Influence of Oral Supplementation with Whey on Egg Quality Characteristics and Fatty Acid Composition

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ABSTRACT— The effects of whey supplementation on egg quality characteristics and fatty acid composition were studied. A total of 240 ISA hens were used and divided into two uniform groups: for the experiment group was used a solution of water 10% and whey 90% while for the control group was used pure water (100 %). Eggs were collected daily after 30 days of feeding and stored at 4 °C during the experiment. Lastly eggs were boiled in water at 100 °C for 10 minutes and then be analyzed (0 days). The advance of the oxidative state was also assessed at 6 days after cooking. The whey supplementation carried out egg yolks with different % of some volatile compounds and similar lipids and fatty acid composition compared to samples coming from the control group. The colour and lipid oxidative stability of egg yolk are significantly different between the two groups. MDA value was lower in the experimental group, while the colour was higher than the control, probably due to an antioxidant effect of some compounds present in the whey. Supplementation of whey in the diet of laying hens has positive effects on the quality of eggs, especially on oxidative stability.

Keywords— Whey; Egg yolks; Hens; Volatile profile; Quality

1. INTRODUCTION

Whey is a co-product of cheese-making and casein manufacture in the dairy industry. After the casein curd separates from the milk, following coagulation of the casein proteins through the action of chymosin (rennet) or mineral/organic acid, the remaining watery and thin liquid is called whey (Zadow, 1994).The economics of processing this liquid fraction is to a great extent dependent on capital investments, which are much more dependent on economies of scale than the cheese making itself (Peters, 2005). Whey has a yellow/green colour, or sometimes even a bluish tinge, but the colour depends on the quality and type of milk used. It can be made from any type of milk, with cows' milk being the most popular in western countries (Yetim et al, 2001). Nowadays, whey is a major co-product of modern cheese and casein industries. On average across the world, volumes of whey are growing at about the same rate as milk volumes (42 % per year; FAO, 2006). This increased quantity of milk is being channelled into the production of larger volumes of cheese, casein/caseinate, and other dairy products, resulting in concomitant increases in the volume of whey. Smithers (2008), on the review, reported a comparison of the proximate analysis of bovine milk and whey. He revealed that about 50 % of the milk solids appear in the whey, together with essentially 100% of the lactose and some 20 % of the protein. The lactose makes up a high proportion (47.5 %) of the total whey solids (Gaenzle et al., 2008), and contributes in large part to dairy whey being considered one of the most polluting food by/co-product streams (biochemical oxygen demand - BOD > 35.000 ppm; chemical oxygen demand - COD > 60.000 ppm) (Siso, 1996).

While the polluting power of whey is well known (Siso, 1996), this dairy stream also represents an excellent source of functional proteins and peptides (Hoffmann, 1961), lipids, vitamins, minerals, and lactose that until relatively recently have been less well recognized. Liquid whey is nutritionally rich, containing a part of the nutrients of the whole milk (93 % water, 5 % lactose, 1 % protein and 1 % minerals) but it is considered an expensive and frustrating disposal problem. Whey constitutes a massive potential food source, but it is not fully utilized by man; this is a tragedy in a world concentrated with a shortage of food (Yetim et al., 2001). Furthermore without any treatment, cheese-whey surplus can be supplied into drinking waters for farm animals; in fact, in addition to high-quality proteins and lactose, whey also provides calcium, phosphorus, sulphur and water-soluble vitamins. For example, egg contains all essential nutrients, such as lipids, amino acids and vitamins, required for a new life; moreover egg composition is suggested to be altered by diet of hens (Cobos et al., 1995). Eggs are considered as one of nature's perfect foods that have been consumed for centuries all over the world, have been collected and eaten for centuries before the domestication of hens and other birds (Fearne and Lavelle, 1996).

They are both an inexpensive food and a food ingredient and are consumed worldwide and accepted in all cultures. Based on the foregoing, we thought to use eggs as functional food. For this reason the object of our study was to value the effect of diet with addition of whey on colour, lipid, fatty acids composition and volatile compounds of egg yolk, as much attention is focused on whey proteins, but there are little knowledge about lipid component and its effect on animal product fed with whey.

