

## EFFECT OF USING NUTS MEAL IN DIET FORMULATIONS ON LAYER PERFORMANCE AND EGG QUALITY

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### Abstract

The nuts meal obtained from oil extraction is among the plant by-products of interest as feed ingredients, due to their high feeding value. The chemical determinations have shown that this batch of nut meal had a high protein content (29.13%), with 11.49 MJ/kg metabolisable energy, also being a natural source of antioxidants (112.536 mM Trolox/g antioxidant capacity). From the total content of crude fat (15.15%) of the nut meal, 70.79% are polyunsaturated fatty acids. An experiment was conducted for 4 weeks on 80 Lohmann Brown layers (75 weeks), assigned to two groups (C and E) according to their body weight. The birds were housed in an experimental hall with controlled micro climate and 16 h light regimen. Feed intake, feed conversion ratio, laying percentage and egg weight were monitored throughout the experiment. At the end of the trial, 18 eggs were collected from each group, and egg quality was determined. The laying percentage was significantly ( $p \leq 0.05$ ) higher in group E (83.66±8.36%), (nut meal treatment), than in group C (77.58±9.25%). Omega 3 fatty acids concentration in the yolk of the eggs from group E (2.44 g/100g total fatty acids) was significantly ( $p \leq 0.05$ ) higher than in group C (1.48 g/100g total fatty acids). The antioxidant capacity in the yolk was higher ( $p \leq 0.05$ ) in group E (88.404 mM Trolox/g) than in group C (79.171 mM Trolox/g), while the pH was higher ( $p \leq 0.05$ ) in group C (6.46) than in group E (6.31).

**Key words:** layers, nut meal, performance, eggs, quality

### INTRODUCTION

The by-products of the food industry can be used in animal feeding on condition that they have feeding value. The nut meal is among the non-conventional by-products used lately in poultry feeding. The nuts differ in their feeding properties depending on genetic and soil-climate conditions [10; 12]. They have high energy values (234 – 268 kJ/g product), while the amount of fat varies from 45 to 75% [2; 13]. The total protein content is rather high, which makes them a good protein source.

The nut meal has been used for the first time in France, in the 19<sup>th</sup> century to feed goose and geese and turkeys. The nutrient levels of the nut meal (protein, lipids and fibre) are extremely variable because of the extraction process. A study of Brunnschwig [4] on the process of nut meal production

shows that the cold pressing produces a high concentration of fat (20%) and a medium concentration of protein (32%), while the hot pressing process produces a yellow-brown product with 10-12% oil and 37% protein. McGregor [8] have shown that the nut meal obtained using an American procedure has little proteins (13-17%) and lipids (6-10%), but a lot of fibre (27-33%). The nut meal is a valuable source of polyphenols and of other components with antioxidant activity.

*Purpose of the paper:* with the purpose to enhance the feeding value of the hen eggs using non-conventional by-products, we conducted a feeding trial on layers treated with nut meal

### MATERIAL AND METHOD

The nut meal has been tested within a 4-week trial conducted on 80 Lohmann Brown layers aged 75 weeks. The hens were weighed individually and assigned to two groups (40 hens/group) depending on their body weight. The average live weight of group C was 1719.3±185.88 g, while the

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average live weight of group E was  $1720.00 \pm 169.19$ g. The layers were housed in modern 3-tier cages (2 hens per cage) which allow the daily recording of the ingesta and leftovers. The environmental conditions in the house was according to Lohmann Brown maintenance manual (temperature:  $23.12 \pm 2.03^\circ\text{C}$  and humidity:  $44.83 \pm 6.20\%$ ). The light regimen (16h daily) was according to the prescriptions for the particular category of poultry. The layers had free access to the feed and water. The laying hens received diet formulations according to the particular species, hybrid and feeding requirements. The basal diet formulation (Table 1) was similar for both groups. Diet E differed from diet C by the inclusion of 8.85% nut meal. The diet formulation for group C accounted for 90% of the diet formulation for group E.

