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The Effect of Inclusion of Chromium Yeast (Co-Fator II, Alltech Inc.) and Folic Acid to the Rations of Laying Hens on Performance, Egg Quality, Egg Yolk Cholesterol, Folic Acid and Chromium Levels

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Abstract: In this trial, the effect of Chromium yeast and folic acid on the egg yolk cholesterol, folic acid and chromium levels with performance and some egg criteria is evaluated. One hundred and eighty Lohmann White line 40 weeks of age layers were separated into 4 groups having 15 layers and trial replicated 3 times. The trial took place for 56 days. The 4 groups were fed basal diet with control group, chromium yeast (150 mg ton^{-1}) group, folic acid (10 mg ton^{-1}) group and folic acid + chromium yeast (10 + 150 mg ton^{-1}) group. The inner and outer quality levels and cholesterol levels of eggs were determined. The feed consumption was decreased in chromium yeast added and folic acid + chromium yeast groups by 1.90 and 1.92% and feed conversion rate was increased by 3.5 and 4.35%. The folic acid group and the control group had the same feed consumption and feed conversion rate. Live weight, egg production, egg weight, shape index, eggshell thickness and Haugh units were not changed in the chromium and folic acid groups but egg white and yolk index was higher in the chromium and folic acid groups than the control group. In comparison to the control and folic acid groups, the egg yolk cholesterol levels of the chromium added groups was decreased in samples taken on the 4th and 8th weeks of the trial ($p<0.001$). Egg yolk folic acid levels in the folic acid suplemented groups. There is no difference on yolk chromium levels among the treatments. As a result, the decrease in feed consumption, increase in the feed conversion and decrease in egg yolk cholesterol by means of inclusion of chromium yeast to layer feeds could be useful in practical feeding strategies.

Key words: Chromium yeast, folic acid, egg quality, cholesterol, egg weight

INTRODUCTION

Chromium (Cr) is considered as an essential trace mineral for normal metabolism of carbonhydrates and lipids. Low availability from feedstuffs and the effect of stress on Cr excretion can affect performance and profitability. Recently, organic Cr from yeast has proven to improve growth, carcass and egg quality characteristics of poultry.

In some studies, chromium increased (Amoikon *et al*., 1995) or decreased (Kitchalong *et al*., 1995; Press *et al*., 1998) plasma cholesterol, whereas in some other studies chromium had no effect on cholesterol concentrations (Cupo and Donaldson, 1987; Uyanik, 2001; Sahin *et al*., 1999). Limited evidence suggests that chromium may interact with other minerals (Kalaycioglu *et al*., 1999; Moonsie-Shageer and Mowat, 1993; Sahin *et al*., 2001a). Chromium requirements increase in humans and in animals as a consequence of factors generally described as stressors such as fatigue, trauma, pregnancy and several forms of nutritional, metabolic, physical, environmental and emotional stress (Burton, 1995). Such conditions increase chromium mobilisation from the tissues that is irreversibly excreted through the urine (Borel *et al*., 1984). It is excreted primarily in urine either freely or bound to low molecular weight organic transporters (Ducros, 1992). The principal route by which chromium enters the body is via the digestive system. Most human diets contain 60% of the minimum suggested daily intake of 50 μg of chromium (Anderson, 1997). Human intestinal absorption of chromium is 0.5-2% of dietary intake, when in the inorganic form (Hallberg *et al*., 1993) and about 25-30% for the organic complexes (Mowat, 1994). Once absorbed, chromium can circulate in the free state, bind to transferring or to other plasma proteins (beta-globulin protein), or circulate as a complex such as the Glucose Tolerance Factor (GTF). Interest in dietary trivalent chromium for laying hens was stimulated, when Jensen *et al*. (1978) reported that $Cr3^{+}$ had a favourable effect on albumen quality (Haugh Unit Score) and

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suggested that this element may be necessary to maintain the physical state of albumen. The need for increased consumption of folate by humans has been realized last years. Increased periconceptional intake of this vitamin by women has proven to reduce the occurrence and recurrence of neural tube defects, such as spina bifida, in children. Folic acid does not occur naturally, in appreciable amounts, in food. Nevertheless, due to its stability and commercial availability, it is the form that is used in vitamin supplements, fortified foodsand vitamin premixes. However, additional strategies may be necessary in order to ensure that all segments of the population are consuming adequate amounts of folate in specific target groups (women of child-bearing age) as well as educating consumers to eat foods that are rich in folate. Eggs naturally contain folate at approximately 22 μg folate per large egg (Cherian, 2006), which is equivalent to 6% of the newly established adult daily requirements for folate (Institute of Medicine, 1998). Increasing the folate content of eggs may position the egg as an important source of dietary folate and lead to an improvement in consumer acceptance of this commodity as a healthy product (House *et al*., 2002).

