*poultry*

MDPI

*Article*

# Effects of Varying Levels of Dietary DL-Methionine Supplementation on Breast Meat Quality of Male and Female Broilers

**Anthony Pokoo-Aikins [1,*], Jennifer Rumsey Timmons [2], Byungrok Rok Min [2], William Robert Lee [3], Samuel Njoroge Mwangi [4], Callie Megan McDonough [1] and Chongxiao Chen [5]**

[1] US National Poultry Research Center, Toxicology & Mycotoxin Research Unit, USDA, ARS, Athens, GA 30605, USA; callie.mcdonough@usda.gov
[2] Food and Agricultural Sciences Program, Department of Agriculture, Food and Resource Sciences, University of Maryland Eastern Shore, Princess Anne, MD 21853, USA; jtimmons1@umes.edu (J.R.T.); bmin@umes.edu (B.R.M.)
[3] Maple Leaf Farms Inc., P.O. Box 167, Leesburg, IN 465384, USA; billlee@mapleleaffarms.com
[4] Department of Agriculture, School of Agriculture and Applied Sciences, Alcorn State University, Lorman, MS 39096, USA; smwangi@alcorn.edu
[5] Prestage Department of Poultry Science, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC 27695, USA; sean_chen@ncsu.edu
* Correspondence: anthony.pokoo-aikins@usda.gov; Tel.: +1-334-552-1494

**Abstract:** This study evaluated the effects of feeding varying levels of dietary DL-methionine (MET) supplementation on breast meat (BM) quality of broilers of different sex. The 1-day-old, sexed chicks (Ross 708, 1552) were randomly allocated into four groups (each with 4 replicates) and were raised with diets supplemented with 0, 0.5, 1, or 2 g MET/kg of feed to a common weight (2.72 kg). Color, pH, drip loss (DL), water-holding capacity, moisture uptake, cooking yield (CY), texture, total antioxidant capacity (TAC), and lipid oxidation (LO) were determined using BM samples harvested 24 h postmortem. The male BM had higher redness, TAC, firmness, and toughness but lower yellowness ($p < 0.01$) than the female BM. In both sexes, birds fed 0.5 g/kg MET had lower DL ($p < 0.01$) than those fed 1 and 2 g/kg MET. For storage up to 3 days, MET suppressed LO in cooked BM ($p < 0.01$) and suppression increased as MET increased. CY for 1 and 2 g/kg MET were higher ($p < 0.01$; 79.04 and 78.60%, respectively) than CY for 0 and 0.5 g/kg MET (66.18 and 68.03%, respectively). These results suggest that MET supplementation at 1 g/kg or higher for broilers can improve oxidative stability, muscle functionality, and breast meat CY.

**Keywords:** DL-methionine; amino acid; meat quality; raw meat; cooked meat



**Citation:** Pokoo-Aikins, A.; Timmons, J.R.; Min, B.R.; Lee, W.R.; Mwangi, S.N.; McDonough, C.M.; Chen, C. Effects of Varying Levels of Dietary DL-Methionine Supplementation on Breast Meat Quality of Male and Female Broilers. *Poultry* **2022**, *1*, 40–53. https://doi.org/10.3390/poultry1010005

Academic Editor: Ilias Giannenas

Received: 24 February 2022
Accepted: 16 March 2022
Published: 17 March 2022



## 1. Introduction

The poultry industry has undergone many changes in recent years in response to increasing demand for both meat and eggs [1]. As a result, nutritionists and geneticists have performed extensive work to improve bird weight, maturity time, growth rate, feed efficiency, and size of breast meat [1]. These changes have affected muscle formation and the chemistry of the modern broiler meat and consequently meat quality traits. Consumers demand high-quality meat, which is often associated with and defined by personal preference of color, texture, and other sensory properties [2]. Proper nutrition of birds has an important effect on meat quality and safety [3]. Both diet and specific feed ingredients influence the color and texture of meat [4,5].

One feed additive that can affect meat quality is methionine (MET). MET supplementation improves carcass traits of chicken [6–8]. MET supplementation can affect shear force, drip loss, pH, and color [9]. For example, pH, water-holding capacity, meat yield, and meat color were improved by MET [9,10]. Other researchers also reported improved pH with

MET supplementation [11,12]. MET supplementation also decreased drip loss [9,12,13] and shear force [13]. Increasing MET levels in broilers' diets increased yellowness (b*) and decreased redness (a*) [3,9]. Increased lightness and redness in broiler breast muscles have also been reported with increased MET supplementation [13]. Consumers prefer meat to be light in color and to have a pinkish to red appearance [14].

Several studies have shown improved oxidative stability with MET supplementation [9,11,15]. According to Fikre [16], incorporating methionine into poultry diets has been shown to improve the oxidative stability of the meat, increase meat color stability, and decrease drip loss.

Although many factors define meat quality, consumers attach great importance to color and texture [2]. Consumers rely heavily on the visual appeal of the product to judge if it is fresh, wholesome, and flavorful [3]. Financial losses incurred from the loss of product and loss of valuable shelf space for unacceptable products result in decreased profits [9]. Therefore, meat producers need to focus on factors that impact important meat quality attributes.

Based on the aforementioned studies, the use of dietary methionine (MET) could be one way to overcome meat quality issues. Methionine is an amino acid, containing sulfur, that is considered essential as poultry cannot synthesize it in great quantity to maintain bodily functions [17]. Methionine is known to improve poultry growth because it takes part in protein synthesis and may influence the functional properties of muscle/protein [10]. The purpose of this study was to evaluate the impact of various levels of DL-methionine on meat quality parameters of broilers of different sex. This study is important because, in addition to investigating the effect of varying MET levels and no added methionine, it also looks at both sexes. Many studies involving dietary methionine supplementation and meat quality look at male broilers only [9,12,18,19]. We also investigated the impact of sex because the poultry industry utilizes both sexes when producing meat and the differences can impact several market factors.

## 2. Materials and Methods

This study was conducted in accordance with the recommendations and guidelines of the University of Maryland Eastern Shore Institutional Animal Care and Use Committee (AUP-Timmons-2021-4).

