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EFFECTS OF THE DIETARY FLAXSEED AND ALFALFA ON THE ORGANOLEPTIC QUALITIES OF THE BROILERS' MEAT

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Abstract

Due to the customers` desire to improve their healthy diet enriched in Omega-3, researchers included ingredients such as flaxseed in poultry feed. The study was conducted on a commercial farm to compare the organoleptic qualities of broilers' meat using dietary flaxseed and alfalfa. The broilers (44.000 heads) divided into two groups (C, E) were fed with compound feed containing 6% flaxseed + 2% alfalfa (growth phase) and 12% flaxseed + 2% alfalfa (finishing phase). At the end of the experiment, the organoleptic and physico-chemical (color and pH) meat parameters of breast and thigh were assessed for storage period (24, 48, 72 and 168 h). The results showed no significant differences ($p \geq 0.05$) concerning the pH of the thighs and breast, but significant differences ($p \leq 0.05$) were observed for all color parameters (L, a*, b*) within the storage period*group interaction. In conclusion, using combined dietary rich Omega-3 sources (flaxseed) and xanthophyll (alfalfa) contributes to meat quality enrichment by increasing the beneficial fatty acid concentration and meat color, important criteria in customer decision.*

***Key words**: alfalfa, broiler, flaxseed, meat quality, pH.*

INTRODUCTION

Numerous scientific studies demonstrate the role of n-3 polyunsaturated fatty acids (PUFA) in the normal development of the central nervous system in children, maintaining its functions in adults, regulating immune functions, and preventing and managing coronary diseases (Simopoulos, 2000; Griffin, 2012; Calder, 2013).

The benefits of PUFAs from the Omega-3 fatty acid family (n-3) are scientifically proven through studies conducted on both animals and human subjects. In human food, the intake of n-3 fatty acids comes from both plants (seeds, nuts, oils, legumes) and marine (fatty fish, seafood) sources. However, modern agro-food industry practices have led to a reduction in n-3 fatty acids in the diet of most people (Stark et al., 2016). Nevertheless, the industry is attempting to readjust and contribute to an increase in the consumption of n-3 fatty acids, especially those with long chains.

The intake of n-3 fatty acids from marine sources is limited in diets due to factors such as cost, availability, and sustainability, as well as other concerns including allergies, taste, and smell (Moghadam et al., 2017; Cherian, 2016).

The alternative would be animal-derived products such as eggs and meat, which can be enriched with n-3 fatty acids by introducing plant sources of Omega-3 into the feed of poultry. The poultry industry has quickly adapted by producing eggs enriched with n-3 fatty acids. This technology primarily utilizes feed sources rich in n-3 fatty acids to enhance the nutritional value of food products (Bourre, 2005).

Flaxseed (*Linum usitatissimum*) serves as a plant source for increasing the level of n-3 PUFA in chicken meat (thigh, breast). Chemically, flaxseeds contain approximately 32-43.6% oil, 19-33.8% fiber, 20.3-26.78% protein, 6% carbohydrates, and 4% ash, depending on the genotype (Iliescu et al., 2023).

Flaxseed is a plant source with a high content of PUFA compared to fish oil, soybean, corn, or marine algae (Jiang et al., 2021). The composition of flaxseed oil highlights its distinct fatty acid content: 57% alpha-linolenic acid, 16% linoleic acid, 18% oleic acid, 4% stearic acid, and 5% palmitic acid (Cloutier et al., 2012).

The protein content (20-30%), metabolizable energy level (3800-3960 kcal/kg), and the alpha-linolenic acid (ALA) content in flaxseed oil make flaxseeds an ideal plant source in poultry feed (Jia et al., 2021; Moghadam & Cherian, 2017; Moghadam et al., 2020).

Although, flaxseed contains up to 50% ALA also contains anti-nutritional factors (trypsin inhibitor, phytic acid, etc.) that can affect the health and muscle development of broilers. These anti-nutritional factors are eliminated through the "extrusion" process (Wu et al., 2008), which also enhances the metabolism of other nutrients, including ALA (Anjum et al., 2013; Kumar et al., 2023;). The high degree of unsaturation, which is desired for its beneficial effects on the health of consumers, can affect the oxidative stability of meat, inevitably leading to the deterioration of its nutritional and functional value (Betti et al., 2009; Cortinas et al., 2005; Juskiewicz et al., 2017). Therefore, it is necessary that alongside sources of n-3 PUFA, there are also antioxidants to slow down lipid oxidation and add nutritional value to the meat (Bernardi et al., 2016; Moghadasian, 2008).

