THE EFFECT OF DIET WITH AMARANTH (AMARANTHUS CRUENTUS) SEEDS ON JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA) PERFORMANCE, SOMATIC DEVELOPMENT, HATCHING RESULTS AND SELECTED BLOOD BIOCHEMICAL PARAMETERS

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Abstract. The aim of this study was to determine whether the inclusion of amaranth seeds in diet for quails would favourably affect their performance, health and reproductive indices. Research material included Japanese quails divided into three groups on the 42nd day of their life, 36 females and 12 males each. Compound feed for experimental groups II and III was supplemented with 0, 4 and 7 % amaranth seeds, respectively. Group I (control) received standard feed. During the experiment, lasting to week 40 of quail life, feed consumption and egg number and weight, as well as deaths and culling, were recorded on a daily basis. Egg hatching was performed on week 26, 32 and 38 of quail life. Egg quality was evaluated at the end of the experiment, determining their basic morphological composition. In the blood collected during slaughter, selected biochemical parameters were determined (glucose, triglycerides, total cholesterol and its low density level (LDL) and high density level (HDL) fractions, total protein, albumin, alanine amino transferase (ALAT) and aspartate amninotransferase (ASPAT)). Eighteen birds of each group were also evaluated in terms of their somatic development. Quail nutrition with a diet containing amaranth seeds improved egg fertilisation and chick hatchability. Higher percentage of the experimental component (7 %) induced a decrease in egg laying production. No changes in egg weight and morphological composition were found, except the yolk percentage which was significantly higher in the quail group receiving a 4 % addition of amaranth seeds in diet. The feeding with experimental diet did not have any effect on the growth, development and morphology of quail internal organs. Increased quantity of this component in compound feed, to 7 %, induced an increase in albumin, triglyceride and LDL cholesterol concentrations, decreasing at the same time the level of liver enzymes in blood serum.

Keywords: quail, amaranth, hatching results, somatic development, blood biochemistry

Introduction. Unconventional plant components, such as herbs, fruits and vegetables, are being used more and more frequently in animal nutrition. Most often, they are applied in a dried form or as extracts and concentrates containing numerous biologically active substances, among others flavonoids, phytosterols, alkaloids, phenols, vitamins, macro- and microelements, or polyunsaturated fatty acids. Bioactive components increase organism resistance to infections, regulate blood pressure and its biochemical parameters, as well as affect gastrointestinal tract microflora composition, ensuring the so called gut health. It is well-known that the presence of functional components in diet has favourable effects not only on bird production traits but also on egg fertilisation and chick hatchability (Bozkurt et al., 2009). An improvement was observed in reproductive indices after the introduction, among others, of black cumin (Szczerbińska et al., 2012), flax seeds (Biesiada-Drzazga, 2009) or common origanum, thyme, rosemary and turmeric (Radwan et al., 2008) into diet for laying hens. The studies using amaranth seeds on poultry nutrition have been also conducted. Specific properties of this plant are determined first of all by a high content of squalene, as well as protein with large amounts of lysine and lipids being rich in unsaturated fatty acid, particularly linoleic acid and oleic acid. The mineral content of amaranth seeds exceeds that of feed components being commonly used. This primarily refers to calcium, magnesium, copper and iron. It is also a rich source of B-group vitamins and vitamins A, E and C (He and Corke, 2003, Venskutonis and Kraujalis, 2013). Results have been obtained confirming the thesis that amaranth can be a valuable component of feeds for broiler chickens (Rouckova et al. 2004, Pisarikova et al., 2006), as a plant being very rich in biologically active substances, particularly those with high antioxidant activity (Khanam and Oba, 2013). In the available literature, however, there is no information referring to the effect of amaranth seeds on bird reproductive performance. Therefore, it becomes important to clearly determine whether biologically active substances contained in that plant show any favourable effect on reproductive indices, in particular at the end of egg laying season when egg fertilisation and chick hatchability significantly deteriorate.

Objective. The aim of this experiment was to determine the effect of amaranth seeds on the performance, selected blood biochemical parameters, hatchability rates and somatic development of Japanese quails.

Material and methods

The study was carried out on an experimental farm of the Department of Poultry and Ornamental Bird Breeding, Western Pomeranian University of Technology in Szczecin. The research material included adult Japanese quails (Coturnix coturnix japonica). The birds were from own hatching and rearing.

