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## Productive Performance, Hatching Egg Quality and Health Indices of Hisex Brown Laying Hens Fed Extruded Grain Amaranth

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**Abstract:** The article presents results proving the feasibility of extruded amaranth grain (*Amaranthus cruentus*) in feeding breeding laying hens of the Hisex Brown cross (aged 25-45 weeks). Due to improvements in the quality indicators of hatching eggs, such as weight (2.09%;  $P<0.01$ ), Haugh units (1.07%;  $P<0.05$ ), and shell thickness strengthened (5.0  $\mu\text{m}$ ;  $P<0.05$ ) to 362  $\mu\text{m}$ , it was possible to increase egg laying intensity by 1.81%, hatching egg yield by 2.20%, and hatching of chickens by 1.33% with the addition of 5% extruded grain to the diet structure. In the test group, there was an 11.66% ( $P<0.05$ ) reduction in cholesterol in the yolk of eggs. The test group's hemoglobin concentration rose by 4.16% ( $P<0.05$ ) in comparison to the control group's blood, while the test group's lymphocyte and segmented neutrophil levels decreased by 1.65% ( $P<0.05$ ) and 1.93% ( $P<0.05$ ), respectively. These results demonstrated the high efficacy of the feed under investigation in preserving the immune status of breeding chickens during the first productivity phase. The chicken body exhibited a high level of antioxidant activity as evidenced by the rise in superoxide dismutase activity by 8.85% ( $P<0.05$ ), the total amount of antioxidants by 21.66% ( $P<0.01$ ), and the decrease in malonaldehyde by 13.52% ( $P<0.05$ ) in the test group. Analysis of the microbiome of the cecum in the colon revealed an increase in bacteria of *Bifidobacteriales* and *Lactobacillales* by 46.93 ( $P<0.01$ ) and 25.54% ( $P<0.01$ ), as well as a rise in Ruminococcaceae by 15.87% ( $P<0.01$ ), in the test group compared with the control group.

**Keywords:** Breeding poultry; Egg productivity; Extruded amaranth grain; Health of laying hens; Quality of hatching eggs.

### Introduction

Amaranth is one of the promising sources of protein and biologically active substances for

feeding animals and poultry (Ulbricht *et al.*, 2009). The use of amaranth grain in feeding

makes the feed more complete and balanced. Due to the high preservation of protein compounds, it can partially replace even corn and soybean (Fasuyi, 2006). However, the presence of anti-nutritional substances in this pseudocereal can reduce the availability and disrupt metabolism (Písařiková *et al.*, 2005; Fasuyi *et al.*, 2007). The anti-nutritional factors are the reason why raw amaranth grain is considered a growth inhibitory feed and limit its acceptability and use by birds and other monogastric animals (Martens *et al.*, 2012). At the same time, Jimenez-Aguilar & Grusak (2017), Peiretti *et al.* (2017), Jimoh *et al.* (2019), Karamać *et al.* (2019) found the antioxidant properties of *Amaranthus caudatus* grain due to its high content of phenolic compounds (including rutin and quercetin). *Amaranthus spinosus* has a pronounced hepatoprotective and immunostimulating effect. Strzelecka *et al.* (2005) note the anti-inflammatory properties of amaranth. For destruction of anti-nutritional factors, heat treatment (autoclaving or cooking under atmospheric conditions) of amaranth grain is necessary. However, Písařiková *et al.* (2005) demonstrated a higher level of *in vitro* protein digestibility in raw amaranth than in thermally processed grain and explained this with a decrease in the biological value of the protein that occurs at high temperatures. There is no research on the use of amaranth grain (*Amaranthus cruentus*, hybridus, and hypochondriacus) as a component of the diets of the parent flock of egg crosses, but it has been shown to be used in the feeding of broilers (Longato *et al.*, 2017) and laying hens (Króliczewska *et al.*, 2008; Popiela *et al.*, 2013; Janmohammadi *et al.*, 2023). This information prompted us to investigate the effects of feeding breeding hens of the Hisex Brown cross with extruded *Amaranthus cruentus* grain on the microbiome of the cecum, the immunological

and antioxidant status of the hens, and the productivity of layers' eggs.

## Materials & Methods

### Ethical aspects

Experiments were conducted in a manner that avoided unnecessary discomfort to the animals by the use of proper management and laboratory techniques. The study was conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (4th edition, 2020). The blood sampling and poultry handling were practiced in accordance with the ethical guidelines of the L.K. Ernst Federal Science Center for Animal Husbandry, the guidelines for keeping established in the farm and local poultry care laws and policies. The farm owner also provided consent for the use of eggs and layers in this study.