The experiment was performed to determine the effect of chromium yeast and folic acid on the egg yolk cholesterol, folic acid, chromium levels with performance and some egg criteria in laying hens.

MATERIALS AND METHODS

One hundred and eighty with Lohmann White laying hens, 40 weeks of age were housed in California cages (50×50×45 cm) in an environmentally controlled, closed-sided poultry house (18-20°C, 75 relative humidity and 16 h of light daily). The birds were randomly divided into four groups having 15 layers and the trial was replicated 3 times. The hens received a typical layer diet containing 2700 ME kcal kg^{-1} and 18% CP to meet or slightly exceed the nutrient recommended by NRC (1994) (Table 1). The four groups of hens were fed the basal diet (Control Group) or the basal diet supplemented with chromium yeast (150 mg ton^{-1}), folic acid (10 mg ton^{-1}) and folic acid + chromium yeast (10 +150 mg ton^{-1}). Feed and water were supplied ad libitum. Egg numbers and egg weight were recorded daily from the 40th week throughout the 8 weeks experiment.

The feed consumption of the hens was calculated weekly by weighing the left over feed in all the feedboxes of the hens. The amount of feed consumed of eggs was calculated by dividing the total amount of feed consumed in that period to the total amount of egg production weight (YDS = Feed Consumed(kg)/for every kg.

Table 1: Ingredients and chemical nutrient composition of basal diet fed to laying hens

Ingredients	%	Chemical nutrient composition	%
Corn	32.0	Crude protein (%)	17.89
Barley	21.1	Calcium (%)	3.7
Soybean meal	16.8	Phosphorus (total) (%)	0.4
Canola meal	10.0	Folate (mg kg^{-1})	0.49
Fish meal	2.0	Chromium (mg kg^{-1})	4.1
Tallow	7.1		
Limestone	8.7		
Monocalcium phosphate	0.8		
Vitamin premix[1]	1		
Mineral premix[2]	0.5		
Calculated analysis			
ME (kcal kg^{-1})	2700		
Crude protein (%)	18		
Calcium (%)	3.75		
Phosphorus (total) (%)	0.4		

[1]Provided (per kg of diet): vitamin A, 8225 IU; vitamin B12, 11.2 μg; calcium panthothenate, 4.4 mg; choline chloride, 110 mg; vitamin D3, 1000 IU; vitamin E, 5.46 IU; ethoxyquin, 125 mg; dl-methionine, 500 mg; niacin, 7.6 mg; riboflavin, 2.2 mg. [2]Provided (per kg of diet): MnO, 165 mg; ZnO, 55 mg; salt (iodized), 4.78 g

Live weight and living effort: The hens were weighed in the beginning and at the end of the trial in order to state any possible weight differences of live hens. The mortality and health conditions of hens were observed throughout the trial.

The egg production records were recorded daily, the same time of day. The eggs that were collected daily were divided into in good condition and damaged (egg shell fractured, broken or without egg shell) groups and also weighed. The egg production of the groups were calculated daily including the repetitions. In every repetition of the groups, the eggs collected from the hens were weighed and divided into the number of eggs collected.

The specific gravity of a whole egg was measured by Archimedes' method with an instrument designed for the measurement of egg weight in air (Wa) and in water (Ww), which was at 15.6°C and specific gravity was calculated with the following equation:

$$\left(\text{Specific gravity} = \frac{\text{Wa}}{(\text{Wa-Ww})}\right)$$

on the same day of egg collection. The other egg quality parameters were measured 24 h later. The shape index was measured by an instrument (BV. Apparatenfabreik Van Doorn, Holland). Shell thickness was measured by a micrometer. Albumen Height (H) was measured by a tripod micrometer, albumen Length (L) and Width (W) by a compass and then the albumen index was calculated with the following equation:

$$\left[\text{Albumen index} = \frac{H}{\left\{\frac{(L+W)}{2}\right\}} \times 100 \right]$$

