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Quality characteristics of *n-3* polyunsaturated fatty acid-enriched eggs*

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ABSTRACT

The aim of this work was to develop a diet suitable for obtaining eggs enriched in omega-3 fatty acids at a sufficient level with minimum disadvantages affecting egg quality parameters or laying hen health and yield. A trial was conducted to study the effects of two previously designed experimental diets on egg quality and fatty acid composition; these effects were compared with those of a standard diet for laying hens. Yolk fatty acid composition was determined and typical egg quality parameters were measured, in particular rheological measurements were carried out to evaluate structural yolk quality. Sensorial quality of eggs was also analysed and necropsies of laying hens were performed. The results show that a diet containing 1.5% Cantabrian blue fish oil and 5% linseed achieved commercially acceptable levels of omega-3 fatty acids in egg yolk avoiding most of the drawbacks reported by many authors.

KEY WORDS: laying hen, *n-3* PUFA, rheological, egg quality

INTRODUCTION

It is currently recognized that omega-3 fatty acids exert a strong, positive influence on human health (Kolanowski, 1999). Dietary intake of omega-3 fatty acids decreases the risk of heart disease (Temple, 1996) and plays an important role in preventing cancer (Pandalai et al., 1996) as well as immunologic reactions (Fernandes, 1995). Omega-3 fatty acids are also essential for normal foetal brain and visual development (Neuringer et al., 1998).

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High amounts of these acids are found in fish and other types of seafood, but in many countries the consumption of marine products is low (Carrillo-Domínguez et al., 2005), so one way to increase the intake of omega-3 fatty acids is through functional foods enriched in them.

The egg yolk fatty-acid profile is clearly affected by the fatty acid profile of hen diets (Meluzzi et al., 2000), so eggs are a product relatively easy to be enriched in omega-3 fatty acids. Common dietary sources of omega-3 fatty acids include fish, crustaceans and mollusks, but there are other sources, such as linseed or marine microalgae. The use of these sources in laying hen diets in order to obtain enriched eggs may have negative effects, not only on hen health (Aymond et al., 1994; Van Elswysk et al., 1994; Bean and Lesson, 2003), but also on egg quality parameters and laying hen yields (Collins et al., 1997; Baucells et al., 2000; Gonzalez-Esquerra and Leeson, 2000; Schreiner et al., 2004).

In this work, two different diets were developed to obtain eggs enriched in omega-3 fatty acids at a sufficient level, but also with the aim of producing minimum disadvantages, affecting either egg quality and structural parameters, or laying hen health and yield. As sources of omega-3, linseed oil was used in the formulation of one of the diets (Diet 1) and Cantabrian blue fish oil and linseed in the other (Diet 2). These raw materials were chosen due to their well-known good results as omega-3 sources, moreover Cantabrian blue fish oil is a by-product obtained in high quantities from the local fishing industry.

In order to evaluate the effects of these experimental diets, yolk fatty-acid composition was determined and typical egg quality parameters were measured. A sensorial evaluation of eggs and necropsies of laying hens were also carried out. Besides, it is important to take into account that in this study, rheological measurements were developed as a new tool to evaluate egg quality, because currently high-resolution rheological measurements are becoming essential to evaluate structural modifications in food industry processes.

MATERIAL AND METHODS

Birds and diets

Ninety Leghorn hens were divided into 3 groups of 30 birds each. One group was fed a standard diet for laying hens and the other two with two different experimental diets enriched in omega-3 fatty acids (diet 1 containing 2.5% linseed oil and diet 2 containing 1.5% fish oil and 5% linseed; fatty acid profiles of these *n*-3 sources are given in Table 1). Diets were balanced with a proportion of 17% crude protein and their metabolizable energy was between 11 and 12 MJ/kg (calculated according to the Directive 86/174/EEC). Hens were housed, three

Table 1. Fatty acid profile of fish oil, linseed oil and linseed used as sources of omega-3

