DIETARY EFFECT OF WHEAT VARIETY AND FAT SOURCE ON PERFORMANCE AND EGG QUALITY CHARACTERISTICS OF LAYING HENS

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ABSTRACT

The importance of keeping laying hens lies in egg production. In terms of quality, the most important feature for the simplistic consumer is the colour of the egg yolk. For this reason, either natural or synthetic carotenoids are often added to mixed feed for laying hens. The aim of this experiment was to evaluate the dietary effect of PEXESO wheat with increased carotenoid concentrations in hen diets on performance and egg quality characteristics. Two hundred forty 42-week-old Lohmann Brown hens were divided into 4 treatment groups according to the wheat variety (TERCIE × PEXESO) and fat source (rapeseed oil × pork lard) in the diet. PEXESO wheat in the diet decreased daily feed

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intake ($P < 0.001$). Higher values of the egg yolk colour ($P < 0.001$) and strength of the eggshell ($P < 0.001$) were found in hens that were fed PEXESO wheat diets. The lutein ($P < 0.001$ and $P = 0.001$) and zeaxanthin ($P < 0.001$ and $P = 0.001$) contents in the egg yolks and the oxidative stability of fresh ($P < 0.001$ and $P = 0.008$) and stored ($P = 0.050$ and $P = 0.021$) eggs were positively influenced by PEXESO wheat and pork lard. The diet with PEXESO wheat and rapeseed oil increased the concentration of α-tocopherol ($P = 0.008$) and γ-tocopherol ($P = 0.012$) in the egg yolk. In conclusion, PEXESO wheat increased the retention of biologically active substances, which was subsequently reflected in the performance and quality of the products, i.e. eggs.

Keywords: poultry nutrition; carotenoids; tocopherols; egg quality; yolk colour

INTRODUCTION

Livestock farming is an important part of the agricultural industry, and domestic poultry farming, as a basic animal husbandry industry, plays an important role in promoting economic development, ensuring market supply, and increasing farmers' income (Ruiz-Garcia et al., 2009; Nkukwana, 2018). The use of poultry products (meat and especially eggs) is an effective way for humans to obtain animal protein in daily life (Scholten et al., 2013). The importance of keeping laying hens lies in egg production. Eggs are one of the most nutritious foods. In terms of quality, the most important feature for the simplistic consumer is the colour of the egg yolk (Berkhoff et al., 2020). Preferences for yolk richness vary considerably from country to country according to consumer requirements (Hernandez et al., 2000).

Also, the yolk colour of farm eggs has always been rated higher than that of industrial eggs. This was likely owing to the fact that industrial eggs had a pale-yellow yolk, while the yolk of farm eggs was a more intense yellow with some orange tones (Titcomb et al. 2019). For this reason, either natural or now exclusively synthetic carotenoids are often added to mixed feed for laying hens.

Carotenoids are one of the widespread and ubiquitous lipid-soluble pigments that produce a wide range of colours which are universally found in various plants, micro-algae, bacteria, yeast, mould and fungi (Nabi et al., 2020). They are categorized into two groups according to their chemical structure, in particular the presence or absence of oxygen in the molecule, and according to their functional properties; carotenes, including α-carotene, β-carotene and lycopene and xanthophylls, such as lutein and zeaxanthin (Saini et al., 2015). The animals and birds are incapable of synthesize carotenoids in vivo, so these pigments must be supplemented through diet (O'Byrne & Blaner, 2013). Dietary addition of carotenoid pigments to poultry diet improves the colour of skin, flesh and especially the egg yolk (Carvalho et al., 2009). Grazing poultry obtain xanthophylls from pasture vegetation. In large farms without the access to grazing for the animals, synthetic xanthophylls are added to poultry mixed feed for economic reasons. Xanthophylls of natural origin are gaining popularity among consumers who refuse the use of synthetic substances in livestock nutrition. On this basis, the number of people interested in what have become known as functional foods is expanding.

