

The effect of increased methionine in broiler chicken diets on the quality of breast muscles at different times of vacuum storage under refrigeration

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The aim of the study was to determine the effect of different levels of DL-methionine in feed for broiler chickens on the quality of vacuum-packed breast muscles stored under refrigeration. The material for the study was 72 breast muscles from 35-day-old broiler chickens fed diets with varying content of DL-methionine. The control group received a basal diet without additional DL-methionine, while the experimental groups received compound feeds supplemented with 0.08% or 0.24% DL-methionine. At 35 days of age, 24 birds from each group were randomly selected and experimentally slaughtered. After the carcasses were dissected, 8 right breast muscles were selected from each feeding group and analysed immediately after cooling. The remaining muscles were vacuum-packed and after 7 and 14 days of storage physicochemical and sensory analyses were performed to assess the influence of storage time on changes in their quality. The methionine level was not found to affect the sensory properties of the breast muscles, but the meat of birds receiving a higher level of this amino acid had better shear force and water-holding capacity in comparison to the control group. After just 7 days, the results of the analyses of vacuum-packed refrigerated muscles showed a negative effect of storage time on the capacity of the muscle tissue to bind water, as well as changes in the proportion of red colour in the muscles. However, it is worth noting that the sensory quality of the muscles deteriorated only after 14 days of storage.

KEY WORDS: broiler chickens / methionine / meat quality / vacuum packing / storage time

Methionine is the first limiting amino acid in the diet of chickens for slaughter, and its level determines that of all other amino acids in the diet. According to Drazbo et al. [8], an appropriate level of methionine in the diet increases assimilation of protein, which results in better rearing results. Both a surplus and a deficiency of methionine reduce the productivity of poultry. The amount of methionine and cystine in feed is often too low to ensure its most beneficial utilization and optimal quality of broiler carcasses. For this reason syn-

thetic methionine is commonly used, which in feeds takes the form of DL-methionine [29]. Amino acids may occur as D- or L-isomers, or as a mixture of these two products. D-isomers are biologically inactive, while the L form is available in most tissues [20]. However, chickens have been shown to be able to effectively convert D-methionine to L-methionine in the liver [2, 7, 35] and then use it for protein synthesis and as an element of other metabolic pathways. Ahmed and Abbas [1] and Wallis [36] found that increasing the methionine level in the diet results in increased fat content in the carcasses of broiler chicken. According to Hickling et al. [14], increased levels of methionine and lysine in the diet improve the yield of breast meat in birds, which may have economic benefits depending on the cost of amino acid supplementation and the price of poultry meat. Similar results were obtained by Drazbo et al. [8], who, in addition to better yield of breast meat, also noted improvement in some meat quality traits. An experiment by Gardzielewska et al. [13] showed that the use of methionine results in meat with a lower pH and darker colour, and increases the water-holding capacity of fresh meat and meat frozen for 4 months.

Recent years have seen greater consumer interest in fresh food, with the least possible processing and the longest possible shelf life [33]. Vacuum packing, i.e. the evacuation of air from packaging, effectively protects meat from the effects of air and microorganisms, thereby extending its shelf life [34]. The removal of oxygen protects the product against its adverse effects on organoleptic characteristics, such as colour, flavour and smell. Vacuum packing of fresh meat also significantly reduces drying out of meat [11, 12, 21].

As the available literature contains little research on the effect of methionine on the quality characteristics of poultry meat, the aim of the study was to determine the effect of different levels of this amino acid in the diet of broiler chickens on the quality of vacuum-packed breast muscles stored under refrigeration.