### Location, animals, facilities and experimental period

The investigation was conducted in the poultry breeding farm, located in Volgograd region of the Russian Federation (48°28'50"N, 44°46'13"E, altitude of 17 m) on chickens of the parent flock of the Hisex brown cross, 25 weeks of age. The duration of the experiment was 20 weeks until the end of the first phase of the productive period (45 weeks). The birds were kept in cage batteries UV 550 (Big Dutchman), cage area of 3316 cm<sup>2</sup>. For the experiment, 10 cages were used for each group (7 head each).

### Experimental design

For the experiment, two groups of chickens (control and test) were formed, 70 head each (Table 1).

**Table (1): Experiment Design.**

Group	Age of birds,	Number of heads	Feeding parameters

	weeks		
Control	25-45	70	Standard feed concentrate
Test	25-45	70	Diet including 5% extruded <i>Amaranthus cruentus</i>

The birds in control group received feed intended for birds at the age indicated in the scheme (feed concentrate-1-1), in accordance with the requirements for the quality of raw materials and feed concentrate. The diet of test breeding hens (starting from their age of 25 weeks) contained extruded amaranth grains in an amount of 5%.

### Studied parameters

The body weight of the birds was determined at the beginning and then weekly until the end of the experiment.

The egg production, livability and feed consumption were recorded every day. Average daily feed intake (ADFI) was measured as the amount of feed eaten in each group (per hen) (Janmohammadi *et al.*, 2023).

The hen-day production (%) was calculated by dividing the total number of eggs by the total number of laying hens per day in each group (Janmohammadi *et al.*, 2023).

Hatching eggs yield was calculated as ratio of difference between the total number of eggs and number of non-hatching eggs by the total number of eggs multiplied by 100 (Janmohammadi *et al.*, 2023).

Average egg weigh (EW) was calculated as ratio of the total eggs weight by total number of eggs (Janmohammadi *et al.*, 2023).

The egg mass (EM) was calculated by multiplying the number of eggs per hen per day by EW (Janmohammadi *et al.*, 2023).

Feed costs for the production of ten eggs

were calculated by dividing ADFI by the number of eggs per hen per day and multiplied by 10 (Janmohammadi *et al.*, 2023). The results were converted to kilograms.

The feed conversion ratio (FCR) was calculated as ADFI divided by EM (Janmohammadi *et al.*, 2023).

To determine the qualitative characteristics of hatching eggs, OST 10 321-2003 "Hatching chicken eggs. Specifications" were applied (within 24 hours after lay). The shell thickness and yolk diameter were measured with a caliper, and egg albumin and yolk heights were found with a tripod micrometer. Haugh units were calculated using the equation (1):

$$\text{Haugh unit} = 100 \cdot \log_{10}(HA - 1.7 \cdot EW^{0.37} + 7.57) \quad (1),$$

where *HA* is the height of the albumin and *WE* is the weight of the egg, g.

The lipid composition of the yolk was determined by the gas chromatographic method, according to GOST 32886 - 2014 "Poultry processing industry. Foodstuffs of processed poultry eggs. Determination of cholesterol content by gas chromatographic method."

Blood for analysis was taken in the morning before feeding. To study hematological parameters (morphological and biochemical), analyzers URIT-3000Vet Plus and URIT-800 (China) were used. To obtain the indicators of antioxidant status, Biochem Sa device (USA) was applied.

Determination of microbiome of the cecum in the intestine. After the period of feeding the studied additive (extruded amaranth grain), 5 chickens were randomly selected from each group. They were euthanized and immediately dissected for cecum to be

selected. The samples were frozen at -20°C for storage until analysis. Microbial DNA was isolated using a QIAcube Connect automated station with a QIAamp Power Fecal DNA Kit (QIAGEN, Germany). The quality of the isolated microbial DNA was quantified using a Qubit 3.0 instrument. The total microbial count was determined by real-time PCR on a LightCycler® 96 System (Roche, Switzerland). The composition of the microbiome of the caecum was found using modern molecular genetic methods of NGS sequencing. The DNA libraries were prepared for NGS sequencing using the Ion 16S Metagenomics Kit and Ion 520 and 530 Kit - OT2 protocols, a sequencing Ion 520™ Chip based on the Ion GeneStudio™ S5 System (Thermo Fisher Scientific, USA).

#### Statistical analysis

All digital data were processed using the statistical software Statistika 12.0 (Statsoft Inc., USA) and Student's t-test to compare the mean values (Johnson and Bhattacharyya,

2010). Differences of  $P < 0.05$  were considered significant.