Recently, carotenoid pigments are of interest to the food and feed industry, researchers, nutritionists and food and feed scientists due to their multiple bioactive and health-promoting benefits including antioxidant, anti-inflammatory, immunomodulatory and also playing an important role against several diseases (Rao & Rao, 2007; Arain et al., 2018). Dietary supplementation of carotenoids to the poultry birds improves the production performance and health of birds, but also enhance the quality of eggs and meat (Langi et al. 2018). Several studies have suggested that carotenoids reduce the heat and oxidative stress in pre-hatched and post-hatched birds (generally in the host body) through different mechanisms, including quench free radicals, activating antioxidant enzymes and inhibiting the signalling pathways (Sahin et al., 2011; Arain et al., 2018; Langi et al., 2018). Xanthophylls (lutein and zeaxanthin) supplementation in hens' diets has been shown to increase antioxidant capacity and decrease malondialdehyde (MDA) production in the liver and serum (Gao et al., 2013).

Many studies have examined the effects of different sources of lutein and zeaxanthin (e.g. alfalfa concentrate, tomato powder, marigold extract, coloured carrots etc.) on poultry metabolism and the enrichment of eggs and meat with these xanthophylls. Currently tested natural carotenoids are too expensive and therefore are not economically feasible for practical applications. The most commonly used organic source of both xanthophylls is the extract from the flowers of marigold (Tagetes erecta), which is also economically acceptable (Skřivan et al., 2016). Biofortified arable crops can be a special alternative to natural sources of carotenoids. Biofortification is a very cost-effective process that increases the concentration of essential nutrients in crops through agronomic intervention or genetic selection (White & Broadley, 2005). Wheat (Triticum aestivum), as one of the most widely grown cereal crops, accounts for more than 1/4 of field food crop production and nearly 2/3 of the daily energy intake of the population in several developing countries. Related to this is the fact that the nutritional value of wheat has a significant impact on human health (Wang et al., 2011). Biofortification of wheat with mineral micronutrients has been successful and thus far has the most widespread use. Examples of successful biofortification in terms of carotenoid content are Golden Rice producing β-carotene (Paine et al., 2005) or transgenic Multivitamin Corn providing β-carotene, lycopene, lutein, zeaxanthin, ascorbic acid and folate (Naqvi et al., 2009).

In this experiment, the biofortified wheat variety PEXESO with increased concentration of lutein and zeaxanthin was compared with the common wheat variety TERCIE in the presence of two dietary sources of fat (rapeseed oil × pork lard) with contrasting fatty acid content in mixed feed for laying hens. Vegetable oils and animal fats, which are usually less expensive but may adversely affect the fatty acid composition of animal products, are added to compound feed to balance the metabolic energy requirements of poultry. Therefore, two dietary sources of fat and two varieties of wheat were assessed in this experiment to answer questions about their relationships with metabolism, performance and quality of eggs produced.

MATERIAL AND METHODS

Animals, Husbandry, Diets and Performance

The experiment was performed with two hundred forty Lohmann Brown laying hens (Enterprise for egg production, Kosičky, Czech Republic) at the age of 42 weeks that were housed in enriched cages (10 hens per cage). The hens were assigned to 4 treatments with 6 replicates according to the wheat variety (TERCIE × PEXESO) and fat source (rapeseed oil × pork lard) in the mixed feed. All mixed feeds in the experiment contained 61% of one or other wheat variety and 3% of one or other fat source. The content of lutein and zeaxanthin in wheat variety TERCIE was 0.45 mg/kg and 0.22 mg/kg, respectively. The content of lutein and zeaxanthin in wheat variety PEXESO was 1.14 mg/kg and 0.79 mg/kg, respectively. Both wheat varieties were bred in the Czech Republic by the company Selgen, a.s. (Prague, Czech Republic). In order to achieve the normal metabolizable energy requirement, it is necessary to add fat to poultry mixed feed. In the experiment, two fat sources with contrasting fatty acids proportions were used. Mixed feeds for hens were mixed in the state enterprise International Poultry Testing (Ústrašice, Czech Republic). Feed and fresh water were provided ad libitum. The technological and microclimatic conditions of the housing corresponded to the management guide of the given Lohmann Brown hybrid. The length of the light day was 16 hours (2.30 – 18.30). The number of hens and their health status were checked daily during the experiment. Hen – day egg production was recorded daily, and feed intake was calculated weekly, both on a per-cage basis. The experiment lasted 10 weeks. The eggs for chemical analyses of the yolk (carotenoid ad vitamin contents and oxidative stability of fat) were collected in the 50th week of age of the hens. Three eggs each replicate were homogenized to form one sample (n = 6). The analyses were carried out in the laboratories of Institute of Animal Science (Prague – Uhříněves, Czech Republic).

