



Influence of Pectin Contained in and Extracted from Phytomass of *Amaranthus cruentus* L. on Erythropoiesis and Growth Performance of Commercial Layer Replacement Chickens

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Key words: Replacement chickens, erythropoiesis, growth intensity, nutrition, amaranth, pectin, hydrolysis-extraction

Abstract: The aim of this study was to investigate the influence of feeding rations with different nutritional value, that included either the grass meal of *Amaranthus cruentus* L. (AGM) or products of AGM hydrolysis-extraction enriched with pectin or purified amaranth pectin, on the indices of erythropoiesis of commercial layer replacement chickens, their growth performance and liveability. Chickens had some dysfunctions in the erythropoiesis during the period of intensive growth, accomplished with the higher spending of iron or due to the change of composition and nutritive value of the ration. The therapeutic and prophylactic effect of all above mentioned products on erythropoiesis of chickens has been revealed. It was indicated by the increased hemoglobin concentration, number of erythrocytes and normalization of a single erythrocyte's indices. Moreover, the use of AGM and its products of hydrolysis-extraction resulted in the elevated growth performance and liveability of young pullets. The dose-dependent effects of investigated feed ingredients: AGM, its products of hydrolysis-extraction and purified amaranth pectin, on the erythropoiesis and growth performance in chickens were evaluated. It was demonstrated, *A. cruentus* pectin, contained in its phytomass as a protopectin or extracted from the phytomass as a soluble pectin have a stimulating effect on the erythropoiesis and positive effect on the liveability of chickens but does not affect their plastic metabolism. The promoting effect of AGM and its products of hydrolysis-extraction on the weight gain of replacement chickens is determined by the other biologically-active substances of non-pectin origin that are present in amaranth. Products of AGM hydrolysis-extraction

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Page No.: 298-308

Volume: 18, Issue 10, 2019

ISSN: 1680-5593

Journal of Animal and Veterinary Advances

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enriched with pectin are considered as a new therapeutic and prophylactic feed additive that is much more effective than the raw materials used for their

manufacturing. These products significantly increase the efficiency of chickens and improve their functional state that is indicated by the recuperation of erythropoiesis.

INTRODUCTION

According to the existed experimental data, chicken anemia virus (Adair, 2000; Davidson *et al.*, 2008) or myeloblastosis-associated virus MAV-2(0) (Cummins and Smith, 1988) cause significant losses in the poultry farming. Moreover, unspecific infection agents such as bacteria *Salmonella gallinarum* or its endotoxin (Assoku and Penhale, 1978) as well as the presence of ectoparasites (Al-Saffar and Al-Mawla, 2008) which are quite common in poultry farming could also be potential causes of anemia. In poultry farms, use of medications is inevitable, although, there is information that some of them such as coccidiostat maduramicin could reduce hemoglobin concentration and amount of red blood cells in chickens (Singh and Gupta, 2003). Stress factors that could take place during rearing period such as a feed restriction (Maxwell *et al.*, 1991) or poultry moving (Minka and Ayo, 2008) could also be a cause of anemia in poultry.

According to the information above anemia cases in industrial poultry farming could be caused by various factors. Since, maintaining of hemoglobin and red blood cells amount on sufficient level is important for optimal tissue oxygenation and metabolism, anemia can provoke a decrease in poultry's efficiency and infection resistance. Therefore, elaboration of veterinary remedies that contribute to erythropoiesis maintenance in poultry is a demanded scientific task.

During the last decades, products of vegetable origin have attracted attention of many scientists as ecologically-safe stimulants and adaptogens for a poultry. It was demonstrated, that the use of wild-ginseng adventitious root meal (Yan *et al.*, 2011), essential oil derived from oregano, anis and citrus peel (Hong *et al.*, 2012), extracts from *Radix astragali*, *Radix codonopsis*, *Herba epimedii* and *Radix glycyrrizae* (Liu *et al.*, 2010), seeds of *Nigella sativa* (Toghyani *et al.*, 2010) causes poultry's efficiency to increase, its immunity to reinforce and erythropoiesis to enhance.

Amaranth is a plant that is rich in biologically active substances and full-value protein and should be distinguished as raw materials for manufacturing of highly efficient feed additives for a poultry. It was shown that amaranth is rich in chlorophyll, β -cyanins, β -xanthins, betalains, carotene and ascorbic acid and total antioxidant (Sarker *et al.*, 2018). It was found that amaranth vitamin-grass meal has a stimulating effect on the development of the reproductive system in commercial layer replacement chickens (Vyshtakalyuk *et al.*, 2010a), on the increase of their body weight gain and liveability (Vyshtakalyuk *et al.*, 2011).

Amaranth seeds cause an increase in egg production and body weight gain of laying hens (Ewa *et al.*, 2013) as well as an increase in weight gain and meat quality of broilers (Orczewska-Dudek *et al.*, 2018). Feed additives obtained by a complex chemical and mechano-acoustical processing of amaranth phytomass in a rotary-pulse apparatus (Minzanova *et al.*, 2007), cause an increase of weight gain and liveability of commercial layer replacement chickens and display a higher efficiency compared to the original raw materials (Vyshtakaliuk *et al.*, 2010).