Products containing higher amounts of n-3 PUFA must have at least equal attractiveness to other products in the same category. Therefore, color and its preservation over time during shelf exposure are important attributes (Janisch et al., 2011).

By combining extruded flaxseeds with various antioxidants such as vitamin E and selenium, the meat oxidation can be stabilized, and a positive contribution can be made to the nutritional value of broiler meat (Parveen et al., 2016; Perez et al., 2010; Leskovec et al., 2019; Taulescu et al., 2010).

The alfalfa (*Medicago sativa*) stands as a valuable feed for livestock. Its high protein content, complemented by a well-balanced amino acids profile, vitamins, and essential minerals, contributes significantly to animal health and growth. Moreover, the presence of biologically active compounds, including beneficial saponins, enhances its value as a feed source (Soto-Zarazúa et al., 2017). Alfalfa is rich in xanthophylls and can be used in poultry feeding to add value to the final products (Yildiz et al., 2020). Alfalfa is well-known for its superior fiber quality, and serves as a beneficial dietary supplement in animal feeding, enhancing production, reproductive performance, health status, and meat quality (Ma et al. 2022).

This study evaluated the effects of including dietary sources rich in Omega-3 fatty acids (flaxseed) and xanthophyll (alfalfa) in poultry feeding on meat quality parameters as pH and color, during different storage periods. These meat quality parameters are considered crucial factors, and manipulating the broilers' diet structure represents a viable feeding strategy for meeting consumers preferences.

MATERIALS AND METHODS

The study was conducted on a commercial farm in southern Romania where broilers (44.000 birds) were divided into two groups (30.000 birds - Control group; 14,000 birds – Experimental group). The experimental group diet structure, presented in Table 1, contained 6% flaxseed + 2% alfalfa (growing phase) and 12% flaxseed + 2% alfalfa (finishing phase).

Table 1. The experimental diet structure composition during different production phases

Ingredients	Starter phase (day 0-10)	Growing phase (day 11-24)	Finishing phase (day 25-42)
Corn	34.41	40	34.09
Wheat	20	13.04	25.00
Alfalfa	-	**2.00**	**2.00**
Flaxseed	-	**6.00**	**12.00**
Soybean meal	37.98	31.82	20.30
L-lysine	0.25	0.16	0.33
DL-methionine	0.35	0.29	0.31
L-threonine	0.11	0.15	0.18
Calcium carbonate	1.28	0.59	0.94
Monocalcium Phosphate	1.24	1.24	0.85
Salt (NaCl)	0.40	0.40	0.36
Vegetable oil	3.47	3.62	2.94
Colourant	0.50	0.18	0.18
Phytase	0.01	0.01	0.01
Vitamin Premix	0.50	0.50	0.50

At the end of the experiment, the chemical-physical and organoleptic parameters of the collected meat samples for breast and thigh were evaluated for storage period (24, 48, 72, and 168 hours, respectively).

For evaluating the color of the meat samples, a portable spectrophotometer 3nh YS3020 (Shenzhen Threenh Technology Co., Ltd, Beijing, China) was used, with a wavelength ranging from 400-700 nm. The measurements conducted with the spectrophotometer were based on the trichromatic system adopted by CIE, utilizing three primary spectral colors: red, green, and blue. The values obtained in the CIE color space were determined by reading the Cartesian parameters (L*, a*, b*). Flaxseed meal was ground using an electric grinder to pass through a 1-mm mesh.

The results were analised using general linear model (GLM) procedures of SAS (Statistical Analysis System, Minitab version 17, SAS Institute Inc., Cary, NC, USA). The storage period effects on meat parameters (pH and color) were analyzed to determine whether the factors studied (diet and storage period) influenced the pH and meat color. The data obtained were analyzed by two-way ANOVA used the Tukey test following the statistical model:

$$Yijk = \mu + \alpha i + \beta j + \alpha i\beta j + eijk$$

where Yijk = variable measured for the kth observation of the ith treatment and jth feeding or storage period; μ is the sample mean, αi is the effect of the ith treatment; βj is the effect of the jth feeding or storage period; αiβj = interaction of ith treatment and jth feeding time, and εijk is the effect of error. The differences were highly significant when $p < 0.001$, significant if $p < 0.05$, and a tendency of influence was considered when $p < 0.10$.