### *2.1. Experimental Design, Birds, Diets, and Housing*

A total of 1552 sex-sorted Ross 708 chicks were used in this study. The experimental design was a randomized complete block with a 2 × 4 factorial arrangement of treatments (TRTs) (2 sexes × 4 MET levels) with four replicates per TRTs. Birds were placed in 32 pens with approximately 1 $ft^2$ per bird and raised on pine shaving bedding. Each pen was blocked based on room location and considered an experimental unit. Broilers were allocated at hatch to respective pens and fed one of the four MET diets in a three-phase feeding program (0–21, 22–35, and 36–46/48 days of age). The dietary treatments were 0 g (0%), 0.5 g (0.05%), 1.0 g (0.1%) and 2.0 g (0.2%) MET per kg of feed. Birds fed the 0 and 0.5 g MET/kg diets were raised to 48 d of age, while the birds fed the 1.0 and 2.0 g MET/kg diets were raised to 46 d of age, to ensure all birds reached a common target weight of 2.72 kg (6 lb). The basal corn-soybean meal diet was formulated to meet or exceed the National Research Council [20] requirement for maximum growth performance, except MET. The MET levels (0, 0.5, 1, and 2 g MET/kg of feed) chosen in this study represented levels above, same as, and below the National Organic Standards Board recommended MET levels [21].

Chicks were group-weighted by pen before placement. Birds were fed diets with DL-methionine (MET) (MetAMINO, 99%, Evonik Degussa GmbH, Hanau-Wolfgang, Germany) until they reached a target weight. The three feed phases used in this study were the starter phase (0–21 days), the grower phase (22–35 days), and the finisher phase (36–46 or 36–48 days). Ingredients, calculated nutrients, and analyzed nutrients in the three-phase

feed are presented in Table 1. Analyzed diets for the basal and MET treatments for the starter, grower, and finisher diets are presented in Table 2.

**Table 1.** Ingredients in the basal diets in the three-phase feed.

| Ingredient | Day 0–21 (Starter) (%) | Day 21–35 (Grower) (%) | Day 35–46/48 (Finisher) (%) |
|---|---|---|---|
| Corn | 47.78 | 53.96 | 60.66 |
| Soybean Meal | 46.75 | 39.80 | 33.35 |
| Filler [1] | 0.20 | 0.20 | 0.20 |
| Vegetable Oil | 2.55 | 3.40 | 3.75 |
| Defluorinated Phosphate | 1.96 | 1.64 | 1.07 |
| Limestone | 0.44 | 0.51 | 0.51 |
| Slat | 0.15 | 0.21 | 0.29 |
| Betaine 32% | 0.00 | 0.09 | 0.05 |
| Trace Minerals [2] | 0.05 | 0.06 | 0.06 |
| DL-Methionine | 0.00 | 0.00 | 0.00 |
| Vitamin Premix [3] | 0.05 | 0.05 | 0.03 |
| Enzyme [4] | 0.05 | 0.05 | 0.00 |
| Calsporin [5] | 0.02 | 0.03 | 0.03 |

[1] Filler: Sand; the filler levels were varied in different treatments (0 g MET/kg of feed had 0% added MET and 0.2% filler, 0.5 g MET/kg had 0.05% added MET and 0.15% filler, 1 g MET/kg of feed had 0.10% added MET in the feed and 0.10% filler, 2 g MET/kg of feed, had 0.20% MET in the feed and 0% filler). [2] Mineral premix per kilogram of diet: 0.264 mg Se as $Na_2SeO_3$; 12.80 mg Cu as $CuSO_4.5H_2O$; 0.24 mg I as $KIO_3$; 106.67 mg Fe as $FeSO_4$-$H_2O$; 81.36 mg Mn as $MnSO_4$-$H_2O$. Determined by analysis of duplicate samples. [3] Vitamin premix per kilogram of diet: 11,025 I.U. vitamin A, 3528 I.U. vitamin D3, 33 I.U. vitamin E, 0.91 mg vitamin K, 2 mg thiamin, 8 mg riboflavin, 55 mg niacin, 18 mg Ca pantothenate, and 5 mg vitamin B. [4] Enzyme refers to endo-1,3(4)-beta-glucanase and endo-1,4-beta-xylanase, used as a feed additive for chickens. [5] Calsporin refers to Bacillus subtilis C-3102 probiotic that is beneficial to gut health.

**Table 2.** Analyzed starter (Str), grower (Gr), and finisher (Fin) diets and MET dietary treatments.

| Chemical Composition | 0 g MET/kg | | | 0.5 g MET/kg | | | 1.0 g MET/kg | | | 2.0 g MET/kg | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Str | Gr | Fin | Str | Gr | Fin | Str | Gr | Fin | Str | Gr | Fin |
| Crude Protein (%) | 26.49 | 22.2 | 20.57 | 26.09 | 22.89 | 21.13 | 25.61 | 22.24 | 21.11 | 25.54 | 23.73 | 20.36 |
| Moisture (%) | 12.38 | 12.62 | 12.44 | 12.16 | 12.83 | 12.42 | 12.31 | 12.75 | 12.52 | 12.45 | 12.68 | 12.58 |
| Fat (Crude) (%) | 4.9 | 5.88 | 6.26 | 4.77 | 5.75 | 6.41 | 5.07 | 5.78 | 6.32 | 4.74 | 5.90 | 6.45 |
| Fiber (Crude) (%) | 2.58 | 2.56 | 2.24 | 2.90 | 3.05 | 1.88 | 2.85 | 2.22 | 2.31 | 2.995 | 2.33 | 2.22 |
| Ash (%) | 6.015 | 5.35 | 4.80 | 6.03 | 5.35 | 4.86 | 6.02 | 5.46 | 4.03 | 5.97 | 5.18 | 4.54 |
| ME Kcal/kg | 3011 | 3075 | 3131 | 3000.5 | 3044 | 3150 | 3012.5 | 3074 | 3156 | 2987.5 | 3088 | 3146 |
| Sulfur (%) | 0.23 | 0.20 | 0.19 | 0.24 | 0.22 | 0.196 | 0.25 | 0.23 | 0.214 | 0.27 | 0.25 | 0.238 |
| Met (%) | 0.37 | 0.31 | 0.33 | 0.40 | 0.38 | 0.33 | 0.44 | 0.39 | 0.39 | 0.53 | 0.47 | 0.43 |
| Cys (%) | 0.32 | 0.36 | 0.26 | 0.42 | 0.32 | 0.30 | 0.40 | 0.32 | 0.28 | 0.39 | 0.28 | 0.31 |
| Lys (%) | 1.46 | 1.42 | 1.21 | 1.45 | 1.41 | 1.26 | 1.48 | 1.41 | 1.28 | 1.47 | 1.30 | 1.27 |

### *2.2. Feed and Feed Analyses*

Feed samples were analyzed in the lab using the AOAC 994.12/985.28 method for amino acid profile (including, methionine, cysteine, and lysine) and the AOAC 923.01 method for sulfur. Energy (calories) was analyzed using a modified Atwater calculation, consisting of carbohydrates by difference and by the following tests: Ash AOAC 942.05, fat AOAC 920.39, fiber AOAC 978.10, moisture AOAC 930.15, and protein AOAC 990.03. The basal diet was provided by a commercial poultry feed mill.