There is a lot of information found in literature regarding therapeutic-and-prophylactic effects of pectin and neutral oligo and polysaccharides that are considered the most important components of vegetal raw materials. It is shown, that the functional oligosaccharides may influence the availability of minerals such as iron, calcium and magnesium (Sakai *et al.*, 2000; Xu *et al.*, 2009). Natural plant polysaccharides stimulate hemopoiesis, modulate the weight and cellular composition of hemopoietic organs (Sychev *et al.*, 2006). Pectin with low molecular weight and high degree of esterification results in improvement in bioavailability of iron (Kim and Atallah, 1992; Kim *et al.*, 1996; Wikiera *et al.*, 2014; Zhang *et al.*, 2017). Iron bound to pectin might be released by microbial degradation and subsequently made available for absorption in the large intestine (Miyada *et al.*, 2011). This iron is well used by animals and causes an increase of body-weight gain, an increase of hemoglobin concentration and prevent a development of iron deficiency anemia (Miyada *et al.*, 2011). Synthesized water-soluble complexes of pectin with iron and calcium (Vyshtakaliuk *et al.*, 2010; Minzanova *et al.*, 2015, 2018) as well as with the other metal microelements (Vyshtakalyuk *et al.*, 2008) are the source of biologically available ions of macro and microelements.

The aim of this disquisition was to study the effect of feeding rations with different nutritional value that included either the dried phytomass of *Amaranthus cruentus* L. or enriched with pectin products of hydrolysis-extraction of amaranth phytomass or purified amaranth pectin obtained from this phytomass on the indices of erythropoiesis of commercial layer replacement chickens on their growth performance and liveability.

MATERIALS AND METHODS

Materials under investigation: *Amaranthus cruentus* dry phytomass manufactured in the form of Amaranth Grass Meal (AGM). Properties: protein 17.5-25%, carotene 120-250 mg kg⁻¹, crude fiber 18.0-19.8%.

Commercial Citrus Pectin (CP) of Herbstreith Fox KG production. Properties: M.W. 17.6 kDa, free carboxyl groups 3.5%, esterification degree 70-75%.

Amaranth Pectin (AP) manufactured in A.E. Arbuzov IOPC from AGM by means of hydrolysis-extraction with an acidified aqueous solution of organic or inorganic acids that convert a protopectin in soluble form which was precipitated by ethanol or acetone and then dried (Minzanova *et al.*, 2014a, b). Properties: M.W. 85-90 kDa, free carboxyl groups 4.5%, esterification degree 70-75%.

Aqueous Hydrolysate (AH): complete product of AGM extraction by water obtained in the form of suspension. Nutritional value (g per 100 g of dry matter): water-soluble protein 1.0; water-soluble pectin 1.4; crude protein 22.5; crude fiber 24.5; *in vitro* digestibility 35.8%. Pectin substances in this product are presented preferable in the form of a water-soluble fraction.

Pectin Hydrolysate (PH): complete product of AGM hydrolysis-extraction by whey or aqueous solutions of any organic acids, obtained in the form of suspension. Nutritional value (g per 100 g of dry matter): water-soluble protein 1.3; soluble pectin 2.7; crude protein 20.3; crude fiber 22.0; *in vitro* digestibility 45.8%. Pectin substances in this and other products, that are obtained by the hydrolysis-extraction with an acidified aqueous solution, contain, besides the water-soluble pectin fraction, a pectin that is converted into a soluble form from the protopectin.

Protein-Pectin Hydrolysate (PPH): complete product of AGM hydrolysis-extraction in series by whey and aqueous solution of alkali, obtained in the form of suspension. Nutritional value (g per 100 g of dry matter): soluble protein 4.5; soluble pectin 3.4; crude protein 20.3; crude fiber 20.5; *in vitro* digestibility 50.6%.

Pectin Extract (PE): a supernatant fraction of pH. Nutritional value (g per 100 g of dry matter): water-soluble protein 4.2; soluble pectin 9.0; *in vitro* digestibility 96.3%. Saccharified Protein-Pectin Hydrolysate (SPPH): complete product of AGM hydrolysis-extraction in series by whey aqueous solutions of alkali and cellulase enzymes (celloviridin and pectofectidin) obtained in the form of suspension. This product is rich with neutral mono and oligosaccharides as well as oligomeric fragments of pectin polysaccharides. Nutritional value (g per 100 g of dry matter): soluble protein 4.5; fermented soluble pectin 8.6; crude protein 20.7; crude fiber 17.8; *in vitro* digestibility 55.4%.

Saccharified Protein-Pectin Extract (SPPE): a supernatant fraction of SPPH. Nutritional value (g per 100 g of dry matter): soluble protein 15.1; fermented soluble pectin 14.4; *in vitro* digestibility 98.7%.

Objects of investigation: The trials were carried out on the commercial layer replacement chickens (immature males and pullets) of the White Leghorn breed. Three trials were conducted. Replacement chickens were kept in cages in accordance with the rules for the studied categories of poultry in industrial conditions at the poultry farm. Feeding and drinking of the poultry was ad libitum with a free access to the feeders and drinkers.