Material and methods

The research material consisted of 72 breast muscles from 35-day-old Ross 308 broiler chickens. The birds (297 in total) were divided into three experimental groups (11 replications of 9 chickens in each), kept in pens on litter and fed compound feeds with different levels of DL-methionine. The methionine content was 0.3% in the basal starter feed and 0.27% in the grower and finisher feeds. The control group (C) received a basal diet without DL-methionine, while the diet of the experimental groups was supplemented with a 0.08% ($DLM_{0.08}$) or 0.24% ($DLM_{0.24}$) addition of DL-methionine. The maximum level of supplementation was calculated so as not to overly exceed the recommended content of digestible methionine and cysteine, i.e. 0.86% and 0.77%, respectively. The nutritional value of the experimental diets, presented in Table 1, corresponded to the nutrient requirements of chickens defined in Poultry Nutrition Standards [31].

Table 1
Composition and nutritional value of starter and grower/finisher basal diets

Item	Starter	Grower/Finisher
Composition (%)		
Maize	36.25	31.58
Soybean meal, 48% CP	33.22	30.00
Wheat	23.18	28.15
Soybean oil	3.20	6.16
Dicalcium phosphate	1.70	1.82
CaCO ₃	1.21	1.17
Sodium chloride	0.34	0.35
L-lysine HCL	0.25	0.18
L-threonine	0.10	0.05
Vitamin-mineral premix	0.55	0.55
Nutritional value (%)		
Metabolizable energy (MJ/kg)	12.6	13.4
Crude protein	22.15	19.59
SID methionine	0.30	0.27
SID methionine+cysteine	0.61	0.54
SID lysine	1.20	1.04
SID threonine	0.78	0.68

SID – standardised ileal digestible

On day 35, 24 birds (3 x 24 = 72 individuals) were randomly selected from each group and experimentally slaughtered [30]. Immediately after slaughter, the carcasses were gutted and cooled in a cooling chamber at 4°C for 24 hours. After cooling, the carcasses were dissected and the breast muscles were used for further analysis.

Eight right breast muscles from each feeding group were selected and analysed immediately after cooling. To assess the effect of storage time on changes in quality, the remaining muscles ($3 \times 16 = 48$) were placed in heat-shrink PVDC/PP laminate packaging from PABEX, made of polyester and non-oriented polypropylene film with a high gas barrier, and then vacuum-packed in a model PP-15 single-chamber machine from TEPRO SA, using a -98% vacuum. Breast muscle quality was assessed after 7 and 14 days of storage in a FROST double-door refrigerator at $4^{\circ}\text{C} \pm 1^{\circ}$ and about 80% humidity. The following characteristics were analysed: pH_u (pH of muscle tissue in a water homogenate), shear force (tenderness), colour, drip loss, cooking loss, and water-holding capacity.

In order to determine the pH_u and water-holding capacity of the meat, muscle samples were ground three times and thoroughly mixed. The acidity was determined based on the pH of water homogenates (1:1 ratio of meat to distilled water) using a Double Pore electrode (Hamilton) and a 340i pH meter (WTW). A method proposed by Honikel [15] was used to determine drip loss and cooking loss, and water-holding capacity by Grau and Hamm's method as modified by Pohja and Niinivaara [26]. Muscle colour was characterized based on the L^* , a^* and b^* parameters in the CIELAB system [6]. For this purpose, three measurements were made using a MiniScan XE Plus apparatus (HunterLab) at various points of the muscle section under standard conditions, i.e. D_{65} and a 10° standard observer. Before each session, the apparatus was calibrated using standard black and white plates, as recommended by the manufacturer.

The shear force was measured after heat treatment using a Warner-Bratzler chamber attached to an INSTRON 5542 testing apparatus. The meat samples were prepared according to Honikel [15], wrapped in aluminium foil, stored at 4°C for 24 h, and then cut into cylinders (three cylinders with a diameter of 1.27 cm and a height of 2 cm). The maximum force needed to cut the samples was recorded [4].

The sensory properties of the breast muscles of the broiler chickens were determined for cooked samples in 0.62% NaCl solution at 96°C , according to the method described by Baryłko-Pikielna and Matuszewska [3]. The meat assessment was carried out by a 5-person panel with proven sensory sensitivity using a 5-point scale, including half-points [25]. For each trait, each point on the scale was assigned a quality definition: 1 – bad, 2 – inadequate, 3 – adequate, 4 – good, 5 – very good [5].