### *2.3. Sample Collection and Processing*

At the end of this study (days 46 and 48), 320 birds were randomly selected (10 birds per pen), weighed, and wing banded for processing. At 12 h prior to processing, the feed was removed, but water access was continued. Birds were euthanized and processed using commercial processing methods. Whole eviscerated carcasses without giblets (WOG) were drained and chilled for about 2 h in a static chiller with ice at 1 °C. The carcasses were

packed into an icebox with ice and stored in a walk-in refrigerator at 5 °C overnight. The WOGs were weighed and deboned, and then the breast fillets were collected from the right pectoralis muscle. Breast samples were individually put into zip-lock bags, placed on ice, and sent to the meat lab to determine various meat quality parameters, including color, pH, drip loss, water-holding capacity (WHC), moisture uptake, texture, cooking yield, total antioxidant capacity (TAC), and lipid oxidation (LO).

### *2.4. Meat Quality Analysis*

#### 2.4.1. Color

The surface color of breast meat was determined using a colorimeter (Minolta Chroma Meter CR-400, Minolta Italia S.p.A., Milano, Italy) with illuminant D65, a 25 mm aperture, and a 2° standard observer. The instrument was calibrated against white reference tiles prior to use. Commission Internationale de I'Eclairage (CIE) L* (lightness), a* (redness), and b* (yellowness) values were recorded.

#### 2.4.2. pH

pH values were recorded in the right breast by sticking the pH meter (Testo 205 pH Probe, Testo Limited, Hampshire, UK) probe into the center of the breast muscle 0.5–1 cm below the surface of the muscle for about two minutes. Color and pH were measured in triplicates at 3 points for each sample and the average values were obtained.

#### 2.4.3. Drip Loss (DL)

The cranial section of the pectoralis muscle was cut, weighed (approximately 100 g), put in individual zip-lock bags, and placed in a 4 °C refrigerator for 48 h. Samples were dried by a paper towel before initial weights were recorded. After storage, the samples were individually drained, dried with a paper towel, and re-weighed. Drip loss was calculated as the difference between final and initial weights and expressed as a percentage of the initial weight (Equation (1)):

$$\text{Drip Loss } (\%) = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100 \quad (1)$$

#### 2.4.4. Water-Holding Capacity (WHC)

WHC was measured using a centrifugal method described by Zhang et al. [22]. A total of 15 g of ground breast samples were weighed in a centrifuge tube and centrifuged at 40,000$\times$ $g$ for 10 min. Subsequently, the supernatant was carefully removed, and the remaining solid portion was weighed. WHC was expressed as the percentage of water loss (Equation (2)):

$$\text{WHC } (\%) = \frac{\text{initial sample weight} - \text{sample weight after centrifugation}}{\text{initial sample weight}} \times 100 \quad (2)$$

#### 2.4.5. Moisture Uptake

Moisture uptake ability of breast meat was determined according to Van Laack et al. [23] with some modification. Briefly, the ground breast sample (6 g) was vigorously mixed with 10 mL of 3.5% NaCl solution in a test tube for 15 s. The mixture was incubated at 25 °C for 30 min and then centrifuged at 3000$\times$ $g$ for 15 min. Afterward, the test tube was thoroughly drained, and the pellet weight was recorded. Moisture uptake (as a percentage) was calculated as follows (Equation (3)):

$$\text{Moisture Uptake } (\%) = \frac{[(\text{weight pellet } + \text{tube}) - \text{weight tube} - 6.00]}{6.00} \times 100 \quad (3)$$

2.4.6. Cooking Yield

The cranial section of breast meat was cut, weighed at approximately 90 g, and packaged in a zip-lock bag. The sample was cooked in a boiling water bath until the internal temperature reached 75 °C. Cooked samples were cooled for 1 h in a refrigerator at 4 °C to allow the temperature to equilibrate prior to weighing. Excess liquid was removed from the meat by patting with a paper towel. Subsequently, the cooked sample was weighed. Cooking yield is calculated as the ratio of cooked weight to raw weight and expressed as a percentage as follows (Equation (4)):

$$\text{Cooking Yield}(\%) = \frac{\text{final weight}}{\text{initial weight}} \times 100 \quad (4)$$

2.4.7. Shear Force and Texture Profile Analysis (TPA)

The cooked breast meat samples prepared for cooking yield were utilized to determine textural properties, including shear force and texture profile.

Shear force was determined using a texture analyzer (Model TAXT2i, Texture Technologies Corp., Scarsdale, NY, USA) with a Warner-Bratzler shear blade. The sample was cut into a rectangular box shape with 10 × 15 × 25 mm in dimension. The blade traveled through the sample at a speed of 100 mm/min. Shear force (kgf) was defined as the maximum force required to shear the sample and determined as the peak force. Firmness (kgf·sec) was defined as the energy required to shear the sample and determined by calculating the area under the force-time curve.

Textural Profile Analysis (TPA) was conducted using the texture analyzer with an aluminum plunger of diameter 90 mm in accordance with the procedure documented by Min and Green [24]. Cooked breast samples were cut into a cylindrical shape with 18 mm in height and 30 mm in diameter. Each sample was compressed at a crosshead speed of 60 mm/min through two cycles with 80% compression of the initial height. Between the two cycles, a 5 s rest period was given to allow the sample to recover its height. The TPA curve was used to calculate hardness, cohesiveness, chewiness, and resilience as defined by Bourne [25].

2.4.8. Total Antioxidant Capacity (TAC)

Antioxidant compounds were extracted from meat samples according to Prior et al. [26] and then the extracts were used for the oxygen radical absorbance capacity (ORAC) assay to determine the total antioxidant capacity (TAC) of the samples. In brief, breast meat (5 g) was mixed with 15 mL of deionized distilled water (DDW). The mixture was homogenized for 15 s and centrifuged at 15,000× *g* for 20 min. The supernatant was filtered through Whatman No. 1 filter paper and collected into a 15 mL tube. The filtrate (400 µL) was used to prepare the meat extract containing antioxidant compounds. The ORAC value of each meat extract sample was determined following the procedure by Min et al. [27]. The ORAC values were calculated by using a linear regression equation (y = ax + b) between a series of Trolox standards (6.25 to 50 µM; x) and the net area under the fluorescence decay curve (net AUC; y). The area under the curve (AUC) was calculated as (Equation (5)):

$$\text{AUC} = \left(0.5 + \frac{f_2}{f_1} + \frac{f_3}{f_1} + \frac{f_4}{f_1} + \cdots + \frac{f_i}{f_1} + \cdots + \frac{f_{35}}{f_1}\right) \times \text{CT} \quad (5)$$

where $f_1$ = the initial fluorescence reading, $f_i$ = the fluorescence reading at cycle i, and CT = cycle time in minutes.

