0 | 7.83±0.45 | 7.09±0.24 | 5.42±0.33 | 5.59±0.45 |
| Oleic | C18:1 | 42.63±1.56 | 42.46±1.15 | 49.13±1.70 | 49.33±1.38 |
| Linoleic | C18:2 | 11.31±0.43 | 11.59±1.46 | 9.91±0.55 | 10.25±1.36 |
| Linoelaidic | C18:2 L | 0.15±0.03 | 0.14±0.04 | - | - |
| Linolenic | C18:3 | 0.10±0.01 | 0.19±0.04 | 0.13±0.03 | 0.13±0.02 |
| Gadoleic | C20:1 | 0.27±0.03 | 0.23±0.03 | 0.16±0.06 | 0.25±0.04 |
| Dihomo-gamma-linolenic | C20:3 | 0.12±0.00 | 0.11±0.03 | 0.14±0.03 | 0.32±0.14 |
| Arachidonic | C20:4 | 2.56±0.20 | 2.36±0.10 | 0.24±0.06 | 0.33±0.03 |
| Eicosapentaenoic | C20:5 | 0.45±0.04 | 0.37±0.04 | 0.48±0.06 | 0.31±0.11 |
| Docosamonoenoic | C22:1 | - | - | 0.31±0.03 | 0.14±0.03 |
| Docosatetraenoic | C22:4 | 0.26±0.06 | 0.22±0.03 | - | - |
| Docosapentaenoic | C22:5 | 0.83±0.13 | 0.82±0.11 | - | - |
| Docosahexaenoic | C22:6 | 0.38±0.03 | 0.46±0.08 | - | - |
| Total | | | | | |
| Saturated fatty acids | SAT | 36.28±1.31 | 36.42±0.70 | 33.93±1.81 | 33.76±1.04 |
| Monounsaturated fatty acids | MONO | 47.56±1.23 | 47.31±1.02 | 55.16±1.36 | 54.89±1.25 |
| Polyunsaturated fatty acids | PUFA | 16.16±0.58 | 16.27±1.55 | 10.91±0.59 | 11.35±1.48 |

At present, dietologists pay a special attention to dietary Ω 3- and Ω 6-fatty acid contents, due to their essential functions in the body and the impossibility for mutual substitution. The optimal Ω 3/ Ω 6 ratio is 1:3-5 as the content of Ω 6-fatty acids in foods is higher. Inadequate nutrition results in ratios of 1:20-30, and during the last century, a dramatic increase in Ω 6-fatty acids dietary intake is observed on the account of lower Ω 3-fatty acids intake. Linolenic acid is of particular importance in human nutrition as precursor for prostaglandin E - coronary vasodilator (dilates coronary blood vessels, inhibits free fatty acids and plaque deposition in blood vessels). Unlike mammals, in which linolenic acid is easily converted into other fatty acids, its content in egg yolk of birds is higher and stable. Eggs laid by experimental chickens had a twice higher linolenic acid content in egg yolk phospholipids ($P<0.01$).

**Serum biochemical parameters**

The blood serum biochemical analysis performed in chickens and hens at 7 and 52 weeks of age, respectively, showed that average concentrations of triglycerides and total cholesterol were lower in birds from the experimental group, supplemented with the herbal mixture with feed compared to controls – $P<0.001$ in chickens and $P<0.01$ in hens for both parameters (Table 6). The results could be attributed to the complex antistress, antioxidant and antimicrobial effects of rosemary, thyme, oregano, yarrow, basil and particularly of garlic, which act towards reduction of cholesterol and triglyceride levels (Khan et al. 2008; Canogulari et al., 2010). Garlic has a hypolipidaemic, hypothrombotic and relaxing effect due to its allicin content. The other studied blood biochemical parameters did not exhibit any significant differences for the respective age. Similar findings were reported also by other researchers (Chowdhury et al., 2002; Khan et al. 2008; Połtowicz and Węśyk, 2009; Canogulari et al., 2010).

Table 6. Serum biochemical parameters of 7$^{th}$ and 52$^{d}$ weeks old chickens (n=6 per group)

| Biochemical parameters | 7$^{th}$ weeks of age | | 52$^{d}$ weeks of age | |
|---|---|---|---|---|
| | Control group | Experimental group | Control group | Experimental group |
| Aspartate aminotransferase (ASAT), U/L | 165.33±15.6 | 167.33±26.9 | 172.80±7.2 | 182.33±20.8 |
| Alanine aminotransferase (ALAT), U/L | 20.67±2.94 | 18.67±2.94 | no data | no data |
| Gamma-glutamyl transferase (GGT),U/L | 19.93±3.45 | 19.56±3.71 | 17.71±1.67 | 13.72±3.48 |
| Alkaline phosphatase (AP),U/L | 1518±776 | 1826±300 | no data | no data |
| Total protein (TP), g/L | 34.70±1.09 | 30.53±4.37 | 57.06±2.01 | 59.37±7.30 |
| Albumin (Alb), g/L | 15.23±1.39 | 12.50±1.04 | no data | no data |
| Triacylglycerols (TG), mmol/L | 0.48±0.08 a | 0.32±0.03 a | 1.99±0.13 b | 1.55±0.03 b |
| Total cholesterol, mmol/L | 2.66±0.16 a | 1.89±0.07 a | 4.75±0.28 b | 3.76±0.34 b |
| HDL cholesterol, mmol/L | 1.31±0.05 | 1.35±0.06 | no data | no data |
| Urea, mmol/L | 1.06±0.16 | 0.86±0.00 | no data | no data |
| Creatinine, µmol/L | 19.00±5.34 | 24.33±2.86 | 26.60±0.76 | 22.67±5.72 |

