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THE EFFECT OF LUTEIN ADDITIVES ON BIOCHEMICAL PARAMETERS IN BLOOD OF LAYING HENS

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SUMMARY

The aim of this study was to present the performance and some biochemical parameters in the blood of hens fed feed mixtures with different levels of lutein. The study involved a total of 291 Tetra SL hybrid laying hens, divided into 3 groups (O_0 with 0 mg/kg lutein added, O_{200} with 200 mg/kg lutein added and O_{400} with 400 mg/kg lutein added), and lasted for 5 weeks. The O_0 group included 93 hens, whereas experimental groups 98 and 100 hens, respectively. The highest egg production and the best feed consumption per egg were recorded in O_{400} group with a total of 3367 pieces of eggs produced and 128.60 g of feed consumed. The best laying intensity (96.68%) and number of eggs per laying hen (34.19) as well as the highest feed consumption per day (129.01 g), was recorded in O_{200} group. Lutein added to feed mixtures for laying hens did not significantly affect the values of biochemical parameters in blood ($P>0.05$). An increase in total cholesterol (CHOL) and HDL cholesterol content as well as the decrease of total protein (PROT) and albumin (ALB) content in the experimental groups can be observed. The content of glucose (GUK) and urea was similar between the experimental groups, while the highest content of triglycerides (TGC) was recorded in O_{200} group. It can be concluded that different levels of lutein added to the feed mixtures for laying hens did not have negative effect on the performance and blood biochemical parameters of laying hens.

Key-words: lutein, omega-3 fatty acids, laying hens, performance, biochemical parameters

INTRODUCTION

Feeding laying hens with designed feed mixtures can influence the content and composition of specific nutrients in the egg. Addition of vegetable or fish oil can increase desirable n-3 polyunsaturated fatty acids in the egg yolk. Adding of various antioxidants, such as vitamin E, selenium, and lutein, enhance stability of fatty acids but also increase the content of those ingredients in the egg, which, as a result, gives an enriched food. Lutein is a plant pigment most commonly found in dark green leafy vegetables (spinach, kale, lettuce) including peppers, tangerines, corn and egg yolk (Sommerburg et al., 1998). It belongs to xanthophyll group of carotenoids and must be consumed with food, since it cannot be synthesized in the body. Lutein is soluble in fats, and in the blood. It is transported by high-density lipoproteins (HDL) (over 50%), while the rest is divided between low-density (LDL) and very low-density lipoproteins (VLDL) (Wang et al., 2007).

In the poultry industry xanthophylls like lutein were used, so far, mainly for pigmentation of meat, skin of broilers and egg yolks, since color is an important factor in the perception of poultry products quality in many countries of the world (Rajput et al., 2012). It is well known that the intensity, as well as

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colour (yellow–red), can be controlled by the concentration and type of dietary xanthophylls (Lai et al., 1996). Although several xanthophylls with biological properties are found in nature, only a few are of industrial importance (mainly astaxanthin, canthaxanthin, and lutein), which was also a reason for an increasing interest in lutein for application in human food as well as for production of animal feed (Breithaupt, 2007) in recent years. Eggs with increased levels of lutein in yolk are very good source of lutein in human nutrition. Since lutein from feed mixtures is absorbed into the hen's bloodstream and further into egg yolks due metabolism, it is necessary to ensure that during the production, i.e. feeding of hens with elevated levels of lutein, hens are healthy and have good performances. Due to very scarce literature on the impact of lutein added to mixtures on blood biochemical parameters in hens, the aim of our study was to determine whether different levels of lutein added to feed mixtures for laying hens, rich in omega-3 fatty acids, have influenced some biochemical parameters in hens' blood, and to present hens' performances when fed such feed mixtures.

MATERIAL AND METHODS

The study involved a total of 291 laying hens of Tetra SL hybrid. Laying hens were divided into 3 groups, which differed depending on the levels of lutein added to the feed mixtures as follows: control O_0 (0 mg/kg lutein), experimental O_{200} (200 mg/kg lutein) and O_{400} (400 mg/kg lutein). Prior experiment start with the addition of lutein to feed mixtures, a 4 weeks long preparatory period of hens habituation to omega mixture was conducted. During that period, mortality in O_0 (7 hens) and O_{200} (2 hens) groups occurred, causing different number of hens in the experimental groups (initial number of hens per group was 100). The control group included 93 hens, and experimental groups 98 and 100 hens, respectively. At the beginning of the study with the addition of lutein to feed mixtures, hens were in the 31st week of age, and the study lasted for five weeks. Hens were fed *ad libitum* with feed mixtures containing 5% of oils with an increased content of omega-3 fatty acids, and different levels of lutein. The composition of feed mixture for laying hens is presented in Table 1.