The net AUC was calculated by subtracting the AUC of the blank from the AUC of the sample or standard. The ORAC was expressed as µmol Trolox equivalents (TE)/g meat.

2.4.9. Lipid Oxidation (LO)

Development of lipid oxidation was determined in raw and cooked breast samples during 10 and 7 days of storage, respectively, in a 4 °C refrigerator, using 2-thiobarbituric

acid reactive substances (TBARS) analysis according to Min and Green [24]. Meat samples (5 g) were mixed with 15 mL DDW and 100 uL butylated hydroxytoluene (BHT) solution (6% in 100% ethanol, $w/v$). The mixture was homogenized for 15 s. A volume of 1 mL of the homogenate was mixed with 2 mL TBA/TCA solution (20 mM TBA/15% trichloroacetic acid [TCA; $w/v$]). The mixture was incubated in a boiling water bath for 15 min. The mixture was cooled and then centrifuged at 3000× $g$ for 15 min. The mixture's absorbance was determined at 531 nm against the reagent blank. Lipid oxidation (LO) values were calculated using the molecular extinction coefficient ($1.56 \times 10^5$ $M^{-1}cm^{-1}$) of malondialdehyde (MDA) and expressed as mg MDA/kg meat.

### *2.5. Statistical Analysis*

The meat quality-dependent variables were meat pH, color, drip loss, WHC, cooking loss, moisture uptake, TPA, TAC, and TBARS. All data were analyzed using the General Linear Models procedure for analysis of variance (ANOVA) (STATISTIX version 10.0). A randomized complete block design with a 2 × 4 factorial arrangement of treatments (2 sexes × 4 methionine levels) and 4 replicates per treatment was used. Mean differences were determined using Tukey's all-pairwise comparison test. The significance level was set at $p \leq 0.05$.

## 3. Results and Discussion

### *3.1. Color and pH*

The effects of dietary MET supplementation on meat color and pH are provided in Table 3. The lightness (L*) and yellowness (b*) of the breast meat were not significantly affected ($p > 0.05$) by the dietary MET levels. The results for L* and b* are consistent with those of Khan et al. [28]. The redness (a*) of the meat from the birds fed the 1 and 2 g MET/kg diets was lower ($p < 0.01$) than the redness of the meat from birds fed the 0 g MET/kg diet (2.22, and 2.04 vs. 2.71, respectively). Meat redness is similar to findings reported in other studies where researchers reported that MET supplementation in birds led to a lower hemoglobin concentration, leading to lighter breast meat color [28–30]. Higher concentrations of oxymyoglobin are responsible for meat redness and when myoglobin is oxidized to metmyoglobin, there is a reduction in meat redness [28,31]. Bird age could also be a contributing factor to meat redness. The birds fed the 0 and 0.5 g MET/kg diets were raised for two extra days than birds fed the 1 and 2 g MET/kg diets to reach the target live weight. Myoglobin, the primary muscle pigment, tends to increase with age in chickens [32].

The sex of the bird also affected the meat color of the breast meat. Male broilers had a more reddish (a*) meat color ($p < 0.01$) compared to the meat color of the female broilers (2.55 vs. 2.11). Meat redness is related to the myoglobin content and its chemical state in meat [33]. Fouad and El-Senousey [34] concluded that male turkeys exhibited a significantly higher myoglobin concentration than females. More activity leads to more myoglobin in the muscle, giving the muscle a reddish color. The color of meat is dependent upon the concentrations of myoglobin and hemoglobin [33]. The yellowness (b*) of the meat from the female broilers was higher ($p < 0.01$) compared to that from the male broilers (8.29 vs. 7.42). This could be because female broilers tend to deposit more fat than male broilers [34].

The ultimate pH of the meat samples from the broilers fed 0, 0.5, 1, and 2 g MET/kg of feed were 5.86 5.88, 5.83, and 5.82, respectively. Birds fed diets supplemented with 0.5 g MET/kg of feed had a significantly higher carcass pH ($p < 0.05$) than those fed 2 g MET/kg of feed. Higher muscle pH is associated with darker meat and when comparing the pH and L*, a*, b* values for birds fed 0.5 and 2 g MET/kg of feed, those with higher pH values tended to have higher L*, a*, and b* values. However, the carcass pH from the birds fed the 2 g MET/kg dietary treatment was not different from the carcass pH from the birds fed the 0 and 1 g MET/kg dietary treatments. The pH range observed in this study is comparable to the pH reported in the existing literature [12,28]. Multiple studies have

reported that high pH (over 6.2) and low pH values (below 5.8) can negatively influence meat quality (dark, firm, and dry (DFD) vs. pale, soft, exudative (PSE), respectively) [33]. The pH values obtained fall between the reported high and low pH values and may be an indicator of good meat quality. Although some of the pH values in this study were significantly different, they were within the pH range acceptable for meat quality. Fresh broiler muscle pH can vary between 5.96 and 6.18 [33]. It has been suggested that the ultimate pH of the breast meat is negatively related to the muscle glycogen stores at the time of death [33].

**Table 3.** The effects of varying levels of DL-methionine (MET) in feed on color, pH, and shelf life of breast meat from broiler chickens [1].