RESULTS AND DISCUSSIONS

Various scientific studies have recommended different inclusion of dietary flaxseed percentages for broilers: 2.5% and 10% (Mridula et al., 2015); 12-15% (Shen et al., 2005; Pekel et al., 2009; Najib & Al-Yousef, 2011) increasing the Omega-3 fatty acid content of meat. The alfalfa utilization in poultry feed reduces oxidative processes (Mattioli et al., 2022) but the high fiber content of alfalfa (25-30%) is a limiting factor in monogastric diets due to its potential antinutritional properties (Wang et al., 2018).

Also recent studies showed that alfalfa inclusion have no negative impact on growth performance if is included less then 4% (Sánchez-Quinche et al., 2023).

More authors stated that an optimum inclusion level can promote the abundance of beneficial microbiota (Zhang et al., 2016).

The pH values

The processing capacity, appearance, and sensory quality of broiler meat are influenced by the pH value (Petracci et al., 2009; Allen et al., 1997; Beauclercq et al., 2016). A normal pH of chicken meat, measured 24 hours after slaughter (final pH), ranges between 5.7 and 6.0. A pH lower than 5.7 is generally associated with a pale appearance and reduced water retention capacity, similar to PSE (pale, soft, exudative) meat, whereas a pH higher than 6.0 is associated with darker, firmer, and drier meat, qualitatively poor and not recommended for storage (Petracci et al., 2014)

Table 2. The pH measured values on breast and thighs samples during different storage period

Estimated time	Group	**pH breast**	**pH thighs**
Initial (T_0)	Control	6.292^a	6.055
	Experimental	6.227^a	5.858
24 h (T_{24})	Control	5.690^b	5.938
	Experimental	5.732^b	5.923
48 h (T_{48})	Control	5.622^b	5.887
	Experimental	5.683^b	5.843
72 h (T_{72})	Control	5.568^b	5.707
	Experimental	5.603^b	5.867
168 h (T_{168})	Control	5.663^b	5.823
	Experimental	5.705^b	5.930
Multiple effects			
Group			
Control		5.767	5.882
Experimental		5.790	5.884
SEM $_{group}$		0.022	0.034
Period			
Initial (T_0)		6.259^a	5.957
24 h (T_{24})		5.711^b	5.931
48 h (T_{48})		5.652^b	5.865
72 h (T_{72})		5.585^b	5.787
168 h (T_{168})		5.684^b	5.877
SEM $_{period}$		0.034	0.053
The interaction (p-Value)			
Group		0.453	0.961
period		0.000	0.206
period*group		0.704	0.161

The average values located on the same column, with different letters as superscripts, show significant differences $p < 0.05$.

In Table 2, no significant intra-lot differences are observed (p=0.453) for the pH value measured at the level of the breast; however, distinct significant differences (p=0.000) are observed for the values recorded at different pH measurement time periods (T0 h, T24 h, T48 h, T72 h, T168 h). It is noted that the T0h pH value of the experimental group for breast is slightly above 6, but subsequently measured values are within the normal pH range, with a minimum of 5.59 (T72 h) and a maximum of 5.71 (T24 h). The measurements taken at the thigh level do not exhibit statistically significant differences ($p>0.05$) in terms of group ($p=0.961$), period ($p=0.206$), or the interaction between group and period ($p=0.161$). Other authors used flaxseed oil in feed (over 35 days) to assess its influence on quail meat and conducted pH measurements at the breast level at different time intervals (0, 7, 14, 21, 28, 35 days) without any differences noticed ($p=0.12$). (Mirshekar et al., 2021).

A correlation is noted between meat pH and various meat quality parameters, such as shelf life, water retention capacity, and color (Gratta et al., 2019). In this study, the fresh pH of broiler breast ranged from 5.89 to 6.23, and the pH of thighs ranged from 5.87 to 5.96. This range considered normal (5.00-6.00) for pH recorded in the broiler breast meat and thighs is due to a proper growth and welfare conditions for poultry (Genchev & Mihaylov, 2008).

In other studies, it is shown that there are differences between various types of poultry meat, including between species. Thus, for the breast meat from broilers, we have more white glycolytic fibers, whereas the composition of quail breast meat's red color is caused by the predominance of oxidative fibers. It has been demonstrated that the pH of the breast is positively correlated with higher yield and is due to the lower glycogen content in the muscle tissues (Berri et al., 2005).