Table 1. The composition of mixture for laying hens

Tablica 1. Sastav smjese za nesilice

Component *Sastojak*	**Sadržaj (%)** *Content (%)*
Corn – *Kukuruz*	47.09
Soybean meal - *Sojina sačma*	21.65
Toasted soy - *Tostirana soja*	4.04
Sunflower meal - *Suncokretova sačma*	6.00
Alfalfa - *Lucerka*	2.50
Livestock yeast - *Stočni kvasac*	1.00
*Lime*stone - *Vapnenac*	10.24
Monocalcium phosphate – *Monokalcijev fosfat*	1.50
Livestock salt - *Sol stočna*	0.32
Synthetic methionine - *Sintetički metionin*	0.16
[1]Oil mixture - *Smjesa ulja*	5.00
[2]Premix – *Premiks*	0.50
Ukupno – *Total*	100.00
Sirovi proteini, % - *Raw proteins, %* ME, MJ/kg	18.00 11.40

[1]There was 5% of oil mixture added to feed, wherein the soybean oil were represented with 1.25%, rapeseed oil 2.00%, linseed oil 1.00% and fish oil 0.75%; [2]Premix, content in 1 kg: vitamin A 2,000,000 UI, vitamin D_3 500,000 UI, vitamin E 20,000 mg, vitamin K_3 400 mg, vitamin B_1 420 mg, vitamin B_2 900 mg, vitamin B_6 540 mg, vitamin B_{12} 2,300 mg, vitamin B_9 170 mg, pantothenic acid 1,400 mg, vitamin B_3 5,000 mg, vitamin H 17,000 μg, vitamin B_4 80,000 mg, vitamin C 4,500 mg, iodine 180 mg, manganese 14,000 mg, zinc 12,500 mg, iron 6,000 mg, copper 1,000 mg, selenium organic 80 mg, butylated hydroxytoluene 3,400 mg, propyl gallate 1,400 mg, canthaxanthin 600 mg, beta-apo-beta-carotenoic acid 200 mg

Lutein added to feed mixtures for laying hens was in the form of a 20% Marigold extract (*Calendula officinalis*), and purchased from the company Phyto Nutraceutical Inc., Changsha, Hunan, China. Egg production in all experimental groups was recorded daily. Based on obtained data, the number of eggs per hen, laying intensity and feed consumption per day and egg was calculated. On the last day of the experimental period, 7 hens from each group were randomly selected for blood sampling in order to determine the biochemical parameters. Blood was taken from the wing vein in the BD Microtainer® SST ™ vacuum tubes (Becton, Dickinson and Company, NJ, USA). Analyses were conducted at the Department of Animal

Husbandry, Faculty of Agriculture in Osijek, using the automatic analyzer "Olympus AU 400". Biochemical parameters that were analyzed in blood serum are as follows: glucose (GUK, mmol/L), urea (UREA, mmol/L), total protein (PROT, g/L), albumin (ALB, g/L), total cholesterol (CHOL, mmol/L), HDL cholesterol (HDL, mmol/L) and triglycerides (TGC, mmol/L).

The research results were analyzed using statistical software Statistica for Windows v.12.0. (StatSoft Inc., 2014). Testing the significance of differences between and within groups was determined with the GLM procedure using the single ANOVA. The calculated F value was compared with the critical theoretical F value at 5% significance level. The significance of differences between means was determined using Fisher's LSD test.

RESULTS AND DISCUSSION

Performances of laying hens monitored during 5 weeks of trial period are shown in Table 2. Lutein added to feed mixtures for laying hens didn't have significant influence on their performances. The highest egg production (3367 eggs) and best feed consumption per egg (128.60 g) and day (124.96 g) were recorded in O_{400} group, followed by O_{200} and O_0 group. The best laying intensity was recorded in the group supplemented with 200 mg/kg of lutein.

Table 2. Performances of laying hens monitored during 5 weeks of the trial period

Tablica 2. Proizvodne osobine kokoši nesilica bilježene tijekom 5 tjedana pokusnoga razdoblja

Indicator - *Pokazatelj*	**O_0**	**O_{200}**	**O_{400}**	**P value**
Number of hens - *Broj nesilica*	93	98	100	P>0.05
Total eggs - *Ukupno jaja*	3108	3316	3367	P>0.05
Number of eggs per laying hen - *Broj jaja po nesilici*	33.42	34.19	34.01	P>0.05
Laying intensity (%) - *Intenzitet nesivosti*	95.48	96.68	96.20	P>0.05
Feed consumption (g/day) - *Konzumacija hrane (g/danu)*	127.80	129.01	124.96	P>0.05
Feed consumption (g/egg) - *Utrošak hrane (g/jajetu)*	133.84	132.07	128.60	P>0.05