2. MATERIALS AND METHODS

2.1. Animal Care and Dietary Treatments

The project has been performed in a small breeding at Mosciano Sant'Angelo, Teramo, Italy). A total of 240 ISA laying hens were randomly distributed into two groups of 120 each hens and were maintained in individual cages for 30 days in a windowed poultry house at a light regimen of 16 h light and 8 h dark. The hens were assigned to two dietary treatments: for the experiment group, in place of water, it was administered whey with an addition of 10% of water, to avoid hen drinking trough occlusion, while for the control group, only pure water was used. Feed was a standard solution for laying hens (SaGeM, Roseto Degli Abruzzi, Teramo Italy) composed by maize, soy extraction meal, calcium carbonate, wheat bran, sunflower extraction meal, soybean vegetable oil, dicalcium phosphate, sodium chloride, sodium bicarbonate and magnesium oxide. It was offered once a day and pure water was provided ad libitum. The chemical composition of the diets are reported in Table 1.

2.2. Sample collection and egg quality

Eggs were collected daily during the experimental time, after 30 days of controlled feeding and stored at 4 °C. One day following collection, all eggs were weighted and 40 of these eggs were boiled in water at 100 °C for 10 min. Some physical parameters of 20 eggs for each group were evaluated and the rest, were stored for 6 days to proceed with the chemical analysis. Egg quality parameters determined were: egg weight, yolk colour, malondialdehyde content (MDA), total fat, fatty acid composition and volatile compounds. Only the parameters related to the oxidative status were carried out up to 6 days of storage.

2.3. Analytical determination

2.3.1.Reagents

All chemicals were reagent grade commercial products and were used without any further purification. TBA: 2-thiobarbituric acid (Sigma-Aldrich, Italy) in acetic acid 90 % (Carlo Erba, Italy); TCA: trichloroacetic acid (Carlo Erba, Italy) in distilled water; BHT: butylated hydroxytoluene (Sigma-Aldrich, Italy) in methanol (Carlo Erba, Italy); standard solution (STD solution): 1,1,3,3-Tetramethoxypropan 99 % (Sigma-Aldrich, Italy) in methanol (Carlo Erba, Italy); KOH: potassium hydroxide (Titolchimica, Roma, Italy); chloroform (Carlo Erba, Italy).

2.3.2. Egg weight and colour

The egg weight was measured using an analytical balance on fresh eggs. The colour of the yolk was evaluated according to the Roche scale on which 14 graded colours are available for comparison with egg yolk samples (Vuilleumier, 1969). Each fan blade (1-14) contains a colour that has been measured objectively and can thus be reproduced in the yolk.

2.3.3. Egg MDA content (TBARS-test)

The susceptibility of egg yolk to oxidation was determined by TBARS-test (Tarladgis et al., 1960) modified, at 0 and 6 days from cooking. The egg yolk was weighted (3-3.5 g), added with BHT, homogenized (Ultra-Turrax T25) with 50 ml of TCA 7% and distilled. Two ml of distilled were mixed with 2 ml of 0.02 M TBA solution prepared in acetic acid 90 %. The mixture was heated in a boiling water bath at 80 °C for 60 min and cooled to room temperature. The absorbance was read at 534 nm with JENWAY 6305 UV/vis Spectrophotometer (Barloworld Scientific, Milano, Italy). Each sample was replicated three times. Standard curve were built through increasing concentrations of STD from 1.25 µg to 20 µg in methanol.

2.3.4. Feed, pasture and eggs total fat and fatty acid composition

Total fat for fatty acid analysis extracted with the method of Folch et al. (1957) was transmethylated into methyl esters (FAME) at room temperature by using KOH 2M in methanol. FAME composition was determined by gas chromatography using Focus GC Thermo Scientific (Milan, Italy) with flame ionisation detection (FID) equipped with a VARIAN column CP-SIL 88 of 100m (Milan, Italy), the carrier gas was hydrogen. Oven temperature programming was as follows: 75 °C held for 6 min; 160 °C at 6 °C/min, held for 13 min; 190 °C at 2.5 °C/min, held for 10 min; 220 °C at 3 °C/min, held for 10 min. Fatty acid identification was carried out with standard mixture and fatty acid values were expressed in percentage.

