NUTRITIONAL QUALITY OF POULTRY EGGS TREATED WITH A MIXTURE OF CEREALS AND OLEAGINOUS CEREALS

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Abstract

The role of essential fatty acids in human and animal health, growth, and development is a topic of continued interes. A 6-wk study was performed on 80 Tetra SL (56 weeks) laying hens, to determine the nutritional quality of the poultry eggs treated with a a mixture of cereal and oleaginous grains (corn, flax oil, barley and peas - VMLO). The laying hens, housed in an experimental hall with controlled environment (16 light regimen, 20.01±1.44°C temperature; 65±6.38% humidity) were assigned to 2 groups (C and E). The poultry received a conventional feed with the same basic structure (2750 kcal/kg metabolizable energy and 16.5% crude protein). Compared to the C diet (2750 kcal/kg metabolizable energy and 16.5% crude protein) characterized by 0.83 g of α-linolenic acid (C18:3n3), the E diet included 15% VMLO, which resulted in an increase in α-linolenic acid concentration in the diet up to 13.80 g /100g total fatty acids. At the end of the study, 18 eggs/group were collected to determine the PUFA and cholesterol concentrations in yolk. The results obtained for E group eggs, whose concentration in PUFA Ω3 (5.07±0.546g % total fatty acids) increased significantly ($P<0.05$) compared to C (1.22±0.139g % total fatty acids), indicated significantly lower values concerning the cholesterol concentration (1.661±0.056 g% dry egg yolk) compared to C (1.865±0.178 g% dry egg yolk). Use of VMLO in poultry feed has positively influenced egg quality without affecting production performance.

Key words: layers, eggs, fatty acids, cholesterol

INTRODUCTION

Today consumers are becoming more concerned about quality and food safety of conventional foods [1,2]. Polyunsaturated fatty acids (PUFA) is important for humman health [3,4] because of the omega-3 (n−3) family have demonstrated a wide range of health benefits [5,6]. In this moment is increasing interest in developing animal foods enriched with n−3 fatty acids [7,8]. So, in this context, chicken egg is a highly targeted and well-researched food item due to its fat content (30–32%). Among the different plant-based sources, flaxseed, owing to its high fat (>38%) and ALA (>50%) contents along with other nutritional properties (e.g., metabolizable energy and protein), is the most common feed ingredient explored for egg n−3 fatty acid enrichment. The fatty acid composition of eggs can be changed by modifying the fatty acid composition of the hens' diet by using flaxseed, chia seed, fish oil, or marine algae in the hens' diet [9,10,11,12,13].

In this context, the objectives of the current study were to determinate the nutritional quality of the poultry eggs treated with a a mixture of cereal and oleaginous grains (corn, flax oil, barley and peas termed vegetable mixture with linseed oil (VMLO) on egg quality, lipid and fatty acid composition, and egg cholesterol contration, during a 6 weeks period of feeding trial.

MATERIAL AND METHODS

The evaluation of the effectiveness of the experimental diets concerning the nutritional quality of laying hen eggs fed a mixed of

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cereals and oilseeds (corn, flaxseed oil, barley and peas) was carried out in an experimental study conducted over 6 weeks on 40 Tetra breeds SL (56 weeks), individually weighted and randomized in two experimental lots (C and E).

The experiment was conducted in accordance with the Romanian legislation (Law 206/2004, ordinance 28/31.08.2011, law 43/11.04.2014, Directive 2010/63/EU according to an experimental protocol approved by the IBNA Ethics Commission regarding the progress of the experiments. The laying hens, housed in cages (4 hens/cage), structured on 3 levels, benefited of the same controlled microclimate conditions (temperature: 20.01±1.44^{0}C, humidity 65± 6.38%, ventilation head/bird: 1.38 ± 0.24 %) and a light program of 16h /24h. The layers had free access to the feed and water.

To elaborate the combined feed formula of this experiment we considered: the objective of the experiment, the species, the hybrid, the age and the nutritional requirements of the hybrid Tetra SL [14]. The basic structure of feeding formula was the same for the two groups (Table 1). The difference between the control group (C) and the experimental group (E) was given by the inclusion in the experimental diet of a mixed cereals and oleaginous, consisting of: corn (30%), barley (30%), peas (20%), linseed oil (20%), linseed oil. This mixture was named in the present paper as (VMLO).