#### Results

Monitoring the body weight of chickens in terms of their age (25-45 weeks) showed that extruded amaranth grain did not have a negative impact on the parameter. The body weight of the hens in test group was at the level of control values and corresponded to the age of the birds throughout the study, and exceeded their analogue indices in the control by 3.17% at values being not statistically significant by the end of the experiment (45 weeks). In our studies, the livability of birds in both groups was 100%, which can be explained by the age of the birds (the first half of productivity) and the high level of technology for growing replacement pullets in the breeding reproducer. The high livability of breeding hens and a stable body weight throughout the experiment made it possible to obtain stable production indices of hatching eggs in both experimental groups (Table 2).

**Table (2): Technological parameters for the production of hatching eggs.**

Parameters	Control (n=70)	Test (n=70)
Number of eggs (140 days)		
Total	9014	9191
Per hen	128.8	131.3
HDP, %	91.98	93.79
ADFI, g day <sup>-1</sup> hen <sup>-1</sup>	119.5	118.9
Livability, %	100	100
Feed costs, kg per 10 eggs	1.30	1.27
FCR	2.08	1.98
Hatching eggs yield, %	93.7	95.9

Note: ADFI = Average daily feed intake; HDP = Hen-day production; FCR = Feed conversion ratio.

HDP in the groups corresponded to the standard values for this cross, with the experiment indices exceeded the control values by 1.81% due to extruded amaranth grain used in feeding the hens of test group,

which made it possible to obtain 177 more eggs than in control group. Of the eggs obtained, the number of eggs that turned out to be suitable for incubation was greater by 2.20% or 368 eggs in test group compared to

control group. The higher yield of hatching eggs in test group was provided by qualitative

indicators shown in table (3).

**Table (3): Main morphological quality parameters of hatching eggs.**

Parameters	Control (n=10)	Test (n=10)
Egg weight, g	62.58±0.39 <sup>a</sup>	63.89±0.32 <sup>b</sup>
Shape index, %	75.79±0.21 <sup>a</sup>	75.43±0.19 <sup>a</sup>
Haugh units	81.69±0.23 <sup>a</sup>	82.77±0.27 <sup>b</sup>
Shell thickness, µm	357.00±1.42 <sup>a</sup>	362.00±1.09 <sup>b</sup>
Acid number, mg KOH <sup>-1</sup>	3.08±0.09 <sup>a</sup>	3.02±0.07 <sup>a</sup>
Content of carotenoids, µg g <sup>-1</sup>	16.7±0.97 <sup>a</sup>	17.2±1.12 <sup>a</sup>

Means with different superscript in each row differ significantly (P<0.05);

Means with same superscript in each row are not significantly different (P>0.05)

Our analysis of egg quality before incubation showed an increase in the weight of hatching eggs by 1.31 g (2.09%; P<0.01) in test group compared with control group. The Haugh units in both groups met the requirements of OST 10 321-2003; however, this indicator in test group exceeded the control by 1.07% (P<0.05). Moreover, the shell thickness strengthened by 5.0 µm (P<0.05) and amounted to 362 microns in

test group. The extruded amaranth grain, rich in biologically active substances in the composition of the feed for breeding hens, insignificantly contributed to a decrease in cholesterol in the yolk of eggs by 4.88% in test group. The carotenoid content in the yolk of the test hatching eggs remained at the control level, which made an absolute value of 17.2 µg.g<sup>-1</sup>. Before incubation, we determined the lipid composition of the yolk of hatching eggs (Table 4).

**Table (4): The composition of lipids in the yolk of hatching eggs.**

Parameters	Control (n=5)		Test (n=5)	
	mg g <sup>-1</sup> of yolk	g per yolk	mg g <sup>-1</sup> of yolk	g per yolk
Total lipids	288.40±4.58 <sup>a</sup>	5.52±0.10 <sup>a</sup>	302.62±3.67 <sup>b</sup>	5.88±0.09 <sup>b</sup>
Triglycerides	181.30±3.21 <sup>a</sup>	3.47±0.08 <sup>a</sup>	193.00±2.98 <sup>b</sup>	3.75±0.06 <sup>b</sup>
incl. Fatty acids	161.44±2.79 <sup>a</sup>	3.09±0.07 <sup>a</sup>	170.36±2.49 <sup>b</sup>	3.31±0.05 <sup>b</sup>
Phospholipids	95.61±1.68 <sup>a</sup>	1.83±0.03 <sup>a</sup>	98.82±1.57 <sup>a</sup>	1.92±0.03 <sup>a</sup>
incl. Lecithin	63.22±1.37 <sup>a</sup>	1.21±0.04 <sup>a</sup>	64.33±1.29 <sup>a</sup>	1.25±0.03 <sup>a</sup>
Cholesterol	11.49±0.29 <sup>a</sup>	0.22±0.007 <sup>a</sup>	10.29±0.32 <sup>b</sup>	0.20±0.004 <sup>b</sup>