2.3.5. Egg volatile profile

To study the volatile profile immediately after cooking and at 6 days from cooking, 4 g of ground egg yolk were weighed into a 30 ml vial, which was screw-capped with a laminated Teflon-rubber disk. The used fiber was divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (50/30 μm thickness; Supelco Inc, Bellafonte, PA, USA). Before the SPME fiber was inserted into the vial, the sample was equilibrated for 30 min at the extraction temperature (40 °C). The extraction was carried out for 30 min at 40 °C. Prior to analysis the SPME fiber was preconditioned in the injection port of the gas chromatograph at the temperature and for the time suggested by the manufacturers (Supelco Inc, Bellafonte, PA, USA). Analyses were performed using Thermo Focus GC-FID. Volatiles were separated using a fused silica capillary column (60 m x 0.25 mm i.d., film thickness 0.25 μm, Perkin Elmer, Monza, Italy). The SPME fiber was desorbed and maintained in the injection port at the temperature. Oven temperature programming was as follows: 40 °C held for 10 min; 200 °C at 5 °C, held for 0 min; 250 °C at 20 °C, held for 30 min. *n*-Alkanes were run under the same chromatographic conditions as the samples to calculate the Kovats indices (KI) of detected compounds (Kovats, 1958; Gianelli et al 2002)

2.4. Statistical analysis

All data were statistically analysed by one-way ANOVA using SPSS 9.0 for windows. ANOVA analysis was carried out to determine the main effects (Whey supplementation) and its interaction on eggs yolk parameters. The significant differences were determined using t-Test at the level of $P < 0.05$.

3. RESULTS

The total fat and the fatty acids results obtained for the whey used in the water are shown in Table 2. Whey was rich of palmitic (C16:0) and oleic acid (C18:1 ω9): 37.21 ± 1.23 and 21.03 ± 1.47, respectively.

Figure 1 shows the york colour values (Roche Scale), at the different times after cooking (T2, T4 and T6) both in the control and the experimental group. At T2, the mean values in the experimental group was significantly higher ($P < 0.05$) than in control one, but this difference was not still observed at T4 and T6. The Roche scale values range between 1, for which the colour is very pale (straw-yellow), to 14, for which the colour is orange. The mean result of colour of all the eggs, both experimental and control, was before cooking 12-13, while after cooking the colour decreased, probably by cause of oxidation and/or denaturation of carotenoid compounds that yield the classic colour to york. Higher levels observed in the eggs of the experimental group are very likely due to a greater protection power through oxidative processes activated by the cooking temperature, probably thanks to the antioxidant effect of some compounds devolved from whey upon eggs. This hypothesis is confirmed by the MDA results (mg/Kg) obtained in the TBARS test and reported in figure 2.

At 2 days from cooking the malonaldehyde content was not different between the control and the experimental group. During storage, this compound remained constant in the control group while decreased in egg yolk of the experimental group (1.07 ± 0.10 vs 0.57 ± 0.09 ppm at 4 days, and 1.01 ± 0.09 vs 0.76 ± 0.08 at 6 days in the control and experimental group, respectively), probably due to the antioxidant compounds presented in the whey.

The total fat and the fatty acids of samples are shown in Table 3.

Egg yolks coming from hens of the experimental group did not show different total fat percentage and fatty acid composition, when compared to samples coming from hens without the whey supplementation.