| | Meat Color and pH | | | | Meat Quality (Shelf Life) | | | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | **L*** | **a*** | **b*** | **Ultimate pH** | **Cooking Yield (% Meat)** | **Drip Loss (%)** | **Water-Holding Capacity (%)** | **Moisture Uptake (%)** |
| **MET Level** | | | | | | | | |
| 0.0 g MET/kg | 55.07 | 2.71 $^{a}$ | 7.82 | 5.86 $^{ab}$ | 66.18 $^{b}$ | 4.56 $^{ab}$ | 10.82 | 38.14 |
| 0.5 g MET/kg | 54.99 | 2.35 $^{ab}$ | 8.16 | 5.88 $^{a}$ | 68.03 $^{b}$ | 3.53 $^{b}$ | 9.87 | 43.47 |
| 1.0 g MET/kg | 54.71 | 2.22 $^{b}$ | 7.76 | 5.83 $^{ab}$ | 79.04 $^{a}$ | 5.78 $^{a}$ | 11.14 | 44.09 |
| 2.0 g MET/kg | 54.54 | 2.04 $^{b}$ | 7.67 | 5.82 $^{b}$ | 78.60 $^{a}$ | 5.82 $^{a}$ | 11.76 | 38.23 |
| SEM | 0.56 | 0.09 | 0.20 | 0.015 | 0.57 | 0.32 | 0.57 | 3.47 |
| **Sex** | | | | | | | | |
| Male | 54.42 | 2.55 $^{a}$ | 7.42 $^{b}$ | 5.85 | 73.06 | 4.77 | 11.10 | 39.42 |
| Female | 55.13 | 2.11 $^{b}$ | 8.29 $^{a}$ | 5.84 | 72.86 | 5.08 | 10.70 | 42.54 |
| SEM | 0.40 | 0.06 | 0.14 | 0.010 | 0.40 | 0.23 | 0.41 | 2.45 |
| **MET Level × Sex** | | | | | | | | |
| 0.0 g/Male | 54.57 | 2.91 | 7.28 | 5.84 | 65.81 | 3.78 | 10.68 | 36.85 |
| 0.5 g/Male | 54.57 | 2.57 | 7.85 | 5.88 | 68.15 | 3.59 | 10.05 | 40.21 |
| 1.0 g/Male | 54.88 | 2.54 | 7.24 | 5.82 | 78.90 | 6.19 | 11.86 | 42.27 |
| 2.0 g/Male | 54.06 | 2.20 | 7.30 | 5.86 | 79.38 | 5.51 | 11.81 | 38.37 |
| 0.0 g/Female | 55.57 | 2.50 | 8.35 | 5.87 | 66.55 | 5.34 | 10.97 | 39.43 |
| 0.5 g/Female | 55.40 | 2.14 | 8.47 | 5.88 | 67.91 | 3.47 | 9.700 | 46.72 |
| 1.0 g/Female | 54.55 | 1.91 | 8.28 | 5.85 | 79.17 | 5.38 | 10.42 | 45.91 |
| 2.0 g/Female | 55.01 | 1.88 | 8.05 | 5.78 | 77.83 | 6.13 | 11.72 | 30.09 |
| SEM | 0.79 | 0.13 | 0.28 | 0.021 | 0.80 | 0.45 | 0.81 | 4.91 |
| ***p*-Value** | | | | | | | | |
| MET Level | 0.9008 | 0.0003 | 0.3474 | 0.0354 | 0.0001 | 0.0001 | 0.1637 | 0.4720 |
| Sex | 0.2869 | 0.0001 | 0.0002 | 0.6460 | 0.7348 | 0.3365 | 0.4968 | 0.3807 |
| MET Level × Sex | 0.8122 | 0.6737 | 0.8168 | 0.0584 | 0.5236 | 0.0868 | 0.7409 | 0.9202 |

[a,b] Means within a column with different superscripts differ significantly by $p \leq 0.05$. MET source: DL MET. 0.0 g = broilers fed diet with 0 g added MET per kg of feed; 0.5 g = broilers fed diet with 0.5 g added MET per kg of feed; 1 g = broilers fed diet with 1 g added MET per kg of feed; 2 g = broilers fed diet with 2 g added MET per kg of feed. L*, lightness; a*, redness; b*, yellowness. [1] Data are the means of four replicate pens with 10 males and 10 females randomly selected per pen.

Differences in muscle glycogen reserves at slaughter and the rate and extent of post-mortem decrease in pH result in variations in meat quality [33]. Dark-colored muscle is associated with high muscle pH at 24 h postmortem, resulting in darker breast meat [35]. A high pH is associated with poor/shorter shelf life because it is more prone to bacteria spoilage due to the high environmental temperature [36,37]. Another study [38] suggested that low muscle pH might indicate decreased glycogen consumption of pre-slaughtered animals. A low pH is associated with poor WHC [36,39,40], which is one of the major factors that affect the characteristics of cooked meat, i.e., cook loss, juiciness, appearance, etc. [38,41]. Betti et al. [42] reported higher Allo–Kramer shear (A-K shear) values (harder

or firmer meat) when pH was below 5.72 and tenderness differences were attributed to decreases in WHC associated with low pH.

### *3.2. Cooking Yield, Water-Holding Capacity, Moisture Uptake, and Drip Loss*

The cooking yields were significantly higher in the breast meat of the birds fed the 1 and 2 g MET/kg diets (79.04 and 78.60%, respectively) compared to those fed the 0 and 0.5 g MET/kg diets (66.18 and 68.03%, respectively) (Table 3). Contrary to these results, other studies found no significant difference in cooking yield for broilers fed diets with various methionine supplementation levels [9,43]. Although the birds fed 0 and 0.5 g MET/kg of feed had extra days prior to slaughter, they still had lower cooking yields than birds fed the higher MET levels. MET contributes to muscle accretion and could have contributed to the higher cooking yields noted for birds fed 1 and 2 g MET/kg of feed. Increased sarcoplasmic protein solubility is an indicator of improvements in meat quality and a possible explanation for improved cooking yield [23]. Van Laack et al. [23] reported a positive correlation with sarcoplasmic protein solubility and increased cooking yield, pH, and moisture uptake. Improved WHC may improve the juiciness of the meat, decrease the cooking loss, and help maintain the appearance of the cooked meat. MET supplementation may also improve the water-binding capacity of meat by lowering drip loss and cooking loss [9]. Genotype, fast muscle growth, preslaughter stress, and slaughtering and processing conditions can impact water-holding capacity [9]. More studies are needed to better understand the relationship between the MET level and cooking yield.

There were no effects of MET levels on WHC and moisture uptake of raw breast meat (Table 3). Khan et al. [28] also reported no effect on moisture content with MET supplementation, suggesting that MET supplementation may not have an impact on moisture uptake. WHC is a function of pH and other factors, which act by altering cellular and extracellular components [3]. There is an overall decline in pH due to postmortem decrease in oxygen and production of lactic acid, leading to an overall reduction in the number of reactive groups on muscle proteins available to bind water, ultimately leading to a decrease in WHC [3]. Drip loss and moisture uptake are different measures of WHC [3]. There was a significant effect of methionine levels on drip loss. Breast meat from birds fed the 1 and 2 g MET/kg diets had significantly higher percentages of drip loss than those fed 0.5 g MET/kg of feed. The drip loss of those fed 0 g MET/kg of feed was not different from the other three treatments (Table 3). The drip loss results seem to be contrary to the cooking yield results. In studies by other researchers, MET level had an effect on drip loss [7,9], where the higher MET supplementation levels led to lower drip loss. Low drip loss is an indicator of higher meat quality [36]. Khan et al. [28] reported lower drip loss with MET supplementation compared with the control group. It was expected that the drip loss would decrease with MET level, but this was not the case in our results. Additionally, studies have reported a decrease in drip loss with increasing age at slaughter [36]. This is consistent with the results in this study because 1 and 2 g MET/kg birds were slaughtered two days earlier than those fed 0.5 and 0 g MET/kg of feed. Although slaughter age is tied to MET level, drip loss is likely more strongly influenced by slaughter age.