Means with different superscript in each row differ significantly (P<0.05);

Means with same superscript in each row are not significantly different (P>0.05); Amount of each parameter g per yolk was calculated by multiplying the yolk weight by this parameter per gram of yolk

The analysis of the obtained results showed a significant increase in total lipids in the yolk of the hatching eggs by 6.53% (P<0.05), triglycerides by 8.07% (P<0.05), including fatty acids by 7.12% (P<0.05) in

test group compared to control group. In addition, our study established a significant decrease in cholesterol in the yolk of hatching eggs by 11.66% (P<0.05) in test group against the background of control group) under the influence of extruded amaranth grain. The

incubation of eggs obtained during the experiment showed a high hatch of chickens both in test and control groups (84.57 and 83.24%), with the test group values exceeding the control values by 1.33%. The available

information on the functional properties of amaranth helped us to study the effect of its extruded grain on the intestinal microbiota of breeding hens (Table 5).

**Table (5): Total microbial count and the taxonomy ratio of microorganisms in the cecum.**

Parameters	Control (n=5)	Test (n=5)
Phylum Actinobacteria, %	3.47±0.18 <sup>a</sup>	4.39±0.23 <sup>b</sup>
Genus <i>Bifidobacteriales</i> , %	2.28±0.17 <sup>a</sup>	3.35±0.14 <sup>b</sup>
Phylum Bacteroidetes, %	32.95±0.42 <sup>a</sup>	34.53±0.33 <sup>b</sup>
Phylum Firmicutes, %	44.73±0.57 <sup>a</sup>	45.68±0.45 <sup>a</sup>
Genus <i>Lactobacillales</i> , %	6.97±0.24 <sup>a</sup>	8.75±0.29 <sup>b</sup>
Genus <i>Clostridiales</i> , %	28.14±0.84 <sup>a</sup>	28.21±0.67 <sup>a</sup>
Family Ruminococcaceae, %	9.64±0.19 <sup>a</sup>	11.17±0.22 <sup>b</sup>
Genus <i>Selenomonadales</i> , %	4.58±0.26 <sup>a</sup>	6.12±0.34 <sup>b</sup>
Phylum Fusobacteria, %	2.23±0.15 <sup>a</sup>	0.78±0.21 <sup>b</sup>
Phylum Proteobacteria, %	12.50±1.21 <sup>a</sup>	12.47±1.36 <sup>a</sup>
Family Enterobacteriaceae, %	1.87±0.54 <sup>a</sup>	2.07±0.43 <sup>a</sup>
Phylum Synergistetes, %	0.53±0.12 <sup>a</sup>	0.38±0.16 <sup>a</sup>
Phylum Tenericutes, %	0.98±0.11 <sup>a</sup>	0.30±0.09 <sup>b</sup>
Family Mycoplasmataceae, %	0.12±0.013 <sup>a</sup>	0.04±0.008 <sup>b</sup>
Normal flora, %	83.38±0.39 <sup>a</sup>	85.40±0.27 <sup>b</sup>
Pathogenic and unwanted flora, %	2.78±0.49 <sup>a</sup>	2.75±0.63 <sup>a</sup>
Total microbial number, units	3.86x10 <sup>7</sup> ±0.14 <sup>a</sup>	4.52x10 <sup>7</sup> ±0.17 <sup>b</sup>

Means with different superscript in each row differ significantly (P<0.05);

Means with same superscript in each row are not significantly different (P>0.05)

Under the influence of the studied feed ingredient, a 17.10% (P<0.05) growth of beneficial microorganisms in the cecum of the intestines of layers was recorded in test group compared with control group. The data obtained indicated an insignificant increase in bacteria of the phyla Actinobacteria and Bacteroidetes by 26.52 (P<0.05) and 4.80% (P<0.05), and the phylum *Firmicutes* by 2.12% in test group. A significant increase in bacteria of the genus *Bifidobacteriales* and *Lactobacillales*, responsible for the suppression of pathogenic microflora, was found in test group and made 46.93 (P<0.01) and 25.54% (P<0.01) compared with control

group. An increase in the Ruminococcaceae family bacteria, responsible for the digestion of fiber, by 15.87% (P<0.01) should be noted in test group versus control group. The total number of normal flora significantly increased by 2.42% (P<0.05) in test group against the background of control group. The amounts of pathogenic and unwanted microflora in both experimental groups were at the level of norms for healthy birds and were not statistically significant. The results of studies of the extruded amaranth grains' effect on the content of formed elements and the leukocyte blood count of breeding chickens obtained during the experiment are presented in table (6).