Two-way analysis of variance of the results was performed in Statistica 10.0 software [32]. Significance of differences between groups was determined using Duncan's test, for a significance level of $p = 0.05$.

Results and discussion

The physiochemical properties of the breast muscles are shown in Table 2. The methionine level in the feeds and the storage time significantly affected the tenderness of the bre-

ast muscles, which consumers regard as the basic feature of meat texture [24]. A reduction in the shear force and thus an improvement in tenderness was noted in the meat of broiler chickens receiving a 0.08% or 0.24% addition of methionine in the diet (p-value = 0.001). Significant improvement in this characteristic was also noted with increased storage time under vacuum conditions (p-value = 0.001). These results differ from those reported by Kondratowicz [17], who found that as storage time increased, whether in an atmosphere of controlled gases or in atmospheric air, the tenderness of the muscles of broiler chickens deteriorated significantly. The statistical analysis showed a significant interaction between the factors (p-value = 0.002), with the best tenderness shown in the muscles of chickens receiving the 0.08% methionine supplement in the diet, stored in vacuum conditions for a period of 14 days. It should be emphasized, however, that the shear force of all muscles analysed, irrespective of the methionine level and storage time, was characteristic of soft and tender meat.

Both the 0.08% and 0.24% addition of methionine to the diet of broiler chickens caused a significant improvement in the water-holding capacity of the breast muscles compared to the control (p-value = 0.022). A study by Edens [9] also showed that the addition of selenium and methionine to the diet can reduce water loss in meat. Storage time, on the other hand, had a negative effect on the water-binding capacity of the protein structure of the muscle tissue; the values obtained after 7 and 14 days were similar and significantly worse than those obtained in fresh muscles (p-value = 0.001). These results are supported by a study by Krala [19], which showed that the water-holding capacity of chicken meat deteriorated as storage time increased. According to Orkusz [24], the water-holding capacity of meat is lowest at the isoelectric point of muscle proteins (pH in the range of 5.1-1.3). The acidity (pH) of meat has a major impact on meat quality, because it determines characteristics responsible for suitability for processing, nutritional properties and shelf-life [38]. At slaughter, the pH of muscle tissue is close to neutral, and then it decreases as a result of glycolysis. The drop in post-mortem pH is one of the most important changes in the ageing of muscle tissue, due to its effect on the texture, colour and water-binding capacity of meat [10]. The changes in pH lead to an increase in water-holding capacity, and consequently to an increase in drip loss and cooking loss. In our study, the pH_u measured in the water homogenate of meat was closest to the isoelectric point (5.84) in the breast muscles of the chickens that had received the highest level of methionine in their feed. These muscles had the best water-holding capacity, while the drip loss and cooking loss were similar in all feeding groups. On the other hand, a significant increase in cooking loss was noted after 14 days of storage (p-value = 0.050). According to Pospiech [28], the drip loss of meat of good quality should range from 2% to 5%, so it should be stressed that the water-holding capacity indicated that all analysed breast muscles were of very good quality, including the vacuum-packed muscles refrigerated for 14 days.

Table 2

Physicochemical properties of vacuum-packed breast muscles depending on methionine level (Me) and storage time (CP)