At the end of the study (week 6), we collected randomly 18 eggs/group. After the external and internal quality parameters of the eggs were measured, we formed 6 yolk samples/group (3 eggs/sample) and assayed them for polyunsaturated fatty acids (PUFA) and cholesterol concentration in yolk.

The methods from Regulation (CE) 152/2009 (Methods of sampling and analysis for the official control of feed) have been used: the gravimetric method for dry matter (DM); the Kjeldahl method for crude protein (CP); extraction in organic solvents for ether extractives (EE). The fatty acids and cholesterol concentration has been determined using the methods described by Panaite et al., (2016).

Table 1 Diet formulation and estimated chemical composition of experimental diet

Diet composition	Control	VMLO
Maize, %	57.16	49.3
Soybean meal, %	21.24	18.4
Sunflower meal	7	4.96
Vegetable mixture with linseed oil, %	-	15
Vegetable (soybean) oil, %	2.02	-
Methionine, %	0.1	0.06
Coline, %	0.05	0.05
Calcium carbonate, %	9.91	9.93
Calcium phosphate, %	1.12	0.9
Sodium chloride (Salt), %	0.35	0.35
Vitamin-mineral premix*, %	1	1
Mycotoxin inhibitor, %	0.05	0.05
Total raw materials	100	100
Chemical composition (calculated)		
Metabolizable energy, kcal/kg	*2750.00*	*2750.00*
Crude protein, %	16.5	16.5
Crude fat, %	3.74	3.79
Lysine, %	0.837	0.813
Methionine, %	0.331	0.311
Chemical composition (analysed)		
Linolenic α (C18:3n3)	1.19	13.80
PUFA (%), of which	57.77	58.39
Σ Ω:3	1.57	14.42
Σ Ω:6	56.20	43.97
Σ Ω:6/ Ω:3	35.87	3.05
Coliform bacteria (E. Coli), Count/g DM	81,2	50
Salmonella	negative	negative

Where: *1 kg premix contains: (1350000 UI/kg vit.A; 300000 UI/kg vit.D3; 2700 UI/kg vit.E; 200 mg/kg Vit.K; 200 mg/kg Vit.B1; 480 mg/kg Vit.B2; 1485 mg/kg A pantothenic acid; 2700 mg/kg Acid nicotinic; 300 mg/kg vitamin B6; 4 mg/kg vitamin B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2500 mg/kg vitamin C; 7190 mg/kg manganese; 6000 mg/kg iron; 600 mg/kg copper; 6000 mg/kg zinc; 50 mg/kg cobalt; 114 mg/kg iodine; 18 mg/kg selenium

Bacteriological analyzes of coliform bacteria (*Escherichia coli*) and salmonella (*Salmonella sp*) were determined using the Colilert-18 probability number method and Quanty Tray/2000 system (SR EN ISO 9308-2/2014) for *Escherichia coli*, EPA 1682 method for *Salmonella* spp. determination, respectively (Method for detecting and quantifying Salmonella spp. in Biosolids, 2005).

The potential health hazard of the compound feeds samples for the layers was evaluated by comparison with the legal limits regarding the quality and salubrity parameters for the manufacture, import, quality parameters for compound feeds and feed additives, as per Order 358/2003 approving the Norms regarding the quality and salubrity inspection, selling and using simple concentrate feeds, compound feeds, feed additives, premixes, energy substances, minerals and special feeds.

Statistically analysis: The analytical data were compared by variance analysis (ANOVA) using STATVIEW for Windows (SAS, version 6.0). The difference between the means was considered significant at $P<0.05$. The results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSIONS

The vegetable product consisting of a cereal and oleaginous mixture (VMLO) was chemically analyzed to determine its nutritional composition to optimize the nutritional diet (Table 1). The primary chemical composition, presented in Table 2, highlights a high protein content (19.75%) and cellulose (10%). Also, the fatty acid profile shows that VMLO is a good source of PUFA fatty acids (Figure 1), especially with respect to α-linolenic acid content (C18: 3n6). This is due to the inclusion of flaxseed oil (20%) in the plant mixture.

