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Bioavailability Comparison of Free and Esterified Lutein for Layer Hens

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ABSTRACT

Lutein supplements are often used to pigment and enrich layer chicken eggs. This experiment was conduced to compare the bioavailability of free and esterified lutein, by depletion method. Forty chickens were randomly divided into two groups. After 2 weeks of washout period, when all birds were fed the same low lutein basal diet, the two groups were fed for another 2 weeks with diets supplemented with free lutein or esterified lutein. Two experimental diets were supplemented with the same amount of 15 mg lutein/kg. On day 0 (baseline) and days 3, 7, and 14, birds fasting morning plasma samples were collected and stored for lutein analysis by high performance liquid chromatography method (HPLC). Results showed that: 1) Plasma lutein concentration increased dramatically after feeding free or esterified lutein; 2) Plasma lutein level in birds fed the diet supplemented with free lutein significantly differed from those fed esterified lutein on day 3. 3) There were no significant differences in plasma lutein levels between free lutein and esterified lutein on days 7 and 14. In conclusion, the lutein bioavailability from free lutein or esterified lutein supplements was comparable.

INTRODUCTION

Lutein is a group of natural pigments known as xanthophyll carotenoids without vitamin A activity, and itis mainly found in fruits, vegetables, grains, and eggs (Johnson, 2004). It has a yellow-orange color, and it has been used for many years in poultry diets as a mean to pigment egg yolks. Recent evidence suggests that lutein can be beneficial for the prevention of a wide list of human diseases (Ribaya-Mercado & Blumberg, 2004). Because lutein in egg yolk is highly bioavailable, as most of fats and fat-soluble compounds (Chung *et al*., 2004), and lutein content in eggs can be manipulated by the use of lutein in layer diets, it is possible to use lutein feed additives to develop lutein-enriched eggs (Leeson & Caston, 2004).

Lutein is mostly found in marigold (*Tagets erecta* L.), and its extraction by solvent has been used to produce lutein additives. Native marigold extracts supply more than 95% of the esterified lutein available (Breithaupt, 2002). A solvent method, following saponification, has been widely used for the extraction of free lutein from marigold (Larsen & Christensen, 2002). However, the hydroxyl groups and multiple double bonds of free lutein make it susceptible to some chemicals problems, such as saponification (Oliver *et al*., 1998). Furthermore, the stability of esterified lutein is superior to that of free lutein, under the same challenge of light and heat (Li *et al*., 2007). Since esterified fat soluble nutrients, such as vitamins A and E, are absorbed in the small intestine as free alcohol (Muller *et al*., 1976), it is hypothesized that lutein esters can be transformed, by lipase catalyzed hydrolysis, in free lutein, in the gut

lumen, before being absorbed. However, there is little information available in literature relative to the digestion, absorption, and metabolism of lutein esters. Therefore, data regarding the bioavailability of lutein from various sources are insufficient.

This study compared the concentration of free lutein in chicken plasma after feeding free or esterified lutein by depletion method to layer hens. It may help selecting the best form of lutein to be used in the poultry industry.

MATERIALS AND METHODS

Hy-Line Grey commercial layers (n=40, 70-wk-old), weighing 1.6±0.2 kg, were randomly divided into two treatments, with 20 birds each. Hens were individually housed in cages measuring 0.2×0.2 m, and were submitted to a lighting program of 16 h light/day, at the Research Farm of Hubei Feed Engineering Technology Research Center. Layers were kept under a temperature of 25±5 °C. Feed and water were supplied *ad libitum*. Care of the animals was in accordance to the "Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching" (Guide, 1988).

After two weeks of washout period, when the hens were fed diets supplemented with the same low-lutein basal diet, two groups were fed the basal diets supplemented either with free lutein or esterified lutein for a period of two weeks. The low-lutein basal diet contained broken rice instead of yellow corn, and was formulated to meet the nutrient requirements of layers proposed by the NRC (1994). The ingredient and nutrient compositions of the basal diet are presented in Table 1. The source of free-lutein and esterified-lutein supplements were commercial premixes of marigold. Lutein contents were determined using a HPLC method, as described by Chen *et al*. (2004). The free-lutein and esterified-lutein supplements contained 45.9±0.4 and 7.5±0.2 g free lutein/kg, respectively. The basal diet contained 0.50±0.02 mg lutein/kg (n=3; determined as is). Other carotenoids were not detected. The two experimental diets were formulated to contain the same level of 15 mg lutein/kg. All feeds were prepared every 3 days. No feed additives were supplemented and all diets were prepared in mash form.

Fasting morning blood samples were taken on day 0 (baseline, before feeding the fortified diets), and days 3, 7, 14 for lutein analysis. The number of eggs laid by each hen was counted during the experiment. Egg production of individual hens did not change during the study. Therefore, plasma lutein levels were not corrected for individual egg production.

Blood samples were obtained by puncture of the vena ulnaris, with an EDTA-monovette. One milliliter of blood was sampled from each chicken. Immediately after collection, the plasma was separated from the erythrocytes by centrifugation (2,200 r/min, 10 min, 4 °C). The plasma was stored -20 °C, in plastic caps, and protected from light. Plasma lutein concentrations have been shown to be stable under these storage conditions (Craft *et al*., 1988).

The plasma samples were analyzed for free lutein as a modification of the HPLC method, as described by Chung *et al*. (2004). The HPLC system comprised a Waters 600S controller, Waters 1525 pump, Waters 717 autosampler, Waters 2487(dual λ absorbance) detector, and Waters *XTerra*^{R}C$_{18}$ column (3.9 mm×150 mm, 5μm, MS). The HPLC mobile phase was methanol: water ($CH_3OH:H_20$)(92:8, vol/vol), The column flow rate

Table 1 - Ingredient and nutrient composition of basal diet (as fed basis).