Group, factor	Shear force (N)	pH _u	Drip loss (%)	Cooking loss (%)	Water-holding capacity (cm ²)	Colour		
						L*	a*	b*
Me ₀ CP ₀	11.9	5.91	2.44	29.2	1.47	63.4	6.50	14.8
Me ₀ CP ₇	10.0	5.86	1.71	31.8	2.70	61.5	6.09	21.8
Me ₀ CP ₁₄	8.71	5.94	2.98	30.4	2.94	62.9	5.82	13.6
Me _{0,08} CP ₀	9.12	5.89	2.29	31.8	0.69	62.5	6.21	14.4
Me _{0,08} CP ₇	8.40	5.90	2.61	30.3	2.52	63.0	5.02	13.7
Me _{0,08} CP ₁₄	5.75	5.91	3.00	29.0	1.70	60.9	5.58	14.5
Me _{0,24} CP ₀	8.31	5.87	2.29	28.4	0.75	62.1	6.86	14.8
Me _{0,24} CP ₇	7.75	5.79	2.20	30.7	2.08	60.6	5.50	16.1
Me _{0,24} CP ₁₄	8.75	5.85	2.67	29.7	1.69	61.3	5.61	15.5
SEM	0.254	0.012	0.123	0.333	0.15	0.296	0.123	0.757
Me level								
0,00		5.90 ^a	2.38	30.4	2.37 ^a	62.6	6.13	16.7
0,08		5.90 ^a	2.63	29.9	1.64 ^b	62.2	5.61	14.2
0,24		5.84 ^b	2.39	29.6	1.51 ^b	61.3	5.99	15.4
P		0.037	0.620	0.571	0.022	0.208	0.151	0.411
Storage time								
0	9.78 ^a	5.89	2.34 ^{ab}	29.3	0.97 ^b	62.7	6.52 ^a	14.7
7	8.73 ^b	5.85	2.17 ^b	30.9	2.43 ^a	61.7	5.54 ^b	17.2
14	7.74 ^c	5.90	2.89 ^a	29.7	2.11 ^a	61.7	5.67 ^b	14.5
P	0.001	0.196	0.050	0.126	0.001	0.292	0.001	0.295
Me x CP ¹	0.002	0.637	0.607	0.537	0.745	0.297	0.532	0.268

P denotes p-value

¹P-value of methionine level × storage time interaction

The methionine level was not shown to affect the individual colour components of the muscles. This is in contrast with a study by Gardzielewska et al. [13], in which a 0.13% and 0.24% addition of methionine caused (besides a decrease in acidity and water-holding capacity in the breast muscles) an increase in red colour and thus a darker muscle

colour than in the control group. Wang et al. [37] also found that methionine content significantly affected the lightness of chicken breast muscles and increased the share of red colour. According to the authors' research, the use of methionine in combination with selenium reduces protein oxidation, which to a certain degree ensures the stability of meat colour. On the other hand, prolonged storage of meat may result in the accumulation of metmyoglobin, leading to adverse changes in meat colour [39]. Interestingly, in our study vacuum-packed, refrigerated muscles had significantly less red colour than fresh muscles (p-value = 0.001). According to Kondratowicz [17], the removal of oxygen from the environment surrounding meat during storage protects haem pigments from oxidation. However, the results of our own research suggest that despite the use of vacuum-packing, the remaining oxygen molecules in the packaging may have affected the colour of the muscles.

The addition of methionine to feed for broiler chickens had no significant effect on the sensory quality of the breast muscles, except for the intensity and desirability of the aroma, which suggests an interaction of this factor with the storage time under vacuum conditions (p-value = 0.001 and p-value = 0.003, respectively). The aroma of fresh meat from chickens receiving a 0.08% supplement of the amino acid was rated highest. According to Zhai et al. [40], an increased level of methionine in combination with lysine may favourably affect characteristics associated with the acceptability of the product for the consumer.

The results of our own research showed a significant effect of storage time on most of the sensory quality parameters of vacuum-packed muscles. Interestingly, the panel noted a significant deterioration in muscle tenderness and juiciness after 14 days of storage (p-value = 0.001). This is puzzling, because the tenderness assessment of the same muscles based on shear force suggested better results (Table 3). Tenderness is an important sensory feature that has a major impact on the consumer's perception of the product. This sensory impression depends mainly on the age of the animals, the collagen content in the muscles, and its solubility [16]. According to Baryłko-Pikielna and Matuszewska [3], the tenderness of muscles corresponds to juiciness. The deterioration of juiciness after 14 days of storage may have been the result of the increased drip loss noted in the present study.