The study was conducted during the growing period of chickens, when they have a higher demand in iron and the chance that they have any disorder of erythropoiesis is more probable. Also, to increase the functional load on the erythropoiesis system, some investigations (trials 2-II and 3) were conducted on the poultry which was fed with a low-protein diets. Trails 1 and 2-I were conducted with the feeds that have the standard nutritional value. The composition of the used rations is given in Table 1, their

Table 1: Composition of the base ration for the experimental poultry groups (g kg⁻¹ of feed)

Components	Trail number			
	1-I, 2-I ^a	1-II ^a	2-II ^a	3-I ^a
Wheat	506	205	191	500
Soy meal (46% of protein)	-	-	-	-
Barley	48	535	574	201
Maize	97	-	-	-
Peas	68	95	96	80
Sunflower meal (protein 40%)	97	-	-	-
Sunflower meal (protein 34%)	-	20	-	115
Wheat bran	-	-	-	-
Fish meal (protein 65%)	55	20	-	-
Meat and bone meal (protein 42%)	-	20	33	17
Fat-free dry milk	29	-	-	-
Fodder yeast (protein 42%)	52	56	57	57
Zeolite	14	15	15	15
Chalk	-	-	-	-
Vitaminous premix	14	15	15	15
Vegetable oil	-	19	19	-
Amaranth Grass Meal (AGM)	-	-	-	-
Motley Grass Meal (MGM)	-	-	-	-
Fresh fish	10	-	-	-
Carrot	10	-	-	-

^aRoman numerals after numbers of trails; the replacement chickens rearing period: I-the first rearing period from 1-45 days; II-the second rearing period from 46-120 days

Table 2: Nutritional value of diets for the experimental poultry groups (per 100 g of feed)

Energy and essential nutrients content	Trail No.			
	1-I, 2-I ^a	1-II ^a	2-II ^a	3-I ^a
Energy value (kcal)	269.10	272.20	271.60	263.70
Crude protein (g)	18.88	15.44	14.34	16.68
Crude fat (g)	2.47	4.15	4.19	2.15
Crude fibre (g)	4.25	4.58	4.37	5.33
Crude ash (g)	6.84	4.59	4.64	4.67

^aThe notation is the same as in Table 1

Table 3: Design of trails

Trial No. (poultry group)	Product under investigation	Dry matter content in feed (g kg ⁻¹)	Dosage ^b per body mass (mg kg ⁻¹)	Period of application (days)	Chickens age at weighing (days)	Chickens age at blood sampling (days)
1-I ^a +1-II ^a (pullets)	AGM	30, 50, 70	-	1-45	45	50
		+30	-	50-115	80	125
2-I ^a (pullets, immature males)+	PH	1, 3, 10	30, 90, 300	1-37	38	47
		1, 3, 10	30, 90, 300	1-37	40	47
2-II ^a (immature males)	SPPH SPPE PE AP CP	6	180	7-37	37	47, 110
		2	200	7-37	35	47
		1, 2	100, 200	4-37	37	47, 110
		0.05, 0.1	10, 20	4-37	35	47, 110
		0.1	20	4-37	35	110
3-I ^a (pullets)	PH PPH	12	360	2-37	37	50
		12	360	2-37	37	50

^aThe notation is the same as in Table 1; ^bthe dosages of dry extractive substances kept in different products of amaranth phytomass hydrolyses-extraction or the dosages of pure pectin are calculated per kg of chicken's body mass

nutritional value in Table 2. The keeping conditions of the control and experimental poultry groups did not differ. The design of the trails is given in Table 3.

Blood sampling and parameters of erythropoiesis evaluated: For the acquiring of blood samples from layer replacement chickens, 3-5 typical individuals from each group were sacrificed. The effect of the preparations and feed materials under investigation on the parameters of erythropoiesis was estimated by evaluation of hemoglobin concentration (Hb, g L⁻¹) which content was determined by hemoglobin-cyanide method of Red Blood Cell count (RBC, ×10¹² L⁻¹), counted in Bürker counting chamber of hematocrit-Packed Cell Volume (PCV, %), determined by using a capillary centrifuge tube and hematocrit reader and by calculation of Mean Corpuscular Hemoglobin (MCH, pg), of Mean Corpuscular Volume (MCV, fL) and of mean corpuscular hemoglobin concentration (MCHC, %).

Parameters of performance evaluated: The body weight gain of layer replacement chickens was measured by the individual weighing of chickens from one or more fixed cages at the end of the 1st rearing period (I, age 1-45 days) or of the 2nd rearing period (II, age 46-125 days). The number of a weighted chickens in each group was 20-100 heads. Flock uniformity in each group was evaluated by the Coefficient of Variation (CV%) in percent of average body mass. The lower is the value of the CV%, the more precise is the estimation of the flock uniformity. Livability of flock in each group for the 1st rearing period was determined by the percentage of retained chickens at the age of 46 days from the number

of day-old chicks in each group. Mortality in each group was also determined by the percentage of the initial number of chicks in each group.

Data analysis: Numeric data was processed statistically in the program Origin 6.0. Average values (M), Standard deviation Error (SE) and Coefficient of Variation in percent of M (CV%) are given. The samples were compared by Student's t-test or by Van der Warden's nonparametric criteria.

RESULTS AND DISCUSSION

In the trials 1-3 performed on commercial layer replacement chickens during the growing period were detected some features of the influence of the investigated products on the erythropoiesis and on the performance of chickens fed balanced diet (trials 1-I, 1-II and 2-I) or low-protein diet (trials 2-II and 3).