**Table (6): Formed elements and leukocyte blood count.**

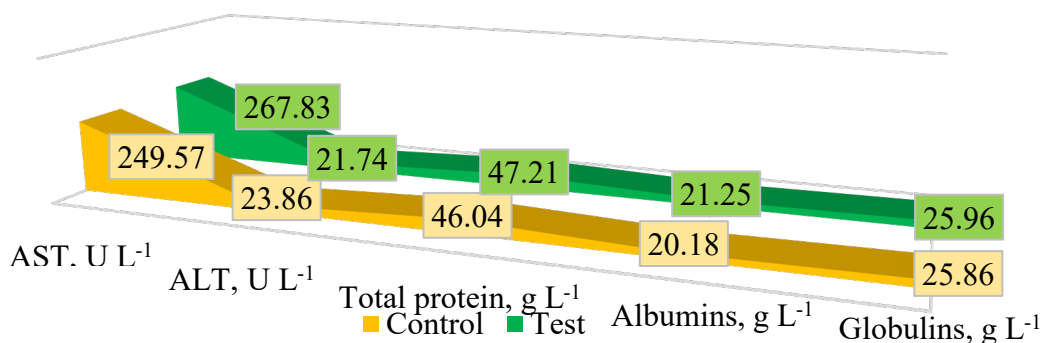
Parameters	Control (n=5)	Test (n=5)
Erythrocytes (RBC), $10^{12} \text{ L}^{-1}$	2.87±0.11 <sup>a</sup>	3.02±0.13 <sup>a</sup>
Hematocrit (HCT), %	32.3±0.33 <sup>a</sup>	33.4±0.47 <sup>a</sup>
Hemoglobin (HGB), $\text{g L}^{-1}$	127.3±1.48 <sup>a</sup>	132.6±1.61 <sup>b</sup>
Leukocytes (WBC), $10^9 \text{ L}^{-1}$	34.2±0.42 <sup>a</sup>	34.9±0.57 <sup>a</sup>
Eosinophils, %	6.75±0.12 <sup>a</sup>	6.51±0.13 <sup>a</sup>
Basophils, %	2.86±0.18 <sup>a</sup>	3.04±0.14 <sup>a</sup>
Pseudo-eosinophils:		
Stab, %	0.21±0.09 <sup>a</sup>	0.16±0.08 <sup>a</sup>
Segmented, %	26.78±0.54 <sup>a</sup>	25.13±0.49 <sup>b</sup>
Lymphocytes, %	58.78±0.51 <sup>a</sup>	60.71±0.59 <sup>b</sup>
Monocytes, %	4.62±0.21 <sup>a</sup>	4.45±0.27 <sup>a</sup>

Means with different superscript in each row differ significantly ( $P < 0.05$ );

Means with same superscript in each row are not significantly different ( $P > 0.05$ )

The studied feed ingredient did not significantly influence the content of formed elements in the blood of breeding chickens in test group compared with control group. However, the hematocrit level in test group increased by 1.1%. The content of hemoglobin significantly increased by 4.16% ( $P < 0.05$ ) in test group versus control group. The leukocyte indices in the blood of chickens in both experimental groups were at the same level, which indicated the absence of inflammatory processes in the bodies of experimental birds. Considering the indices of the leukocyte formula, a significant increase in lymphocytes by 1.93% ( $P < 0.05$ ) and a decrease in segmented neutrophils by 1.65% ( $P < 0.05$ ) were found in the blood of hens in test group compared with control group, which proved the high efficiency of the