Table 1. **Composition of compound feed**

Item	Group		
	I	II	III
Feed components [%]			
Amaranth meal	0	4	7
Maize meal	24.00	44.00	49.00
Wheat meal	41.14	16.51	8.56
Soybean oil	0.50	0.50	0.50
Extracted soybean meal	26.40	27.00	26.95
NaCl	0.21	0.21	0.21
Monocalcium phosphate	1.25	1.25	1.25
Limestone	5.10	5.10	5,10
DL-Methionine (technically pure)	0.11	0.12	0.12
L-Lysine hydrochloride	0.24	0.26	0.26
Kemzyme X DRY [1)]	0.05	0.05	0.05
Toxfin [2)]	0.10	0.10	0.10
Lutamix DJR [3)]	0.50	0.50	0.50
Sodium hydrogen carbonate	0.20	0.20	0.20
Natuphos 5 % Layer [4)]	0.20	0.20	0.20
Chemical composition			
Dry matter [g]	883	883	884
Metabolisable energy [MJ/kg] – calculated	11.7	11.5	11.3
Total protein [g]	201.5	197.6	197.3
Crude fibre [g]	20.7	19.9	18.9
Crude ash [g]	6.62	6.47	6.61
Total phosphorus [g] – calculated	8.15	7.95	9.20
Assimilable phosphorus [g] – calculated	5.75	5.67	5.62
Lysine [g] – calculated	11.48	11.44	11.27

*1) dry stabilised enzyme preparation containing α-amylase, β--xylanase, β--glucanase *2) preparation against mycotoxins *3) vitamin-mineral premix *4) enzyme preparation containing phytase

On day 42 of life, the quails were weighed and divided into 3 groups with equal weight. Each of the groups comprised 36 females and 12 males. Control group (I) received a feed with standard composition, being produced from maize, wheat and soybean meal (Table 1). Compound feeds for groups II and III were supplemented with 4 and 7 % amaranth seeds, respectively. The quails were kept in group cages, under controlled micro-climate conditions (temp. 20 ºC, relative humidity 65–75 %), applying a proper day-light programme (17L:7D).

During the experiment lasting to week 40 of quail life, feed consumption and egg number and weight, as well as deaths and culling, were recorded. On week 26, 32 and 38 of quail life, the analysis of egg hatching was performed. Eggs were collected over a 10-day period and candled to eliminate those being unfit for incubation and then stored in a dark room at 18 ºC and relative humidity 60–65 %. On the day of setting, eggs were disinfected with formaldehyde vapours, using 21 cm^3 40 % formaldehyde, 17g $KMnO_4$ and 21 cm^3 water per 1 m^3 of disinfection chamber. Hatches were carried out in a box-type hatching apparatus, following the technique appropriate for that poultry species. After completion of each hatch, the analysis of dead embryos was performed, paying attention to the condition of foetal membranes, position defects, morphological deformations, degree of yolk sac absorption, as well as appearance of skin (hyperaemia) and internal organs.

On week 40 of quail life, the quality of eggs was evaluated, determining their basic morphological composition. From each group, 15 eggs were sampled at random, in which albumen, yolk and eggshell weights were determined after boiling.

At the end of the experiment, 12 females and 6 males from each group were slaughtered and dissected to analyse somatic development. Female livers, stomachs and hearts and male testicles were weighted and evaluated in terms of their morphology (size, colour, shape and tissue consistency).

From the blood being sampled during the slaughter of 12 females from each group, blood serum was separated, in which the content of selected biochemical constituents was thereafter determined using BIOLABO (France) reagents. Total cholesterol was assayed colorimetrically by the enzymatic method according to Allain et al. (1974). The same method was used to determine the percentage of HDL cholesterol fraction, after prior precipitation of LDL, VLDL (very low density lipoprotein) and sample chylomicrons with phosphomolybdic acid and magnesium chloride and then centrifugation of sediment. Blood serum triglycerides were also assayed colorimetrically with enzymatic reactions, in accordance with the method of Fossati and Prencipe (1982). Knowing the contents of total cholesterol, its HDL fraction and triglycerides, it was possible to determine the percentage of LDL cholesterol fraction by the estimation method of Friedewald et al. (1972). Total blood serum protein was assayed colorimetrically by the biuret method described by Guobing et al. (2001). Glucose in blood serum was assayed by the enzymatic method according to Trinder (1969). The basis for colorimetric assay of the albumin content in blood serum according to Rodkey (1965) was the reaction of coloured albumin complex formation with bromocresol green (BCG). The activity of aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) was assayed with coupled enzymatic kinetic methods according to Karmen et al. (1955) and Wroblewski and Ladue (1956), respectively, with further modifications in both methods being recommended by International Federation of Clinical Chemistry (IFCC).

