

Walnut Meal as Feed Additive in Broilers Nutrition: Effects on Performance and Thigh Meat Quality

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Abstract

The aim of the study was to evaluate the effect of dietary walnut meal inclusion on productive parameters of broiler chickens' and thigh meat quality. A nutritional trial was conducted on 80 Ross 308 broilers, raised in semi-intensive system conditions, reared on permanent shave litter (10–12 cm thick) in boxes of 3 m² (40 broilers / each group, housed in a single box), fed *ad libitum*, having free access to the water. The diets given to the broilers, consisted of a control diet (C group) and an experimental diet with 6% walnut meal (E group). During the experimental period, production performances were recorded. At 42 days of age, 6 birds from each group were slaughtered and thigh meat samples were collected. The results showed that walnut meal supplement negatively influenced the final body weight, when compared with C group. Regarding the proximate composition of thigh meat, a decrease of crude fat concentrations was registered at the end of experiment (12.66 vs 10.49%) but also a significant improvement of quality of lipids in terms of omega 3 concentrations and omega 6 to omega 3 ratio (2.22 vs 2.90% omega 3 and 3.28 vs 2.66 omega6/omega3). The synthesis of long chain fatty acids was noticed to be stimulated, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) recording increased concentrations for experimental group compared to control. In conclusion, the walnut meal supplements positively influenced the nutritional composition of broilers meat (thigh meat) in terms of crude fat deposition and quality of lipids, beneficial for human consumers.

Keywords: broilers; crude fat; fatty acids; meat quality; productive parameters; walnut meal.

1. Introduction

Following the ban on antibiotics as growth promoters, research has focused on identifying unconventional feed additives that ensure animal health and productivity without contributing to bacterial resistance. These additives play a role in improving digestion, supporting gut flora, and enhancing the immune system [1].

In addition to probiotics, prebiotics, enzymes, and organic acids, another studied category includes plant extracts and phytochemicals. The latter comprise a variety of essential oils, polyphenols, and other plant compounds that exhibit antimicrobial, antioxidant, and anti-inflammatory properties [2]. They can help reduce oxidative stress and maintain a healthy gut environment. The implementation of these unconventional additives requires in-depth studies to determine optimal dosages, synergistic effects between different components, and their long-term cost-effectiveness. At the same time, potential side

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effects or interactions with other feed components must be monitored.

Playing a crucial role in the body's function, fatty acids are essential for human health. Foods rich in omega-3 fatty acids have gained popularity among consumers due to their numerous health benefits. Omega-3 fatty acids support cardiovascular health by lowering blood pressure and heart rate, reducing serum triglycerides, inflammatory markers [3], and arrhythmias [4]. They are also vital for brain development and the prevention of neurodegenerative diseases, improving endothelial function, insulin sensitivity, and even slowing or reducing atherosclerotic plaque buildup [5].

Key sources of omega-3 include fish and fish oil, oilseeds (rapeseed, flaxseed, hempseed, sesame) and their by-products, walnuts, wheat germ, and dietary supplements [6,7].

Walnuts (family *Juglandaceae*) are a good source of protein and fiber [8] in human nutrition, thanks to their rich content of nutrients such as magnesium, zinc, phosphorus, iron, and calcium, as well as vitamin E and B-complex vitamins [9]. These nutrients support bone, skin, and hair health while also strengthening the immune system [10]. Archaeological discoveries have shown that humans have been consuming walnuts for at least 10,000 years [11] and have been cultivating them for 6,000 years. Research on the health benefits of walnuts has focused on the prevention of cardiovascular diseases, particularly coronary heart disease, due to their omega-3 fatty acid content, which helps lower LDL cholesterol and increase HDL cholesterol [12].

Walnut consumption is also beneficial for the brain, as walnuts contain antioxidants [13], as well as omega-3 fatty acids and vitamin E [14]. Walnut meal is a byproduct obtained after extracting oil from walnut kernels. Its chemical composition is influenced by factors such as the walnut variety, growing conditions, and the technological process used. After oil extraction, whether through cold pressing, solvent extraction, or other methods, walnut meal is produced and can be used in both human and animal nutrition [15]. The fat content of walnut meal ranges from 9% to 20%, depending on the extraction method [16]. It is a valuable source of protein, containing up to 50%, along with 6 - 7% dietary fiber [16]. Other studies have reported a composition of approximately 30% crude protein, 16% of crude fat, and 18% crude fiber [15].

In this context, the aim of the study was to evaluate the effects of walnut meal on broilers production performances and its potential to improve the nutritional and lipids quality of thigh meat.