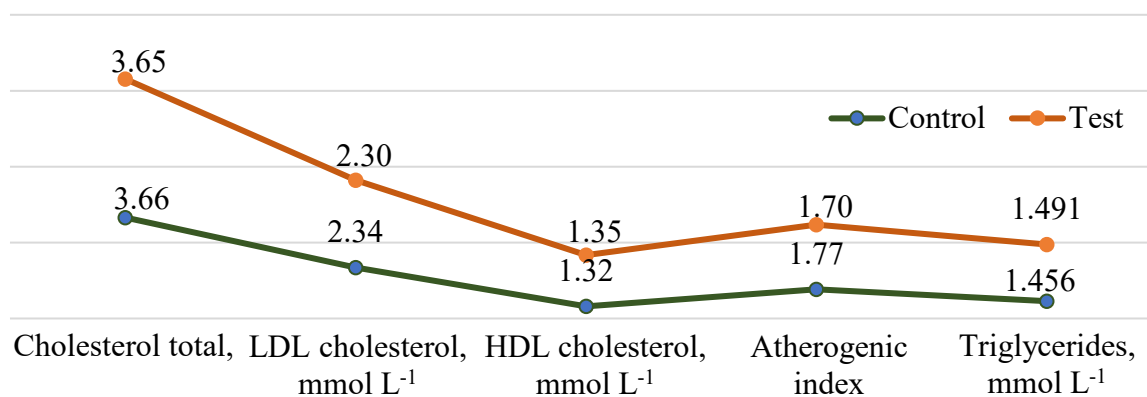
studied feed for maintaining the immune status of breeding chickens in the first phase of productivity. Changes in protein metabolism in the chicken body are shown in Figure 1. The total protein content in the blood serum increased by  $1.17 \text{ g L}^{-1}$  (2.54%;  $P < 0.05$ ) and albumin protein fraction by  $1.07 \text{ g L}^{-1}$  (5.30%;  $P < 0.05$ ) in test group compared to control group, which is likely to be explained by the most optimal content of amino acids in the amaranth protein compared to other cereals used in the diet of control chickens. An increase in protein metabolism in the bodies of test birds had a positive effect on the physiological status of the liver, as evidenced by a decrease in the ALT enzyme activity by 9.75% ( $P < 0.05$ ) in test group compared to control group, with the AST enzyme activity increasing by 7.32% ( $P < 0.05$ ) in test group.



**Fig. (1): Intensity of protein metabolism.**

The contents of triglycerides, total cholesterol and low- and high-density lipoproteins are shown in fig. (2). Despite a slight decrease in the level of total cholesterol and low-density lipoprotein cholesterol and a

certain increase in high density lipoprotein cholesterol (the difference was not statistically significant), the value of the atherogenic index improved (1.70) in test group compared to control group (1.77).



**Fig. (2): The content of cholesterol and triglycerides in the blood serum of hens.**

In our opinion, biologically active substances with antioxidant properties in amaranth grains should contribute to an increase in the antioxidant status of laying hens in test group. In this regard, we studied the activity of superoxide dismutase,

glutathione peroxidase, ceruloplasmin and the content of the total amount of antioxidants and TBA that are active substances (malonic dialdehyde). As expected, extruded amaranth grain used in feeding chickens contributed to the activation of enzymes of the antioxidant status (Table 7).

**Table (7): Indices of the antioxidant statuses of laying hens.**

Parameters	Control (n=5)	Test (n=5)
Superoxide dismutase, U per g (Hb)	943.6±22.41 <sup>a</sup>	1027.2±25.14 <sup>b</sup>
Glutathione peroxidase, U per g (Hb)	50.49±0.44 <sup>a</sup>	52.35±0.59 <sup>a</sup>
Ceruloplasmin, mmol (ml h) <sup>-1</sup>	2.12±0.05 <sup>a</sup>	2.26±0.06 <sup>a</sup>
Total amount of antioxidants, mmol L <sup>-1</sup>	1.57±0.06 <sup>a</sup>	1.91±0.08 <sup>b</sup>
TBA that are active substances (malondialdehyde), μmol L <sup>-1</sup>	3.61±0.06 <sup>a</sup>	3.18±0.09 <sup>b</sup>

Means with different superscript in each row differ significantly (P<0.05);

Means with same superscript in each row are not significantly different (P>0.05)



The activity of superoxide dismutase significantly increased by 8.85% ( $P < 0.05$ ), and glutathione peroxidase and ceruloplasmin tended to increase in test group compared to control group. The total amount of antioxidants also significantly exceeded the control by 21.66% ( $P < 0.01$ ). The level of active substances decreased in terms of thiobarbituric acid and, in particular, malonaldehyde by 13.52% ( $P < 0.05$ ) in test group, which indicated a high antioxidant activity of the organism.

