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Effects of bread waste and *Calendula officinalis* inclusion on egg production and egg quality

Svetlana Grigorova[1] and Dimo Penkov[2*]

[1] *Agricultural Academy, Institute of Animal Science, 2232 Kostinbrod, Bulgaria*
[2]*Agricultural University-Plovdiv, 4000 Plovdiv, Bulgaria*
[*]*Corresponding author:* dimopenkov@gmail.com

Abstract

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Study of the effect of 20% substitution of maize with bread waste and 0.5% *Calendula officinalis* inclusion to the laying hen's diets on their productivity, egg quality and chemical composition of egg yolk and egg albumen was made. A total of 90 layers (42 weeks old) from Lohman Klassik Brown breed were randomly allocated into 3 groups (n = 30 hens/group//each group = 3 replications(subgroups)*10 birds in replication) – control group (main energetic source in the diet – maize); I experimental group – maize replacement with 20% extruded bread waste; II experimental group – maize replacement with 20% extruded bread waste+0.5% marigold (*Calendula officinalis*) flowers. The compound feeds for all the groups were isoenergetic and isoprotein balanced. The hens from II experimental group had the best feed conversion (159.80 g of feed for one egg), followed by the first experimental group – 164.17 g. It was found significant lower total yolk cholesterol content in hens from II-nd experimental group than other groups. Extruded bread waste can replace 20% of maize in laying hens diet, without negatively effect on their egg productivity and egg quality, but it's way saved expensive maize grain, but also bread not consumed by humans is utilized.

***Keywords*: **laying hens; egg quality; compound feed; bread waste; *Calendula officinalis*

Introduction

The food wastes production covers all the food life cycle: from agriculture, up to industrial manufacturing and processing, retail and households (Mirabella et al., 2014). The Food and Agriculture Organization (FAO) reported approximately 1.3 billion tons of food including bakery (bread wastes), meat, dairy products, fresh fruits and vegetables are lost along the food supply chain each year (FAO, 2012). Furthermore, food wastes ending up in landfills can cause serious environmental pollution (Dao et al., 2020). The use of food waste has been one of the panacea to high feed cost, because they are cheap (Omole et al., 2011). Bread wastes are rich in energy, low in fiber (Dabron et al., 1999; Abdulatif et al., 2004; Penkov & Chobanova, 2020). They have been used to replace grain comopunds in the broilers' diet (Epao et al., 2013; Oke & Samson, 2013) which has led to reduction in feed cost. Dimitrova et al. (2020), established significantly egg weight increase ($P < 0.05$) and higher average laying intensity by replacement a part of wheat in the guinea fowl diet with 10% extruded bread wastes. According Grigorova & Penkov (2020), extruded bread waste can be used in the compound feed of laying hens at the expense of part of the maize (5% and 10%), without problems with the egg productivity.

Data in the available literature on the replacement of maize part in the laying hens' diets with larger amount (over 15%) of bread waste. However, it is possible that this will reduce the yolk color intensity. This expected side effect can be corrected by adding plant pigments' sources.

The flowers of annual herb marigold (*Calendula officinalis* L., *Asteraceae* family) possess many active substances, such as saponins, flavonoids (Vahed et al., 2016), polyphenols (Frum, 2017; Demasi et al., 2021), carotenoids (Kljak et al., 2021), which have antioxidant effect and are essential for the immune system (Jung et al., 2012). As natural source of pigments marigold flowers have been used in hens' nutrition for enhancement of egg yolk color (Čolović et al., 2016).

The aim of the present work was to evaluate the use of higher bread wastes amount (20%) with/without marigold flowers addition in hen's diet and their effects on their egg productivity and egg quality.

Material and Methods

An experiment was carried out at the Poultry Experimental Center of Institute of Animal Science-Kostinbrod, Bulgaria, with a total of 90 layers (42 weeks old) from Lohman Klassik Brown breed. The hens were randomly allocated into 3 groups * 3 replications, raised in separate boxes on a deep litter pen on a 16 h lighting schedule (n = total 30 hens/group and 10 hens each replication/subgroup). Water was supplied via nipple drinkers. The diet of the control group had following composition: maize; sunpro®; sunflower meal; soybean meal; sunflower oil; salt; limestone; monocalcium phosphate; L lysine; mineral premix; vitamin premix and Sinergin®. The compound feeds of all three groups were formulated to contain: 11.05 MJ metabolizable energy; 16.7% crude protein; 4.3% crude fats; 3.5% crude fiber; 12.14% crude ash; 3.70% Ca; 0.47% available P. In the diet of the hens from I experimental group 20% of maize was supplemented with extruded bread waste from Bulgarian bakery. Layers from II experimental group received 20% bread waste + 0.5% dried marigold (C*alendula officinalis*) flowers. The marigold flowers were added as compensation of the expected decrease in the yolk color pigmentation by addition of higher bread waste amounts at the expense of corn. The poultry from all groups received 115 g/hen/day of their diets (acc. to the guidelines of the hybrid). The trial duration was 68 days.