The glycogen content and low levels of phosphocreatine result in the white color of muscles. In the red-colored muscle fibers of quail breast, there are large deposits of phosphocreatine that provide the necessary energy for ATP recycling and their high capacity to supply oxygen through their mitochondria, resulting in slower aerobic glycolysis. Rigor mortis occurs, and a lower rate will be observed, thus resulting in a higher final pH (Velleman & McFarland, 2015).

Broiler meat color

The meat color measurements, shown in Table 3, were carried out using the trichromatic system adopted by the CIE, with three primary spectral colors: red, green, and indigo.

Table 3. The evaluation of broilers' meat parameters

Parameter		Breast			Thigh		
Estimated time	Group	L*	a*	b*	L*	a*	b*
Initial (T_0)	Control	39.187^{bcd}	-0.859^{b}	4.972^{b}	44.238^{ab}	-0.956	4.719
	Experimental	37.010^{d}	0.649^{a}	11.373^{a}	44.451^{ab}	4.543	2.400
24 h (T_{24})	Control	42.828^{ab}	0.172^{ab}	3.882^{bc}	45.531^{a}	-0.610	3.679
	Experimental	41.770^{abc}	0.036^{ab}	4.882^{b}	41.281bc	0.506	4.076
48 h (T_{48})	Control	38.421^{cd}	0.277^{ab}	2.222^{c}	39.641^{c}	-0.244	1.788
	Experimental	41.541^{abc}	1.089^{a}	5.809^{b}	42.742^{abc}	0.231	2.456
72 h (T_{72})	Control	41.191^{abc}	0.235^{ab}	2.008^{c}	41.554^{bc}	-0.147	1.029
	Experimental	43.415^{a}	-0.650^{b}	3.909^{bc}	44.496^{ab}	0.198	3.937
168 h (T_{168})	Control	40.822^{abcd}	-0.805^{b}	2.177c	41.187^{bc}	-0.404	0.992
	Experimental	41.408^{abc}	-0.757^{b}	6.321b	41.344^{bc}	-0.206	4.568
Multiple - effects							
Group							
Control		40.490	-0.196	3.052^{b}	42.430	-0.472	2.442
Experimental		41.029	0.073	6.459^{a}	42.863	1.054	3.488
SEM lot		0.384	0.114	0.260	0.334	0.593	0.918
Period							
Initial (T_0)		38.099^{b}	-0.105^{bc}	8.173^{a}	44.344^{a}	1.793	3.56
24 h (T_{24})		42.299^{a}	0.104^{ab}	4.382^{b}	43.406^{a}	-0.052	3.88
48 h (T_{48})		39.981^{ab}	0.683^{a}	4.016^{b}	41.192^{b}	-0.007	2.12
72 h (T_{72})		42.303^{a}	-0.208^{bc}	2.958^{b}	43.025^{ab}	0.026	2.48
168 h (T_{168})		41.115^{a}	-0.105^{c}	4.249^{b}	41.266^{b}	-0.305	2.78
SEM period		0.604	0.179	0.408	0.527	0.937	1.45
Interaction (p-Value)							
lot		0.321	0.096	0.000	0.361	0.070	0.422
period		0.000	0.000	0.000	0.000	0.512	0.905
period*group		0.013	0.000	0.000	0.000	0.223	0.631

The average values located on the same row, with different letters as superscripts, show significant differences $p < 0.05$.

The values obtained in the CIE space were determined by reading the Cartesian parameters (L*, a*, b*). Thus, when the parameters a* and b* are positive, the color of the sample will fall within the red-orange-yellow range; when the parameter a* is negative and b* is positive, the color of the sample will fall within the yellow-yellowish-green-green range; when the parameters a* and b* are negative, the color of the sample will fall within the green-turquoise-blue range; when a* is positive and b* is negative, the color of the sample will fall within the blue-purple-red range.

The luminosity axis L* is perpendicular to the axes a* (green/red) and b* (blue/yellow) and extends from the ideal black domain (L*=0), passing through the neutral point (gray) (N) (achromatic), to the ideal white (L*=100). The parameter L* refers to the brightness of the sample, a* to the intensity of the red color of the sample, and b* to the intensity of the yellow color of the sample. Each meat sample (breast, thigh) was measured three times, and the final values were obtained by calculating the average of the three measured values.