## Discussion

The extrusion method for the production of feed is considered one of the resource-saving and promising ways of animal husbandry development. Extrusion is the processing of raw materials with simultaneous short-term exposure to high pressure and temperature. At the same time, the digestibility of feed nutrients increases (Nikmaram *et al.*, 2017). Rathod & Annapure (2016) found that extrusion led to a reduction in the content of tannins and polyphenols, with protein digestibility improving *in vitro*. The increasing *in vitro* protein digestibility resulted from degradation of protein complexes in extruded samples and protein denaturation due to heating (Kumar *et al.*, 2018). According to Popiela *et al.* (2013), the extruded amaranth grains supplied had no effect on the fatty acids in the laying egg yolk, but the layers' final body weight did increase. In that study, the fatty acid composition of the egg yolk, egg features, blood indicators, and production of Lohmann Brown laying hens were all determined. Taking into account the new feed ingredient in the diets, we, first of all, monitored the change in the body weight of chickens during the experiment. Results obtained in this study are consistent with the data obtained by Rouckova *et al.* (2004), Fasuyi *et al.* (2007),

Longato *et al.* (2017). According to Longato *et al.* (2017), 10% amaranth in the diet of broilers is more suitable for improving productive characteristics, including the body weight, while Rouckova *et al.* (2004) reported on a decrease in the final body weight when broiler chickens were fed 50 and 100 g of amaranth grain per kg of feed. As determined by Popiela *et al.* (2013), egg production was increased by feeding laying hens with amaranth grains in the diet structure. Similarly, in our study, indices of test group productivity were higher than control. In studies by Popiela *et al.* (2013), laying hens fed a diet containing amaranth grain required significantly less feed to produce a given number of eggs than laying hens fed a control diet. No negative effect on the strength and thickness of the shell, Haugh units (an egg quality indicator), height of dense protein, weights of egg and shell or content of fatty acids in the egg yolk was found, but, on the contrary, improved indices were recorded. According to the authors, extruded amaranth grain can be effectively used in the diets of laying hens, without the production characteristics being deteriorated, but pigment must be added to the feed to improve the color of the yolk. Tillman & Waldroup (1987) reported, that if grain amaranth is properly steam extruded, it can be effectively used in layer rations at up to 30% without any adverse consequences on production, egg mass, feed per dozen eggs, shell strength, shell thickness, hen weights, blood spots, or Haugh units. The level of phospholipids, including lecithin, in the yolk of the hatching eggs tended to increase by 4.92 and 3.31% versus the control. In our opinion, this was due to the high content of unsaturated fatty acids in amaranth grain. The basis of amaranth grain lipids are unsaturated fatty acids. Of the total amount of fatty acids, linoleic acid made more than 50%, oleic

about 25%, palmitic 20% and linolenic 1%. The total unsaturation of amaranth lipids was more than 75%. Phospholipids made about 5% of the lipid fraction; lecithin, cephalin and phosphoinositol were the most common. Despite its high level of unsaturation, amaranth oil is resistant to oxidation due to the protective effect of tocopherols. It also contains squalene, a carotenoid hydrocarbon that has been credited with numerous health benefits. According to Alegbejo (2014), amaranth contains 8% squalene, 2% tocopherols, 10% phospholipids and 2% phytosterols. The amount of non-food components in amaranth is lower than in cereal grains (Ravindran *et al.*, 1996; Jacob *et al.*, 2008). A decreasing of cholesterol level in the yolk of hatching eggs was consistent with the results of studies in Janmohammadi *et al.* (2023). High rates of production and quality of hatching eggs in Test group are highly likely to be provided by the activated metabolism resulted from feeding chickens with the additive under study. *Amaranthus cruentus* has a pronounced hepatoprotective and immunostimulating effect. Some authors note the anti-inflammatory properties of amaranth (Strzelecka *et al.*, 2005; Janmohammadi *et al.*, 2023). The extruded amaranth grain in the diets of breeding chickens had a stabilizing effect on the intestinal microbiota and showed, to a certain extent, antibacterial properties. Our results are consistent with Pineda-Quiroga's *et al.* (2019) study. Li *et al.* (2015) and Peiretti *et al.* (2017) found that amaranth grain has a higher content of total phenolic compounds that are secondary metabolites than leaves. Longato *et al.* (2017) reported that the antioxidant capacity of the serum was significantly higher, and the levels of cholesterol, triglycerides and lipid peroxidation in serum were significantly lower in broilers fed diets supplemented with 5% and 10% amaranth