Bread wastes were extruded using an extruder AL 150 (particle size of the outlet is 1.5-2 mm). The chemical composition of the diets, extruded bread wastes and *Calendula officinalis* was determined by the conventional Weende analysis (AOAC, 2007). The total carotenoids content of marigold flowers was determined by the method described by Petrova et al. (2016). The metabolizable energy of the diets was calculated according to Todorov et al. (2021).

During the trial, on daily basis, the following indicators were controlled: health status, laying capacity (in egg number /group), laying intensity (in percent/group), and the mortality (in hen number). Also, the feed intake and feed conversion were calculated for each group.

Live body weight of the poultry as indicator for hens' health status was controlled at the beginning and at the end of the trial.

At the beginning, and at the end of the experiment, 30 eggs from each group were submitted to the following measurements: the weights of the egg, yolk, albumen and egg shell (using an electronic scale OHAUS 2000 with the tolerance of 0.01 g); eggshell thickness (in millimeters) without the shell membrane (using a micrometer Amer 25 EE); shape index (measured by index meter); yolk index (determined by the formula: YI (%) = (h/d)×100); albumen index (determined by the formula: I_{al} (%) = (h/[D+d]/2)×100); Haugh units (measured with index meter) and yolk colour (according to the 15 Roche Color Fan having 15 degrees scale).

The content of Ca and P in the egg shell were determined (AOAC, 2007) at the beginning and at the end of the trial.

At the end of the treatment ten eggs per each group were collected for determination of total lipid content of the egg yolk by the method of Bligh and Dyer (1959) and total yolk cholesterol content by the method of Schoenheimer-Sperry modified by Sperry and Webb (1950).

At the end of the experiment, the content of protein and ash in egg yolk and albumen as well as the fat content in the yolk of 6 raw eggs from each group were determined by the Weende method (AOAC, 2007).

The dates obtained in this work were statistically processed by Excel 2000, single factor, ANOVA program. All results are presented as means with their standard errors.

Results and Discussion

When analyzing the tested in this work bread wastes from Bulgarian bakery, the following results (in % of dry matter) were obtained: 13.72% crude protein; 2.07% crude fats; 1.63% crude fiber; 80.23% NFE. Similar results reported Penkov & Chobanova (2020). The used in our study marigold flowers content (in % of dry matter) 18.82% crude protein; 7.42% crude fats; 16.26% crude fiber; 11.47% crude ash; 46.03% NFE; 0.766% Ca; 0.324% P. The content of total carotenoids in the herb is 51.8 μg/g.

The results about initial and final live body weight and laying intensity of hens from all the groups are given in Table 1. The live body weight varied in close range both at the beginning (1753.33g; 1738.67; 1766 for control, I and II experimental groups, respectively) and at the end (1753.33g; 1762.67g; 1786g for control, I and II experimental groups respectively) of the trial. At the treatment beginning the hens

Table 1. Live body weight (g) and laying intensity (%) of hens from control and experimental groups (X ± SE)

Groups Indices	Control n = 30	I experimental n = 30	II experimental n = 30
Body weight at the beginning of experiment, g	1753.33±23.84	1738.67±25.76	1766±23.39
Body weight at the end of experiment, g	1753.33±23.82	1762.67±26.34	1786±30.34
Laying intensity at the beginning of experiment, %	64.17±2.50	66.67±5.44	70.00±6.80
Laying intensity at the end of experiment, %	73.72±0.91	74.94±0.94	76.89±0.97

laying intensity was as follows: 64.17%; 66.67%; 70.00% for control, I and II experimental groups respectively. At the trial end the following laying intensity was established: 73.72%; 74.94%; 76.89%. There were no statistically significant differences between the groups about these parameters ($P < 0.05$). Similar findings reported Grigorova & Penkov (2020) by maize replacement with 5% and 10% bread wastes in laying hen's diet and the data are compatible with the parameters of the hybrid used (Tierzucht, 2019).