Indices of erythropoiesis in commercial layer replacement chickens: In the 1st trial where replacement chickens were kept on a balanced diet during two growing periods (trial 1-I and 1-II), some erythropoiesis features in the control group which were measured at the end of the 1st and 2nd growing periods (7 and 18 weeks, respectively) were undergoing some particular age-related changes: Hb between the two measurements increases significantly from 82.0±3.9-88.1±1.0 g L⁻¹ (Fig. 1A, a), RBC increases from 3.09±0.08 to 3.61±0.34×10¹² L⁻¹, although not significantly (Fig. 1C, a) and MCV significantly decreases from 110.6±3.5 to 96.9±0.8 fL

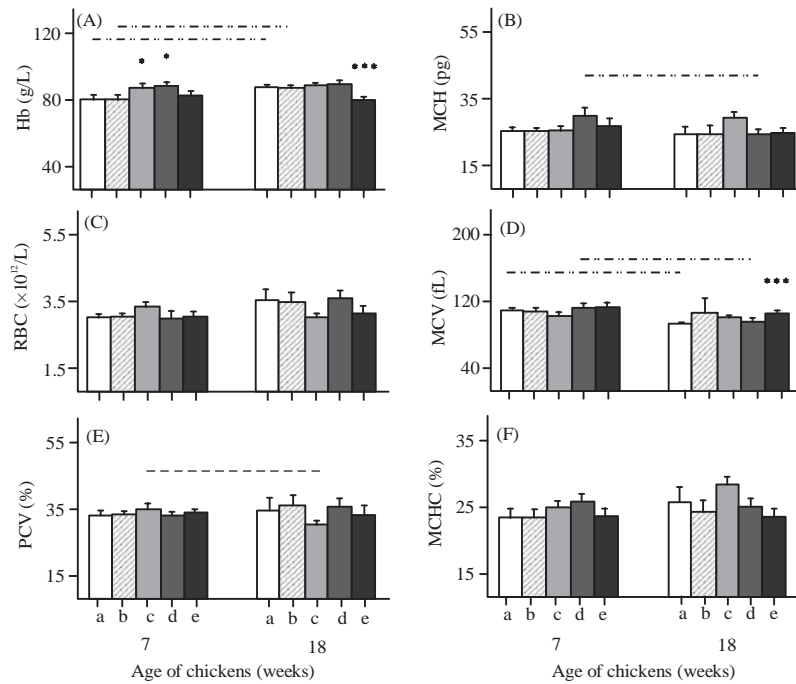


Fig. 1: Hematological indices of commercial layer replacement chickens in the 1st trial after the I rearing period (7th week) and after the II rearing period (18th week). Asterisks (*) above the bars indicate statistically significant differences between a different test groups and the control group within the same rearing period: *statistically significant differences at $p < 0.05$; **the same at $p < 0.01$; ***the same at $p < 0.001$. Horizontal lines, respectively, indicate statistically significant differences of corresponding indices between different rearing periods within the same test group: dash-dotted line-statistically significant differences at $p < 0.05$; dashed line-the same at $p < 0.01$; solid line-the same at $p < 0.001$ (Groups: a-control; b-e-AGM, $g\ kg^{-1}$ in age 1-45 days (I)+50-115 days (II): b-0(I)+30(II); c-30(I)+30(II); d-50(I)+30(II); e-70(I)+30(II) $g\ kg^{-1}$)

(Fig. 1D, a). Remaining features of erythropoiesis (PCV, MCH and MCHC) on the control diet did not change with age (Fig. 1b-f).

In the trial 1-I, experimental diet with AGM in dosage of 30-50 $g\ kg^{-1}$ caused a significant increase of Hb by 7-7.7% (Fig. 1A, c-d), however, at a higher dosage (70 $g\ kg^{-1}$) no longer had a positive effect on this feature (Fig. 1A, e) which could happen due to a fiber content overdose for the chickens of this age group. AGM with neither of the dosages studied did not have an effect on the other erythropoiesis features of chickens at the 7-weeks-old age (Fig. 1B-F, c-e).

In the trial 1-II, all the chickens, including some from the control group were appointed to a diet with a supplemented AGM at the dosage of 30 $g\ kg^{-1}$. However, in the group that was appointed from the control diet in the trial 1-I to the diet with AGM at the beginning of trial 1-II, Hb at the age of 18 weeks was significantly higher than at the age of 7 weeks (Fig. 1A, b) but did not differ from the level of Hb in the control group at the age of 18 weeks (Fig. 1A, a). It means that the use of AGM that starts at the 2-nd growing period does not have a stimulating effect on the Hb level and the increase of this feature between 7 and 18 weeks of chickens' life is due to the age-related changes in erythropoiesis. Either there

was any effect on the rest of erythropoiesis features because all values in this group at the age of 18 weeks (Fig. 1B-F, b) did not differ from the control group's level (Fig. 1B-F, a).

In groups where AGM was used from the very beginning (1-I and 1-II), its effects on the erythropoiesis in the trial 1-II was dependent on the effects that it had produced in the trial 1-I. If in trial 1-I, AGM was added to the diet at the optimal dosages (30 and 50 $g\ kg^{-1}$), its effect in the trial 1-II was displayed by the fact that the level of Hb achieved in these groups at the age of 7 weeks was preserved at the age norm level up to the 18-week-old age (Fig. 1A, c-d) and the remaining features (Fig. 1B-F, c-d) at the period of 18 weeks did not differ from the control group's level for this age (Fig. 1B-F, a); although, in comparison with the trial 1-I, there was a decrease in some parameters of erythropoiesis. For instance, at 18 weeks the value of MCH (Fig. 1B, d), MCV (Fig. 1D, d) and PCV (Fig. 1E, c) were significantly lower than at 7 weeks.