By including VMLO in the diet of the experimental group, the dietary α-linolenic acid concentration increased to 13.80 g/100 g total fatty acids compared to the conventional C diet, characterized by 0.83 g α-linolenic acid (C18: 3n3) table no.1. Also, from the data presented in Table 2, VMLO is also a valuable source of minerals, characterized by a high content of calcium in particular.

Table 2 Feeding characterization of the vegetable mixture with linseed oil

Specification	*Vegetable mixture with linseed oil (VMLO)*
Primary chemical composition, %	
Dry matter	88.41
Crude protein	19.75
Ether extractives	13.32
Fibre	10.00
Ash	5.12
Fatty acid profile, g /100g total fatty acids	
Lauric acid (C12:0)	0.04
Miristic acid (C14:0)	0.14
Palmitic acid (C16:0)	8.41
Palmitoleic acid (C16:1)	0.27
Stearic acid (C18:0)	3.77
Oleic acid (C18:1)	22.79
Linoleic acid (C18:2)	35.86
Arachidic acid (C20:0)	0.07
Linolenic acid (C18:3n6)	0.13
Alpha Linolenic acid (C18:3n3)	26.79
Eicosadienoic acid (C20:2n6)	0.26
Eicosatrienoic acid (C20:3n6)	0.72
Erucic acid (C22:1n9)	0.07
Eicosatrienoic acid (C20:3n3)	0.10
Arachidonic acid (C20:4n6)	0.48
Other fatty acids	0.11
Mineral profile, mg/kg DM	
Calcium	4200
Phosphorus	3420
Iron	183
Manganese	22.8
Copper	19.3
Zinc	20.5
Lead	0.45
Nickel	702
Selenium	< 0.30
Cadmium	0.03
Chromium	2

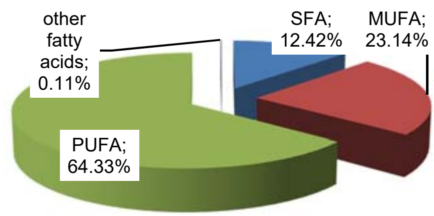

Fig. 1 Classes of fatty acids

Regarding the content of the main nutrients of the yolk (table 3) it was found that the dry E group eggs. Instead, there were no differences registered between the 2 groups with regard to fat and protein percentages. Also, the dry matter of the C group egg white (Table 3), was significantly (P≤0.05) higher than in E group, the same trend being recorded for protein content.

Table 3 Chemical composition of the eggs collected at the end of the experiments (average values/group)

Specification		Control	VMLO
Yolk, %	DM	96.34±0.43^b	95.33±0.05^a
	CP	31.66±0.18	32.26±0.20
	CF	52.27±0.31	52.28±0.10
	Ash	3.38±0.02^b	3.62±0.07^a
White, %	DM	94.19±0.38^b	90.46±0.33^a
	CP	81.59±0.32^b	80.35±0.20^b
	CF	0.04±0.01^b	0.06±0.002^a
	Ash	4.76±0.16	4.82±0.09
Shell, %	Ash	54.80±0.42	53.88±0.24
	Ca	33.62±0.09	33.54±0.03

Where: a,b= significant differences (P≤0.05) compared to C and E

Analyzing the values of the physical quality parameters of the egg (Table 4) and its components, follows were observed: the weight of the egg recorded close values to an average of 63.06 g; the weight of the whites recorded values close to an average of 38.60 g; the weight of the yolk recorded values between 15-17 g, with statistically significant differences between the two groups; and the egg shell recorded close values, on average 8.45 g; the shell thickness recorded values between 0.3-0.4 mm, with statistically significant differences between the two groups; the pH of the whites recorded values between 8-9, with statistically significant differences between the two groups; the color of the yolk recorded values between 4-5.5, with statistically significant differences between the two groups; Haugh unit recorded close values to an average of 68.04. Regarding the degree of freshness, it was found that the highest percentage of 33.33% for AA class was recorded in eggs collected from E group, 66.67% for class A was recorded for eggs collected from C group and 11.11 % for class B was also recorded in eggs collected from C group.