### *3.3. Textural Properties*

Meat firmness is a question of consumer preference. The textural properties of cooked meat (chewiness, cohesiveness, hardness, and resilience) were not significantly affected by the dietary methionine level and the sex of the bird and there were no interactions between sex and dietary MET level (Table 4). There was a significant effect of methionine levels on meat firmness (Table 4). Birds fed diets with 1 g MET/kg (shear force of 3.20 kgf) had meat that was significantly firmer than those fed 0 and 0.5 g MET levels (2.88 and 2.87 kgf shear force, respectively). The birds fed diets with higher MET levels likely had firmer meat due to the effects of MET on muscle accretion. In addition, meat tenderness tends to decrease as birds age [44]. Older birds are more mature at the time of harvest and have more cross-linking of collagen [44]. This can result in making their meat firmer. Similar

to this study, Belloir et al. [45] found that nutrient level did not affect the texture of breast meat. In our study, the birds that were younger at slaughter had firmer meat. This may be due to the interplay between MET level and bird age.

**Table 4.** The effects of feeding varying levels of DL-methionine (MET) to broiler chickens on texture profile analysis (TPA), shear force and total antioxidant capacities (TAC) of breast meat [1].

| Variable | TPA | | | | Shear Force | | TAC |
|---|---|---|---|---|---|---|---|
| | Hardness (kgf) | Cohesiveness (%) | Chewiness (kgf·sec) | Resilience (%) | Firmness (kgf) | Toughness (kgf·sec) | Muscle TAC (μmol TE/g Meat) |
| **MET Level** | | | | | | | |
| 0.0 g MET/kg | 47.43 | 0.46 | 8.69 | 0.24 | 2.88 $^{b}$ | 32.23 | 12.17 |
| 0.5 g MET/kg | 42.37 | 0.43 | 7.13 | 0.23 | 2.87 $^{b}$ | 32.07 | 12.49 |
| 1.0 g MET/kg | 46.77 | 0.41 | 6.94 | 0.23 | 3.20 $^{a}$ | 33.98 | 11.28 |
| 2.0 g MET/kg | 44.58 | 0.64 | 10.04 | 0.24 | 3.09 $^{ab}$ | 33.58 | 11.41 |
| SEM | 1.79 | 1.12 | 2.23 | 0.02 | 0.06 | 0.73 | 0.38 |
| **Sex** | | | | | | | |
| Male | 44.95 | 0.55 | 9.60 | 0.25 | 3.31 $^{a}$ | 36.05 $^{a}$ | 12.31 $^{a}$ |
| Female | 45.62 | 0.41 | 6.80 | 0.22 | 2.72 $^{b}$ | 29.88 $^{b}$ | 11.37 $^{b}$ |
| SEM | 1.26 | 0.09 | 1.57 | 0.01 | 0.04 | 0.51 | 0.27 |
| **MET Level × Sex** | | | | | | | |
| 0.0 g/Male | 50.13 | 0.44 | 10.18 | 0.26 | 3.23 | 35.71 | 11.80 $^{ab}$ |
| 0.5 g/Male | 41.43 | 0.46 | 8.20 | 0.24 | 3.06 | 34.17 | 12.70 $^{a}$ |
| 1.0 g/Male | 45.43 | 0.41 | 6.27 | 0.21 | 3.56 | 37.63 | 12.31 $^{a}$ |
| 2.0 g/Male | 42.80 | 0.88 | 13.77 | 0.28 | 3.39 | 36.69 | 12.43 $^{a}$ |
| 0.0 g/Female | 44.73 | 0.44 | 7.20 | 0.23 | 2.54 | 28.76 | 12.54 $^{a}$ |
| 0.5 g/Female | 43.30 | 0.41 | 6.06 | 0.22 | 2.69 | 29.97 | 12.29 $^{a}$ |
| 1.0 g/Female | 48.10 | 0.41 | 7.62 | 0.22 | 2.85 | 30.33 | 10.26 $^{b}$ |
| 2.0 g/Female | 46.36 | 0.39 | 6.32 | 0.21 | 2.79 | 30.46 | 10.39 $^{b}$ |
| SEM | 2.53 | 0.17 | 3.15 | 0.02 | 0.08 | 1.03 | 0.54 |
| ***p*-Value** | | | | | | | |
| MET Level | 0.2082 | 0.5532 | 0.7356 | 0.6361 | 0.0009 | 0.1919 | 0.10 |
| Sex | 0.7102 | 0.2653 | 0.2218 | 0.1430 | 0.0001 | 0.0001 | 0.02 |
| MET Level × Sex | 0.2936 | 0.4644 | 0.5848 | 0.4858 | 0.1573 | 0.4532 | 0.04 |

$^{a,b}$ Means within a column with different superscripts differ significantly by $p \leq 0.05$. MET source: DL MET. 0.0 g = broilers fed diet with 0 g added MET per kg of feed; 0.5 g = broilers fed diet with 0.5 g added MET per kg of feed; 1 g = broilers fed diet with 1 g added MET per kg of feed; 2 g = broilers fed diet with 2 g added MET per kg of feed. [1] Data are the means of four replicate pens with 10 males and 10 females randomly selected per pen.

Based on sex, there were significant differences in the firmness and toughness of the meat. The firmness of the meat from male birds was higher (3.31 kgf) compared to that of female birds (2.72 kgf). Regarding meat toughness, the meat from male broilers was tougher (36.05 kgf·sec) ($p \leq 0.05$) compared to the toughness of the meat from female broilers (29.88 kgf·sec). Low shear force is an indicator of higher meat quality [36]. These results could be due to the fact that male broilers grow at a faster rate than females, thus resulting in faster muscle growth or accretion.

