

Natural sources of β -carotene and lycopene in laying hens' nutrition

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SUMMARY

Eggs morphological characteristics under influence of natural products *VITATON* (biomass of microscopic fungi *Blakeslea trispora* with 7.2% β -carotene) and *TOMATO OIL* (with 1.5% β -carotene, lycopene 0.5%, 1.7% phytofluene), produced by *LYCORED Ltd*, Israel was made. Special attention was paid to the egg yolk colour. An experiment was conducted with one hundred laying hens at the age of 38 weeks from hybrid combination *LOHMAN BROWN*, randomly distributed in five groups – control and four experimental groups. All birds received 100g/day of one and the same compound feed for laying hens. The diet was formulated to contain 19.33 % crude protein, 4.81% crude fibre, 2.71% crude fat and metabolisable energy 2584 kcal/kg. The hens from Ist and IInd experimental groups received as daily dose 0.07% and 0.035% respectively *VITATON* and those from IIIrd and IVth groups – 0.2% and 0.1% respectively *TOMATO OIL* with the diet for a period of 30 days. The indices yolk colour (Roche), shape index, Haugh unit, the weights (of egg, egg shell, yolk and albumen) as well as shell thickness, were controlled once weekly on 30 eggs from each group. The both content of Ca and P in the egg shell (mixed samples) were determined at the beginning and at the end of the trial. The yolk pigmentation obtained in experimental groups was more intensive by 1.37, 0.85, 5.80, 5.13 units (the Roche's scale) for Ist, IInd, IIIrd and IVth treated groups respectively in comparison to the control group. The differences on this parameter were significant ($P < 0.001$ for Ist, IIIrd and IVth groups and $P < 0.01$ for IInd group). Egg shell weight in all treated groups was significantly higher ($P < 0.001$) in comparison to control group. There are significantly increase of egg weight ($P < 0.001$ for Ist, IInd and IVth experimental groups), albumen weight ($P < 0.05$ for the Ist experimental group and $P < 0.001$ for IInd and IVth experimental groups) and yolk weight ($P < 0.05$ for Ist, experimental group and $P < 0.001$ for IInd experimental group) relative to the control group. The tested additives

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have no effects on the shape index, Haugh unit, egg shell thickness and content of Ca and P.

Keywords: β -carotene, lycopene, microscopic fungi *Blakeslea trispora*, Tomato oil, laying hens, egg morphological characteristic

INTRODUCTION

The increased demand of safety animal products during the recent years requires further investigations on the possibilities to include various natural sources of carotenoids as supplement to the laying hens' diet. Carotenoids are pigments that have primary responsibility for egg yolk colour intensity. Egg yolk colour is one of the important factors for marketing in many countries (Sikder et al., 1997; Calislar and Uygur, 2010). Hens are not able to synthesize colour pigments, but have the ability to transport them to the egg yolk from the diet (Surdjiiska, 1996; Karadas et al., 2006). To achieve desirable egg yolk colour intensity hens' diet is often supplemented with synthetic carotenoids because they are cheaper. But, the controversial topic of synthetic dyes both in foods and feeds had been discussed more recently. And their application was not allowed in certain countries (Lokaewmanee et al., 2010). Some of the most widely used carotenoids in the laying hens' diet are β -carotene and lycopene. In addition it bears mentioning their antioxidant and immunomodulatory properties, widely reported in the literature which may result in improved production parameters. (Bohm et al., 1995; Gerster, 1997; Mascarell et al., 2012).

β -carotene is the yellow/orange pigment, which is a precursor of vitamin A. It is contained in the corn, green feed, algae, marigold, nettle etc. (Kljak et al., 2012). β -carotene can also be synthesized by certain microscopic fungi like *Blakeslea trispora* (Dufosse, 2006). Waste from the juice production (pulp from carrots, grapefruit, apricot, etc.) could be successfully used as natural sources of β -carotene in laying hens compound food because of the lower price (Sikder et al., 1998; Mascarell et al., 2012).