2. Materials and methods

Ethical consideration

The study was approved by the Ethical Committee of the National and Development Institute for Biology and Animal Nutrition (INCDBNA-IBNA), Balotesti according to experimental protocol no. 1375/ 2020. Also, the study complied with the principles of Romanian Law 43/2014 ordinance 28/31.08.2011 and Directive 2010/63/EU concerning the protection of animals used for scientific purposes.

Experimental design, broiler management and diets

A nutritional trial was conducted on 80 Ross 308 broiler chickens, raised in semi-intensive system conditions, reared on permanent shave litter (10–12 cm thick) in boxes of 3 m² (40 broilers / each group, housed in a single box), fed *ad libitum*, having free access to the water.

The broiler chickens were reared under controlled environmental conditions for a standard 42-day production cycle. Upon placement (day 1), chicks were housed in a temperature-controlled facility with a starting ambient temperature of 32–34 °C, which was gradually reduced by approximately 2–3 °C per week to reach 20–22 °C by day 42, in accordance with commercial management guidelines. Relative humidity was maintained between 50–70%. A 23 hours light and 1 hour dark photoperiod was applied during the first 7 days to promote feed intake and adaptation, followed by a 20 hours light and 4 hours light regime until slaughter. Birds had *ad libitum* access to feed and water throughout the trial. The feeding program implemented: starter (day 1–14), grower (day 15–28), and finisher (day 29–42), was formulated to meet or exceed the nutrient requirements specified in the Ross 308 performance objectives. A conventional diet based on corn, wheat, and soybean meal, was used for the control group and the experimental ratio included 6% walnut meal (WM) as presented in Table 1.

Production performances, such as, average daily gain (ADG), average daily feed intake (ADFI) and

feed conversion ratio (FCR) were calculated from the weekly records of the body weights and daily feed intake. At the end of the experiment, 6 broilers/groups were randomly selected and

slaughtered by cervical dislocation and tissue samples (thigh meat) were collected, for laboratory analyses.

Table 1. Dietary ingredients of the diets given to broilers during experimental period

. Ingredients, %	Grower phase		Finisher phase	
	C	E	C	E
Maize	42.00	42.00	42.00	42.00
Wheat	18.73	15.91	20.56	17.75
Soybean meal	30.53	27.37	28.10	25.03
Oil	4.10	3.96	5.11	4.97
Walnut meal	0.00	6.00	0.00	6.00
Lysine	0.19	0.13	0.09	0.03
Methionine	0.24	0.30	0.20	0.25
Threonine	0.03	0.10	0.10	0.07
Calcium carbonate	1.29	1.30	1.17	1.18
Monocalcium phosphate	1.48	1.52	1.30	1.35
Salt	0.36	0.36	0.33	0.33
Choline	0.05	0.05	0.04	0.04
Premix	1.00	1.00	1.00	1.00
Total	100	100	100	100
Chemical composition				
ME (Kcal/kg)	3086	3086	3167	3167
Crude protein (%)	20.00	20.00	19.00	19.00
Crude fat (%)	5.93	6.68	6.92	7.67
Crude fiber (%)	3.78	4.62	3.75	4.55
Calcium (%)	0.84	0.84	0.76	0.76
Phosphorus (%)	0.42	0.42	0.38	0.38

1 kg of premix contains 1,100,000 IU/kg vitamin A; 200,000 IU/kg vitamin D3; 2700 IU/kg vitamin E; 300 mg/kg vitamin K; 200 mg/kg Vit. B1; 400 mg/kg vitamin B2; 1485 mg/kg pantothenic acid; 2700 mg/kg nicotinic acid; 300 mg/kg vitamin B6; 4 mg/kg Vit. B7; 100 mg/kg vitamin B9; 1.8 mg/kg vitamin B12; 2000 mg/kg vitamin C; 8000 mg/kg Mn; 8000 mg/kg Fe; 500 mg/kg Cu; 6000 mg/kg Zn; 37 mg/kg Co; 152 mg/kg I; 18 mg/kg Se.

Chemical analysis

Proximate composition

For the crude protein determination, a Kjeldahl reference method and a semiautomatic Kjeltak auto 1030—FOSS Tecator AB (Höganäs, Sweden) was used. Crude fat was determined by continuous extraction in solvent, followed by fat measurement with Soxhlet FOSS Tecator AB (Höganäs, Sweden) and gravimetric methods according to Regulation (CE) no. 152/2009 were applied for dry matter content and crude ash. The methods were described by Untea et al. [17]

Fatty acids determination

Perkin Elmer Clarus 500 (Massachusetts, United States) gas chromatograph was used for the determination of fatty acids profile from meat samples and vegetal materials. The method used aims to Fatty acids from the original matrix were transformed into methyl esters, separated on the chromatographic column, and identify them by

reference to standard chromatograms. FID detector and capillary separation column with high stationary polar phase TRACE TR-Fame, (Termo Electron, Massachusetts, United States), measuring 60 m×0.25 mm×0.25 µm flm43 were the technical parameters of the method. The preparation of samples as well as the determination steps were described by Turcu et al. [18].