Fatty acid	Percentage of total fatty acids		
	fish oil	linseed oil	linseed
Myristic	3.41	-	0.04
Myristoleic	0.14	-	-
Pentadecilic	0.87	-	-
Palmitic	19.10	4.49	5.40
Palmitoleic	6.33	-	-
Margaric	0.87	-	-
Margaroleic	1.05	-	-
Stearic	5.14	3.45	4.20
Oleic	18.20	19.10	21.20
Linoleic	2.37	15.80	17.00
Linolenic	0.86	56.10	50.90
Araquidic	0.48	0.26	0.18
Gadoleic	3.72	0.28	-
Eicosadienoic	2.99	0.18	0.05
Araquidonic	2.16	-	-
EPA	6.52	-	-
Behenic	0.36	0.08	0.18
DHA	20.80	-	-
Others	4.63	0.26	0.85
Saturated fatty acids	30.23	8.28	10.35
Unsaturated fatty acids	69.77	91.72	89.65

external laboratory data

Table 2. Composition of standard and experimental diets, % w/w

Components	Standard	Diet 1	Diet 2
Barley	8.90	4.20	-
Maize	49.20	49.0	50.60
Soya oilmeal	27.91	26.81	27.71
Palm oil	0.50	-	1.70
Sunflower meal	-	4.0	-
Fish oil	-	-	1.5
Linseed oil	-	2.5	-
Linseed	-	-	5
Salt	0.30	0.30	0.30
Calcium carbonate	9.30	9.30	9.30
Dicalcic phosphate	0.80	0.80	0.80
Copper sulphate pentahydrate	0.5	0.5	0.5
Antioxidant	2.10	2.10	2.10
DL-methionine	0.12	0.12	0.12
Vitamins, A, D_3, E	0.07	0.07	0.07
Red pigment	0.30	0.30	0.30

ranges of total concentrations for all diets, % w/w: lysine 0.8-0.9, methionine 0.4-0.5, calcium 3.6-4.0, avalaible phosphorus 0.2-0.3

to a cage, in a commercial egg production facility located in Asturias (Spain). A detailed description of the composition of standard and experimental diets is given in Table 2. At the start of the experiment the hens were 18 weeks old and were fed with the experimental diets for 15 days prior to collecting the samples. The hens received 130 g per day of feed, water was provided *ad libitum*. Egg production was summarized daily during three months.

Sample preparation

Egg yolks were prepared from fresh eggs. The shelling of the eggs and the separation of the yolk from the albumen were performed manually. The albumen residuals were eliminated from the yolk using blotting paper, and the vitelline membrane was removed using tweezers. The yolks were mixed manually with a spatula for the different analyses.

Fatty acid composition

To evaluate the effectiveness of the tested diets, yolk fatty-acid composition was determined. These analyses were performed following the chloroform-methanol extraction method described by Wang et al. (2000). A fused silica capillary column (Omegawax 320, 30 m × 0.32 mm ID, 0.25 μm) on a Shimadzu GC-14B chromatograph, equipped with an autosampler and flame ionization detector, was used to separate and quantify the fatty acid methyl esters. The initial column temperature was set at 180ºC for 5 min, increased to 200ºC by 1ºC/min and held for 40 min. The column temperature was elevated to 260ºC at 10ºC/min and held at the final temperature for 10 min. Helium was used as the carrier gas. The detector was set at 260ºC. Fatty acid methyl esters were identified by comparison with retention times of standards. A Chromatopac data integration system C-R46 was used to integrate peak areas. The determined fatty acids were: stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2*n-6*), EPA (C20:5*n-3*) and DHA (C22:6*n-3*). The fatty acid content of egg yolk was calculated using tricosanoic acid (C23:0) as an internal standard. Thirty samples of each treatment were measured and the analyses were carried out in triplicate.

Egg quality parameters

In order to determine the possible drawbacks of the experimental diets, the main egg quality parameters were evaluated: yolk colour, yolk volume, yolk weight, yolk pH, yolk dry matter content, albumen volume, albumen weight, albumen pH, egg size, egg weight, shell weight and shell thickness. Thirty samples of each treatment were measured.

pH was measured at 20ºC using a Crisom micro 2002 pH meter. Dry matter was assessed with a HR73 Halogen Moisture Analyzer using an approximately 0.25 g sample. Yolk colour was evaluated using the Roche colour fan.