Lycopene is the red pigment that is abundant in tomatoes, hips, paprika etc. It is the most powerful antioxidant that is found in food and feed sources. In photosynthetic organisms, lycopene is an important intermediate in the biosynthesis of many carotenoids, including β -carotene. Inexpensive alternative of natural sources of lycopene are by-products like tomato pulp and pomace, red grapefruit pulp, etc.

The information about the effect of microbial pigment sources and tomato oil on egg yolk pigmentation and eggs' morphological characteristics is limited. That's why, the objective of our present research was to assess the efficacy of natural sources of β -carotene and lycopene from biomass of microscopic fungi

Blakeslea trispora and tomato oil in laying hens' diet concerning egg yolk pigmentation, egg morphological characteristics and Ca and P content in the egg shell.

MATERIAL AND METHODS

The used feed additives *VITATON* and *TOMATO OIL*, produced by *LICORED Ltd.*, Israel are standardized. They are harmless for humans and animals. The tested product *VITATON* is biomass of microscopic fungi *Blakeslea trispora* and contains 7.2% β -carotene (Spectrophotometry) and < 10 ppm heavy metals. The additive *TOMATO OIL* contains 1.5% β -carotene, 0.5% lycopene (HPLC Method), 1.7% phytoene and phytofluene, 2.9% Vitamin E and < 10 ppm heavy metals (ICP Analysis). The present experiment was carried out in the period February – March 2013 in the Poultry Experimental Base of Institute of Animal Science – Kostinbrod, Bulgaria with a total of one hundred laying hens at the initial age of 38 weeks from hybrid combination LOHMAN BROWN, raised on a deep litter pen. The hens were randomly allocated in five groups – control and four experimental groups, 20 birds in each. Water was supplied via nipple watering trough. All groups received 100g/ day one and the same diet for laying hens (Table 1).

Table 1 Ingredients and chemical composition of laying hens compound feed

Ingredients	Percent
Maize	10.00
Wheat	42.25
Wheat bran	15.00
Soybean meal	3.00
Sunflower expeller	20.00
Limestone	8.60
Monocalcium Phosphate	0.50
Salt	0.30
Premix 15 C	0.20
Enzymes 4 AKT	0.10
Choline chloride	0.05
Total	100.00
Chemical composition	%
Moisture	8,73
Crude protein	19.33
Crude fibre	4.81
Crude fat	2.71
Ca, total	2.45
P, total	0.548
Metabolizable energy, kcal/kg	2584

The chemical composition was determined by the conventional Weende analysis (AOAC, 1984). The metabolisable energy was calculated according to WPSA (1989). The hens received high dose (0.07%, Ist experimental group) and low dose (0.035%, IInd experimental group) *VITATON* and those hens from IIIrd and IVth groups – 0.2% (high dose) and 0.1% (low dose) respectively *TOMATO OIL* with the diet for a period of 30 days (Table 2).

Table 2. Experimental design

Control group	I Experimental Group (I EG)	II Experimental group (II EG)	III Experimental Group (III EG)	IV Experimental group (IV EG)
Without additives	0.07% VITATON	0.035% VITATON	0.2% TOMATO OIL	0.1% TOMATO OIL

Thirty eggs from each group, laid within two consecutive days were taken ones weekly and following measurement were made:

- The egg yolk colour was determined visually (according to the 15 Roche Color Fan having 15 degrees scale);

- The weight of the egg, egg shell with shell membrane, egg yolk was measured with electronic scales *BOECO* within 0.001g;

- The albumen weight was determined for greater precision in the following way: the sum of the yolk weight with the shell membrane was deducted from the value of the egg weight;

- The shape index was measured by index meter;

- Haugh unit was calculated by the formula: $HU = 100 \lg (h + 7.17 - 1.7 W^{0.37})$, where h is the height of the thick albumen (in mm), W – the egg weight;

- The shell thickness (mm) without the shell membrane was measured at three locations (at the middle and at both poles, the average of the three measurements being retained) by a micrometer;

- Visually were also accounted the colour of egg albumen and the availability of blood stains and other inclusions;

The both content of Ca (BSS 11 374-86, 1990) and P (BSS 4336-73, 1990) in the egg shell (mixed samples) were determined at the beginning and at the end of the trial.