The volatile compounds content of samples was shown in Table 4. A total of 5 volatile compounds were tentatively identified in this study. Identified compounds belonged to the following chemical groups: alcohols (1 compound), aldehydes (3 compound), ketones (1 compound). Aldehydes (hexanal, decanal and nonanal) were the most significant flavour compounds both in the control and in the experimental group, at 2 and 6 days from cooking. Content of hexanal and decanal was higher in the control group than the experimental one, at 6 days (6.15 ± 4.50 vs 4.55 ± 3.49 % and 21.65 ± 1.11 vs 13.75 ± 4.78 % for hexanal and decanal, in the control and experimental group, respectively). However, these last differences were not significant, while at 0 days, hexanal and decanal were significantly lower ($P < 0.05$) in the control group than in the experimental one (3.29 ± 0.58 vs 3.47 ± 2.09 %, and 21.01 ± 2.69 vs 22.37 ± 3.57 % for hexanal and decanal, in the control and experimental group, respectively). Nonanal, the primary oxidation products of oleic acid, was present in the major content in the experimental group at 0 days (40.22 ± 17.86 vs 55.53 ± 8.40 % in the control and experimental group, respectively) from cooking. Significant differences ($P < 0.01$) were also reported for nonanal at 0 and 6 days from cooking for the experimental group (55.53 ± 8.40 and 30.17 ± 7.90 %, in the experimental group at 0 and 6 days respectively).

1-hexen-3-olo did not show significant differences ($P > 0.05$). 2-Butanone, an aliphatic ketone forming by autoxidation of lipids, was present in higher contents in the control group at 0 days and at 6 days from cooking, with a statistically significant ($P < 0.01$) difference (6.42 ± 1.06 vs 2.68 ± 1.46 %,(in the control and experimental group respectively), probably due to the oxidation effect of whey compound.

4. DISCUSSION

Our results show that the whey supplemented diet positively influences the quality of cooked eggs. The colour and the lipid content were not different between the two groups, even if in the experimental group colour was always higher than in the control, probably due to an antioxidant effect of some compounds present in the whey (Jackson et al., 1978). The same could be said for the value of lipid oxidative status. Molecules that act as protective agents against oxidation could be lactoferrin and lactoferricin, two minor protein in whey, that function as antioxidants via their iron binding capacity. Lactoferrin is only 8-30 % saturated in its native state, a condition that enables chelation of iron and subsequent inhibition of bacterial growth or oxidative reactions. Whey might also enhance antioxidant capacity by contributing cysteine rich proteins which are pivotal in the synthesis of glutathione, a major intracellular antioxidant (Ha and Zemel, 2003).

Egg yolks from hens of the experimental group did not produce statistically significant differences about total fat percentage and fatty acid composition, when compared with samples coming from hens without the whey supplementation (control group), although the values obtained are slightly higher (table 3).

Lipids, proteins, and carbohydrates, the major structural components of living cells, are also the major source of flavour in foods. Generally, the negative qualities of food flavour are associated more closely with lipids than with proteins and carbohydrates. Strecker degradation of methionine, phenylalanine and proline, as well as autoxidation of phospholipid-bound linoleic and arachidonic acids are proposed as the major factors for egg yolk flavour formation. According to literature search (Cherian et al., 2002), results of volatile profile of samples showed remarkable variability in the same group. 1-hexen-3-olo did not show any difference between the groups and any significant increases during storage.

Contents of hexanal and decanal were higher in the control group than the experimental one, at 6 days; these differences were not significant, while at 0 day, hexanal and decanal levels were lower in the control group than in the experimental one. The content of these compounds is suggested to be a good indicator of oxidation (Ahn et al., 1998). Indeed, hexanal is the primary oxidation product of linoleic acid (Meynier et al., 1999). The autoxidation of linoleic acid generates 13-hydroperoxide of linoleic acid. Cleavage of 13-hydroperoxide will lead to hexanal. Whereas, the amount of linoleic acid in the egg yolk, between the two groups, was similar (14.56 ± 2.98 % vs 15.28 ± 2.50 %, in control and experimental group, respectively). We can assume a protective effect of whey component on egg yolk, given the lower concentration of hexanal obtained in the experimental group, when compared to the control one, during storage.