Physical Analysis of Eggs

A whole day of egg production (50^{th} week of age of the hens) was analysed for the determination of physical parameters. A total of 438 eggs were analysed. The values were averaged per cage (n = 6). The yolk colour was evaluated visually with a DSM yolk colour fan (DSM Nutritional Products, Basel, Switzerland) from 1 to 15 and further expressed as a* and b* values using the Minolta SpectraMagicTM NX (Konica Minolta Sensing, Inc., Osaka, Japan). The value a* correspond to redness (– 100 = green, 100 = red) and the value b* to yellowness (– 100 = blue, 100 = yellow). The shell breaking strength was evaluated on the vertical axis using an Instron 3360 apparatus (Instron, Norwood, MA, USA).

Chemical Analyses

The egg yolks were lyophilised before the analytical determination of carotenoids and vitamins. The concentrations of carotenoids, lutein and zeaxanthin, were analysed by a high-performance liquid chromatography (HPLC) system (VP series; Shimadzu, Kyoto, Japan) according to a modified method of Froescheis et al. (2000). The HPLC instrument was equipped with a diode array detector. A Kinetex C18 column (100 × 4.6 mm; 2.6 μm) supplied by Phenomenex (Torrace, CA, USA) was used. A gradient system was established, where eluent A was acetonitrile:water:ethyl acetate (88:10:2) and eluent B was acetonitrile:water:ethyl acetate (88:0:15). The carotenoid content was expressed in mg per kg of egg yolk dry matter. The retention of carotenoids in the yolk was calculated using the following formula:

$$R = \frac{A \times B \times C}{D}$$

R = retention (%)

A = egg mass (g/hen/day)

B = proportion of yolk (%)

C = carotenoid content in fresh egg yolk (mg/g)

D = carotenoid intake (mg/hen/day)

The α-tocopherol and γ-tocopherol contents were evaluated in accordance with the European standards EN 12822-1 (2000) and EN 12823-1 (2000), respectively, by a HPLC system (VP series; Shimadzu, Kyoto, Japan) equipped with diode array detector. Samples were subjected to alkaline saponification with 60% potassium hydroxide (KOH) followed by appropriate diethyl ether extraction. The lipid peroxidation levels in the yolk of fresh eggs and eggs stored on paper trays for 28 days at a temperature of 18 °C and relative humidity of 50 – 55 % were measured using the modified method of Czauderna et al. (2011). A Phenomenex C18 column (Synergi 2.5 μm, Hydro-RP, 100 Å, 100 mm × 3 mm, Torrace, CA, USA) was used for chromatographic analysis (Shimadzu HPLC system (VP series; Shimadzu, Kyoto, Japan) equipped with a diode array detector). Solvent A consisted of water-acetonitrile (95:5, v/v), and solvent B consisted of acetonitrile. The lipid oxidative stability was expressed in mg of malondialdehyde (MDA) per kg of eggs.

Statistical Analyses

The obtained experimental results were analysed by two-way analysis of variance (ANOVA) with the general linear model (GLM) procedure using SAS software (SAS v 9.3 Institute, 2003). The main effects were the wheat variety, the fat source and the interaction between these two factors (wheat*fat). The cage was the experimental unit (n = 6). The results in the Table 1 are presented as the mean, standard error of the mean (SEM) and probability (P) value. A statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

The selected results of dietary effect of wheat variety and fat source on performance and egg quality characteristics of laying hens are summarized and presented in Table 1.