During the trial layers from all the groups consumed the diets willingly**.** In Table 2 are presented total eggs' numbers during the whole experiment, feed intake and mortality of the hens from control and experimental groups. All layers were healthy, throughout the study and there is no mortality. The highest amount of eggs from 1 layer (48.9) was obtained in the group received 20% bread wastes + 0.5% *Calendula officinalis* (II experimental group) in the diet and the lowest amount (46.8) – in control group. Feed conversion was the best in layers from II experimental group, where 159.80 g of feed were spent for obtaining one egg. The next best result (164.17 g) achieved in I-st experimental group, receiving 20% bread wastes.

Due to the relative higher variation, within the subgroups, the differences under the groups were statistically insignificant ($P \geq 0.05$). However, it could be concluded that the replacement of grain components with bread wastes up to 20% does not adversely affect the laying capacity, especially when the inclusion of 0.5% marigold as a biologically active substance.

Our results show higher fodder conversion than the cited for the hybrid parameters (Kabakchiev et al., 2014) and closer to those for common breeds (Lukanov et al., 2016). The tendence of better feed conversion in experimental hens might be explained by high content of biologically active substances in marigold flowers, as well as by higher digestibility of bread waste. Dimitrova et al. (2020) also established better feed conversion by 10% wheat substitution in guinea fowl diet with extruded bread waste.

Data regarding eggs' morphological characteristics are shown in Table 3. There were no significant differences about the weights of egg, yolk, albumen and egg shell; egg shell thickness; shape index; Haugh units; albumen index and yolk index. About Ca and P content in egg shell in our experiment, there are no differences under the groups also (Table 4). Similar results were obtained when replacing maize in the hen's diet with 5% and 10 % bread wastes (Grigorova & Penkov, 2020).

The egg yolk color in hens from I experimental group (maize replacement with 20% bread wastes) significantly decreased ($P < 0.01$). The reason for this reduction of yolk color pigmentation is probably the higher percentage of maize replacement with pigment-poor bread waste, the main component, of which is wheat. The addition of 0.5% marigold flowers (II experimental group) corrected the decrease of this parameter. In our previous study replacing maize with 5% and 10% bread wastes did no change yolk color intensity (Grigorova & Penkov, 2020). It could be concluded, that higher than 15% replacement of the maize with bread wastes decreases the egg yolk color and in this case the addition of pigment enhancers is recommended.

Table 5 presents the contents of total yolk lipids and total yolk cholesterol. There is not significant difference

Table 2. Feed intake, total eggs number during the whole experiment and mortality of the hens from control and experimental groups

Indices	Groups		
	Control	I experimental	II experimental
Feed intake during the whole experiment (together with the spills), kg	234.600	234.600	234.600
Total eggs number from 1 layer for the whole experimental period (68 days)	46.79±0.52	47.62±0.50	48.93±0.93
Fodder intake/hen/day, g	115	115	115
Feed intake per one egg, g	167.9±1.90	164.17±1.79	159.80±3.05
Mortality, hen's number	0	0	0

Table 3. Egg's morphological characteristics (X ± SE)

Indexes \ Groups	Control n = 30	I experimental n = 30	II Experimental n = 30
At the beginning of the experiment			
Egg weight, g	58.44±0.34	57.54±0.37	60.59±0.89
Albumen weight, g	36.94±0.29	36.01±0.35	38.65±0.76
Yolk weight, g	14.74±0.23	14.87±0.22	15.08±0.18
Eggshell weight, g	6.76±0.09	6.68±0.08	7.02±0.12
Eggshell thickness, mm	0.39±0.006	0.40±0.005	0.41±0.004
Shape index%	78.94±0.43	79.20±0.43	79.41±0.36
Haugh units	89.40±1.02	86.67±1.62	86.45±1.30
Albumen index, %	11.93±0.42	12.11±0.54	11.59±0.44
Yolk index, %	45.93±0.87	45.32±0.84	44.43±0.66
Egg yolk color	10.07±0.22	10.12±0.21	10.18±0.21`
At the end of the experiment			
Egg weight, g	59.62±0.84	59.34±0.72	60.70±0.72
Albumen weight, g	37.12±0.65	36.70±0.53	38.29±0.97
Yolk weight, g	15.63±0.25	16.10±0.23	15.57±0.21
Eggshell weight, g	6.96±0.09	6.69±0.10	6.84±0.07
Eggshell thickness, mm	0.41±0.004	0.40±0.004	0.41±0.004
Shape index%	79.45±0.53	79.17±0.36	79.33±0.36
Haugh units	66.10±1.98	70.90±1.56	66.13±1.64
Albumen index, %	5.90±0.42	6.80±0.37	5.92±0.33
Yolk index, %	36.40±0.54	36.48±0.57	36.88±0.60
Egg yolk color	10.10±0.17	9.23±0.20A*C*	10.03±0.18