Statistical analysis

One-way ANOVA (XLStat, Addinsof, New York, USA) was used for performing statistical analysis (p values below 5% were considered significant). Performance parameters like ADFI and FCR were analyzed considering all broilers in a box as an experimental unit. For the study of body weight evolution and ADG every broiler was considered an experimental unit. From each group of animals, six broilers were randomly selected, and each of

them become an experimental unit for the meat quality parameter evaluation.

3. Results and discussion

Data presented in Table 2 showed that the supplementation of broilers diets with walnut meal, affected the productive performances, in terms of final body weight and average daily gain. The two before mentioned parameters significantly decreased ($P < 0.05$) for the walnut

meal supplemented group, compared with the control one. Similar results were reported by Jiang et al. [19] which noticed that a 5% walnut meal inclusion rate in broilers diets led to significant decrease of final weight and daily gain. In a nutrition study on laying hens, the influence of dietary bilberry and walnut leaves on average egg weight was noticed. The reported results and the negative influence of walnut products on some productive parameters, can be related to the inclusion rate in diets.

Table 2. Productive parameters registered during experimental period

Parameter	C	E	SEM	P value
BWi (g)	352.7	347.3	5.882	0.5130
BWf (g)	3140 ^a	2821 ^b	45.65	0.0001
ADG (g)	92.91 ^a	82.47 ^b	1.371	0.0001
ADFI (g/broiler/day)	151.7	139.0	5.659	0.1160
FCR (kg feed/kg weight)	1.618	1.619	0.019	0.9590

Bwi – initial body weight (g); Bwf – final body weight (g); ADG – average daily gain (g); ADFI – average daily feed intake (g/broiler/day); FCR – feed conversion ratio (kg feed/kg weight). Means within a raw with no common superscript differ ($p < 0.05$); SEM—standard error of the mean; C – control diet; E – experimental diet with 6% walnut meal.

In the Table 3 are presented results regarding the proximate composition of thigh meat samples. Only crude fat was significantly influenced ($P < 0.05$) by the walnut meal supplement present in broilers diets. In a similar study, the proximate composition of breast meat was inversely affected by the supplements, the crude fat concentrations

being significantly increased ($P < 0.05$) in walnut meal supplemented diet [15]. The decreased concentrations of fats recorded in the experimental group are associated with the quality of lipids determined as fatty acids profile and presented in Table 4.

Table 3. Proximate composition of thigh samples

Parameter	C	E	SEM	P value
Dry matter (%)	32.39	30.32	1.286	0.282
Crude protein (%)	17.73	17.82	0.829	0.938
Crude fat (%)	12.66 ^a	10.49 ^b	0.491	0.011
Crude ash (%)	1.199	1.141	0.051	0.439

Means within a raw with no common superscript differ ($p < 0.05$); SEM—standard error of the mean; C – control diet; E – experimental diet with 6% walnut meal.

The fatty acid profile (Table 4) revealed a positive effect of the phyto additive used as a nutritional supplement in this study. In the experimental group, saturated fatty acid levels decreased, while monounsaturated fatty acid levels increased. With regard to polyunsaturated fatty acids, omega-3 concentrations were higher in the group supplemented with walnut meal. Additionally, the omega-6 to omega-3 ratio shifted closer to the ideal value of 1, indicating an improved lipid profile. The long chain PUFA synthesis was stimulated by the nutritional supplements, as it is proved by the values recorded for eicosapentaenoic acid (EPA) and docosahexaenoic

acid (DHA). In a study regarding the use of African walnut seed meal in broilers diets on 2.5 and 5% inclusion level, the authors noticed the omega 6 fatty acids like linoleic acid or its synthesis product arachidonic acid were significantly lower in thigh meat samples [20, 21]. The effects of walnut supplementation on the fatty acid profile did not strictly follow the dietary patterns. This observation may be explained by the presence of long-chain fatty acids in the lipid composition of thigh muscle samples, which can result from processes such as desaturation, de novo synthesis, and elongation of precursor fatty acids like linolenic and linoleic acids. The

enhanced synthesis capacity observed in the birds derived dietary supplements.
 may be associated with the influence of walnut-