Body weight of the birds as indicator for hens' health status was controlled at the beginning and at the end of the trial.

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

RESULTS AND DISCUSSION

The body weight of laying hens during experimental period did not change significantly (Table 3). This parameter decreased with 106g and 51g for both the control and IIIrd experimental group respectively at the end of the trial. But, the values in IInd and IVth experimental groups were increased by 151 and 104 g respectively. There is no change in the body weight of births at Ist group. Similar results are reported by Salajegheh et al. (2012) by adding of different levels of dried tomato pomace.

Table 3. Body weight of laying hens at the beginning and at the end of the trial (mean±SEM)

Groups/Periods	CG	I EG	II EG	III EG	IV EG
At the beginning	1.78 ±56.12	1.84 ±57.31	1.71 ±47.67	1.79 ±60.84	1.61 ±44.58
At the end	1.67 ±46.78	1.84 ±65.53	1.86 ± 68.92	1.40 ±64.79	1.70 ± 48.26

Table 4. Morphological characteristics of hens' eggs from the control and experimental groups during experimental period as a whole (mean ± SEM, n=5 measurements x 30 eggs =150)

Items/Groups	Egg weight, g	Shape index	Albumen weight, g	Haugh unit	Yolk weight, g	Yolk colour Roche	Egg shell weight, g	Egg shell thickness mm
CG	57.62 ±0.32	78.30± 0.22	36.84± 0.28	76.24± 1.27	14.74± 0.12	5.52± 0.16	6.10± 0.05	0.34± 0.003
I EG	59.19 ±0.32 A*	78.80± 0.19	37.69± 0.24 A*	77.85± 1.22	15.15± 0.11 A*	6.63± 0.15 A***	6.31± 0.05 A**	0.35± 0.002
II EG	62.12 ±0.43 B*** E***	78.63± 0.21	40.36± 0.36 B*** E***	76.42± 0.82	15.55± 0.10 B*** E***	6.10± 0.17 B**	6.41± 0.05 B***	0.35± 0.002
III EG	57.58 ±0.31	78.11± 0.23	36.20± 0.23	76.62± 0.98	14.88± 0.10	11.05± 0.19 C***	6.45± 0.06 C***	0.36± 0.003
V EG	60.42 ±0.39 D*** F***	78.61± 0.22	38.95± 0.34 D*** F***	76.11± 1.18	14.90± 0.09	10.39± 0.18 D*** F*	6.56± 0.05 D***	0.35± 0.35

* - P≤0.05; * - P≤0.01; *** - P≤0.001

A - CG vs. I EG

B - CG vs. II EG

C - CG vs. III EG

D - CG vs. IV EG

E - I EG vs. II EG

F - III EG vs. IV EG

The average values of total estimations of morphological parameters of all laying hens` groups are presented in Table 4. But, follow parameters: egg colour, weights of egg, egg shell, yolk and albumen with their average weekly values are illustrated on figures 1-5.

The yolk colour intensity into the groups varied in close range - from 5.4 to 5.67 points on the Roche Colour Fan (Fig1). Corn in the control diet was the only source of xanthophylls as compare to the other groups which are supplemented with the natural carotenoids. That was why, the yolk colour in this group remained constant during the trial. One week after addition of the tested products in both doses was observed significantly increase of this parameter ($P < 0.001$). At the end of the trial significant increase of yolk intensity by 1.37, 0.85, 5.80, (Roche) for Ist, IIIrd and IVth experimental groups ($P < 0.001$) and by 5.13 points for the IInd group ($P < 0.01$) compared with the control group was registered. Dotas et al. (1999) and Mansoori et al. (2008) reported for the similar findings as ours by feeding diets with dry tomato pulp and dry tomato pomace. It is important to note that similarly good effect of tested additives (*VITATON* and *TOMATO OIL*) in the condition of our experiment we achieved by the both doses. This fact is very important for feed manufacturers and eggs producers. To consumers, which prefer deep yellow colour of egg yolk it is recommended *VITATON*, which contains only β -carotene. To other consumers, which prefer deep orange-coloured yolk it could be recommended products with lycopene (*TOMATO OIL*). Based on the fact supported by various scientific studies (Kang et al., 2003; Karadas, 2006), that the carotenoids from the diet passed unchanged in egg yolk can be concluded that used in our study natural sources of β -carotene and lycopene are suitable applicants for pigmentation of egg yolk and for making functional eggs. A strategy has been proposed for partial replacement of yellow pigments by red pigment over the last years. The use of natural red pigment ensures also a percentage close to 15% of yellow xanthophylls (Mascarell et al., 2012).

