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Feeding *Rhodotorula rubra* Yeast in Egg Yolk Pigmentation (II)

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Abstract

Rhodotorula rubra yeast effect upon egg yolk color and layer hens' productive performances is mounted on 450 Albo SL-2000. The layer hens were divited in three experimental groups. The birds were 20 weeks old and the experimental period was of five weeks The three groups (one control and two experimental groups) received: the control group 3% Saccharomyces cerevisiae feed-grade yeasts, while the experimental groups received 1.5% and 3% Rhodotorula rubra (groups E_1 and E_2, respectively). The feeding was made with standard diets that contained 2750 kcal/kg metabolisable energy and 17.5% crude protein. The productive performances were not influenced by feeding on Rhodotorula rubra yeast ($p \geq 0.05$). The egg yolk pigmentation was significantly influenced by the use of Rhodotorula rubra yeast. The highest values of RYCF were 11 on group E_1 (with 44% of total eggs) and 13 on group E2 (with 47% of total eggs). After the end of the experiment, the yolk pigmentation started to fade two days later on E_1, stabilising at the level of the control group. On E_2 group, the yolk pigmentation started to fade three days later, changing slowly for seven days stabilising at the level of the other two groups.

Keywords: yolk pigmentation, *Rhodotorula rubra* yeast

1. Introduction

The color of egg yolk plays a very important role in appreciation of eggs as food. Recent surveys in a number of different European countries (France, Germany, Italy, UK, Spain, Poland and Greece) have confirmed that the pigmentation of yolk is one of the main parameters by which the commercial quality of eggs is judged (1).

When samples of eggs with the pigmentation of yolk corresponding to Color Fan of 8, 10, 12 and 14 scores were presented most of the consumers questioned in the European surveys expressed a preference for the darkest colors. The preference of consumers for eggs with strongly coloured eggs is one of the concerns of the producers, nutritionists and of other related specialists

The pigmentation of egg yolk is determined by dietary carotenoids, mainly xanthophylls. The relationship between carotenoid content of feed and egg yolk color was first demonstrated by J.S. Hughes and L.F. Payne in a 1936 paper entitled *The Relation of the Carotenoid Pigments of Feed to the Carotenoid Pigments of Egg Yolk* (1). Then combined feed manufacturers have started to add the carotenoid to the diet of layer hens, but it took another 20 years up to developing an accurate tool for the egg yolk color measurement.

The most important sources of egg yolk pigmenting carotenoids used in poultry feed are corn grains, maize gluten, alfalfa leaves and grass meals. They contain lutein and zeaxanthine, which, together with other oxygen-containing carotenoids, are known by the collective name

of xanthophylls. Xanthophylls added to the diet are traditionally obtained from the petals of Marigold flowers (*Tagetes erecta)*, which are very rich especially in lutein and zeaxanthine (2,3). This source of xanthophylls can supplement or replace the ones originally derived from corn, alfalfa and other minor sources thereby xanthophyll content in the mentioned raw materials is highly variable. These xanthophylls, known as "yellow xanthophylls", are responsible for the basic colour in chickens and eggs. In addition to yellow xanthophylls, for egg yolk coloration it have been used the "red xanthophylls" typically being mainly the paprika (*Capsicum annum*) capsanthin from. A range of orange hues can be achieved by combining yellow and red xanthophylls, which allows adapting colour characteristics of eggs to the variety of consumers' requierments on different markets.

Recent studies have shown that other natural xanthophylls, like the ones contained by dried tomato peel or blue lupin can be used in the diets of layer hens to improve the yolk coloration, as well (4,5). The development of carotenoid-producing bioprocesses is regarded as a competition. *Rhodotorula rubra* able to provide important quantities of pigments such as *torularhodin* and *β-carotene* and *Phaffia rhodozyma* producing *astaxanthin,* entered in competition without facing the typical problems generated by the weather dependency of the agriculture production (6,7).

The aim of this study is to evaluate the effect of including *Rhodotorula rubra* yeast in the diets of layers hens on the commercial quality of eggs and to verify if their productive performances are depressed.

2. Materials and methods

2.1 Diets and Treatments

Three experimental diets (C, E_1, E_2) are compared.. The diets were formulated to have the same metabolisable content energy of (2750 kcal/kg) and the same protein rate (17.5%). The control group (C) received 3% *Saccharomyces cerevisiae* yeast, while the experimental groups received 1.5% and 3% *Rhodotorula rubra* ICCF (groups E_1 and E2, respectively).
Their composition is shown in Table 1

Table1.

Experimental diets (%)

Ingredients	C	E1	E2
Corn	34.5	33.7	33.4
Barley	34.0	34.0	34.0
Soybean meal	23.5	25.8	24.6
Saccharomyces cerevisiae yeast	3.0	-	-
Rhodotorula rubra yeast	-	1.5	3.0
Calcium Carbonate	3.0	3.0	3.0
Dicalcium Phosphate	1.0	1.0	1.0
Vitamin - Mineral premix	1.0	1.0	1.0
TOTAL	100.0	100.0	100.0
Calculated content			
Metabolisalbe energy ME (kcal/kg)	2750	2750	2750
Crude protein CP (%)	17.5	17.5	17.5
Lysine (%)	1.0	1.0	1.0
Methionine + cystine (%)	0.75	0.75	0.75
Tryptophan (%)	0.2	0.2	0.2

The diets were provided *ad libitum* in non-granulated form.