Table 4. Fatty acids composition of thigh meat samples

Item, %	C	E	SEM	P value
C 4:0	0.480	0.378	0.050	0.179
C 6:0	0.303	0.302	0.022	0.958
C 8:0	0.165	0.140	0.012	0.165
C 10:0	0.047	0.048	0.004	0.756
C 12:0	0.063 ^a	0.020 ^b	0.007	0.002
C 14:0	0.808 ^a	0.743 ^b	0.018	0.025
C 14:1	0.138	0.145	0.010	0.631
C 15:0	0.720	0.632	0.021	0.015
C 15:1	2.022	2.082	0.111	0.709
C 16:0	29.27	27.40	0.202	0.0001
C 16:1	4.932	4.297	0.059	0.0001
C 17:0	0.085	0.085	0.020	1.000
C 17:1	0.187	0.187	0.028	0.872
C 18:0	8.375	8.630	0.095	0.087
C 18:1n9c	40.29 ^b	41.71 ^a	0.264	0.003
C 18:2n6	2.507 ^b	3.215 ^a	0.221	0.046
C 18:3n6	0.042	0.038	0.014	0.873
C 18:3n3	1.880	2.293	0.157	0.093
C 18:2	1.610	1.232	0.204	0.219
C 18:4n3	0.100	0.080	0.011	0.214
C 20:2n6	3.992	3.735	0.173	0.318
C20:(4n6)	0.052	0.055	0.011	0.832
C22:(2n6)	0.268	0.282	0.035	0.794
C22:(3n6)	0.203	0.265	0.040	0.301
C 20:5n3	0.242 ^b	0.383 ^a	0.015	0.0001
C24:0	0.317 ^b	0.398 ^a	0.023	0.029
C 24:1n9	0.178	0.215	0.052	0.627
C22 (4n6)	0.077	0.100	0.012	0.215
C22 (5n3)	0.000 ^b	0.088 ^a	0.009	0.0001
C22 (6n3)	0.000 ^b	0.062 ^a	0.005	0.0001
Other FA	0.665	0.727	-	-
SFA (%)	40.61 ^a	38.81 ^b	0.253	0.0001
MUFA (%)	47.75 ^b	48.64 ^a	0.236	0.024
PUFA (%)	10.57	11.54	0.821	0.424
Ω3 (%)	2.222 ^b	2.907 ^a	0.150	0.009
Ω6 (%)	7.140	7.690	0.192	0.070
Ω6/Ω3	3.286 ^a	2.667 ^b	0.171	0.029

Means within a raw with no common superscript differ ($p < 0.05$); SEM—standard error of the mean. SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; Ω3 – total omega 3 fatty acids; Ω6 – total omega 6 fatty acids; Ω6/ Ω3 – ratio of total omega 3 to omega 6; C – control diet; E – experimental diet with 6% walnut meal.

4. Conclusions

According to the results presented in this study, walnut meal supplementation positively influenced the nutritional composition of broiler thigh meat, particularly in terms of crude fat deposition and lipid quality. The reduction in crude fat content, combined with an improved

omega-3 fatty acid profile, results in leaner chicken meat with enhanced nutritional value. Moreover, the presence of long-chain fatty acids such as EPA and DHA in the lipid profile of samples from the experimental group highlights the relevance and significance of these findings for both human nutrition and medical applications.