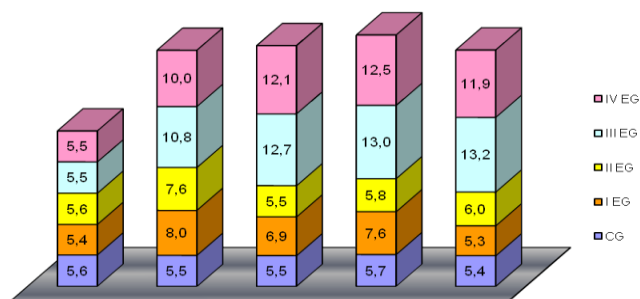


Fig.1 Yolk colour (Roche)

The visual evaluation of yolk and albumen colour did not establish any deviations from normal colour of hens' eggs in all groups. These eggs did not have any blood stains and other atypical inclusions.

The egg weight of the experimental groups except for IIIrd group is significant higher ($P < 0.001$ for Ist, IInd and IVth groups), relative to the control group (Fig. 2, Table 4). Safamehr et al. (2011) mentioned that supplement of 4, 8 and 12% tomato pulp to the laying hens diets significantly increases the values of this parameter.

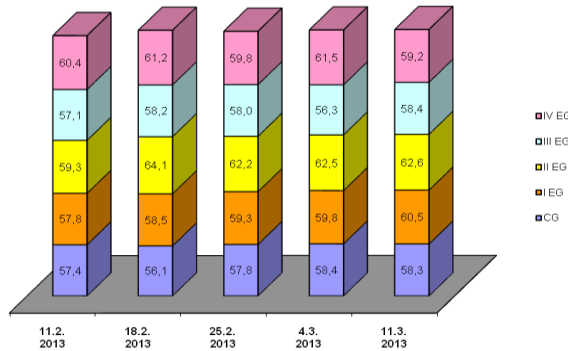


Fig 2 Egg weight, g

It was found significant higher values in all treated groups than the control group regarding shell weight (Fig. 3, Table 4). These our results are not confirm by Salajegheh et al. (2012) which used different levels of dry tomato pomace in laying hens' diet.

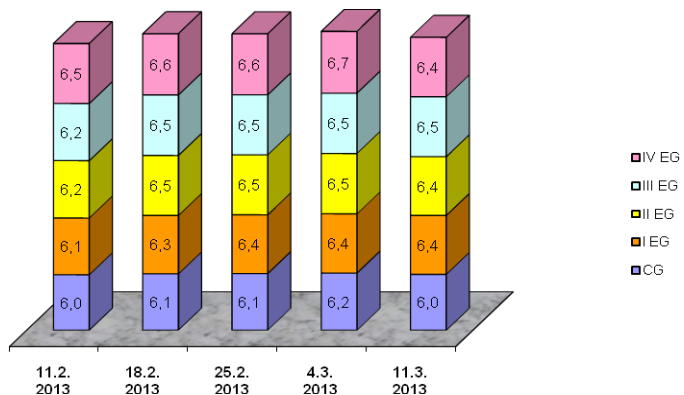


Fig.3 Egg shell weight, g

The hens from experimental groups received high and low doses of *VITATON* had significant higher yolk weight than control group (Fig. 4, Table 4). But, in opposite, the values of yolk weight in the other experimental groups received *TOMATO OIL* are not significant that than the control, which are similar to Calislar and Uygur (2010) used tomato pulp as a component of hens` diet.