There were no significant differences between groups in the hen – day egg production. Hen – day egg production was slightly higher only in the combination of PEXESO wheat with pork lard (94.70 %) as a source of fat in the mixed feed. Significant differences in egg mass production were due to the fat source in the diet ($P < 0.001$). Higher egg mass production ($P = 0.007$) was found in hens mixed feed with PEXESO wheat in combination with rapeseed oil (59.03 g/hen/day) and pork lard (58.56 g/hen/day) compared to mixed feed with TERCIE and rapeseed oil (58.00 g/hen/day). The main result within the performance indicators in favour of PEXESO wheat was lower daily feed intake ($P < 0.001$). Dietary supplementation with carotenoids improves the production performance and health of poultry birds (Nabi et al., 2020). In addition, carotenoids have antioxidant, anti-inflammatory and immunomodulatory effects, which may also affect the performance of birds (Arain et al., 2018). The positive effect of carotenoids on the performance of broiler chickens is also evident, for example, in a study by Fathi et al. (2022). PEXESO wheat positively increased the final egg yolk colour ($P < 0.001$) according to the DSM yolk colour fan (PEXESO*RO 3.48 DSM; PEXESO*PL 3.52 DSM). The parameters of redness (a*) and yellowness (b*) were influenced by wheat variety ($P < 0.001$) and wheat*fat interaction (a* $P = 0.030$; b* $P = 0.007$), but not by the fat source itself. In particular, PEXESO wheat in hens mixed feed significantly increased the parameter of yellowness (b*), both in combination with rapeseed oil (26.8) and pork lard (28.7) as a source of fat in the diet. The colour of the egg yolk varies considerably in terms of individual preferences across the European Union (Hernandez et al., 2000). Central European consumers prefer colouring between DSM levels 10 and 14 (Galobart et al., 2004), and therefore the inclusion of PEXESO wheat in the wheat-soybean type of mixed feeds, which, among other things, are common in Europe, will not provide optimal colouration of egg yolk according to the requirements of many consumers. In this case, it is necessary to add another source of colour pigments, such as marigold. The concentration of lutein and zeaxanthin in commercial marigold-based products are many times higher (Skřivan et al., 2016) than in the biofortified PEXESO wheat used in this experiment. The ideal source of natural carotenoids is pasture vegetation. In a study by Skřivan & Englmaierová (2014) it was found that the access of hens to natural grazing together with sequential feeding of wheat increased the concentration of carotenoids in the yolk and significantly raised its colour score to 10.3 according to the Roche Yolk Colour Fan (RYCF). PEXESO wheat increased strength of the eggshell ($P < 0.001$), which is the main technological characteristics of the egg, mainly in terms of production economics. Lutein ($P < 0.001$) and zeaxanthin ($P < 0.001$) levels in egg yolk were consistent with their several-fold higher concentrations in the diets with PEXESO wheat. The deposition of the carotenoids in the yolk was also affected by the source of the fat ($P = 0.001$) in the diet. Higher levels of carotenoids were measured in the case of hens fed pork lard. The higher deposition of lutein and zeaxanthin in the egg yolk was due to the higher dietary concentration of both xanthophylls in PEXESO wheat and was also supported by their higher retention. The retention of carotenoids, calculated according to above formula, was affected by wheat variety ($P < 0.001$) and fat source ($P < 0.001$). Also, the wheat*fat interaction was significant for the retention of lutein ($P < 0.001$) and zeaxanthin ($P = 0.002$). The highest retention of lutein was observed by PEXESO wheat in combination with both rapeseed oil (55.6 %) and pork lard (55.8 %). On the other hand, the retention of zeaxanthin was highest only in PEXESO wheat with pork lard (69.4 %) compared to the other groups. Lipids have a positive effect on the absorption of lipophilic carotenoids. Dietary fats rich in saturated fatty acids lead to a higher availability of lutein and zeaxanthin compared with fats rich in monounsaturated and polyunsaturated fatty acids (Gleize et al., 2013), which is consistent with the findings of this experiment. The hens mixed feed with PEXESO wheat and rapeseed oil increased the concentration of α-tocopherol (174.1 mg/kg DM (dry matter); $P = 0.008$) and γ-tocopherol (22.98 mg/kg DM; $P = 0.012$) in the egg yolk. The significant increase in tocopherol concentrations was most likely due to rapeseed oil, which is a rich source of these vitamins. The availability of lipophilic vitamins may be affected by the unsaturation of dietary lipids. Prévéraud et al. (2015) showed that a diet containing flaxseed oil resulted in better α-tocopherol bioavailability in adult roosters than a diet containing hydrogenated coconut oil. The increased concentration of α-tocopherol and γ-tocopherol may also be related to the antioxidant properties of carotenoids, which protect these lipophilic compounds against oxidative degradation in the upper gastrointestinal tract (Mortensen et al., 2001). PEXESO wheat, used in this experiment, significantly reduced the process of oxidation of yolk lipids. The process of reducing the oxidation was undoubtedly contributed by lutein and zeaxanthin, because these natural lipophilic pigments are also powerful antioxidants. The better shelf life of the eggs produced is confirmed by the fact that the carotenoids absorbed from the feed are preferentially deposited in the eggs by the hens. The increased oxidative stability of egg yolk due to lutein and zeaxanthin has also been demonstrated by studies (Englmaierová et al., 2013; Skřivan et al., 2016; Kljak et al., 2021). The formation of MDA oxidation products in fresh and stored eggs decreased in hens fed mixed feed containing PEXESO wheat ($P < 0.001$ and $P = 0.050$) and pork lard ($P = 0.008$ and $P = 0.021$). As measured by the decrease in MDA production, the increased effect of egg storability resulting from lard compared to rapeseed oil in the diet was due to higher proportion of saturated fatty acids. In addition, a significant wheat*fat interaction was found for MDA content in fresh eggs ($P = 0.041$). Increased lipid oxidation in fresh egg yolks (0.448 mg MDA/kg eggs) was observed in the TERCIE wheat and rapeseed oil diet group compared to the other groups. This was due to the lower content of natural carotenoids in TERCIE wheat and probably also to the easier oxidation of the more unsaturated rapeseed oil compared to saturated pork lard.