Table 1 Diet formulations

Item	C	E
	%	
Corn	30.14	27.10
Wheat	20.00	18.00
Barley bran	10.00	9.00
Nut meal	-	8.85
Soybean meal	14.70	13.23
Sunflower meal	10.00	9.00
Oil	3.38	3.04
Lysine	0.09	0.09
DL - methionine	0.13	0.13
Calcium carbonate	9.09	9.09
Monocalcium phosphate	1.15	1.15
Salt	0.32	0.32
Premix A6	1.00	1.00
Total	100.00	100.00
Chemical composition		
ME, kcal/kg	2749,12	2718,62
CP, %	16,53	16,47
EE, %	4,84	5,67
Fibre, %	4,83	6,64
Fatty acids $\omega$ :3, g%fat	1,51	2,64
Lysine, g/100 g DM	0,812	0,839
Methionine	0,360	0,391
Polyphenols (mg EAG/g)	1,69	2,28
Antioxidant capacity TEAC (mM ET/g)	7,64	9,48

Zoofort A6 = (1350000 UI/kg vitamin A; 300000 UI/kg vit.D3; 2700 UI/kg vitamin E; 200 mg/kg vitamin K; 200 mg/kg vitamin B1; 480 mg/kg vitamin B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 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.

Throughout the trial we monitored layer performance (average daily feed intake, feed conversion ratio, laying percentage and egg weight) and egg quality parameters.

Samples of nut meal, feed ingredients, finished compound feeds and eggs were collected and analysed chemically. The basic chemical composition determinations were done according to Regulation (CE) 152/ 2009 (sampling and analytical methods for the official inspection of feeds).

In the end of the trial we collected 18 eggs/group. Measurements were performed on egg weight, albumen, yolk and eggshell weight (Kerm scales, 0.001precision); colour intensity (Egg Analyzer TM); egg freshness using the Haugh unit and the Egg Analyzer TM); eggshell thickness (Egg Shell Thicknes Gauge); eggshell breaking strength (Egg Force Reader). Six yolk samples (3 eggs/sample) were formed from the collected eggs (18/group) and assayed for the fatty acids content and antioxidant capacity.

Fatty acids were determined by gas chromatography, whose working principle is the transformation of the fatty acids from the sample into methyl esters, followed by their separation in chromatographic column, identification by comparison with standard chromatograms and percent determination of the fatty acids esters from the sample. For the preparation of the fatty acids methyl esters (FAME) in agreement with the standard ISO 5508: 2002, we weighed a sample of about 1 g of fat extracted from the dried yolk ( $65^\circ\text{C}$ ). The analysis of the methyl esters was done according to standard SR EN ISO 5509:2002. We used a Perkin Elmer-Clarus 500 chromatograph fitted with flame ionization detector (FID) and BPX70 capillary column, with medium or high polar stationary phase, 60 m in length, and 0.25mm inner diameter, 0.25 $\mu\text{m}$  thick film. The carrier gas was  $\text{H}_2$ , while the burning gas was air of analytical purity. The amount of fatty acids esters from the sample (fat) is calculated by the relation between the sample area, the standard area and dilution. The result is expressed in g (grams) of fatty acids in 100 g fat.

The polyphenols content of the methanolic extracts was determined by spectrophotometry using the modified

method of [9], while the antioxidant capacity of the methanolic extracts was determined using the DPPH, method of [7].

**Statistical analysis:** The analytical results have been compared with the variance analysis (ANOVA), with WINDOWS StatView (SAS, version 6.0). The differences of the average values were considered significant for  $P < 0.05$ . The results have been expressed as mean  $\pm$  SD for all measurements.

## RESULTS AND DISCUSSIONS

Table 2 data show that the nut meal is a feed ingredient rich in crude protein (29.13% CP), but with low energy concentration (11.49 MJ ME) because of the large level of fibre (25.89% CF). It also is a rich source of fats (15.15% EE), 70.79% of which being polyunsaturated fatty acids (PUFA). Of the PUFA from the nut meal fat, 10.08% are  $\omega:3$  fatty acids, and 60.71% are  $\omega:6$  fatty acids (Table 2).

Table 2 Feeding characteristics of the nut meal

Chemical composition	DM, %	93.43
	OM, %	88.63
	CP, %	29.13
	EE, %	15.15
	CF, %	25.89
	Ash, %	4.8
	NFE, %	18.46
	ME, MJ	11.49
Mineral content	Ca, %	0.2
	P, %	0.92
	Cu, ppm	24.80
	Fe, ppm	93.17
	Mn, ppm	39.47
Linolenic acid content and fatty acids profile	Zn, ppm	65.41
	Linolenic acid, (C 18:3n3)	10.04
	SFA, %	9.82
	MUFA, %	19.31
	PUFA (%), of which:	70.79
	$\Omega 3$	10.08
	$\Omega 6$	60.71
$\Omega 6/\Omega 3$	6.021	
Antioxidant compounds	Polyphenols (mg EAG/g)	11.28
	Antioxidant capacity TEAC (mM ET/g)	112.54

Table 2 shows that the very high value of the antioxidant capacity (112.54 mM ET/g) is associated to a large content of polyphenols (11,28 mg EAG/g).