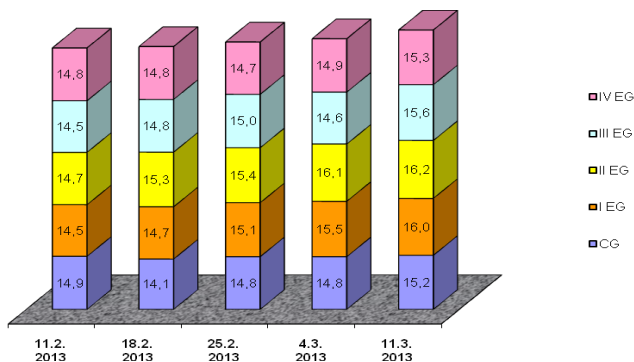


Fig.4 Yolk weight, g

The albumen weight of the hens from the treated groups (except the IIIrd group) was significant higher than control group - $P < 0.05$ for the Ist experimental group and $P < 0.001$ for IInd and IVth experimental groups (Fig. 5, Table 4). In contrast to our results are the finding of Calislar and Uygur (2010).

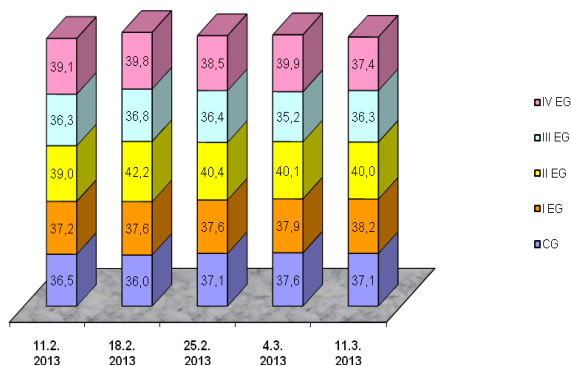


Fig.5 Albumen weight, g

The inclusion of the both concentrations of *VITATON* and *TOMATO OIL* to the laying hens` diet had no effect on shape index, Haugh unit and egg shell

thickness. Similar results observed Safamehr et al. (2011). Calislar and Uydur (2010), above mentioned, did not establish significant effects of tomato pulp supplementation to the laying hens' diet on shell thickness and Haugh unit also, but they reported a significant effects of tomato pulp on shape index as opposite of our findings. About Ca and P content in egg shell in our experiment there are no differences between the groups also (Table 5).

Table 5. Ca and P content in the egg shell (mixed samples) at the beginning and at the end of the trial

Groups/Periods	CG	I EG	II EG	III EG	IV EG
Ca at the beginning, %	32.24	34.32	34.21	34.54	34.14
P at the beginning, %	0.09	0.09	0.11	0.10	0.11
Ca at the end, %	33.84	34.04	32.80	32.61	31.18
P at the end, %	0.07	0.10	0.10	0.11	0.11

CONCLUSIONS

VITATON (0.035% and 0.07%) and *TOMATO OIL* (1% and 2%) added to the laying hens' diet had no adverse effect on hens' body weight and eggs morphological characteristics.

The supplementation of the both natural sources of β -carotene and lycopene benefited the birds to lay eggs with deeper yolk colour - in yellow (6.10 and 6.63 by low and high doses of *VITATON*) and orange (10.39 and 11.05 points on the Roche Colour Fan by low and high doses *TOMATO OIL*).

VITATON and *TOMATO OIL* significantly increase shell weight in all treated groups ($P < 0.001$).

There are significantly increase of egg weight ($P < 0.001$ for Ist, IInd and IVth experimental groups), albumen weight ($P < 0.05$ for the Ist experimental group and $P < 0.001$ for IInd and IVth experimental groups) and yolk weight ($P < 0.05$ for Ist, experimental group and $P < 0.001$ for IInd experimental group).

The form index, Haugh unit, egg shell thickness and content of Ca and P were not change by *VITATON* and *TOMATO OIL*.

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