Rheological measurements

To verify possible changes in gelation properties of egg yolk due to the experimental diets, rheological tests were developed. The assays were carried out with a Haake MARS II rotational rheometer with a plate/plate measuring system (PP60), a gap of 1 mm, and sample amount of about 2.9 g. Cure tests were performed in the linear viscoelasticity range that was previously established. The frequency employed for them was 1 Hz, the temperature ramp went from 20 to 90ºC at a heating rate of 2ºC/min; the tests were carried out in CD mode at a constant deformation of 8%. Experimental detection of the gel point is not easy, however Cordobés et al. (2004) suggested that the gel point occurs at the time at which G' (storage modulus) and G'' (loss modulus) cross each other at a given frequency, in this work the gel point was determined in this way. Samples were analysed in triplicate.

Sensorial evaluation

In the organoleptic assessment the following methodological aspects were established:

- Panel. The panel was composed of 9 healthy subjects between 20 and 30 years of age without any sensorial defects. None of them were taking any medication. Subjects were chosen *via* taste and olfactory tests from among the laboratory staff.
- Definition of attributes and methodology used in organoleptic assessment of these attibutes. The panel was duly instructed about methodology, in particular, concerning the ways of avoiding physiological and environmental causes that might affect the results.
- Detailed description of the quality standard references.
- Preparation and codification of the samples. Samples were prepared in the laboratory using fresh eggs on the same day as the tests. Taking into account the main ways of consumption, three sorts of samples were prepared: raw, boiled, and in a plain omelette using eggs from hens fed control and experimental diets. The samples were presented on dishes in three groups named A, B and C and in each group samples were numbered from 1 to 3.
- Test conditions. The test was conducted in a room next to the laboratory with artificial lighting, during the early afternoon, with a room temperature of 20°C and 80% humidity in isolated, noise-free work stations.

Hen necropsy

Necropsies were carried out on laying hens fed experimental diets to evaluate possible anatomical changes due to the diets. Hens were fed with these diets for 5 months prior to being analysed. Necropsies were performed in duplicate.

Statistical analyses

Data were analysed running t-tests to compare means, at a 5% probability level. Previously F-tests at a 5% probability level were carried out to compare standard deviations. In case the data presented heterogeneity, they were statistically homogenized. Standardized skewness and standardized kurtosis were used to assess if the samples came from normal distributions. The software used was STATGRAPHICS Plus 3.1.

RESULTS

The fatty acid compositions of control and experimental diets are shown in Table 3. As can be seen, although the contents of stearic acid (C18:0) and oleic

Table 3. Comparison of fatty acid composition (mg/g yolk) of yolks from hens fed diet 1 and diet 2 with control, average values ± SD

Item	Control	Diet 1	Diet 2
C18:0	19.4 ± 5.9	21.5 ± 3.5[a]	20.9 ± 4.0[a]
C18:1	178.3 ± 51.0	169.6 ± 49.3[a]	157.0 ± 21.7[a]
C18:2	44.9 ± 14.4	67.6 ± 20.0[b]	57.6 ± 10.1[b]
EPA	0.15 ± 0.09	0.32 ± 0.20[b]	0.48 ± 0.22[b]
DHA	1.54 ± 0.30	3.62 ± 0.47[b]	5.91 ± 0.57[b]

[a] no significant differences respect to the control for the t-tests run to compare means, [b] significant differences respect to the control for the t-tests run to compare means. Confidence interval of 95%

acid (C18:1) of eggs from hens fed diets 1 and 2 are similar compared with the control, the experimental diets had increased linoleic acid (C18:2*n-6*), EPA (C20:5*n-3*) and DHA (C22:6*n-3*) yolk contents, and t-tests showed significant differences in respect to the control (confidence interval of 95%).