compared to broilers fed no supplements. Króliczewska *et al.* (2008) found that increasing level of amaranth did not affect erythrocytes, leukocytes, hematocrit, hemoglobin or iron content in blood of laying hens. There was noted a positive effect of the supplemented diet on blood lipids, with low-density lipoprotein cholesterol and triglycerides being decreased and changed activity of aspartate aminotransferase and alanine aminotransferase compared with the group without supplements. Similarly, in our study, an increase in protein metabolism in the bodies of Test birds had a positive effect on the physiological status of their liver. According to Janmohammadi *et al.* (2023), the content of total lipids in the blood serum is significantly influenced not only by the physiological state of the organism, but also by nutritional factors. The decrease in total lipids, which occurs under the influence of some feed additives, indicates the activation of metabolism and their more intensive consumption for the product formation and metabolism. The contents of different classes of lipids and cholesterol in the blood serum are directly dependent on the level of total lipids. Cholesterol plays an important role in metabolism and, in a certain amount, is absolutely necessary for normal life, as it serves as a material for the synthesis of hormones and bile acids. The atherogenic index as an indicator characterizing the ratio between low-density lipoproteins (LDL) and high-density lipoproteins (HDL) did not go beyond the physiological norm in our studies. The ratio between atherogenic and anti-atherogenic lipids should not normally exceed 3.5.

## Conclusion

Our research proved the effectiveness of extruded amaranth grain *Amaranthus cruentus* used in feeding of breeding hens of

the Hisex Brown cross. The 5% extruded grain added to the diet structure made it possible to increase the egg production by 1.81%, the hatching eggs yield by 2.20% and the hatching of chickens by 1.33% by improving the quality indicators of hatching eggs and strengthening the immunological and antioxidant status of laying hens, which was confirmed by the experiment results in the article.

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## Conflicts of interest

The authors declare that they have no conflict of interests.

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## الأداء الإنتاجي وجودة بيض التفقيس والمؤشرات الصحية لدجاج الهيسكس البني المغذى على حبوب القطفة المبتوقة

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**المستخلص:** يعرض هذا المقال نتائج تثبت جدوى حبوب القطفة المبتوقة (*Amaranthus cruentus*) في تغذية الدجاج البياض من هجين الهيسكس البني (العمر 25-45 أسبوع). أتاحت الحبوب المبتوقة بنسبة 5% المضافة إلى العليقة زيادة كثافة وضع البيض بنسبة 1.81% وإنتاجية بيض التفقيس بنسبة 2.20% وتقريخ الدجاج بنسبة 1.33% بسبب تحسين مؤشرات جودة بيض التفقيس، أي أن وزن بيض التفقيس زاد بمقدار 1.31 غم (2.09%؛  $P < 0.01$ )، ووحدهات هيو بنسبة 1.07 ( $P < 0.05$ )، وتم تعزيز سمك القشرة بمقدار 5.0 ميكرومتر ( $P < 0.05$ ) إلى 362 ميكرومتر. انخفض مستوى الكوليسترول في صفار البيض بنسبة 11.66 ( $P < 0.05$ ) في مجموعة الاختبار. ارتفع تركيز الهيموغلوبين في دم الدجاج بنسبة 4.16% في مجموعة الاختبار مقارنة بمجموعة السيطرة ( $P < 0.05$ )، كما انخفض مستوى الخلايا الليمفاوية بنسبة 1.93 ( $P < 0.05$ )، كما انخفض مستوى الخلايا المتغايرة بنسبة 1.65 ( $P < 0.05$ ). مما أثبت الكفاءة العالية للعلف المدروس في الحفاظ على الحالة المناعية لدجاج التربية في المرحلة الإنتاجية الأولى. في مجموعة الاختبار، زاد نشاط انزيم سوبر اوكسيد ديسموتاز بنسبة 8.85 ( $P < 0.05$ )، وزاد إجمالي كمية مضادات الأكسدة بنسبة 21.66 ( $P < 0.01$ )، وانخفض مستوى المالونالدهيد بنسبة 13.52 ( $P < 0.05$ )، مما يدل على وجود نشاط مضاد للأكسدة عالي لجسم الدجاج. تم تحقيق زيادة في بكتيريا *Bifidobacteriales* و *Lactobacillales* بنسبة 46.93 ( $P < 0.01$ ) و 25.54% ( $P < 0.01$ ) وكذلك بكتيريا *Ruminococcaceae* بنسبة 15.87% ( $P < 0.01$ ) في مجموعة الاختبار مقارنة بمجموعة السيطرة من خلال تحليل ميكروبيوم الأعور في الأمعاء.

**الكلمات المفتاحية:** تربية الدواجن، إنتاجية البيض، حبوب القطفة المبتوقة، صحة الدجاج البياض، جودة بيض التفقيس.