If in the trial 1-I, AGM was included to the diet at a higher dosage (70 $g\ kg^{-1}$), then parameters of erythropoiesis that are the most sensitive to AGM (Hb and MCV), showed a negative effect in the trial 1-II which was caused by the overdose of AGM

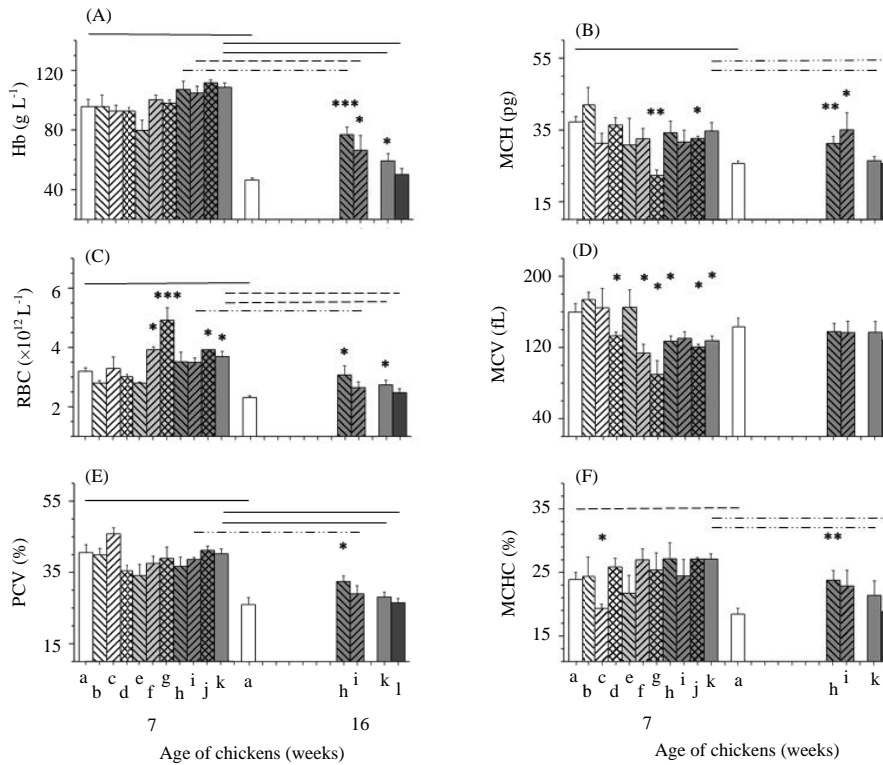


Fig. 2: Hematological indices of commercial layer replacement chickens in the 2nd trail after the I rearing period (7th week), against the background of a balanced diet and after the II rearing period (16th week), against the background of a low protein diet. The asterisks (*) above the bars indicate statistically significant differences with the control group, horizontal lines-the same differences with corresponding indices between different periods within the same test group. The notations with asterisks and horizontal lines are similar to Fig. 1. (Groups: a-control; b, c, d-AH 1, 3, 10 g kg⁻¹; e, f, g-PH 1, 3, 10 g kg⁻¹; h-PE 1-2 g kg⁻¹; i-SPPH 6 g kg⁻¹; j-SPPE 2 g kg⁻¹; k-AP 0.05-0.1 g kg⁻¹; l-CP 0.1 g kg⁻¹)

in the trial 1-I and was reflected by the significant decrease of Hb (Fig. 1A, e) and an increase of MCV (Fig. 1D, e) at the age of 18 weeks. Other parameters of erythropoiesis in this group did not differ from the levels of control group.

In the 2nd trial performed on the mixed replacement chickens flock which (that) included besides of pullets some amounts of immature males, the effects of aqueous and pectin-rich products of AGM hydrolysis-extraction and pure AP supplemented at balanced diet (trial 2-I) was investigated. After that, rebound effect (after-effect) of the use of these substances was examined on the immature males that were fed with a low-protein diet (trial 2-II).

Unlike AH which did not affect the basic indices of erythropoiesis (Hb and RBC), significantly reducing only MCV (Fig. 2D, d) and MCHC (Fig. 2F, c), that are pectin-rich hydrolysates and extracts, caused a dose-dependent increase of RBC from 10.7-63.7%, if compared to the control group (Fig. 2C, f-g, j) which was accompanied by a significant decrease in MCV (Fig. 2D, f-h, j) and MCH (Fig. 2C, g, j). Purified AP at a concentration of 50-100 mg kg⁻¹ that is equivalent to the

content of soluble pectin in the products of AGM hydrolysis-extraction which has also caused an increase of RBC by 18.2% (Fig. 2C, k) and a decrease of MCV by 20% (Fig. 2D, k).

The low-protein diet of the control group between the age of 7 and 16 weeks (Table 2, trial 2-II) caused a statistically significant decrease in all of the erythropoiesis indices, except for MCV, the reduction of which was not significant (Fig. 2D, a). Moreover, were decreasing not only those parameters of the erythropoiesis that shouldn't have been changing significantly with age PCV (Fig. 2E, a), MCH (Fig. 2B, a) and MCHC (Fig. 2F, a) but also those that should have been increasing with age on the balanced diet Hb (Fig. 2A, a) and RBC (Fig. 2C, a). Such a dramatic violation of erythropoiesis can be explained not only by a decrease in the nutritional value of the diet in trial 2-II but also by the higher growth rates of the immature males on which this trial was conducted.

Nevertheless, in those groups where pectin-rich products of AGM hydrolysis-extraction process were used in the diet up to the age of 37 days, Hb remained

significantly higher by 43-65% than in the control group (Fig. 2 A, hi) up to the 16-week-old age and the remaining erythropoiesis indices in this time did not differ significantly from the values they had at the 7-week age for RBC (Fig. 2C, h), PCV (Fig. 2E, h), MCH (Fig. 2B, h-I), MCV (Fig. 2D, h-I) and MCHC (Fig. 2F, h). Besides, at 16 weeks the values of most of these indices were significantly higher than in control group.