The results reported in Table 4 show that of all the egg quality parameters analysed, only the value of yolk pH and yolk colour were statistically different in diet 1 compared with the control. Concerning diet 2, only yolk dry matter percentage and yolk colour were statistically different compared with the control (confidence interval of 95%).

Table 4. Comparison of egg yolk quality parameters from hens fed diet 1 and diet 2 with control, average values ± SD

Item	Control	Diet 1	Diet 2
Shell weight, g	7.8 ± 1.1	8.2 ± 1.0[a]	7.8 ± 0.8[a]
Yolk volume, ml	10.4 ± 0.8	10.4 ± 0.5[a]	9.9 ± 0.9[a]
Yolk weight, g	12.3 ± 1.1	12.6 ± 1.0[a]	11.8 ± 1.1[a]
Yolk pH	6.2 ± 0.2	6.1 ± 0.1[b]	6.2 ± 0.2[a]
Albumen volume, ml	37.2 ± 2.7	36.5 ± 1.9[a]	37.7 ± 2.8[a]
Albumen weight, g	35.6 ± 2.3	35.1 ± 1.6[a]	36.2 ± 1.9[a]
Albumen pH	8.7 ± 0.2	8.7 ± 0.2[a]	8.6 ± 0.2[a]
Egg weight, g	57.8 ± 3.7	57.2 ± 2.7[a]	57.4 ± 4.2[a]
Egg size, cm	5.4 ± 0.2	5.4 ± 0.1[a]	5.5 ± 0.2[a]
Shell thickness, mm	0.41 ± 0.07	0.39 ± 0.08[a]	0.42 ± 0.07[a]
Yolk colour	12.3 ± 0.5	10.8 ± 0.6[b]	11.1 ± 0.5[b]
Yolk dry matter, %	60.5 ± 3.8	58.2 ± 6.2[a]	55.2 ± 2.2[b]

[a] no significant differences respect to the control for the t-tests run to compare means, [b] significant differences respect to the control for the t-tests run to compare means. Confidence interval of 95%

Feed intake was the same for the three diets and hen yield was not influenced by experimental dietary treatments compared with the control. There were no significant differences in yields on diet 1 (0.91±0.12 eggs produced/hen d) and diet 2 (0.94±0.12 eggs produced/hen d) in respect to the control (0.93±0.13 eggs produced/hen d) (confidence interval of 95%).

As stated above, in order to verify that some structural properties of yolk, mainly coagulation capacity, were not affected by the experimental diets, gel points were rheologically determined by means of "cure tests".

During gelation, yolk undergoes a phase transition from a liquid to a gel. The sol-gel transition is a critical point where the transition variable would be related to the yolk chemical composition. The graphics obtained from the cure tests are given in Figure 1; there were no statistically significant differences between the gel point temperature obtained from the cure tests of diet 1 (76.0±0.9ºC) and diet 2 (75.0±1.0ºC) samples respect to the control (74.5±0.3ºC) (confidence interval of 95%).

In this sensorial evaluation, unpleasant aroma and taste refer mainly to "fishy", while unpleasant appearance refers to less coloured yolks (Table 5). The panelists clearly associated the experimental diets with slightly less coloured yolks.

Surprisingly, a percentage of panelists found unpleasant aromas in the control raw egg sample. This percentage was lower in the control boiled egg sample and the same as in the diet 2 sample, the largest percentage was in the diet 1 sample. In case of a plain omelet, none of the panelists found an unpleasant aroma in the control sample and the percentage was the same in diet 1 and 2 samples. Concerning unpleasant aromas, panelists reported more confusing results with