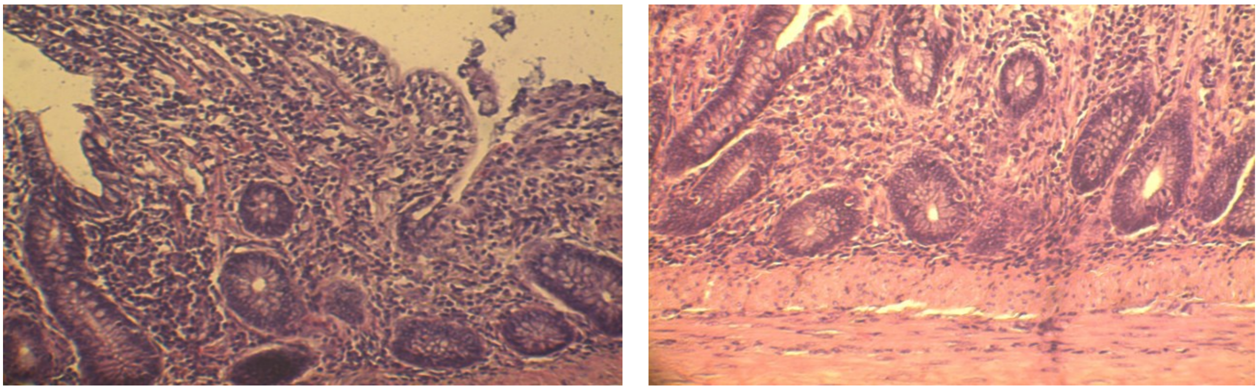
Note: a – $P<0.001$; b- $P<0.01$ within the same row

The comparison of blood biochemical results at both ages of sampling showed that blood serum triglycerides in non-supplemented controls increased 4.15 times in one-year-old hens vs 6-week-old chicks, whereas in experimental birds – 4.84 times. The respective increase in total serum cholesterol was 1.79 and 1.99 times, total serum proteins: 1.64 and 1.94 times. Although blood total cholesterol and triglycerides increased at a slightly higher extent in the experimental group, they remained lower compared to respective values in the control group. The other studied parameters have also changed but inconsistently and insignificantly – aspartate aminotransferase (ASAT) values increased and gamma-glutamyl transferase (GGT) tended to decrease.

**Histological evaluation of visceral organs**

The light microscopy study did not reveal any differences in the structure of studied organs between groups at 7 weeks of age. The histology of intestines and liver was normal for the species and age of birds.

At 52 weeks of age, control birds showed signs of inflammation of the ileal mucosa. This was manifested by desquamation of some areas of the lining epithelium and a heterogeneous cell mass within the intestinal lumen. In the mucosal propria, an extensive infiltration mainly of lymphocytes and monocytes was observed (Figure 3 a). In experimental birds such alterations were absent, but a larger proportion of goblet cells in Lieberkühn glands and the lining epithelium were present (Figure 3 b).



(a) (b)

Figure 3. Cross section through the ileal wall of 52-week-old chickens – (a) control group and (b) experimental group, H/E, 12.5x

The histological picture in control birds showed identical changes in the caecal wall – desquamation of large isolated areas of the lining epithelium into the intestinal lumen together with leukocytes. The intestinal propria exhibited a considerable extent of cell infiltration (Figure 4). These changes were not demonstrated in the experimental group.