The effect of the use of purified AP was also preserved for 60 days after the end of its use, in particular for Hb (Fig. 2A, k) and RBC (Fig. 2B, k), although, citrus pectin at the same dosage did not cause the same effect (Fig. 2A, i and Fig. 2B, i).

In the 3rd trial conducted on the replacement chickens of the I age group with the increased functional load that is linked to the use of a diet of reduced nutritional value (Table 2, trial 3), the control group showed a characteristic changes compare to the standard for this age group: RBC decreased to $2.48 \pm 0.13 \times 10^{12}/L$, Hb decreased to $69.8 \pm 4.0 \text{ g L}^{-1}$ and MCV increased to 133.5 fL. Pectin-rich products of amaranth hydrolysis-extraction in this trial showed the therapeutic and prophylactic effect on the erythropoiesis which lies in the recuperation of erythropoiesis indices in a significant increase of RBC (Fig. 3C) and a decrease of MCV (Fig. 3D). Also, in this trial, there was a significant decrease of MCH (Fig. 3 B). Investigated products did not have a significant effect on the other features of erythropoiesis (Hb, PCV and MCHC).

Body weight gain and liveability of commercial layer replacement chickens: In the 1st trial, conducted with a balanced diet, the use of AGM in the first part of trial at the amounts of 30-70 g kg⁻¹ (trial 1-I) did not affect the average weight of the young chickens at the age of 40 days (Fig. 4 A, b-d). However, group with the maximal dosage of AGM (70 g kg⁻¹) had a higher chicken's live weight uniformity (CV% = 3.02) than in the control group (CV% = 3.82). In all trial groups with an AGM dosage of 30, 50 and 70 g kg⁻¹, flock livability was higher than in the control group (96.67%) by 0.8, 0.9 and 0.6%, respectively but if compare these groups to the mortality level in control group (3.33%), the mortality rates in relation to the control group are reduced by 24.0, 25.8 and 17.4%, respectively (Table 4, 1-I).

In the 2nd part of this trial (2nd rearing period) when chickens from all experimental groups were appointed to a diet with an AGM at dosage of 30 g kg⁻¹ (trial 1-II), the effect of the average weight increase at the age of 80 days was maintained only in the group in which the AGM in the trial 1-I also was in dosage of 30 g kg⁻¹ (Fig. 4A, b) and was significantly higher (by 3.5%) compare to the control group. The flock uniformity in this group (CV% = 3.31) was higher in compare to a control group

(CV% = 6.29). In those groups in which the AGM dosages in the trial 1-I was higher (50 and 70 g kg⁻¹), the live weight of chickens at the age of 80 days did not differ from the control group level (Fig. 4A, c-d) also as a flock uniformity indicator (CV%).

The 2nd trial, that was also conducted with a balanced diet, showed that the use of AGM hydrolysis-extraction products from the first days of life up to 37-days age has a promoting effect on the body weight of young chickens and leads to an increase of liveability up to 40 days at the much lower dosages (calculated in terms of dry weight) than the initial raw feedstock (AGM) and their effectiveness vastly varies depending on the product obtaining method.

AH showed a growth-promoting effect at a dosage of 10 g kg⁻¹, significantly increasing the body weight of the chickens by 17% (Fig. 4B-1, g) as well as the flock uniformity (CV% = 13.30), compare to a control (CV% = 13.65). PH at a dosage of 10 g kg⁻¹ increased the body weight of young chickens by only

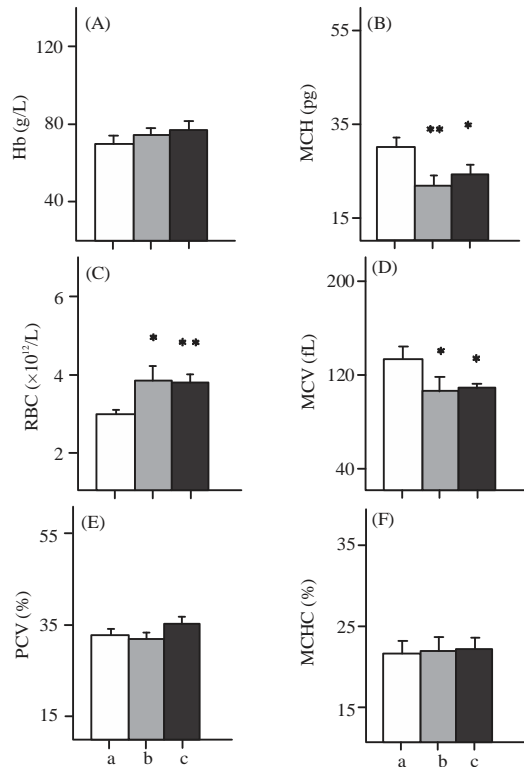


Fig. 3: Hematological indices for commercial layer replacement chickens in the 3rd trail (7th week) against the background of a low protein diet. The asterisks (*) above the bars indicate statistically significant differences with the control group. The notations with asterisks are similar to Fig. 1. (Groups: a-control ration; b-PH, 12 g kg⁻¹; c-PPH, 12 g kg⁻¹)

Table 4: Effects of the products examined on the liveability and on the mortality parameters of commercial layer replacement chickens