For the luminosity parameter L* measured at different storage periods at the breast level, distinct significant differences were recorded (p=0.000). The values recorded at 24 h, 72 h, 168 h are higher compared to the initial pH measured at T0h. Additionally, a significant interaction (p=0.013) between period and group can be observed for this parameter. For the parameter a* measured at different storage periods at the breast level, distinct significant differences were recorded (p=0.000) both for the period and the period*group interaction.

Based on the luminosity values (L*) measured in the breast samples, the authors (Lee et al. 2022) classified the color shades as follows: dark (L*<56), normal (L* 56-62), and pale (L*>62). More open values were observed in the E group for the luminosity parameter L* measured in breast samples, however without statistical significance (p>0.05). Distinct statistically significant higher values for L* (p=0.000) were recorded at intervals of 24 h, 72 h, 168 h. Also, in the case of breast samples, distinct statistically significant differences (p=0.000) were recorded for the parameter a* (negative values) max. at 72 h (-0.208) and positive values of the same parameter at 48 h (0.683), giving a yellowish hue to the sample. Distinct statistically significant differences (p=0.000) were also recorded for the group*period interaction, breast sample.

For the b* color parameter, distinct statistically significant differences (p=0.000) are recorded for group, period, as well as for the interaction between group*period. Thus, the b parameter, measured at the level of the breast sample, records significantly higher positive values in batch E (6.459) compared to C group (3.052). Once again, an increased value of the b* parameter indicates a higher intensity of the yellow color. For the L* parameter measured at the level of the thigh, distinct statistically significant differences (p=0.000) are observed in terms of period and the interaction between group*period. Maximum values were recorded initially at T0 (44.344), at T24 h (43.406), and at T72 h (43.025) compared to T48 h (41.192) and T168 h (41.266). For the other two colors parameters, a* and b* measured at the thigh, no significant differences found (p > 0.05).

The color of meat is generally chromatically correlated with the quantity of compounds containing heme, such as myoglobin in all its forms and variations, especially regarding hue, which can be caused by a variety of intrinsic and extrinsic factors affecting the content of heme pigment. (Boulianne & King, 1995). Furthermore, the color of meat is determined by the degree of light scattering through the microstructure of the meat and its easy modification depending on post-mortem muscular events (Hughes et al., 2020).

In the case of poultry meat, color is influenced to a lesser extent by the content of heme pigment but is affected by numerous other intrinsic factors (age at slaughter, sex, and genotype) and extrinsic factors (feed formula, rearing system) with a greater impact (Wideman et al., 2016).

For determining meat color, the luminosity parameter (L*) can represent a very important indicator of meat quality (poultry breast) for subsequent processing (Petracci et al., 2004). Generally, an L* value between 50 and 56 indicates normal values for poultry breast, while L* < 50 and L* > 56 present a darker or paler color of the poultry breast. In the present study, L* values were within the normal range, considering all investigated variables. In the literature, a negative correlation between pH and the L* parameter measured at the level of the breast has been reported in the case of darker poultry breast color. (Le Bihan-Duval et al., 1999). In the case of meat derived from poultry breast, the parameter a* (redness) is inversely correlated with the L* parameter and directly related to the pH level in the muscle tissues, so that a higher redness with higher values is generally recorded in darker-colored meat with a higher pH (6.53). Specialized literature has reported that the value of the b* parameter (yellow color) of poultry breast decreases concurrently with the L* parameter, while the pH value and the values of the a* parameter increase (Allen et al., 1997).

This means that chicken meat with a higher pH generally has a darker and redder color. On the other hand, paler meat with a lower pH records higher values of the b* parameter (Hughes et al., 2020).

In the literature, there are presented very different values of color parameters for poultry breast measured depending on age (Bianchi et al. 2014) focused on meat chickens from the native system and observed a decrease in L*, a*, and b* values when transitioning from light to heavy breeds (Bianchi et al., 2007; Bosco et al. 2014) observed an increase in L*, a*, and b* values in measurements taken on breast samples from chickens of different genotypes (aged from 70 to 81 days).

On the other hand, (Połtowicz & Doktor, 2013) noticed an increase in the value of the L* parameter and decreasing trends in the values of the a* and b* parameters in breast samples from laying hens (aged from 35 to 42 days).

CONCLUSIONS

During the different time storage periods, variations in physicochemical and organoleptic parameters were observed between the two groups, attributed to the dietary inclusion of xanthophyll sources which mitigated the PUFA’s oxidation. Importantly, the incorporation of flaxseed and alfalfa in broilers' diets did not adversely impact poultry meat quality across various storage periods.

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