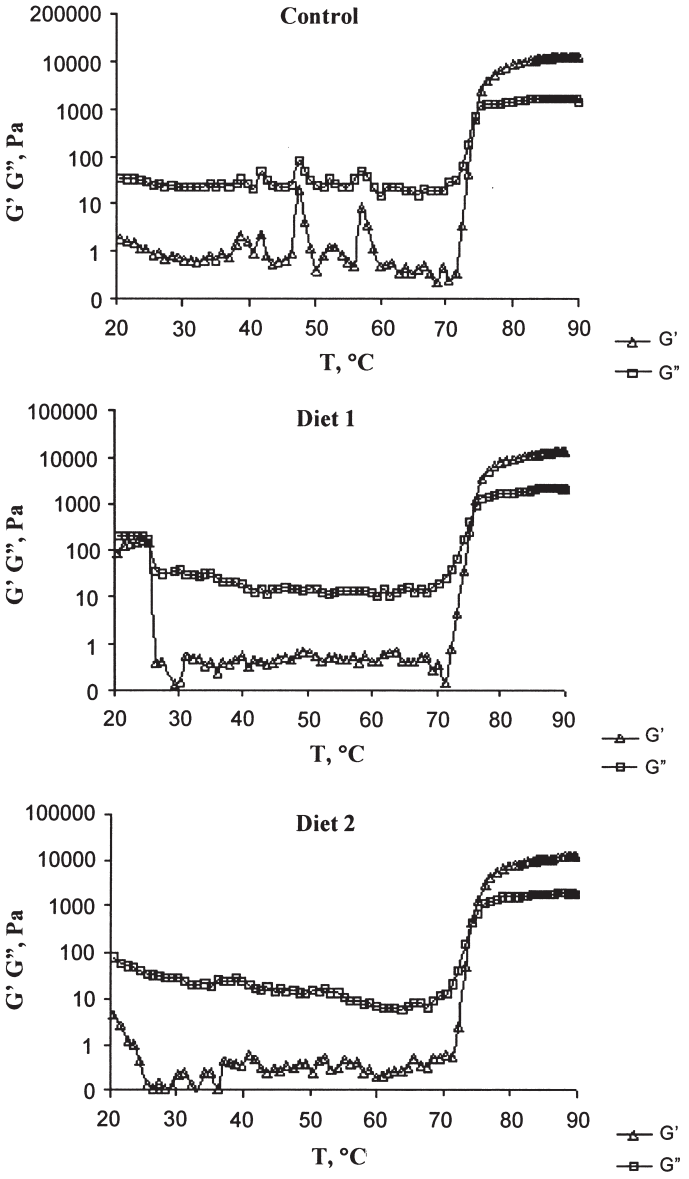

Figure 1. Variation of the storage modulus (G') and the loss modulus (G'') during the heating from 20 to 90ºC of yolks eggs coming from hens fed control, diet 1 and diet 2

Table 5. Evaluation of sensory attributes of raw eggs, boiled eggs and eggs in plain omelette from hens fed control, diet 1 and diet 2

Egg	Sensory evaluation	Panellist that detected unpleasant attributes, %		
		control	diet 1	diet 2
Raw eggs	Aroma	22	0	11
	Appearance	0	77	77
Boiled eggs	Aroma	11	33	11
	Taste	0	0	0
	Appearance	0	67	67
Plain omelette	Aroma	0	11	11
	Taste	0	0	11
	Appearance	0	56	56

raw eggs than with boiled eggs or eggs in a plain omelet. In respect to yolk colour, the more an egg is cooked, the more difficult it is to detect colour differences.

The results of the necropsies are reported in Table 6. As can be seen, diet 1 caused fewer changes in hens than diet 2, where some were observed in the liver.

Table 6. Results for necropsies of laying hens fed experimental diets

Item	Diet 1		Diet 2	
	sample 1	sample 2	sample 1	sample 2
Conformation	Normal	Normal	Normal	Normal
Liver size	Normal	Normal	Light hypertrophy	Light hypertrophy
Liver colour	Normal	Normal	Light	Normal
Liver consistency	Normal	Normal	Slighly mushy	Slightly friable
Amount of abdominal fat	Low	Low	Normal	Normal
Reproductive system	Functional ovary	Functional ovary	Functional ovary	Functional ovary