Trial No.	Group, product, content in feed (g kg ⁻¹)	Liveability		Mortality	
		From the original population (%)	Difference from the corresponding control group (%)	From the original population (%)	Difference from the corresponding control group (%)
1-I	Control	96.67	-	3.33	-
	AGM (30 g kg ⁻¹)	97.47	+0.8	2.53	-24.0
	AGM (50 g kg ⁻¹)	97.53	+0.9	2.47	-25.8
	AGM (70 g kg ⁻¹)	97.25	+0.6	2.75	-17.4
2/1-I	Control	94.94	-	5.06	-
	AH (1 g kg ⁻¹)	95.46	+0.6	4.54	-10.3
	AH (3 g kg ⁻¹)	96.84	+2.0	3.16	-37.6
	AH (10 g kg ⁻¹)	97.46	+2.7	2.54	-48.8
2/2-I	Control	93.75	-	6.25	-
	PH (1 g kg ⁻¹)	96.23	+2.7	3.77	-39.7
	PH (3 g kg ⁻¹)	96.08	+2.5	3.92	-37.3
	PH (10 g kg ⁻¹)	96.18	+2.6	3.82	-38.9
2/3-I	Control	97.23	-	2.77	-
	PE (1 g kg ⁻¹)	98.86	+1.7	1.14	-58.8
	PE (2 g kg ⁻¹)	97.18	-0.1	2.82	+1.8
	AP (0.05 g kg ⁻¹)	97.95	+0.7	2.05	-26.0
	AP (0.1 g kg ⁻¹)	97.85	+0.6	2.15	-22.4
	CP (0.1 g kg ⁻¹)	97.91	+0.7	2.09	-24.6
2/4-I	Control	97.71	-	2.29	-
	SPPH (6 g kg ⁻¹)	97.44	-0.3	2.56	+11.8
	SPPE (2 g kg ⁻¹)	98.84	+1.2	1.16	-49.3

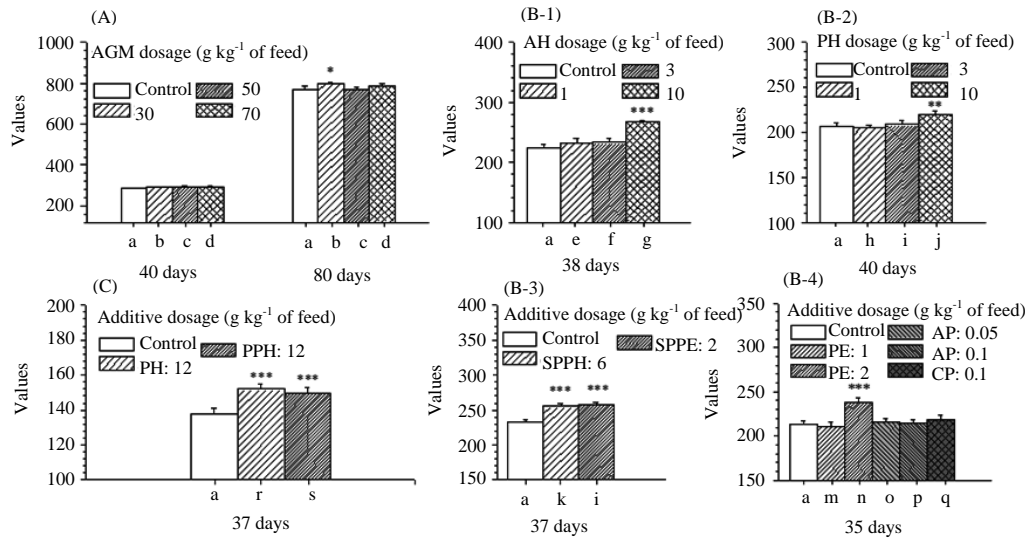


Fig. 4: Influence of the tested products from amaranth phytomass on the live weight of commercial layer replacement chickens in the 1st trial (A), in four variations of the 2nd trial (B-1-B-4) and in the 3rd trial (C). The notations under each figure indicate the age of the chickens at the time of weighing. The asterisks (*) above the bars indicate statistically significant differences with the corresponding parameters in the control groups. The notations with asterisks are similar to Fig. 1

6.4% (Fig. 4B-2, j), although, notably increased the flock uniformity (CV% = 14.24) compare to a control group (CV% = 16.89). However, PH had a more pronounced effect on a flock liveability, decreasing a mortality rate by 37.3-39.7% in relation to the control group at any dosages of 1, 3 and 10 g kg⁻¹ (Table 4, 2/2-I) while AH has had a similar effect at the dosage of 3 g kg⁻¹ (37.6%) and 10 g kg⁻¹ (48.8%) and at the dosage of 1 g kg⁻¹ was less effective, decreasing mortality rate only by 10.3% (Table 4, 2/1-I).

Effective dosages for hydrolysates (calculated in terms of dry weight) were significantly higher than for the extracts because a significant part of the dry matter in the hydrolysates (at least 70%) is the non-extracted components of the phytomass. For example, equivalent doses of SPPH (6 g kg⁻¹) and SPPE (2 g kg⁻¹), that increased the body weight of young chicks by 9.5 and 10.6%, respectively (Fig. 4B-3, k-l). Flock uniformity was increasing a bit more under the influence of the extract (CV% = 12.25), than under the hydrolysate (CV% =

13.36), compare to the control (CV% = 14.08). At the same time, SPPH did not affect the flock liveability but the use of SPPE resulted in the increase of liveability by 1.2% in respect of control group level (97.7%). It means that a mortality rate in group with SPPE is reduced by almost a half from the reference level of 2.29% (Table 4, 2/4-I).