Table 4 Quality physical parameters of the egg (average values/group)

Specification	Control	VMLO
Egg weight (g), of which:	63.22±0.292	62.90±0.302
white (g)	38.30±0.355	38.90±0.42
yolk (g);	16.41±0.208^b	15.62±0.272^a
eggshell (g)	8.51±0.146	8.38±0.19
eggshell thickness (mm)	0.35±0.01^b	0.33±0.01^a
eggshell breaking strength (kgF)	4.00±0.236	3.74±0.206
pH - white	9.00±0.046^b	8.76±0.061^a
pH - yolk	6.20±0.02	6.24±0.01
Yolk colour	5.06±0.151^b	4.56±0.121^a
HU	67.23±1.67	68.85±1.44
Egg freshness,		
AA	22.22	33.33
A	66.67	61.11
B	11.11	5.56
total	100 %	100 %

Where: a,b= significant differences (P≤0.05) compared to C and E

The inclusion of flaxseed oil (20%) in the VMLO structure resulted in a significant increase (P≤0.05) of α-linolenic acid in egg yolk fat along with a reduction in saturated fatty acids (Table 5). Thus, palmitic acid (C16: 0), stearic acid (C18: 0), oleic acid (C18: 1n-9) and linoleic acid (C18: 2n-6) were found in the largest amount of total fatty acids determined in egg yolks. Similar findings have been presented in other studies [2,15,16]. Both, palmitic acid (C16: 0) and linoleic acid (C18: 2n6) decreased significantly (P≤0.05) in the experimental group compared to C group. Thus, compared to C group, the decreasing concentration of palmitic acid for E group was 4.58% lower, and 8.12% for linoleic acid, respectively. There were no differences in the amount of oleic acid (C18:1n-9).

The concentration of α-linolenic acid (C18: 3n3) in the egg yolk of the E group was significantly (P≤0.05) higher compared to the C group, recording an increase of 6.94 times greater than C group. The results are consistent with those obtained by [17] or [18]. Also, docosahexaenoic acid (C22: 6n3) recorded an increase of 348% in E group vs. C group, that is 4.48 times higher compared to C. These results are comparable to those obtained by [4] which obtained an increase in docosahexaenoic acid (DHA) and α-linolenic acid (AAA) in egg yolk by using seed and grain in laying hens. In other studies, the use of fish oil has increased the content of EPA, DHA and total omega 3 in eggs [16,19,20,21,22].

Analyzing the classes of polyunsaturated fatty acids in egg yolk (Table 5), it was observed that by using VMLO in laying hens diet, the concentrations of SFA (saturated fatty acids) and MUFA (monounsaturated fatty acids) determined in egg yolks of E group did not registered significant differences compared to lot C. However, the content of polyunsaturated fatty acids (PUFAs) from dry egg yolk was found in the largest quantity, in group E with a significant increase ($P \le 0.05$) of polyunsaturated fatty acids compared to group C by increasing concentrations of omega-3 polyunsaturated fatty acids. Similar results have been obtained by [23]. Some researchers, like [24] believe that SFAs and MUFAs in egg yolk are also hardly affected by the feed in contrast to PUFAs

The ratio between omega 6 fatty acids (Linoleic, γ-linolenic, Eicosadienoic, Eicosatrienoic, Arachidonic, Docosatetraenoic) and omega 3 fatty acids (α-linolenic, Eicosatrienoic, Docosapentaenoic, Docosahexaenoic) at the group E registered a significant decrease ($P \le 0.05$) compared to C group.

Both n-3 and n-6 PUFAs have many important functions in the human body, e.g. building and repairing cells and regulating blood pressure, functioning kidneys and our immune system [25]. The ratio between n-6 and n-3 fatty acids have also become a factor for preventing many chronic diseases [16]. High n-6:n-3 fatty acid ratio have shown to be related to many chronic diseases [16,23,25,26]. Therefore the increasing concentration of n-3 PUFAs lowers the n-6:n-3 fatty acid ratio. At the same time, it has been scientifically proven that by using PUFA fatty acids enriched diets, cholesterol levels in the egg are reduced.