The collected data were subjected to one-way analysis of variance and Duncan's multiple range test using STATISTICA 10 PL computer software package.

Results. Introduction of amaranth seeds into diet for quails did not affect the weight of quail eggs (Table 2). The highest egg laying performance was characteristic of the quails receiving control feed (80 %), whereas the lowest one of the birds from the group being fed a compound feed with a 7 % addition of the component under evaluation (76 %). Daily feed consumption in the experimental groups was significantly lower than in the control group. Probably, the application of amaranth seeds in complete feed negatively affected its tastiness, which could decrease the quail appetite (Table 2).

The flock health did not raise any objections. Deaths and culling were a consequence of mechanical injuries, which happens in quails kept under caged system. It therefore can be concluded that they were of accidental nature and were not related to the experimental factor (Table 2).

No significant effect of the experimental component on the morphological composition of quail eggs was found (Table 3). Only in the quail group with a 4 % addition of amaranth seeds in their diet, a significantly higher relative yolk weight was observed.

Introduction of amaranth seeds into diet for quails had a favourable effect on their reproduction results (Table 4). The fertilisation rate in the experimental groups amounted to 98–92 % and was significantly higher when compared to the control group (82 %). The best hatchability from the set eggs (86 %) was found in group II with a lower percentage of the experimental component.

Table 2. **Quail production traits** (mean ± SD)

ITEM		Egg weight [g]	Egg production [%]	Feed consumption [g/day]	Mortality (n)
I	control	12.08 ± 0.23	80.00 a ± 8.85	29.5 A ± 2.91	3*
II	4 % amaranth	11.80 ± 0.35	79.59 a ± 5.84	28.6 B ± 2.79	3*
III	7% amaranth	12.02 ± 0.26	75.96 b ± 5.54	28.7 B ± 2.74	2*
A, B – mean values within columns marked with different letters differ significantly (P≤ 0.01); a, b – mean values within columns marked with different letters differ significantly (P≤ 0.05); * – females					

Table 3. **Morphological composition (%) of eggs** (mean ± SD)

Item		Egg weight [g]	Yolk	Albumen	Eggshell
I	control	12.36 ± 0.60	28.60 a ± 1.99	61.20 a ± 2.75	10.61 a ± 0.98
II	4% amaranth	11.87 ± 1.00	30.41 b ± 4.39	60.30 a ± 1.55	10.15 a ± 0.79
III	7% amaranth	11.95 ± 0.87	29.31 a ± 1.50	60.47 a ± 2.74	10.17 a ± 2.01
a, b – mean values within columns marked with different letters differ significantly (P ≤ 0.05)					

Table 4. **Quail reproductive traits – results from three egg settings on the 26th, 32nd and 38th week of quail life** (mean ± SD)

ITEM		Set eggs [n]	Rates [%]		
			Fertilization	Hatchability of set eggs	Hatchability of fertilised eggs
I	control	392	82.14 Aa ± 5.56	67.24 Aa ± 4.09	81.56 ± 5.13
II	4% amaranth	368	97.82 Bb ± 1.99	86.28 Bb ± 6.38	88.01 ± 5.97
III	7% amaranth	410	91.95 ABb ± 7.65	78.14 ABb ± 9.00	85.69 ± 7.35
A, B – mean values within columns marked with different letters differ significantly (P ≤ 0.01); a, b – mean values within columns marked with different letters differ significantly (P ≤ 0.05)					

The highest final body weight (Table 5) was found in the control group females (211 g), whereas the lowest one in those of the quail group with a 7 % addition of amaranth seeds in their diet (205 g). It was probably related to a smaller feed uptake by the quails from the experimental groups. Inverse relationships were observed in the case of males. In the quail group receiving a 7 % addition of amaranth seeds in a feed ration, the males were characterised by the highest body weight (176 g) when compared to other groups (approx. 160 g) but the differences proved to be non-significant. No differences in the percentages of internal organs in quail body weight were shown either. As a matter of fact, the relative weight of liver in both experimental groups was much larger but its statistical analysis did not confirm the significance of these differences.