Ingredients %		Nutrient composition[2]	
Broken rice	60.30	Metabolizable Energy, MJ/kg	11.37
Wheat bran	10.00	Crude protein, g/kg	157.7
Soybean meal	15.70	Calcium, g/kg	32.6
Rapeseed meal	3.00	Available phosphorus, g/kg	4.0
Soybean oil	1.00	Lysine, g/kg	7.6
Limestone	8.00	Methionine + cysteine, g/kg	5.2
Dicalcium phosphate	1.20		
Choline chloride (50%)	0.10		
DL-methionine(98%)	0.10		
Salt	0.25		
Premix[1]	0.35		
Total	100		

1 - Premix supplied the following per kg of diet: Fe (as ferrous sulfate), 70 mg; Cu (as copper sulfate), 7mg; Zn (as zinc sulfate), 70mg; Mn (as manganese sulfate), 70mg; Se (as sodium selenite), 0.36mg; I (as potassium iodide), 1.4mg. vitamin A (retinylacetate), 8,000IU; cholecalciferol, 2,750IU; vitamin E (α-tocopherylacetate), 15 IUμvitaminK3, 3.0mgμthiamin, 1.5mg; riboflavin, 4.0mg; pantothenic acid, 10mg; niacin, 25mg; pyridoxine, 3.0mg; biotin, 50μg; folic acid, 0.4mg; vitamin B12, 10μg. 2 - Obtained by calculation.

was 1 mL/min, and the column temperature was maintained at 16.4 °C, with a water jacket and a circulator.

Lutein was quantified by determining the peak area at 445 nm, in the HPLC calibrated against a known amount of standard. The lutein standard was obtained from Sigma Chemical Co (St Louis).

The limit of detection for this method is 0.245-9.8 µg lutein /mL. The precision was 1.91% (n=6). Recovery of the internal standard averaged 98.95% and the RSD was 1.08%.

All data were analyzed by ANOVA using the GLM procedure of SAS (Statistical, 2000) as a completely randomized design. Data are reported as means (±SE). All statements of significance were based on probability p<0.05.

RESULTS AND DISCUSSION

After feeding the low-lutein basal diet for two weeks, the chickens' plasma contained only small amounts of free lutein (no detection of lutein, lower than method detection limit -0.245µg lutein /mL). These results were in agreement with the proposed experimental design, and implied that the baseline values of day 0 were set up correctly.

Three days later, after feeding the diets supplemented with lutein, plasma free lutein changed remarkably in both free-lutein and esterified-lutein fed layers (Table 2); plasma lutein concentration with the free-lutein diet was 1.90±0.17 µg lutein/mL, s higher (P<0.05) than the level of 1.47±0.13 µg lutein/mL obtained with the diet supplemented with esterified lutein. An explanation for these results might be that the dietary free lutein was absorbed easier by the gut, as compared to lutein ester, which may have needed to be hydrolyzed lutein, before it was absorbed through the intestinal wall into the blood stream.

The plasma lutein level of birds fed the diet with free lutein on day 7 did not differ (P>0.05) from day 3, but the plasma lutein levels of those fed the esterified lutein diet on day 7 differed (P <0.05) from day 3. This difference maybe is a reason of the pigmentation variation of egg yolk. However, this is not consistent with the experimental results of Bowen *et al*. (2002), who reported higher bioavailability from lutein ester formulation when compared to the free form, although this difference was not significant.

On day 14, there were no differences (P >0.05) of the plasma lutein values from day 7 and between both treatments. These results indicate that the plasma lutein levels of layer hens reached approximately a stable value independently of lutein dietary source, although the curves of plasma lutein level after feeding different forms of lutein were different. This might be due to deposition and excretion rates (Tyczkowski & Hamilton, 1987), because plasma analyses reflect not only intestinal absorption of dietary lutein, but also other physiological processes.

There are many reports on egg yolk pigmentation using free or originally esterified lutein. Sometimes, this is a point of controversy, each trying to justify that one form is more efficiently absorbed than the other. However, little attention has been given to the influence of the chemical form of lutein on its absorption and biological availability.

Papa *et al*. (1985) reported that saponification had no effect on egg yolk color. Lai *et al*. (1996) observed that the saponification of paprika did not affect egg yolk color as compared to a non-saponified paprika oleoresin. Galobart *et al*. (2004) reported that saponification of both marigold and paprika extracts improved pigmenting efficiency.

CONCLUSION

According to the results of this study, both free and esterified lutein added to a low-lutein basal diet may increase plasma lutein levels of layer hens to a comparable level. Therefore, it is suggested that esterified lutein can be used to produce lutein-enriched eggs by the poultry industry since the stability of esterified lutein is superior than that of free lutein.

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Table 2 - Mean (± s.e.) plasma lutein levels (µg/mL) after feeding diets supplemented with free or esterified lutein*.

Treatment	day 3	day 7	Day 14	SEM	Probability
Free lutein	1.90(x) ± 0.17	1.89± 0.17	2.19 ± 0.16	0.24	0.247
Esterified lutein	1.47 b(y)± 0.13	1.77a± 0.15	2.14a± 0.19	0.23	0.036

*Data are the means of 20 replicates per treatment. a,b Means in the same row with different superscripts significantly differ at P <0.05. (x), (y). Means in the same column with different superscripts significantly differ at P <0.05

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