*Significance by: $P < 0.05$: A – control/I experimental; C – I experimental/II experimental

concerning the values of total yolk lipids between the groups ($P>0.05$), but a statistically significant decline ($P < 0.05$) in total cholesterol content was observed in the yolk of hens from II experimental group receiving 0.5% *Calendula officinalis* in the feed. Eggs are thought to be a major source of dietary cholesterol, which can cause coronary heart disease (Bragagnolo & Rodriguez-Amaya, 2003; Sharma et al., 2020). There is a lot of examinations to reduce of egg yolk cholesterol by adding plants and herbs (Chowdhury et al., 2002; Chen et al., 2005; Moula et al., 2019). With similar focus, in the present work, the reduced level of total yolk cholesterol showed the potential effect of marigold flowers to regulate/reduce this parameter in hens' eggs.

The data about total chemical composition of egg albumen and egg yolk of the hens from control and experimental groups are presented in Table 6. The current findings demonstrated insignificant influence of maize replacement with 20% bread wastes (with or without marigold combination) on the values of protein in egg albumen and egg yolk as well as on fat content in the yolk. All the differences under the groups are statistical insignificant ($P > 0.05$). A normal difference is observed only between egg yolk and egg white within the same group.

The chemical composition of the egg yolk and egg white do not show statistical significant differences under the groups and the results, obtained by us are closer to the standard (Tierzucht, 2019), which is an indicator, that there is not negative effect of the tested products on the chemical composition of both consumable egg parts.

Table 4. Ca and P content in the eggshell at the beginning and at the end of experiment

Periods \ Groups	Control	I experimental	II experimental
Ca at the beginning, %	34.669±0.47	34.334±0.60	34.897±0.59
P at the beginning, %	0.150±0.03	0.187±0.47±0.02	0.152±0.03
Ca at the end,%	34.922±0.52	34.400±0.47	34.820±0.41
P at the end, %	0.160± 0.03	0.171±0.03	0.158±0.03

Table 5. The contents of total lipids and total cholesterol in egg yolk (X±SE)

Indices \ Groups	Control	I experimental	II experimental
Total lipids, g/100g yolk	34.93±0.46	34.35±0.47	35.42±0.35
Total cholesterol, mg /100 g yolk	1470.56±54.12	1471.85±45.00	1331.12±37.39B*C*

*Significance by: P < 0.05: B-/ control/II experimental; C – I experimental/II experimental

Table 6. Total chemical composition of egg albumen and egg yolk of the hens from control and experimental groups (X±SE).

Indices \ Groups	Control		I experimental		II experimental	
	Albumen n = 6	Yolk n = 6	Albumen n = 6	Yolk n = 6	Albumen n = 6	Yolk n = 6
Dry matter, % in native	13.004± 0.006	46.68±0.35	12.72±0.22	45.72±0.68	12.49±0.25	46.03±0.23
Crude protein, % in native	11.21±0.07	16.06±0.06	10.91±0.08	16.22±0.20	11.06±0.07	16.22±0.20
Crude fats, % in native	0.54±0.04	29.89±0.23	0.43±0.02	29.68±0.17	0.50±0.02	28.98±0.12
Ash, % in native	0.57±0.15	0.11±0.004	0.43±0.11	0.16±0.03	0.60±0.02	0.16±0.03

Conclusions

The results obtained in present work, show that the maize replacement in hen's diet with 20% bread wastes (with, or without marigold flowers combination), did not change significantly egg intensity and egg morphological characteristics. There are not significant differences in contents of Ca and P in egg shell, protein and fats content in egg yolk and albumen.

The inclusion of 20% extruded bread wastes as a maize substitute in laying hens diet led to significant decrease of yolk color intensity (P < 0.05), but the addition of 0.5% marigold flowers corrects this parameter.

The hens receiving combination of extruded bread waste + 0.5% dried marigold flowers (II-nd experimental group) have significantly lower yolk cholesterol content (P < 0.05) compared to the control and I-st experimental groups.

Extruded bread waste can replace 20% of maize in laying hens diets, without negatively effect on their egg productivity and egg's chemical composition. In this way saving on maize grain are realized, and also bread that is not consumed by humans is utilized.

By the substitution of maize content in the combined fodders with high percentage of bread wastes (20 and more %), it's necessary to add pigment sources for correction the yolk color intensity.

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