Yolk height (H) was measured by a tripod micrometer screw and yolk Diameter (D) by a calliper, then the yolk index was calculated with the following equation (Wells, 1968):

$$\left(\text{Yolk index} = \frac{H}{D} \times 100 \right)$$

Feed analysis were performed according to the methods stated by the AOAC (2000). On the 4th and 8th weeks of the experiment, 10 eggs were chosen randomly among the ones that were picked 3 times a day for 2 days in a row and after extracting them (Washburn and Nix, 1974), their egg cholesterol levels were detected spectrophotometrically with the use of a kit (Weingand and Daggy, 1990). In addition, in order to determine the folic acid and chromium levels in egg yolk, on the 4th and 8th weeks of the experiment, 2 eggs were chosen among the ones that were picked 2 times a day for 2 days in a row. They were boiled in boiling water for 10 min, the yolk was extracted, lyophilized, weighed and stored at -20°C. Later, the folic acid composition of this dry egg yolk was detected by using a fluorescent detector in a HPLC device (Vahteristo *et al*., 1997). The chromium levels in the egg yolks were determined by atomic absorption spectrophotometry with the method as stated by AOAC (2000).

The data of the research have been achieved by using the importance test of the procedure effect variance analysis method (Düzgünes *et al*., 1993), the differences among the experimental group averages were evaluated with Duncan (1955) test ($\alpha = 0.05$). Chi-square method was used to compare the egg production.

RESULTS AND DISCUSSION

No significant differences in body weight change were observed between control and the treatment groups (Table 2). Average daily feed consumption was 124.44 g in the contro group and respectively 122.08, 124.40, 122.05 g in the treatment groups (Table 3) thus a decrease of 1.90 and 1.92% in the chromium yeast and folic acid + chromium yeast groups. However, supplemental chromium did not affect overall mean egg production (Table 4).

Feed efficiency was 1.78 in the control group and respectively 1.72, 1.78, 1.70 in the treatment groups (Table 5) thus, feed conversation rate was imroved of 3.50 and 4.50% in the chromium yeast added and folic acid + chromium yeast groups. The folic acid supplemented group and control group had the same feed consumption and feed conversion rate. Egg weight (Table 6) and egg quality parameters (Table 7) were not changed in the chromium yeast and folic acid groups but egg white and yolk index was higher in the chromium yeast and folic acid groups than the control group.

In the treatment groups, the albumen index value increased ($p<0.5$) at week 47 but there were no differences at weeks 40 and 43. Yolk index values increased at weeks 40 and 47. In comparison to the control and folic acid groups, the egg yolk cholesterol levels of the chromium yeast group was decreased in samples taken on the 4th and 8th weeks of the trial ($p<0.001$) (Table 8). In the eggs obtained from the groups that were fed with added folic acid; the egg yolk folic acid level has increased, when compared with the control and chromium yeast fed group (Table 9). As shown in Table 10, no major difference of egg yolk chromium level was detected in the chromium yeast fed group eggs that were picked on the 4th and 8th week of the experiment when compared with the control group. The chromium levels detected on the 4th week of the experiment in egg yolk belonging to the experimental groups ranged between 37.6-39.3 μg. At the end of the experiment, the results ranged between 43.9-44.6 μg. While a considerable number of experiments have been conducted to study the effect of chromium on broiler performances (Hossain *et al*., 1998; Lien *et al*., 1996), there is still a lack of information about the efficiency of dietary chromium supplementation on laying hen performance and the chromium requirements of laying hens. Kim *et al*. (1997) reported that feeding 800 μg chromium as chromium picolinate per kg diet to hens resulted in higher egg production, egg mass, however a

Table 2: The effects of supplemental chromium yeast and folic acid on body weight

	Treatment groups												
	Control			Chromium yeast ($150\ mg\ ton^{-1}$)			Folic acid ($10\ mg\ ton^{-1}$)			Folic acid + Chromium yeast ($10+150\ mg\ ton^{-1}$)			
Weeks	$\bar{X}$	n	$S\bar{X}$	$\bar{X}$	n	$S\bar{X}$	$\bar{X}$	n	$S\bar{X}$	$\bar{X}$	n	$S\bar{X}$	F
40	1892.75	15	24.45	1871.83	15	32.00	1846.42	15	33.01	1823.83	15	20.78	1.16^{NS}
47	1994.29	15	28.60	1992.00	15	28.32	1990.39	15	28.65	1988.17	15	28.30	1.24^{NS}