Nonanal, the primary oxidation products of oleic acid, was present in greater content in the experimental group, at 0 days from cooking. Significant differences ($P < 0.05$) were observed for nonanal, at 0 and 6 days from cooking, for the experimental group, decreasing during storage (55.53 ± 8.40 % and 30.17 ± 7.90 %, at 0 and 6 days respectively); probably nonanal undergo further chemical degradations taking into consideration anthropogenic inputs (Volkamer et al., 2006). Decanal and nonanal are the primary oxidation products of oleic acid. This chemical group can be produced by scission of the lipid molecules on either side of the radical. The products formed by these scission reactions depend on the fatty acids present, the hydroperoxide isomers formed, and the stability of the decomposition products. Indeed, from the oxidation of these two aldehydes, carboxylic acids may have originated (Volkamer et al., 2006).

5. CONCLUSION

The susceptibility of animal lipid to oxidation depends on a number of additional factors, including the concentration of prooxidants and antioxidants. This study was carried out to value the effect of dietary whey supplementation on oxidative stability in cooked egg yolk. The results obtained show that the supplementation of whey in the diet of laying hens has positive effects on the quality of the eggs produced, especially on oxidative stability. Further studies are needed to evaluate which molecules, presented in whey, are involved for this purpose and how these molecules act on the free radical chain, with particular attention to the study of volatile components.

Table 1. Formulation and chemical composition of standard diet.

Diet			
Proximate composition (% fresh matter)		fatty acid	%
Crude protein	16	C12:0	0.03
Ether extract	3	C14:0	0.24
Cellulolose	3.8	C15:0	0.05
Ash	13.50	C16:0	15.37
Lysine	0.85	C18	3.66
Methionine	0.38	SFA	15.69
Calcium	4.10	C16:1	0.27
Phosphorus	0.48	C18:1 ω9	23.78
Sodium	0.14	C18:1 ω7	0.63
Additives per kg		MUFA	24.68
Vitamin A UI	9000	C18:2 ω6	50.15
Vitamin D3 UI	3000	C18:3 ω3	3.22
Vitamin E mg	24	PUFA	53.37
Trace elements tot[a] mg	382.69	Others	2.60

[a]Added per kg: ferrous carbonate 114.89 mg, manganous oxide 67.73 g, manganous sulphate monohydrate 161.70 mg, zinc oxide 111.60 mg, sodium selenite 0.66 mg, cupric sulfate pentahydrate 23.58 mg, potassium iodide 2.36 mg, potassium basic , carbonatemonohydrate 0.27mg.

Table 2. Total fat (%) and fatty acids composition (%) of the whey.

	WHEY
Total fat (%)	0.89 ± 0.06
C4:0 (Butyric acid)	2.09 ± 0.37
C6:0 (Caproic acid)	1.65 ± 0.29
C8:0 (Caprylic acid)	1.11 ± 0.09
C10:0 (Capric acid)	2.45 ± 0.43
C12:0 (Lauric acid)	3.32 ± 0.39
C14:0 (Myristic acid)	11.89 ± 0.03
C16:0 (Palmitic acid)	37.21 ± 1.23
C16:1 (Palmitoleic acid)	1.15 ± 0.04
C18:0 (Stearic acid)	8.90 ± 0.80
C18:1ω9 (Oleic acid)	21.03 ± 1.47
C18:1ω7 (Vaccenic acid)	0.33 ± 0.05
C18:2ω6 (Linoleic acid)	1.67 ± 0.14

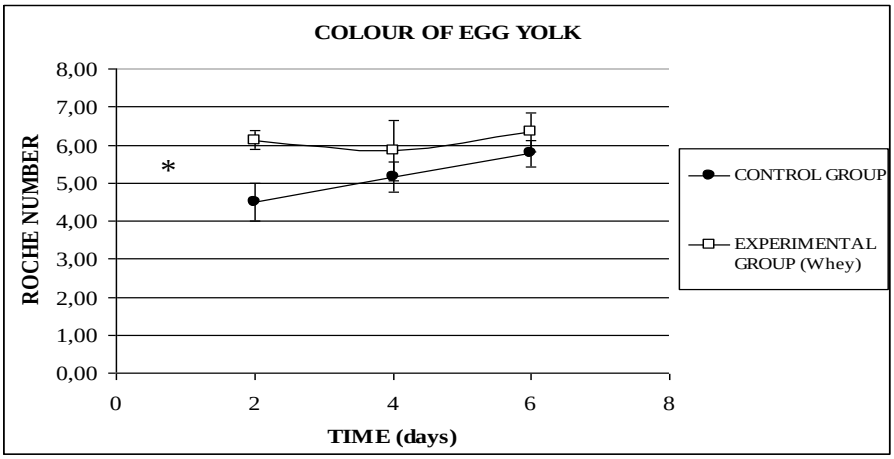
Table 3. Total fat (%) and fatty acids (%) in egg yolks of the control and the experimental groups.