### *3.4. Total Antioxidant Capacity (TAC)*

There was no significant effect of MET on TAC but there was a significant effect on TAC based on sex and there were significant effects on interaction ($p < 0.05$) between diet and sex for muscle TAC (Table 4). TAC values for the meat from female birds fed the 1 and 2 g MET/kg diets (10.26 and 10.39 mmol TE/g meat, respectively) were significantly different from the TAC for meat from females fed the 0 and 0.5 g MET/kg diets (12.54 and 12.28 nmol TE/g meat, respectively). For the males, there was no difference ($p > 0.05$) between the males fed different MET levels. The meat from the males fed all MET levels had higher TAC values than meat from females fed 1 and 2 g MET/kg. The

potential of food to withstand oxidative deterioration is indicated by higher TAC values [30]. de Freitas Dionzio et al. [30] found that broilers fed diets supplemented with MET had higher TAC values than broilers fed diets not supplemented with MET. On the other hand, Jiang et al. [46] reported no significant effect of MET supplementation on TAC (total antioxidant capacity or TOAC). Although the reason is unclear, meat from females that were fed the two higher MET levels may be more prone to deterioration than meat from the males and females in the other treatment groups. More studies on the effects of MET supplementation of broilers on TAC should be performed in order to further explore the factors involved with MET supplementation, TAC, diet, and sex. In this study, we found a difference between male and female broilers with respect to color (a* and b*), shear force (firmness and toughness), and muscle TAC. MET supplementation can impact the meat quality parameters of male and female broilers.

### *3.5. Lipid Oxidation (LO)*

There were no significant effects of MET levels on lipid oxidation (LO) of raw meat at days 0 and 5 and of cooked meat at day 7 (Table 5). However, there was a significant interaction between MET level and sex for raw meat LO measured on day 10. Male broilers fed MET diets with 0.5 and 1 g MET/kg had significantly lower LO values than male birds fed 0 g MET/kg (0.23 and 0.23 vs. 0.36 mg MDA/kg meat). MET supplementation in broiler diets has been reported to have a good effect on broiler meat by decreasing lipid oxidation during storage [11,47]. Additionally, MET supplementation assists in protein synthesis and averts oxidative stress [48]. MET supplementation may serve to improve the shelf life of raw meat.

There was a significant effect of MET levels on LO in cooked meat (Table 5). For day 0, there were significant differences in LO for cooked meat between birds fed 1, 2 and 0 g MET/kg of feed, (2.54, 1.53. and 3.89, respectively). Compared to the LO in raw breast meat, these results indicated that cooking significantly increased lipid oxidation in breast meat (day 0). LO in cooked meat increased during 7 days of storage. However, increasing LO rates in cooked meat decreased as MET levels increased up to 3 days of storage, suggesting that the addition of MET may contribute to improved oxidative stability, and thus be used to control lipid oxidation in poultry breast meat. This improvement in oxidative stability caused by MET supplementation has been previously reported by Wang et al. [15] and Moghadam et al. [49]. MET supplementation increases oxidative stability even in heat-stressed broiler chickens [50]. Lipid oxidation requires the production of free radicals and can be impacted by elements such as the combination of prooxidants, myoglobin, pH, temperature, oxygen, and fatty acid of the meat [51].

Although the setup of this trial was not organic, the MET levels used were on par with the levels recommended or projected by the National Organic Standards Board for rearing organic broilers. Currently, the use of synthetic MET such as DL-methionine in organic broiler diets for supplementation is restricted by the National Organic Standards Board in the U.S. and they have come up with a step-down program to reduce and eventually eliminate the use of MET by 2040 for newer organic poultry operations [52]. The use of synthetic MET has already been banned in broiler production in some parts of the world such as the European Union [53]. The results of this study provided insight into the supplemental use of lower levels or no dietary DL-MET on meat quality.

**Table 5.** The main effects and interactions between feed and sex of various DL MET levels on lipid oxidation of raw and cooked broiler breast meat [1].

| | Lipid Oxidation (mg MDA/kg Meat) | | | | | |
|---|---|---|---|---|---|---|
| | Raw Meat | | | Cooked Meat | | |
| **Variable** | **Day 0** | **Day 5** | **Day 10** | **Day 0** | **Day 3** | **Day 7** |
| **MET Level** | | | | | | |
| 0.0 g | 0.23 | 0.32 | 0.31 $^{a}$ | 3.90 $^{a}$ | 5.32 $^{a}$ | 7.74 |
| 0.5 g | 0.19 | 0.32 | 0.25 $^{b}$ | 3.13 $^{ab}$ | 4.33 $^{bc}$ | 6.90 |
| 1.0 g | 0.19 | 0.35 | 0.26 $^{ab}$ | 2.54 $^{b}$ | 4.66 $^{ab}$ | 8.00 |
| 2.0 g | 0.21 | 0.35 | 0.31 $^{a}$ | 1.53 $^{c}$ | 3.62 $^{c}$ | 8.23 |
| SEM | 0.01 | 0.02 | 0.02 | 0.20 | 0.24 | 0.35 |
| **Sex** | | | | | | |
| Male | 0.21 | 0.33 | 0.28 | 2.91 | 4.69 | 7.85 |
| Female | 0.20 | 0.33 | 0.29 | 2.64 | 4.27 | 7.59 |
| SEM | 0.01 | 0.02 | 0.01 | 0.14 | 0.17 | 0.25 |
| **MET Level × Sex** | | | | | | |
| 0.0 g/Male | 0.23 | 0.31 | 0.36 $^{a}$ | 4.32 | 5.59 | 7.54 |
| 0.5 g/Male | 0.20 | 0.27 | 0.23 $^{b}$ | 2.90 | 4.20 | 6.86 |
| 1.0 g/Male | 0.20 | 0.36 | 0.23 $^{b}$ | 2.75 | 5.23 | 8.03 |
| 2.0 g/Male | 0.22 | 0.40 | 0.30 $^{ab}$ | 1.67 | 3.75 | 7.93 |
| 0.0 g/Female | 0.23 | 0.32 | 0.26 $^{ab}$ | 3.47 | 5.04 | 7.95 |
| 0.5 g/Female | 0.19 | 0.37 | 0.27 $^{ab}$ | 3.36 | 4.47 | 6.94 |
| 1.0 g/Female | 0.19 | 0.35 | 0.29 $^{ab}$ | 2.33 | 4.08 | 7.97 |
| 2.0 g/Female | 0.20 | 0.31 | 0.33 $^{ab}$ | 1.40 | 3.49 | 8.54 |
| SEM | 0.02 | 0.03 | 0.03 | 0.28 | 0.34 | 0.50 |
| ***p*-Value** | | | | | | |
| MET Level | 0.1215 | 0.5350 | 0.0459 | 0.0001 | 0.0007 | 0.0705 |
| Sex | 0.3903 | 0.8718 | 0.6802 | 0.1923 | 0.0987 | 0.4715 |
| MET Level × Sex | 0.9597 | 0.0618 | 0.0215 | 0.1639 | 0.2477 | 0.9007 |

$^{a,b,c}$ Means within a column with different superscripts differ significantly by $p \leq 0.05$. MET source: DL MET. 0.0 g = broilers fed diet with 0 g added MET per kg of feed; 0.5 g = broilers fed diet with 0.5 g added MET per kg of feed; 1 g = broilers fed diet with 1 g added MET per kg of feed; 2 g = broilers fed diet with 2 g added MET per kg of feed. [1] Data are the means of four replicate pens with 10 males and 10 females randomly selected per pen.