The level of pectin by itself, which has determined the efficiency of the products of AGM hydrolysis-extraction on erythropoiesis did not affect the body weight of the young chickens. Thus, the growth-promoting effect of PE which was 11.9% (Fig.4B-4, m-n) at the dosage of 2 g kg⁻¹ was not reproduced with the equivalent doses of AP or citrus pectin (0.1 g kg⁻¹) which did not affect the chickens' body weight (Fig. 4B-4, o-q). Nevertheless, the increase of the flock uniformity, compare to the control (CV% = 16.72) for PE (CV% = 15.36) did not differ much from the effects of the pure amaranth pectin in equivalent dosage (CV% = 15.42), though the effect of citrus pectin on the flock uniformity at the same dosage (0.1 g kg⁻¹) was slightly higher (CV% = 13.83). The increase in flock liveability under the influence of pure pectin was 0.6-0.7% in relation to the control group (97.23%), that corresponds to a decrease of mortality rate at 22.4-26.0% (Table 4, 2/3-I). This effect was substantially different from the effect of PE at a dosage of 1 g kg⁻¹ which increased the flock liveability by 1.7% and the mortality rate was reduced by 58.8% from a reference level of 2.77% (Table 4, 2/3-I). The absence of effect of PE at a dosage of 2 g kg⁻¹ on the flock liveability can be explained by an overdose of the other extractive substances of the non-pectin nature (Table 4, 2/3-I).

In the 3rd trial with a low-protein diet at which the chickens were significantly lagging behind in growth compare to the peer chicken with the balanced diet, even high doses of pectin-rich hydrolysates (12 g kg⁻¹ for PH and PPH) could not provide a complete normalization of body weight, even though chickens increased own body weight by 10.2 and 8.6% (Fig. 4C, r-s) in comparison with the control group (137.9±3.0 r). Body weight uniformity was increasing due to the effects of diets with PH (CV% = 15.74) as well as with PPH (CV% = 18.95) in comparison to the control group (CV% = 19.69). This indicates that the growth-promoting effect of the products of hydrolysis-extraction of amaranth phytomass in young chickens is restricted by the amount of the nutritive substances supplies in the diet; however, improvement in the poultry performance may take place not solely with the balanced diet but also with a low-protein diet.

The results of this study made it possible to reveal that even when a layer replacement chickens during an industrial farm rearing were fed with a balanced diet, a hemoglobin level and the erythrocyte concentration were substantially lower in comparison with the reference

values obtained for the poultry in the experimental conditions: hemoglobin 92-131 g L⁻¹, erythrocyte concentration 2.8-3.0×10¹²/L (Fernandez *et al.*, 1995; Silveira *et al.*, 2009; Toghyani *et al.*, 2010). This demonstrates that the functional disruptions of the erythropoiesis in the replacement chickens do lastingly exist in the most of our trials effectuated in farm conditions, even in the control groups. Control chickens at the age of 7 weeks had a mild form of hypochromic anemia (trial 1-I) or macrocytic normochromic anemia (trials 2-I and 3-I) which then consequently progressed into a severe form of hypochromic anemia in the immature males in the trial 2-II after appointing them to a less nutritious diet.

The use of AGM, pectin-rich products of its hydrolysis-extraction and pure pectin had a therapeutic and prophylactic effect on the chickens, shown by the prevention from this or that forms of erythropoiesis disorders, by reducing their severity and in some cases by the complete recovery of the hematological indices, that characterize a normal erythropoiesis. In addition to the therapeutic and prophylactic effect on the chicken's hematopoietic system, AGM and products of its hydrolysis-extraction had a promoting effect on the poultry growth performance, flock uniformity and liveability. The results of this study showed, that biologically active substances of amaranth phytomass, including pectin, increase their own biological activity when transformed into a soluble form.

The results of this study also revealed the optimal amount of pectin as one of the vegetal components of poultry diet and allowed to evaluate pectin contribution to the effects that AGM and its hydrolysis-extraction products have on the performance of layer replacement chickens and their functional state which is rated by indices of erythropoiesis. Such a low dose of pectin (0.05-0.1 g kg⁻¹) that was used in this study and is equivalent to the amount of pectin in the effective dosages of AGM or its hydrolysis-extraction products in the replacement chickens diet was dramatically lower than the doses of pectin used earlier in poultry rations (10-80 g kg⁻¹) and led to a body weight loss (Saki *et al.*, 2011; Buraczewska *et al.*, 2007; Feltrin *et al.*, 2009) or a decrease of the egg laying performance in poultry (Rotenberg and Mason, 1977). The results obtained from these trials make it possible to recommend the use of low pectin dosage in poultry feeding that improves both the functional condition of the poultry and its hematopoiesis function but still does not impair their growth performance.

The complex effect of AGM and its hydrolysis-extraction products on the growth performance of chickens and their erythropoiesis is driven by the presence of other classes of biologically active substances of non-pectin nature along with the pectin itself.

CONCLUSION

Pectin from phytomass of *Amaranthus cruentus* L., contained therein mainly in the form of protopectin and extracted therefrom in the process of hydrolysis-extraction in the form of soluble pectin has a promoting effect on erythropoiesis in layer replacement chickens but does not affect the processes of their plastic metabolism.

Pectin-rich products from amaranth phytomass obtained by the hydrolysis-extraction process could be considered as the new therapeutic and prophylactic feed additives that are much more efficient than the primary feedstock from which they are manufactured. These additives significantly increase the growth performance of poultry and improve its functional state which results in the recuperation of erythropoiesis.

ACKNOWLEDGEMENT

This research was supported by the government assignment for FRC Kazan Scientific Center of RAS.

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