Table 5. **Quail body weight and the percentage of some internal organs** (mean ± SD)

ITEM	Group		
	I control	II 4% amaranth	III 7%amaranth
Females body weight [g]	211.4 ± 16.20	208.4 ± 15.70	205.4 ± 18.06
heart weight [%]	0.91 ± 0.09	0.89 ± 0.09	0.92 ± 0.12
stomach weight [%]	2.5 ± 0.46	2.4 ± 0.21	2.7 ± 0.26
liver weight [%]	2.5 ± 0.28	2.6 ± 0.35	3.0 ± 0.40
Males body weight [g]	160.5 ± 29.48	163.8 ± 14.16	176.3 ± 10.91
testicles weight [%]	3.4 ± 0.72	3.3 ± 0.75	3.2 ± 0.35

Inclusion of amaranth seeds in diet for quails did not have any effect on the concentration of total protein, total cholesterol and HDL cholesterol in blood serum, whereas significantly decreased the glucose level (Table 6). The highest concentration of that sugar (15.64 mmol/L) was recorded in the control group but the significantly lowest one in the experimental groups (12.65 and 13.35 mmol/L).

The lowest albumin content in blood serum was found in the control group (12.0 g/L) but a significantly higher one in the quails being feed a compound feed supplemented with a 7 % addition of amaranth seeds (15.3 g/L). Moreover, a significant decrease in the quantity of liver enzymes (ALAT and ASPAT) in blood serum was found in this group. It was demonstrated that application of a larger dose of the component under evaluation in compound feed significantly increased the concentration of triglycerides and LDL cholesterol fraction.

Table 6. **Quail blood biochemical parameters** (mean ± SD)

ITEM	Group		
	I control	II 4 % amaranth	III 7 % amaranth
ALAT [IU/L]	43.94 A ± 19.98	54.48 A ± 16.79	21.60 B ± 13.53
ASPAT [IU/L]	92.90 AB ± 25.82	113.27 A ± 29.83	67.80 B ± 33.16
GLUCOSE [mmol/L]	15.64 A ± 1.53	12.65 B ± 1.65	13.35 B ± 1.93
ALBUMIN [g/L]	12.0 A ± 2.2	13.0 A ± 0.7	15.3 B ± 1.8
TOTAL PROTEIN [g/L]	39.5 ± 7.6	43.6 ± 6.1	42.7 ± 5.0
CHOLESTEROL [mmol/L]	5.43 ± 2.32	6.18 ± 1.56	6.17 ± 2.31
HDL [mmol/L]	2.34 ± 0.75	2.47 ± 0.48	1.87 ± 0.87
LDL [mmol/L]	1.67 A ± 0.48	1.43 A ± 0.60	2.44 B ± 0.86
TRIGLYCERIDES [mmol/L]	2.61 A ± 1.40	4.58 B ± 1.43	4.03 B ± 1.93
A, B – mean values within columns marked with different letters differ significantly ($P \leq 0.01$)			

Disscusion. An important aspect in the management of breeding flocks in all poultry species is to obtain a large number of eggs with a high biological value. The studies conducted so far have suggested that bird nutrition with a diet containing amaranth results in improvement of their performance traits (Vishtakalyuk et al., 2001). The cited authors have found the highest egg weight and the best egg laying performance in the group of hens being fed with a compound feed containing a 3 and 7 % addition of amaranth seeds. They have observed a higher mortality and a significant deterioration of egg laying performance, particularly in the egg weight, when applying its higher addition, i.e. 17 %. In the next experiment by these authors (Vishtakalyuk et al., 2010), an acceleration of sexual maturity and an increase in the egg number and weight have been observed in hens after application of amaranth in the amount of 3, 5 and 7 %. These results have not been confirmed in the study by Fasuyi et al. (2007), who have obtained the highest egg weight in the group of birds being fed a feed ration with a 20 % addition of amaranth leaf meal. According to the present study, the application of much smaller quantities of amaranth seeds (7 %) worsens not only the egg laying production of quails but also their appetite. Similar results have been obtained by Tillman and Waldroup (1987) when feeding lying hens with amaranth. In the case of broiler chickens, a smaller feed consumption has been also observed, with a simultaneous increase in the body weight (Kabuage et al., 2002). On the other hand, it has been found in the experiment by Vishtakalyuk et al. (2001) that feed intake is similar in the whole experimental flock, irrespective of the diet being applied. Differences in the findings of different authors may be a consequence of varied doses of the experimental component. Moreover, it should be kept in mind that the studies being mentioned above have been conducted on different bird species and production groups (Japanese quails, laying hens, broiler chickens) which could differently respond to feeding with amaranth.