O_0= without addition of lutein to the mixture, O_{200}= 200 mg/kg of lutein added in the mixture, O_{400}= 400 mg/kg of lutein added in the mixture

Leeson et al. (2007) investigated the influence of lutein (0, 125 and 250 mg/kg) added to feed for laying hens rich in a flax seed (10%) on their performance. Different combinations of lutein in feed did not affect either feed consumption, or the production of eggs (P>0.05), but slightly lower egg production was observed in group of hens fed with 250 mg/kg lutein compared to groups with 125 mg/kg of lutein and no added lutein. In the previous research by Leeson et al. (2004) in which hens were fed diets with added ground flaxseed (8%) as a source of omega-3 fatty acids and different levels of lutein (0, 125, 250 and 500 mg/kg), authors have found that dietary lutein supplementation had no effect (P>0.05) on egg production, egg weight, or any other egg characteristics measured. In our research, the production of eggs in groups with added lutein was very similar and even a little higher compared to group without added lutein. Our results showing that addition of different levels of lutein to feed mixture rich in oils do not prove negative effect on the performance of laying hens, thus being consistent with the results of the above mentioned authors.

Results of biochemical parameters determination in blood of laying hens are shown in Table 3. Addition of lutein in feed mixtures didn't significantly affect the values of biochemical parameters in the blood of laying hens (P>0.05). Values of glucose and urea were uniform between experimental groups and in accordance with reference values for chicken (9-18 mmol/L for glucose and 0.3-0.9 mmol/L for urea) stated by Kaneko et al. (2008). The content of total protein and albumin decreased ($O_0>O_{200}>O_{400}$) and content of cholesterol and HDL cholesterol increased ($O_0<O_{200}<O_{400}$) with the addition of lutein in the feed mixtures, but there were no statistically significant differences. Values of PROT were between 49.7 g/L (O_{400}) and 52.1 g/L (O_0) while ALB ranged from 17.6 g/L (O_{400}) to 18.8 g/L (O_0). These values correspond to the reference values for chickens (Kaneko et al., 2008). Cholesterol values ranged from 2.78 mmol/L (O_0) to 3.72 mmol/L (O_{400}), and HDL cholesterol from 0.68 mmol/L (O_0) to 0.90 mmol/L (O_{400}). Cholesterol values are in accordance with the reference values (2.0-4.7 mmol/L) while HDL values are below the lower limit of the reference range (0.9-2.5 mmol/L) for chickens (Kaneko et al., 2008). The highest TGC value was observed in O_{200} group (19.44 mmol/L), followed by O_{400} (18.45 mmol/L) and O_0 (17.57 mmol/L). Those values are much higher than the reference values for chickens (0.6-1.6 mmol/L; Kaneko et al., 2008).

Table 3. Average values of biochemical parameters in hens' blood serum ($\bar{x}$; n=7 per group)

Tablica 3. Prosječne vrijednosti biokemijskih pokazatelja u krvnome serumu nesilica ($\bar{x}$; n=7 po skupini)

Indicator *Pokazatelj*	**Group**			**SEM**	**P value**
	O_0	O_{200}	O_{400}		
GUK (mmol/L)	10.51	10.21	10.37	0.396	0.866
UREA (mmol/L)	0.57	0.55	0.58	0.027	0.703
PROT (g/L)	52.1	50.3	49.7	2.228	0.727
ALB (g/L)	18.8	18.1	17.6	0.701	0.516
CHOL (mmol/L)	2.78	3.10	3.72	0.427	0.303
HDL (mmol/L)	0.68	0.72	0.90	0.118	0.386
TGC (mmol/L)	17.57	19.44	18.45	1.402	0.648

$\bar{x}$=mean; SEM=standard error of mean; O_0= without addition of lutein to the mixture, O_{200}= 200 mg/kg of lutein added in the mixture, O_{400}= 400 mg/kg of lutein added in the mixture