CONCLUSION

Increasing the content of natural carotenoids in mixed feed for laying hens is highly desirable, both in terms of promoting the health of the farmed animals themselves and the production of quality animal products that are beneficial to human health. The wheat variety PEXESO, with an increased content of lutein and zeaxanthin, included in the hen’s diet positively influenced the performance and some quality parameters of the eggs produced. This was probably related to the higher retention of biologically active substances. However, the colouring effect of the wheat variety PEXESO was insufficient, and the resulting colour of the egg yolk would clearly not satisfy consumer requirements. This wheat can be recommended in combination with rapeseed oil as a source of fat in the diet and also with the addition of another source of natural carotenoids (e.g. from the marigold extract or natural pasture), which together with this wheat will provide the consumer with a quality product. Particularly in terms of preference and optimum colouring of the egg yolk.

Table 1. Selected performance characteristics of laying hens, physical characteristics of egg quality, and vitamin, carotenoid and MDA concentrations in egg yolks

Group	I	II	III	IV	SEM	Probability		
Wheat variety	TERCIE		PEXESO					
Fat source	RO	PL	RO	PL		Wheat	Fat	Wheat*fat
Hen – day egg production (%)	93.48	93.93	93.77	94.70	0.268	NS	NS	NS
Egg mass (g/hen/day)	58.00[b]	58.15[ab]	59.03[a]	58.56[a]	0.184	NS	<0.001	0.007
Feed intake (g/hen/day)	115.7	114.7	111.6	111.2	0.382	<0.001	NS	NS
DSM yolk color fan	1.62	1.61	3.48	3.52	0.051	<0.001	NS	NS
CIE a* (redness)	-1.57[c]	-1.59[c]	-0.93[b]	-0.80[a]	0.025	<0.001	NS	0.030
CIE b* (yellowness)	21.9[c]	21.2[c]	26.8[b]	28.7[a]	0.29	<0.001	NS	0.007
Shell breaking strength (g/cm^2)	4504	4439	4757	4659	33.1	<0.001	NS	NS
Lutein in yolk (mg/kg DM)	1.69	1.91	5.49	6.04	0.368	<0.001	0.001	NS
Lutein retention in yolk (%)	28.5[c]	39.5[b]	55.6[a]	55.8[a]	2.48	<0.001	<0.001	<0.001
Zeaxanthin in yolk (mg/kg DM)	0.94	1.53	3.98	4.25	0.2677	<0.001	0.001	NS
Zeaxanthin retention in yolk (%)	28.2[d]	49.6[c]	63.1[b]	69.4[a]	3.45	<0.001	<0.001	0.002
α-tocopherol (mg/kg DM)	156.3[b]	136.0[c]	174.1[a]	141.2[c]	3.28	<0.001	<0.001	0.008
γ-tocopherol (mg/kg DM)	17.54[b]	10.13[c]	22.98[a]	8.31[c]	1.104	<0.001	<0.001	0.012
MDA 0 D (mg/kg)	0.448[a]	0.375[b]	0.347[b]	0.337[b]	0.0109	<0.001	0.008	0.041
MDA 28 D (mg/kg)	0.458	0.416	0.425	0.363	0.0098	0.050	0.021	NS

RO = rapeseed oil; PL = pork lard; SEM = standard error of the mean; [a,b,c,d] means in the same row with different superscripts differ significantly; NS = not significant; CIE = International Commission on Illumination; DM = dry matter; MDA = malondialdehyde; D = day

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