The palatability of meat is a combination of its aroma and taste. This sensory effect is created by volatile and non-volatile compounds that occur as natural components of fresh meat, as products produced during meat storage and ageing, and as compounds released during cooking [23]. According to Połtowicz et al. [27], the meat of broiler chickens has a light flavour and aroma profile, which is strengthened by heat treatment. Our research indicates that the storage time had a significant effect on the intensity of the aroma of the muscle samples. Muscles stored for 14 days received the lowest score,

Table 3

Sensory properties (points) of vacuum-packed breast muscles depending on methionine level (Me) and storage time (CP)

Group, factor	Tenderness	Juiciness	Aroma		Palatability	
			intensity	desirability	intensity	desirability
Me ₀ CP ₀	4.44	4.06	3.75 ^d	3.75 ^c	3.88	3.88
Me ₀ CP ₇	4.00	4.00	4.44 ^a	4.31 ^{ab}	4.25	4.13
Me ₀ CP ₁₄	3.75	3.56	3.94 ^{bcd}	4.00 ^{bc}	3.69	3.63
Me _{0,08} CP ₀	4.31	4.19	4.38 ^a	4.38 ^a	4.19	4.19
Me _{0,08} CP ₇	4.25	4.25	4.00 ^{bcd}	4.06 ^{abc}	4.19	3.94
Me _{0,08} CP ₁₄	3.75	3.56	3.94 ^{bcd}	3.94 ^{bc}	3.88	3.69
Me _{0,24} CP ₀	4.00	3.69	4.19 ^{ab}	4.19 ^{ab}	3.75	3.75
Me _{0,24} CP ₇	4.25	4.50	4.13 ^{abc}	4.00 ^{bc}	4.25	4.00
Me _{0,24} CP ₁₄	3.63	3.44	3.81 ^{cd}	3.94 ^{bc}	3.94	3.81
SEM	0.057	0.069	0.042	0.043	0.053	0.055
Me level						
0	4.06	3.88	4.04	4.02	3.94	3.88
0,08	4.10	4.00	4.10	4.13	4.08	3.94
0,24	3.96	3.88	4.04	4.04	3.98	3.85
P	0.467	0.600	0.693	0.521	0.477	0.807
Storage time						
0	4.25 ^a	3.98 ^a	4.10 ^a	4.10	3.94 ^b	3.94
7	4.17 ^a	4.25 ^a	4.19 ^a	4.13	4.23 ^a	4.02
14	3.71 ^b	3.52 ^b	3.90 ^b	3.96	3.83 ^b	3.71
P	0.001	0.001	0.003	0.175	0.006	0.058
Me x CP ¹	0.256	0.110	0.001	0.003	0.340	0.317

P denotes p-value

¹P-value of methionine level × storage time interaction

but it was only 0.3 percentage points lower than for the other samples. As mentioned above, there was also an interaction of this factor with the level of methionine in the diet. According to Kondratowicz et al. [18], the deterioration in the aroma may be due to the start of the microbial process of meat spoilage. The sensory analysis also showed

the best intensity of palatability for the muscles stored in vacuum-packaging for 7 days (p -value = 0.006); longer storage significantly worsened this feature. The results are supported by research by Marusiak and Michalska-Požoga [22], who found a negative impact of storage time on the sensory characteristics of poultry meat stored in a vacuum under refrigeration conditions.

To sum up, the results of the study showed that a higher level of methionine in the diet of broiler chickens improved muscle tenderness expressed as shear force and water-holding capacity, but did not affect the sensory quality parameters of the meat. Despite the use of vacuum-packaging, the storage time negatively affected the water-binding capacity of the muscle tissue, and also reduced the proportion of red colour in the muscles. Analysis of the impact of storage time on the sensory quality of the meat showed a significant deterioration in most features after 14 days of storage. It is worth noting, however, that after 7 days of storage, the sensory evaluation of the vacuum-packed muscles was comparable to that of fresh muscles. In summary, the use of vacuum-packing preserves most of the quality characteristics of breast muscles refrigerated for 7 days.

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