The studies being conducted so far have suggested that application of amaranth seeds in bird nutrition has an effect on egg morphological composition. Vishtakalyuk et al. (2001) have found a higher percentage of albumen and yolk, by 4.7 and 4.5 %, in the eggs of experimental groups. Khiroug et al. (2001), after adding polysaccharides being obtained from amaranth seeds to the feed for hens, have observed an increase in the egg weight and the albumen and yolk percentages, with a simultaneous decrease in the relative weigh of eggshell.

The present study shows that quail nutrition with a diet containing amaranth seeds has a favourable effect on reproduction results. Similarly, Vishtakalyuk et al. (2001) have obtained a higher fertilisation rate, by 13.6 %, and better chick hatchability, by 28.4 %, when introducing this component into a feed ration for hens. It has been demonstrated that amaranth is a plant being rich in biologically active substances and its seeds contain large amounts of squalene and polyunsaturated fatty acids, which show a positive effect on reproduction, also in the case of other animal species (Zhang et al., 2008; Li et al., 2010). Improvement in the fertilisation and hatchability rates may be a consequence of better male semen quality. Essential fatty acids (EFA) have a positive effect on spermatozoa motility, as well as reduce the risk of defects in their structure. Omega-3 and omega-6 fatty acids considerably protect chromosomes against the occurrence of abnormalities. Squalene counteracts disturbances in the function of mitochondria, also those being found in the middle piece of spermatozoon (Singh et al., 2003).

Results similar to the present ones, referring to somatic development of birds, have been obtained by Fasuyi et al. (2008). The study conducted by Kabuage et al. (2002) shows minor morphological changes in the livers of birds being fed with amaranth. In the present study, no anomalies have been observed within quail internal organs, or differences in their relative weight depending on the diet being applied, which gives us the basis to think that the component being examined does not have a negative effect on the growth and development of birds.

An important aspect of the studies being conducted with non-conventional feed components is to evaluate their effect on the bird metabolism. Scarce data in this field suggest that this plant affects carbohydrate and lipid metabolism in birds. Roučkova et al. (2004) has found a significant increase in the glucose content in the blood of chickens after application of amaranth seeds in their diet. According to Qureshi et al. (1996), feeding a feed with an addition of amaranth oil to laying hens has induced a considerable decrease in the concentration of total cholesterol and its LDL fraction. The present findings suggest that biologically active substances contained in amaranth have an effect on the quail liver and thereby also on blood biochemical parameters, in the synthesis or metabolism of which the liver takes part. The elevated level of LDL cholesterol in blood serum has not had a negative effect on the health of quails since linoleic and linolenic acids contained in amaranth seeds support the metabolism of lipids and eliminate its harmful effect to some extent.

Conclusion. Introduction of amaranth seeds into diet in the amount of 7 % significantly deteriorated the results of egg laying performance in quails. No changes were observed in the weight of eggs and their morphological composition, except the yolk percentage which was significantly higher in the quail group receiving a compound feed supplemented with a 4 % addition of amaranth seeds. The feeding with experimental diet did not have any effect on the growth, somatic development and morphology of quail internal organs. The best egg fertilisation and chick hatchability rates were obtained in the quail group receiving a compound feed supplemented with a 4 % addition of amaranth seeds. Increased quantity of the feed component under evaluation, to 7 %, induced an increase in the concentration of albumin, triglycerides and LDL cholesterol fraction, decreasing at the same time the level of liver enzymes.

In summary, it can be concluded that amaranth seeds may be used in quail nutrition in the amount to 4 % of feed ration. A higher percentage of that component induces a drop in egg laying production in quails, having no significant effect on the improvement of their reproductive traits.

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Received 19 May 2014
Accepted 16 October 2014