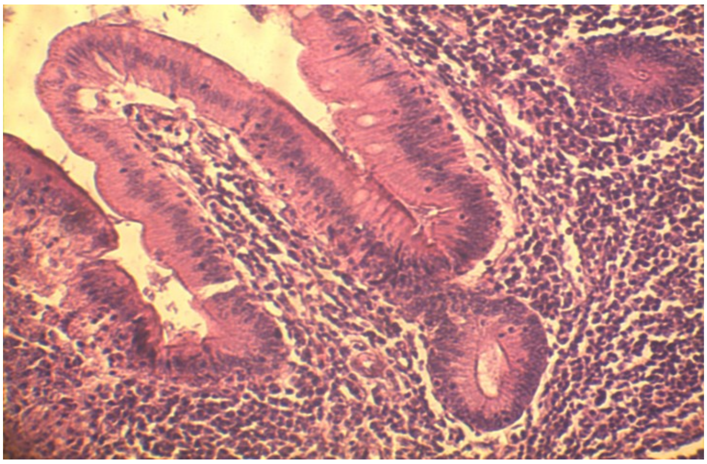
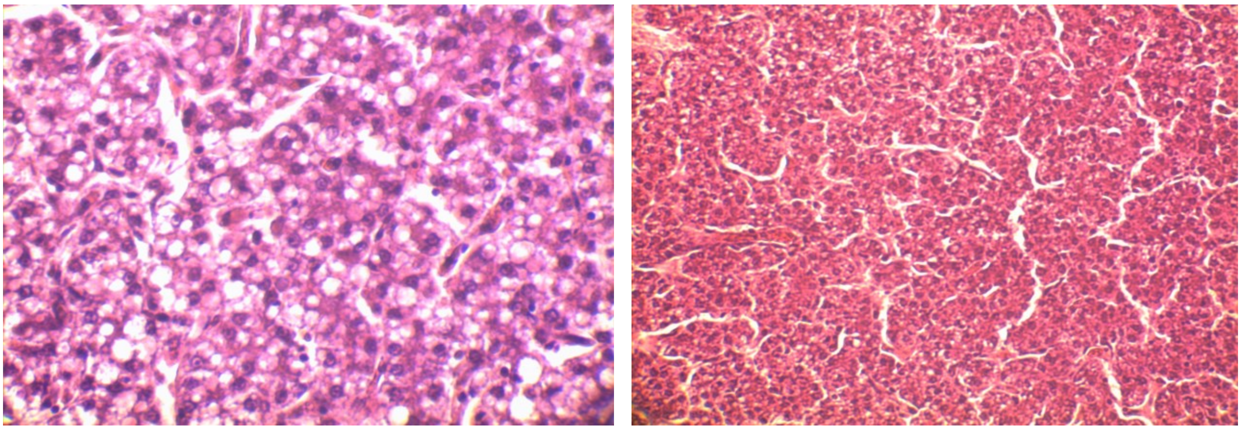


Figure 4. Cross section through the caecal wall
at 52 weeks of age – experimental group, H/E, 12.5x

At the age of 52 weeks, non-supplemented control chickens showed a generalised fatty degeneration of the liver parenchyma. Fat droplets of various sizes were present in the cytoplasm of all hepatocytes without concurrent degenerative changes in their nuclei (Figure 5 a). The hepatocytes of experimental birds did not exhibit cytoplasmic fat droplets. There were however single small fat droplets in separate glandular tubules in the cytoplasm (Figure 5 b).



(a) (b)

Figure 5. Liver of 52-week-old chickens – (a) control group and (b) experimental group, H/E, 12.5x

**Livability of birds**

The highest death rate in the control group of chickens was observed during the first seven weeks of life (Table 7). Afterwards, until the beginning of lay, death rate in controls was 3.27 times higher than in experimental birds. The analysis of the high death rate, described in other our article (Hristev et al., 2010) has shown that a primary cause for mortality up to 7 weeks of age was coccidiosis. Eimeriosis was also found out, which followed a protracted (chronic) course with single cases of watery, brownish faeces in experimental birds, which then recovered, unlike controls. Over the entire period of the trial, no bacterial or viral diseases were observed in both groups.

Table 7. Mortality of chickens, %

| Weeks of age | Control group | Experimental group |
|---|---|---|
| 0-7 | 18.33 | 1.00 |
| 8-21 | 0 | 0.26 |
| 22-52 | 8.57 | 2.62 |

In our opinion, the used herbal combination was efficient and with a complex influence for health promotion and coccidiosis prevention in birds.

The antiseptic properties of essential oils of these herbs separately or in combination with others exhibited their prophylactic effect through inhibition of secondary infections and promotion of normal intestinal microflora growth (Gahnyan-Mircheva, 2003; Arczewska and Świątkiewicz, 2013; Scheurer et al., 2013).

## Conclusion

For the duration of the experiment (from hatching to 52 weeks of age) the feed intake in chickens from experimental group was with 3.37% lower compared to control group.

The beginning of lay in experimental chickens was at 20 weeks of age, and in controls – at 21 weeks of age. The average egg production was 85.5 and 84.0 eggs respectively, i.e. a difference of 1.79 %.

The total blood serum cholesterol concentration in experimental chickens at 7 and 52 weeks of age was statistically significantly lower than respective values in controls. There were no differences in egg yolk cholesterol content between both groups.

Phospholipid and triacylglycerol content, as well as some unsaturated fatty acids in egg yolk (C18:3; C20:3) of experimental chickens was higher.

At 52 weeks of age, chickens from the control group exhibited generalised fatty degeneration of liver parenchyma, while in experimental chickens, the major part of hepatocytes had no fat droplets in their cytoplasm.

The death rate in control chickens until 7 weeks of age was significantly higher than in experimental ones – 18.33% vs 1%. It could be therefore affirmed that the tested combination of herbs and garlic had a beneficial and health promoting effect.

## Acknowledgments

Research was financed by the Ministry of Education and Science of the Republic of Bulgaria (Project N 18-08, University Fund for Scientific Research – Agricultural University, Plovdiv).

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