DISCUSSION

The contents of stearic acid (C18:0) and oleic acid (C18:1) of eggs from hens fed diets 1 and 2 are similar compared with the control diet; this was also reported by Cachaldora et al. (2006). The high contents of EPA and DHA should be pointed out, since these are the two most important essential fatty acids for human health (Vaccaro et al., 2005). Although with diet 1 the amount of EPA and DHA in yolk was twice that of controls, the best results were achieved with diet 2, i.e. triple the amount of EPA and DHA in the control. These levels are even higher than reported by other authors employing sources with equivalent quantities of omega-3 fatty acids (Bean and Leeson, 2003; Pardío et al., 2005). As

linseed oil does not contain EPA or DHA, the two times higher contents of these fatty acids in yolks of hens fed diet 1 in comparison with the control are due to fatty acid metabolism. It is well known (Du et al., 1999; Baucells et al., 2000) that dietary supplementation of linolenic acid increases the synthesis and deposition of long-chain *n-3* fatty acids. Raes et al. (2002) suggested that linolenic acid may influence the activity of the desaturases to a different extent in the synthesis of *n-6* and *n-3* long chain fatty acids, depending on the feed fatty acid profile.

The yolk pH was significantly different in diet 1 and control. However, as both values of yolk pH are similar to the pH characteristic of hen egg yolks which is approximately 6 (Stadelman, 1995), this does not mean that the internal quality of the egg changed. All groups had slightly higher yolk dry matter levels than typical values, 52% (Stadelman, 1995). This could be due to the fact that the analysed eggs were fresh and the transfer of water from albumen to the yolk occurs during the storage of eggs (Kiosseoglou, 1989). Even when all of the analysed eggs were the same age, diet 2 showed a value of dry matter significantly different in respect to the control. As stated above, all feedings showed a higher amount of yolk dry matter than reported values and it is known that the lower the water content, the better the yolk quality.

Both experimental diets showed significant differences in yolk colour from the control; these differences are probably related to interference of pigment deposition with fatty acid metabolism (Zaghini et al., 2005). This effect on yolk colour was reported in studies concerning enriched eggs (González-Esquerra and Leeson, 2000; Cachaldora et al., 2006). Although these authors also described other negative effects (shell thickness and lower egg weights), in this work and, as shown above, these negative effects were not found. The number of eggs produced was similar with all the diets, and egg weight and size were not altered by diets 1 or 2 (Table 4). Thus, none of the experimental diets modified hen productivity.

Coagulation properties of egg yolk are very important for some domestic uses and egg industry processes. Rheological measurements showed that there were no statistically significant differences in coagulation kinetics on experimental diets in respect to the controls.

In all cases, the panelists clearly associated the experimental diets with slightly less coloured yolks, but in no case was this fact named as an unacceptable attribute. It is also important to point out that for general consumers of omega-3 products, small differences in colour, if detected, would be of secondary importance because colour is a minor important parameter in functional foods. The association between low sensory quality of eggs and dietary fish oils has long been recognized (Holdas and May, 1966): a negative effect on yolk aroma, taste and flavour when laying hens were fed with a fish oil diet were reported recently by different authors (Gonzalez-Esquerra and Leeson, 2000; Schreiner et al., 2004; Carrillo-Domínguez et al., 2005). In some cases, a small percentage of panelists

reported an unpleasant aroma and/or taste in the experimental diets, but this was also the case in the control diet, so these data are not conclusive.

Van Elswysk et al. (1994) found that long-term feeding of menhaden oil produced hepatic lipidosis in hens, Aymond et al. (1994) reported reproductive alterations in young hens fed linseed, and Bean and Lesson (2003) showed that hens fed linseed had a higher incidence of liver haemorrhages. In this work, with the experimental diets studied here, none of the modifications found in the necropsies were symptoms of these diseases.

CONCLUSIONS

Our results show that the diet containing 1.5% Cantabrian blue fish oil and 5% linseed as sources of omega-3 fatty acids allows obtaining commercially acceptable levels of EPA and DHA, avoiding most of the drawbacks reported by many authors. Rheological measurements of egg yolks showed that the enriched eggs maintained their gelation properties, so the experimental diets did not affect structural yolk quality, which is very important in egg processing.

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