2.2 Birds, Housing and Experimental Design

The experiments were conducted on 450 Albo SL-2000 hyrid layer hens 20 weeks old. They were random assigned to three equal groups (one control and two experimental groups). This commercial hybrid is characterized by white shell eggs, high performance and a best quality of eggs. The experimental period was of five weeks.

The layer hens were kept in improved batteries, having free access to feed and water. The lighting programme was increased weekly on 10L:14D (20 weeks) to 16L:8D (28 weeks), then remained constant at 16L:8D (L = hours of light; D = hours of dark).

All birds were fed with standard layer diets, having 2750 kcal/kg metabolisable energy and 17.5% crude protein.

The following indices were registerd throughout the experiment: compound feed intake, laying percentage, laying intensity, egg weight, egg-mass production, yolk pigmentation. Feed intake and egg production were determined on a daily basis. Laying intensity was assessed weekly. Egg weight was determined twice a month for continuous periods of one week. The egg-mass production was calculated from the overall egg number and their weight.

The experimental data were statistically processed using ANOVA.

2.3. Chemical Analysis of Ingredients and Diets

The crude composition of ingredients and diets was determined by standard methods. The dry matter content was estimated by gravimetric method at 105°C drying for 8 hours. The protein (N x 6.25) was determined by Kjeldahl method using Tecator KJELTEC. The fat was extracted by Tecator SOXTEC-HT. The cellulose was determined by Tecator FIBRETEC. The ash was determined by gravimetric method. The starch was determined by polarmetric method and the sugar by Bertrand method. The organic matter was calculated.

The metabolisable energy (ME) was deduced based on the EU Regulation (EC 152/2009) from the chemical composition of the feed mixture

ME (MJ/kg) = (15.51 CP + 34.31 CFat + 13.01 sugar + 16.69 starch)/1000, where CP = crude protein; CFat = crude fat.

Table 2 shows the chemical composition of *Rhodotorula rubra* feed-grade yeast atomised preparation.

Table 2.

Chemical composition of *Rhodotorula rubra*

	DM %	OM %	CP %	EE %	CF %	NFE %
Rhodotorula rubra	91.3	86.1	33.7	1.9	1.6	48.9

The weight of eggs was measured by gravimetric method using Egg Analyzer. Yolk pigmentation was assessed using the La Roche Yolk Color Fan (RYCF)

3. Results and discussion

3.1. Productive performances

The layer hens' performances were not influenced by the dietaries containing different kinds of yeasts (Table 3). During the experimental period there were no significant differences in feed intake, egg production, egg weight and egg mass ($p \geq 0.05$).

Table3.

Layer performances

	C	E1	E2	
Feed intake (g/layer/day)	107.33±14.2	105.39±10.2	108.72±11.3	NS
%	100	98.2	101.3	
Egg production, (eggs/layer)	65.22±7.1	66.03±6.1	64.27±7.7	NS
%	100	101.2	98.5	
Egg weight (g)	60.12±4.2	59.33±4.2	61.50±4.8	NS
%	100	98.7	102.3	
Egg mass (kg)	3.921±0.28	3.918±0.43	3.953±0.47	NS
%	100	99.9	100.8	

The literature mentions that the use of different carotenoid pigments has no influence on compound feed intake, egg production or egg weight (8).

3.2. Yolk pigmentation

Yolk pigmentation (Table 4) was influenced differently by the dietary use of *Rhodotorula rubra* yeast. On the control group, the values of RYCF score were less than 10. On the E1 group, the highest percents were obtained for 11 and 10 RYCF score (with 44% and 30%, respectively). On the group E2, The highest percent was obtained at the score 13 (47%), follow by score 12 (36%). The differences were significant ($p \leq 0.01$).

Table 4.

Egg yolk pigmentation

RYCF score	<10	10	11	12	13	14
RYCF %						
C	100	0	0	0	0	0
E1	3	30*	44*	15	8	0
E2	0	2	10	36*	47*	5

*$p \leq 0.01$

After the end of experiment, the three groups were received the same diet like the control group. The yolk pigmentation in the E_1 group started to fade two days later, stabilising at the level of the control group. The yolk pigmentation in the E_2 group started to fade three days later on, changing slowly for seven days stabilising finally at the level of the other two groups.

4. Conclusions

1. The use of *Rhodotorula rubra* yeast in layer hen diets did not affect adversely layer performance.
2. *Rhodotorula rubra* yeast influenced significantly yolk pigmentation, which enhanced according to the dietary level of the protein biomass.
3. The highest values of RYCF were 11 on group E1 (with 44% of total eggs) and 13 on group E2 (with 47% of total eggs).

4. After the end of the experiment, the yolk pigmentation started to fade two days later on E1, and three days later on E2 group, changing slowly for seven days an stabilising at the level of the control group.

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