Since studies on the effect of lutein addition in combination with oils rich in omega-3 fatty acids in feed for laying hens on the blood biochemical parameters are not available in the literature, our results are compared with the results of some authors who have studied the impact of similar level of oils rich in omega-3 fatty acids added to feed mixtures on biochemical parameters in the blood of hens or quails. Ahmad et al. (2014) investigated the influence of rapeseed oil addition on concentration of total, HDL- and LDL-cholesterol and glucose in serum of hens. In the group with 4% oil there was 3.6 mmol/L of total CHOL, 1.4 mmol/L of HDL, and 1.8 mmol/L of LDL. Listed CHOL values correspond to the values measured in our O_{400} group while HDL cholesterol with a value of 1.4 mmol/L is almost twice higher than in our study (0.68 to 0.9 mmol/L). The glucose concentration in the group with 4% of oil was 14 mmol/L, being higher than those measured in our study. Effect of different oils sources on blood lipid parameters of commercial laying hens was the subject of the research conducted by Murata et al. (2003). The authors added 3% of soy, fish or canola oil in mixtures. Cholesterol levels ranged between 2.49 and 3.26 mmol/L, corresponding to our results. On the other hand, levels of HDL (0.11-0.16 mmol/L) and TGC (11.1-14.9 mmol/L) are considerably lower than in our study. Basmacıoğlu et al. (2003) investigated the influence of fish oil and flaxseed addition on blood parameters in 34 weeks old laying hens. In a group with the combination of fish oil and flaxseed, content of TGC was 16 mmol/L, CHOL 2.71 mmol/L and HDL1.01 mmol/L. Values of TGC and CHOL are lower and the value of HDL higher than in our study. Al-Daraji et al. (2010) have investigated the effect of different oil sources on the biochemical parameters in quail's blood. Quails were receiving 3% of sunflower, linseed, corn or fish oil in the feed. Average values of PROT (60.3 g/L), ALB (39.2 g/L), GUK (17.54 mmol/L), CHOL (4.45 mmol/L) and HDL (2.48 mmol/L) determined in quail's blood in groups with linseed and fish oil are much higher, and only the contents of TGC (1.72 mmol/L) is significantly lower in quails than in laying hens in our study. Hajizadeh and Shahryar (2014) conducted a study on the impact of rapeseed oil on blood parameters in quails. In the group with 4% oil, the content of glucose was 17.4 mmol/L, being considerably higher than the average value of glucose in our study (10.36 mmol/L). Values of TGC, CHOL and HDL cholesterol were: 1.2, 5.6 and 2.6 mmol/L. The content of TGC in quails' blood is much lower than in laying hens' blood measured in our study (average 18.49 mmol/L), while the values of CHOL and HDL are on the average by 2 units higher than in our study.

CONCLUSION

The results showed that the addition of different levels of lutein in feed mixtures enriched in omega-3 fatty acids have no statistically significant effect on egg production, laying intensity and feed intake ($P>0.05$). Although addition of lutein did not have significant influence ($P>0.05$) on biochemical parameters in the blood, reduction in content of total protein and albumin in the experimental groups can be observed. On the other hand, the content of total CHOL and HDL cholesterol in the experimental groups was higher compared to the control group. Based on the results, it can be concluded that lutein added to the feed mixtures for laying hens enriched in omega-3 fatty acids did not have negative effect on the performance and blood biochemical parameters of laying hens. Also, the increased levels of lutein can be safely applied in production of eggs enriched with lutein without adverse effect on hens' health and performances.

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UTJECAJ DODATKA LUTEINA NA BIOKEMIJSKE PARAMETRE U KRVI NESILICA

SAŽETAK

Cilj ovog istraživanja bio je predstaviti proizvodne pokazatelje i neke biokemijske parametre u krvi kokoši nesilica hranjenih smjesama s različitim razinama luteina. Istraživanje je provedeno na ukupno 291 nesilici Tetra SL hibrida, podijeljenih u 3 skupine (O_0 s 0 mg/kg dodanog luteina, O_{200} s 200 mg/kg dodanoga luteina i O_{400} s 400 mg/kg dodanoga luteina), a trajalo je 5 tjedana. U O_0 skupini bilo je 93 kokoši, a u eksperimetnalnim skupinama 98, odnosno 100 kokoši. Najveća proizvodnja jaja i najpovoljniji utrošak hrane po jajetu zabilježeni su u O_{400} skupini, s ukupno proizvedenih 3367 komada jaja i utrošenih 128,60 g hrane. Najbolji intenzitet nesivosti (96,68%) i broj jaja po nesilici (34,19), ali i najveća konzumacija hrane po danu (129,01 g), zabilježeni su u O_{200} skupini. Lutein dodan u smjese za nesilice nije značajno utjecao na vrijednosti biokemijskih parametara u krvi ($P>0,05$). Može se primijetiti povećanje sadržaja ukupnoga (CHOL) i HDL kolesterola te smanjenje sadržaja proteina (PROT) i albumina (ALB) u pokusnim skupinama. Sadržaj glukoze (GUK) i ureje bio je ujednačen između pokusnih skupina, dok je najveći sadržaj triglicerida (TGC) izmjeren u O_{200} skupini. Može se zaključiti da dodatak različitih razina luteina u smjese za nesilice nije negativno djelovao na proizvodne pokazatelje i biokemijske pokazatelje u krvi nesilica.

Ključne riječi: lutein, omega-3 masne kiseline, kokoši nesilice, proizvodni pokazatelji, biokemijski parametri

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