Layer performance data (Table 3) show that the nut meal had a positive influence on the laying percentage and in the average egg weight, but also increased by 3.78% the average daily feed intake. This higher intake is the influence of the low energy level (11.49 MJ/kg nut meal) and high fibre content (25.89%) of the nut meal (Table 2).

Table 3 Layer performance

Item	C	E
Average daily feed intake, (g CF/hen/day)	104.78 $\pm$ 3.90 <sup>b</sup>	108.75 $\pm$ 5.76 <sup>a</sup>
Feed conversion ratio, (kg CF/kg egg)	2.09 $\pm$ 0.23	2.02 $\pm$ 0.26
Laying percentage, (%)	77.58 $\pm$ 9.25 <sup>b</sup>	83.66 $\pm$ 8.36 <sup>a</sup>
Egg weight (g)	66.40 $\pm$ 1.22 <sup>b</sup>	67.04 $\pm$ 1.13 <sup>a</sup>

<sup>a</sup>where a and b show significant differences ( $P \leq 0.05$ ) compared to C and E

Similar results have been reported by [3] in a trial on layers, using 5; 10; 15; 20 and 25% cashew meal in the compound feed. They reported that over the 10% inclusion level, the egg production, egg mass, feed conversion and yolk colour were affected.

The dietary nut meal for group E made the diet 2.62% cheaper (0.986 RON/kg CF) compared to the price for C diet (1.013 RON/kg CF). Some researchers [1] reported similar results with the cashew or peanuts meal and recommend their utilization in the regions where these products come in large amounts.

The physical quality parameters of the eggs harvested in the end of the trial (Table 4) didn't show any significant differences in egg weight or in the weight of its components, even if the eggs collected for egg quality measurements had a higher average weight than the average egg weight per experimental period. On the other hand, the albumen and yolk pH was significantly ( $P \leq 0.05$ ) different between the two groups. Group E had slightly higher values, compared to the reference values, for albumen pH (pH = 7.8 – 8.2) and yolk (pH = 6). Table 4 also shows that the eggs from group E, treated with nut meal, had twice as much AA eggs (11.76%) than the control group. Researchers like [5] tested the effect

of 0; 5; 10; 15; 20 and 25% dietary cashew nuts meal on egg quality and found that the inclusion rates up to 25% didn't affect egg quality and freshness.

Table 4 Physical parameters of the eggs (average values)

Item	C	E
Egg weight,(g) of which:		
- albumen;	71.78±6.14	74.72±3.05
- yolk;	45.48±5.49	47.27±3.60
- eggshell	17.39±2.36	18.69±1.56
Eggshell thickness, (mm)	8.90±1.03	8.76±0.73
Eggshell breaking strength, (kgF)	0.36±0.03	0.37±0.02
pH albumen	4.29±0.91	3.98±0.59
pH yolk	9.31±0.10	9.27±0.07
Haugh units	6.46±0.09 <sup>b</sup>	6.31±0.03 <sup>a</sup>
Egg freshness, (%)	56.13±10.2	55.06±11.9
AA	5.88	11.76
A	23.53	23.53
B	70.59	64.71

\* where a and b show significant differences ( $P \leq 0.05$ ) compared to C and E

The fatty acids concentrations determined in the albumen samples (Table 5), show a significant increase ( $P \leq 0.05$ ) for all omega-3 polyunsaturated fatty acids determined for group E compared to group C. the higher concentration of omega 3 PUFA was particularly due to concentration of docosahexaenoic acid, which doubled in the experimental group (1.57%) compared to the control group (0.84%).

Researchers like [5] showed that the use of different nut meals increased the ratio of the monounsaturated/saturated fatty acids in the yolk, changing for the better the fatty acids composition of the egg yolk.