^{NS}: Not Significant

Table 3: The effects of supplemental chromium yeast and folic acid on feed consumption of laying hens by weeks (g/bird/day)

Weeks	Control	Chromium yeast (150 mg ton^{-1})	Folic acid (10 mg ton^{-1})	Folic acid + Chromium yeast (10 mg ton^{-1} +150 mg ton^{-1})
40	122.62	119.61	119.35	119.39
41	127.08	129.05	128.75	127.18
42	121.37	120.45	124.7	121.59
43	129.91	124.51	131.52	122.04
44	119.64	116.23	120.83	118.13
45	117.56	112.28	117.08	115.59
46	129.38	128.33	127.08	126.26
47	127.98	126.23	125.92	126.25
Mean	124.44	122.08	124.40	122.05

Table 4: The effects of supplemental chromium yeast and folic acid on egg production of laying hens by weeks (%)

Weeks	Control	Chromium yeast (150 mg ton^{-1})	Folic acid (10 mg ton^{-1})	Folic acid + Chromium yeast (10 + 150 mg ton^{-1})	χ^2
40	84.14	84.52	84.52	85.48	8.72^{NS}
41	82.36	81.55	82.14	82.38	0.90^{NS}
42	81.76	79.17	80.29	81.07	8.26^{NS}
43	80.76	81.55	80.33	81.71	2.48^{NS}
44	82.14	82.52	82.1	83.29	4.36^{NS}
45	84.52	82.86	82.86	83.45	5.48^{NS}
46	82.74	83.93	83.33	83.52	1.31^{NS}
47	80.36	80.36	82.14	82.33	3.80^{NS}
Mean	82.35	82.06	82.21	82.9	0.48^{NS}

^{NS}: Not Significant

Table 5: The effects of supplemental chromium yeast and folic acid on feed efficiency of laying hens by weeks (kg diet/ a dozen eggs)

Weeks	Control	Chromium yeast (150 mg ton^{-1})	Folic acid (10 mg ton^{-1})	Folic acid + chromium yeast (10 + 150 mg ton^{-1})
40	1.92	1.88	1.92	1.87
41	1.75	1.66	1.74	1.65
42	1.67	1.5	1.65	1.51
43	1.88	1.81	1.88	1.8
44	1.91	1.86	1.88	1.82
45	1.7	1.69	1.72	1.62
46	1.7	1.65	1.71	1.62
47	1.7	1.7	1.72	1.7
Mean	1.78	1.72	1.78	1.70

Table 6: The effects of supplemental chromium yeast and folic acid on egg weights of laying hens by weeks (g)

	Treatment groups												
	Control			Chromium yeast (150 mg ton^{-1})			Folic acid (10 mg ton^{-1})			Folic acid+ chromium yeast (10 mg ton^{-1} +150 mg ton^{-1})			
Weeks	$\bar{X}$	n	$S\bar{X}$	$\bar{X}$	n	$S\bar{X}$	$\bar{X}$	n	$S\bar{X}$	$\bar{X}$	n	$S\bar{X}$	F
40	60.90	15	0.86	59.53	15	0.54	58.84	15	0.62	59.50	15	0.44	1.75^{NS}
41	60.60	15	0.85	61.41	15	0.63	60.37	15	0.56	60.02	15	0.60	1.28^{NS}
42	61.49	15	0.62	60.87	15	0.53	59.75	15	0.49	60.30	15	0.64	1.78^{NS}
43	60.97	15	0.43	62.48	15	0.54	62.33	15	0.51	61.53	15	0.70	1.62^{NS}
44	61.13	15	0.50	61.96	15	0.52	63.47	15	0.48	62.50	15	0.47	2.41^{NS}
45	62.75	15	0.54	62.13	15	0.46	61.99	15	0.45	62.31	15	0.60	1.11^{NS}
46	62.17	15	0.54	62.51	15	0.36	62.30	15	0.39	62.61	15	0.35	0.21^{NS}
47	63.26	15	0.79	63.57	15	0.74	63.37	15	0.71	63.86	15	0.67	0.39^{NS}
Mean	61.72			61.80			61.55			61.58			