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References

1. Roca-Saavedra, P., Mendez-Vilabrille, V., Miranda, J. M., Nebot, C., Cardelle-Cobas, A., Franco, C. M., & Cepeda, A. (2018). Food additives, contaminants and other minor components: Effects on human gut microbiota—A review. *Journal of physiology and biochemistry*, 74, 69-83. <https://doi.org/10.1007/s13105-017-0564-2>
2. Valdivieso-Ugarte, M., Gomez-Llorente, C., Plaza-Diaz, J., & Gil, A. (2019). Antimicrobial, antioxidant, and immunomodulatory properties of essential oils: A systematic review. *Nutrients*, 11(11), 2786.
3. Tortosa-Caparrós, E., Navas-Carrillo, D., Marín, F., & Orenes-Piñero, E. (2017). Anti-inflammatory effects of omega 3 and omega 6 polyunsaturated fatty acids in cardiovascular disease and metabolic syndrome. *Critical reviews in food science and nutrition*, 57(16), 3421-3429.
4. Elagizi, A., Lavie, C. J., Marshall, K., DiNicolantonio, J. J., O'Keefe, J. H., & Milani, R. V. (2018). Omega-3 polyunsaturated fatty acids and cardiovascular health: a comprehensive review. *Progress in cardiovascular diseases*, 61(1), 76-85.
5. Thies, F., Garry, J. M., Yaqoob, P., Rerkasem, K., Williams, J., Shearman, C. P., Gallagher, P. J., Calder, P.C., Grimble, R. F. (2003). Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *The Lancet*, 361(9356), 477-485.
6. Das, U. N. (2006). Essential fatty acids: biochemistry, physiology and pathology. *Biotechnology Journal: Healthcare Nutrition Technology*, 1(4), 420-439.
7. Vlaicu, P. A., Untea, A. E., & Oancea, A. G. (2024). Sustainable poultry feeding strategies for achieving zero hunger and enhancing food quality. *Agriculture*, 14(10), 1811.
8. Cosmulescu, S. N., Baci, A., Achim, G., Mihai, B. O. T. U., & Trandafir, I. (2009). Mineral composition of fruits in different walnut (*Juglans regia* L.) cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 37(2), 156-160.
9. Vieira, B. M., de Mello e Silva, G. N., & Silva, M. I. (2025). Nutritional Elements II: Vitamins and Minerals. In *Fundamentals of Drug and Non-Drug Interactions: Physiopathological Perspectives and Clinical Approaches* (pp. 57-86). Cham: Springer Nature Switzerland.
10. Hayes, D., Angove, M. J., Tucci, J., & Dennis, C. (2016). Walnuts (*Juglans regia*) chemical composition and research in human health. *Critical reviews in food science and nutrition*, 56(8), 1231-1241.
11. Carrion, J. S., Fuentes, N., Gonzalez-Samperiz, P., Sanchez Quirante, L., Finlayson, J. C., Fernandez, S. and Andrade, A. (2007). Holocene environmental change in a montane region of southern Europe with a long history of human settlement. *Quat. Sci. Rev.* 26(11–12):1455–1475.
12. Tufail, S., Fatima, A., Niaz, K. H. A. L. I. D., Qusoos, A., & Murad, S. (2015). Walnuts increase good cholesterol (HDL-Cholesterol) and prevent coronary artery disease. *Pakistan Journal of Medical & Health Sciences*, 9(4), 1244-1246.
13. Chauhan, A., & Chauhan, V. (2020). Beneficial effects of walnuts on cognition and brain health. *Nutrients*, 12(2), 550.
14. Arslan, J., Gilani, A. U. H., Jamshed, H., Khan, S. F., & Kamal, M. A. (2020). Edible nuts for memory. *Current pharmaceutical design*, 26(37), 4712-4720.
15. Untea, A. E., Varzaru, I., Saracila, M., Panaite, T. D., Oancea, A. G., Vlaicu, P. A., & Grosu, I. A. (2023). Antioxidant properties of cranberry leaves and walnut meal and their effect on nutritional quality and oxidative stability of broiler breast meat. *Antioxidants*, 12(5), 1084.
16. Grosu, C., Gorbuleac, V., Scripcari, I., Ciurac, J., & Tatarov, P. (2016). Proprietatile functionale ale srotului de nuci *Juglans Regia* L. In *Modern Technologies in the Food Industry* (pp. 198-202).
17. Untea, A.E.; Panaite, T.D.; Dragomir, C.; Ropota, M.; Olteanu, M.; Varzaru, I. Effect of dietary chromium supplementation on meat nutritional quality and antioxidant status from broilers fed with Camelina-meal-supplemented diets. *Animal* 2019, 13, 2939–2947.
18. Turcu, R. P. et al. Effects of grape seed oil supplementation to broilers diets on growth performance, meat fatty acids, health lipid indices and lipid oxidation parameters. *Agriculture* 11(5), 404 (2021).
19. Jiang, X., Yang, J., Yu, L., Zhou, Z., Yu, L., Luo, Y., & Fang, C. (2024). Walnut meal improves meat quality by modulating intestinal microbes in white feather broilers. *Heliyon*, 10(15).
20. Adeyemi, K. D., Abdulrahman, A., Ibrahim, S. O., Zubair, M. F., Atolani, O., & Badmos, A. A. (2020). Dietary supplementation of *Tetracarpidium conophorum* (African Walnut) seed enhances muscle n-3 fatty acids in broiler chickens. *European Journal of Lipid Science and Technology*, 122(6), 1900418.
21. Untea, A. E., Varzaru, I., Panaite, T. D., Gavris, T., Lupu, A., & Ropota, M. (2020). The effects of dietary inclusion of bilberry and walnut leaves in laying hens' diets on the antioxidant properties of eggs. *Animals*, 10(2), 191.