Table 5 Yolk fatty acids concentration

Item	g/100 g fat	
	C	E
Myristic C14:0	0.27±0.01 <sup>b</sup>	0.23±0.01 <sup>a</sup>
Pentadecanoic C15:0	0.07±0.01	0.07±0.01
Pentadecenoic C15:1	0.12±0.02	0.16±0.01
Palmitic C16:0	22.55±0.46	22.31±0.48
Palmitoleic C16:1	2.29±0.22 <sup>b</sup>	1.92±0.18 <sup>a</sup>
Heptadecanoic C17:0	0.17±0.02	0.18±0.01
Heptadecenoic C17:1	0.13±0.03 <sup>b</sup>	0.19±0.03 <sup>a</sup>
Stearic C18:0	10.67±0.69 <sup>b</sup>	12.14±0.49 <sup>a</sup>
Oleic C18:1	32.61±1.76 <sup>b</sup>	29.82±0.62 <sup>a</sup>
Linoleic C18:2	23.34±1.54	23.89±0.30
Linolenic $\gamma$ C18:3n6	0.17±0.05 <sup>b</sup>	0.10±0.06 <sup>a</sup>
Linolenic $\alpha$ C18:3n3	0.32±0.04 <sup>b</sup>	0.47±0.02 <sup>a</sup>
Eicosadienoic C20(2n6)	0.17±0.05	0.18±0.03
Eicosatrienoic C20(3n6)	0.34±0.03	0.38±0.02
Erucic C22 (1n9)	0.07±0.02	0.08±0.02
Eicosatrienoic C20(3n3)	0.23±0.03	0.27±0.04
Arachidonic C20 (4n6)	3.93±0.44 <sup>b</sup>	4.40±0.25 <sup>a</sup>
Nervonic C24 (1n9)	0.32±0.03 <sup>b</sup>	0.37±0.04 <sup>a</sup>
Docosatetraenoic C22 (4n6)	1.22±0.14	1.16±0.15
Docosapentaenoic C22 (5n3)	0.09±0.02 <sup>b</sup>	0.13±0.02 <sup>a</sup>
Docosahexaenoic C22 (6n3)	0.84±0.13 <sup>b</sup>	1.57±0.09 <sup>a</sup>

*Fatty acids profile of the egg yolk*

SFA	33.74	34.93
MUFA	35.55	32.54
PUFA	30.65	32.53
UFA	66.20	65.07
SFA/UFA	0.510	0.537
PUFA/MUFA	0.862	1.000
$\Omega 3$	1.48	2.44
$\Omega 6$	29.18	30.09
$\Omega 6/\Omega 3$	19.77	12.36

\* where a and b show significant differences ( $P \leq 0.05$ ) compared to C and E

Table 6 data show that both the polyphenols concentration and the antioxidant capacity of the eggs increased significantly ( $P \leq 0.05$ ) in the experimental group (nut meal) compared to the control group. The compounds with antioxidant activity in eggs may be the lipophilic compounds (carotenoids and  $\alpha$ -tocopherol), which can be found in large amounts in the egg yolk as well as the hydrophilic compound, such as the free amino acids [11]; the peptides and oligopeptides resulting from albumen [14] or yolk hydrolysis [15], and high phosphorylating proteins such as the phosvitin [6].

Table 6 Antioxidant capacity of the eggs

Item		Polyphenols concentration, $\mu\text{g EAG/g}$	Antioxidant capacity, mM Trolox/g
Yolk	C	0.88±0.02 <sup>a</sup>	79.17±1.59 <sup>a</sup>
	E	1.46±0.32 <sup>b</sup>	88.40±3.45 <sup>b</sup>
Albumen	C	0.44±0.01 <sup>a</sup>	75.33±5.12 <sup>a</sup>
	E	0.60±0.07 <sup>b</sup>	104.53±8.55 <sup>b</sup>

\* where a and b show significant differences ( $P \leq 0.05$ ) compared to C and E

## CONCLUSIONS

- The nut meal is a rich source of protein (29.13), fats (15.15% EE) and has antioxidant properties (11.28 mg EAG/g polyphenols and 112.54 mM ET/g antioxidant capacity). From the total fat, 70.79% are polyunsaturated fatty acids (PUFA)

- Used in layer diets, the nut meal increased significantly the laying percentage and egg weight, without affecting the feed conversion ratio.

- The feeding quality of the eggs improved due to the higher concentration of omega 3 polyunsaturated fatty acids from the egg yolk; egg freshness also improved, as shown by the 11.76 % AA eggs;

- The antioxidant capacity and the polyphenols concentration both in the albumen and yolk increased significantly (8.85%) in the group treated with nut meal.

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