^{NS}: Not significant

later study reported that the same amount of organic or inorganic chromium did not influence the hens production performances (Lin and Lin, 1999). In the present experiment chromium, yeast and folic acid suplementation did not affect body weight as in the results of Cupo and Donaldson (1987) in chicks and Sahin *et al.* (2001a) in rabbits, but reduced feed consumption and improved feed efficiency. Egg production and egg weight were not affected by chromium supplementation, consistent with the results of Lien *et al.* (1996). Shell thickness was not affected by Chromium supplementation as indicated by Lien *et al.* (1996). However, this effect does not seem to be a result of chromium supplementation, because similar results were not obtained at the following sampling times as well as no differences in other shell quality parameters. Thus, it is possible to say that chromium did not affect the parameters related to egg shell quality investigated in the study. However, chromium yeast suplementation

Table 7: The effects of supplemental chromium yeast and folic acid on egg quality parameters

Parameters	Weeks	Control	Chromium yeast (150 mg ton^{-1})	Folic acid (10 mg ton^{-1})	Folic acid + Chromium yeast (10+150 mg ton^{-1})	p-value
Specific gravity (g/cm^3)	40	1.0662±0.0008	1.0663±0.0008	1.0662±0.0008	1.0664±0.0009	NS
	43	1.0659±0.0008	1.0669±0.0009	1.0667±0.0009	1.0676±0.0011	NS
	47	1.0665±0.0008	1.0667±0.0008	1.0661±0.0010	1.0675±0.0011	NS
Egg shape index	40	79.65±0.38	78.85±0.48	79.87±0.35	78.95±0.33	NS
	43	78.97±0.42	78.27±0.32	78.95±0.44	78.29±0.62	NS
	47	77.65±0.51	77.77±0.30	77.71±0.47	77.79±0.25	NS
Shell thickness (mmx10^2)	40	38.70±0.57	40.10±0.47	39.17±0.23	40.02±0.45	NS
	43	39.63±0.40	39.83±0.49	39.46±0.46	39.97±0.49	NS
	47	39.46±0.46	39.51±0.35	39.26±0.41	39.55±0.39	NS
Haugh unit	40	94.36±1.15	93.20±1.55	94.39±1.07	93.35±1.30	NS
	43	88.10±2.17	87.00±1.77	89.07±1.87	88.80±1.51	NS
	47	87.35±1.39	90.05±1.47	87.49±1.25	90.75±1.64	NS
Albumen index	40	12.40±0.35	12.44±0.43	12.37±0.39	12.47±0.42	NS
	43	10.85±0.58	10.45±0.46	11.02±0.49	10.47±0.47	NS
	47	9.79±0.35	11.00±0.48	10.19±0.30	11.07±0.49	*
Egg yolk index	40	48.81±0.45	50.70±0.77	48.79±0.43	50.68±0.75	*
	43	48.67±0.51	47.60±0.63	48.60±0.52	47.75±0.60	NS
	47	44.70±0.38	47.44±0.53	44.72±0.39	47.50±0.54	**

p<0.05, **p<0.001

Table 8: Egg yolk cholesterol levels in treatment groups

Parameters	Weeks	Control	Chromium yeast (150 mg ton^{-1})	Folic acid (10 mg ton^{-1})	Folic acid + Chromium yeast (10+150 mg ton^{-1})	p-value
Egg yolk cholesterol (mg g^{-1})	40	16.21±0.67	16.41±0.45	16.17±0.61	16.00±0.70	NS
	43	16.60±0.48	13.61±0.52	16.65±0.45	13.96±0.55	**
	47	17.50±0.43	13.42±0.51	16.87±0.48	13.21±0.51	**

*p<0.05, **p<0.001

Table 9: Egg yolk folic acid levels in treatment groups (µg/egg yolk)

		Control		Chromium yeast (150 mg ton^{-1})		Folic acid (10 mg ton^{-1})		Folic acid + Chromium yeast (10+150 mg ton^{1})		
Weeks	n	$\bar{X}$	S $\bar{X}$	$\bar{X}$	S $\bar{X}$	$\bar{X}$	S $\bar{X}$	$\bar{X}$	S $\bar{X}$	F
43	24	16,7	0.95	16,9	1.73	40.6	4.38	40.4	4.01	1.19^{NS}
47	24	17.1	1.10	18.9	1.09	44.2	3.18	43.8	4.92	1.08^{NS}