Table 5 Fatty acids profile from egg (average values/ egg; g/100g total fatty acids)

Fatty acids	Control	VMLO
Miristic (C14:0)	0.319±0.04^b	0.229±0.02^a
Miristoleic (C14:1)	0.076±0.01^b	0.048±0.01^a
Pentadecanoic (C15:0)	0.066±0,01	0.077±0.01
Pentadecenoic (C15:1)	0.113±0.04	0.116±0.03
Palmitic (C16:0)	25.193±0.57^b	24.040±0.33^a
Palmitoleic (C16:1)	3.129±0.50	2.869±0.33
Heptadecanoic (C17:0)	0.112±0.04^b	0.155±0.02^a
Heptadecenoic (C17:1)	0.083±0.03	0.107±0.03
Stearic (C18:0)	10.704±1.45	11.307±0,76
Oleic (C18:1)	33.993±2.74	34.222±1.84
Linoleic (C18:2n6)	18.373±1.06^b	16.881±0.68^a
Linolenic γ (C18:3n6)	0.126±0.02^b	0.083±0.01^a
Linolenic α (C18:3n3)	0.205±0.03^b	1.422±0.18^a
Eicosadienoic (C20:2n6)	0.127±0.06	0.141±0.02
Eicosatrienoic (C20:3n6)	0.245±0.06	0.216±0.07
Erucic (C22:1n9)	0.109±0.04^b	0.070±0.02^a
Eicosatrienoic (C20:3n3)	0.230±0.05	0.223±0.04
Arachidonic (C20:4n6)	4.087±0.58	3.940±1.08
Nervonic (C24:1n9)	0.369±0.05^b	0.212±0.06^a
Docosatetraenoic (C22:4n6)	1.617±0.29^b	0.268±0.05^a
Docosapentaenoic (C22:5n3)	0.075±0.03^b	0.266±0.09^a
Docosahexaenoic (C22:6n3)	0.705±0.12^b	3.159±0.46^a
Total	100	100
Fatty acids classes from yolk (g% total fatty acids)		
SFA	36.39±1.81	35.81±0.61
MUFA	37.78±2.89	37.58±1.90
UFA	25.79±1.47	26.60±1.53
PUFA, of which:	63.57±0.13^b	64.18±0.50^a
Ω6	24.58±1.58^b	21.53±0.34^a
Ω3	1.22±1.38^b	5.07±1.14^a
Ω6: Ω3	20.36±1.58^b	4.28±0.34^a

Where:a,b= significant differences ($P \le 0.05$) compared to C and E

Eggs have traditionally been associated with numerous unfavorable factors for health because of their high content of cholesterol [27, 28] but recent research have shown that healthy individuals consuming up to three eggs each day have an overall beneficial effect on biomarkers associated with cardiovascular disease risk [29]. The results obtained in the present study have shown that by using VMLO, cholesterol concentration in the yolk has decreased significantly. From data presented in fig.2, the results obtained for E group eggs, whose concentration in PUFA Ω3 (5.07±0.546g % total fatty acids) increased significantly ($P<0.05$) compared to C (1.22±0.139g % total fatty acids), indicated significantly lower values concerning the cholesterol concentration (1.661±0.056 g% dry egg yolk) compared to C (1.865±0.178 g% dry egg yolk). The results are consistent with those obtained by [30]. Other studies have also affirmed that the dietary fat source does of the laying hen diet don't influence cholesterol content in the egg yolk or in the total egg [31].

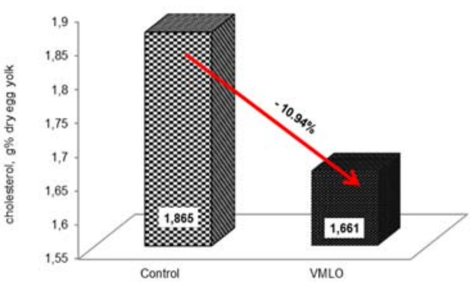

Fig. 2 Cholesterol concentration in yolk eggs

CONCLUSIONS

Use of VMLO in poultry feed has positively influenced egg quality both in the basic chemical composition of the eggs and in the fatty acids profile and cholesterol without affecting production performance. The high protein, energy and omega 3 polyunsaturated fatty acid content of the cereal and oleaginous mixture, situates VMLO as an appropriate source of vegetable protein and omega 3 fatty acids in poultry feed with beneficial effects on the nutritional egg quality by increasing the content of PUFA fatty acids, essential to human health, decreasing the omega 6: omega 3 ratio and a significant decrease of the cholesterol content in egg yolk.

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