	CONTROL GROUP	EXPERIMENTAL GROUP (Whey)
Total fat (%)	20.39 ± 3.08	21.80 ± 2.15
C14:0	0.22 ± 0.07	0.26 ± 0.04
C16:0	23.99 ± 2.41	25.24 ± 1.26
C16:1	1.11 ± 0.26	1.65 ± 0.38
C18:0	7.09 ± 0.74	7.04 ± 0.61
C18:1ω9	45.13 ± 4.44	45.89 ± 2.47
C18:1ω7	1.25 ± 0.20	1.26 ± 0.17
C18:2ω6	14.56 ± 2.98	15.28 ± 2.50

Table 4. Volatile compounds (mean. min. and max area %) in egg yolks of the control and the experimental groups at 2 and 6 days from cooking.

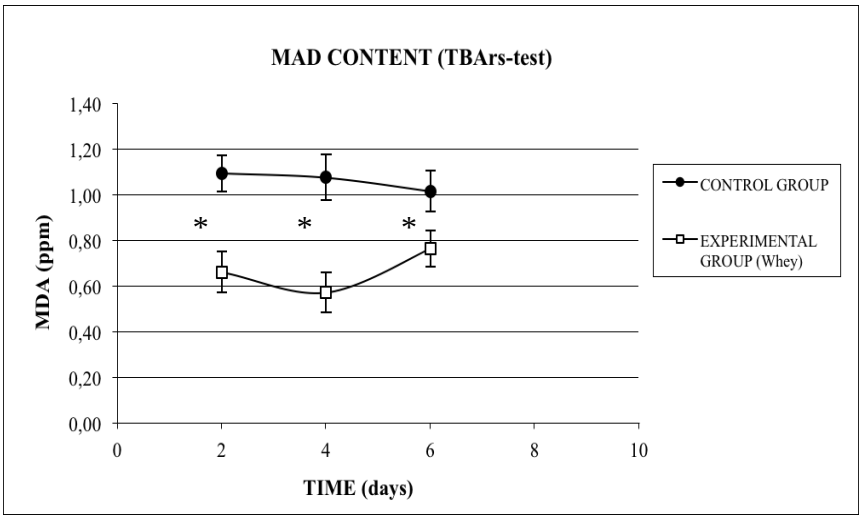
	0 days		6 days	
	Control Group	Whey Group	Control Group	Whey Group
Hexanal	3.29 ± 0.58 ^{a}	3.47 ± 2.09 ^{b}	6.15 ± 4.50 ^{a}	4.55 ± 3.49 ^{a}
Decanal	21.01 ± 2.96 ^{a}	22.37 ± 3.57 ^{b}	21.65 ± 1.11 ^{a}	13.75 ± 4.78 ^{a}
1-Hexen-3-olo	5.39 ± 4.37 ^{a}	3.03 ± 2.17 ^{a}	3.91 ± 0.69 ^{a}	5.06 ± 1.82 ^{a}
Nonanal	40.22 ± 17.86 ^{A}	55.53 ± 8.40 ^{B}	40.85 ± 14.49 ^{A}	30.17 ± 7.90 ^{B}
2-Butanone	11.59 ± 10.07 ^{a}	3.73 ± 1.93 ^{b}	6.42 ± 1.06 ^{A}	2.68 ± 1.46 ^{B}

n = 20 per group, a. b: P < 0.05 on the same row between groups, A. B: P < 0.01 on the same row between days.



*: P<0.05

Figure 1. Colour (Roche Scale) of egg yolk of the control and the experimental groups.



*: P<0.05

Figure 2. MDA content (ppm) in egg yolk of the control and the experimental groups.

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