^{NS}: Not Significant

Table 10: Egg yolk chromium levels in treatment groups (µg egg yolk^{-1})

		Control		Chromium yeast (150 mg ton^{-1})		Folic acid (10 mg ton^{-1})		Folic acid + Chromium yeast (10+150 mg ton^{-1})		
Weeks	n	$\bar{X}$	S $\bar{X}$	$\bar{X}$	S $\bar{X}$	$\bar{X}$	S $\bar{X}$	$\bar{X}$	S $\bar{X}$	F
43	24	38.8	1.18	38.4	0.82	37.6	1.07	39.3	1.57	1.21^{NS}
47	24	44.1	1.19	44.6	1.15	43.9	1.20	44.2	1.64	1.36^{NS}

^{NS}: Not Significant

increased albumen and yolk quality. In the present experiments, the addition of supplemental folic acid to laying hen diets did not impact the performance of the birds, as reflected by no significant differences in egg production or egg weights (House *et al*., 2002). In the study, folic acid supplemented group and control group had same feed consumption and feed conversion rate.

Many studies have been done to reduce yolk cholesterol by supplementing diet with different substances (Elkin and Rogler, 1990; Mohan *et al*., 1995). Reports on chicken egg cholesterol values show considerable variation. Lower yolk cholesterol content in the eggs collected from chromium yeast supplemented diet fed hens than that of the controls is in agreement with the results of Lien *et al*. (1996), who found a dose dependent reduction in the yolk cholesterol of hens fed the chromium supplemented diet.

Sahin *et al*. (2001b) fed Japanese egg quails, 0, 200, 400, 800 and 1200 ppb chromium picolinate feed. As the chromium amount in the feed increased, the amount of insulin in the serum increased too (p = 0.05 linear). As the serum, glucose and cholesterol concentration increased in the treatment groups, which were fed with the highest amount of chromium picolinate (p = 0.01), it was determined that the serum protein concentration decreased.

Suksombat and Kanchanatawee (2005) placed 324 one-day old male and female broiler chicks into randomly groups, 200, 400 and 800 ppb of chromium picolinate and inorganic chromium added to the basal rations. It was determined that the total cholesterol and triglyceride amount in the blood of the treatment groups, which were fed the 200 and 400 ppb chromium picolinate diets had decreased.

The treatment groups with the lowest total cholesterol were bed the 200 and 400 ppb chromium picolinate. The higher the level of chromium picolinate in the feed, the higher the LDL amount in the blood. For good growth performance and carcass composition; 200 ppb of chromium picolinate addition to the feed is advised.

Kheiri and Toghyani (2009) was conducted to investigate the effect of different levels of chromium chloride (0-1600 μg kg^{-1}) on performance and immune responses of broiler chicks. The results of this study showed that dietary supplementation of Cr chloride, at level of 1600 μg kg^{-1} improved performance, carcass yield and some immune responses in broiler chicks, but feed intake, feed conversation were not affected by supplemental chromium ($p>0.05$).

Stanley *et al*. (1996) demonstrated that the egg cholesterol levels of groups which were fed chromium mannan oligosaccaride rations, were lower than the control group egg cholesterol levels. These results are consistant with the cholesterol rates. Results are different from the research due to the different chromium source added.

In the studies of Hebert *et al*. (2005) and Welch *et al*. (1954) with laying chicken; it was shown that different rates of folic acid were used and they achieved a significant amount of increase of folic acid in egg yolk. These studies are in agreement with the results of this study.

In a study of Piva *et al*. (2003) different chromium sources (chromium chloride, yeast chromium and chromium aminoniaciat) were added to egg chicken feed to detect egg yolk chromium levels and no major abnormal change in the chromium level of the egg yolk was detected. This evidence is coherent with the research results.

CONCLUSION

The folic acid level of eggs that are served to human consumption has changed positively related to the feeding of the hens. Also, the chromium level of the eggs was not affected but the usage of chromium yeast caused a distinctive decline of cholesterol level of egg yolk, leading to the idea that it is possible to produce designed eggs that are enriched with feed substances.

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