## 4. Conclusions

Meat quality is important to both the meat industry and consumers. Supplementation of MET at or above 1 g has positive implications for drip loss, cooking yield, meat firmness, and TAC. Furthermore, increased cooking yield with increased MET supplementation implies that there was less water loss after cooking the meat from birds fed the higher MET levels. MET supplementation at 1.0 and 2.0 g/kg levels increased meat firmness. Methionine levels influenced LO values for cooked meat such that the LO rates decreased with increasing MET supplementation. This resulted in decreased deterioration rates in meat from broilers fed diets with supplemental MET compared to meat from broilers fed diets with 0 g/kg MET. Supplementation of MET at 1.0 and 2.0 g/kg improved WHC and increased drip loss. Based on the results in this study, it can be concluded that MET improves the oxidative stability of breast meat, leading to improved shelf life.

**Author Contributions:** Conceptualization, W.R.L., A.P.-A., J.R.T. and B.R.M.; methodology, A.P.-A., B.R.M., W.R.L. and J.R.T.; formal analysis, A.P.-A., S.N.M. and C.C.; investigation, A.P-A., S.N.M. and B.R.M.; resources, A.P.-A., J.R.T. and B.R.M.; data curation, A.P.-A. and B.R.M.; writing—original draft preparation, A.P.-A.; writing—review and editing, A.P.-A., S.N.M., C.C., J.R.T., C.M.M., W.R.L. and B.R.M.; visualization, A.P.-A. and C.C.; supervision, A.P.-A., B.R.M. and J.R.T.; project administration, A.P.-A.; funding acquisition, A.P.-A., W.R.L., J.R.T. and B.R.M. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research work was not externally funded but resources were provided by Perdue Farms and University of Maryland Eastern Shore.

**Institutional Review Board Statement:** The animal experiment was conducted in accordance with the recommendations and guidelines of the University of Maryland Eastern Shore Institutional Animal Care and Use Committee (AUP-Timmons-2021-4) and agreed with the standards for experiments held by the research farm.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available within this article.

**Acknowledgments:** The authors are also grateful to Randy Mitchell at Perdue Farms for his help and Scott Gold and Anthony Glenn at the U.S. National Poultry Research Center unit of Toxicology and Mycotoxin Research of USDA, ARS in Athens, GA, and Grace Pokoo-Aikins for their contributions to this paper.

**Conflicts of Interest:** The corresponding author (Anthony Pokoo-Aikins) worked at Perdue Farms in the past.

## References

1. Davis, C.G.; Harvey, D.; Zahniser, S.; Gale, F.; Liefert, W. *Assessing the Growth of Broiler and Poultry Meat Exports*; USDA Economic Research Service: Washington, DC, USA, 2013.
2. Katiyo, W.; Coorey, R.; Buys, E.M.; de Kock, H.L. Consumers' perceptions of intrinsic and extrinsic attributes as indicators of safety and quality of chicken meat: Actionable information for public health authorities and the chicken industry. *J. Food Sci.* **2020**, *85*, 1845–1855. [CrossRef] [PubMed]
3. Mir, N.A.; Rafiq, A.; Kumar, F.; Singh, V.; Shukla, V. Determinants of broiler chicken meat quality and factors affecting them: A review. *J. Food Sci. Technol.* **2017**, *54*, 2997–3009. [CrossRef]
4. Escobedo del Bosque, C.I.; Altmann, B.A.; Ciulu, M.; Halle, I.; Jansen, S.; Nolte, T.; Weigend, S.; Mörlein, D. Meat Quality Parameters and Sensory Properties of One High-Performing and Two Local Chicken Breeds Fed with Vicia faba. *Foods* **2020**, *9*, 1052. [CrossRef]
5. Cui, Y.-m.; Wang, J.; Lu, W.; Zhang, H.-j.; Wu, S.-g.; Qi, G.-h. Effect of dietary supplementation with Moringa oleifera leaf on performance, meat quality, and oxidative stability of meat in broilers. *Poult. Sci.* **2018**, *97*, 2836–2844. [CrossRef] [PubMed]
6. Hayat, Z.; Rehman, A.U.; Akram, K.; Farooq, U.; Saleem, G. Evaluation of a natural methionine source on broiler growth performance. *J. Sci. Food. Agric.* **2015**, *95*, 2462–2466. [CrossRef] [PubMed]
7. Wen, C.; Jiang, X.; Ding, L.; Wang, T.; Zhou, Y. Effects of dietary methionine on breast muscle growth, myogenic gene expression and IGF-I signaling in fast- and slow-growing broilers. *Sci. Rep.* **2017**, *7*, 1924. [CrossRef] [PubMed]
8. Zhang, S.; Saremi, B.; Gilbert, E.R.; Wong, E.A. Physiological and biochemical aspects of methionine isomers and a methionine analogue in broilers. *Poult. Sci.* **2017**, *96*, 425–439. [CrossRef]
9. Albrecht, A.; Hebel, M.; Heinemann, C.; Herbert, U.; Miskel, D.; Saremi, B.; Kreyenschmidt, J. Assessment of Meat Quality and Shelf Life from Broilers Fed with Different Sources and Concentrations of Methionine. *J. Food Qual.* **2019**, *2019*, 6182580. [CrossRef]
10. Conde-Aguilera, J.A.; Cholet, J.C.; Lessire, M.; Mercier, Y.; Tesseraud, S.; van Milgen, J. The level and source of free-methionine affect body composition and breast muscle traits in growing broilers. *Poult. Sci.* **2016**, *95*, 2322–2331. [CrossRef] [PubMed]
11. Aksu, M.I.; Imik, H.; Karaoğlu, M. Influence of dietary sorghum (*sorghum vulgare*) and corn supplemented with methionine on cut-up pieces weights of broiler carcass and quality properties of breast and drumsticks meat. *Food Sci. Tech. Int.* **2016**, *13*, 361–367. [CrossRef]
12. Albrecht, A.; Herbert, U.; Miskel, D.; Heinemann, C.; Braun, C.; Dohlen, S.; Zeitz, J.O.; Eder, K.; Saremi, B.; Kreyenschmidt, J. Effect of methionine supplementation in chicken feed on the quality and shelf life of fresh poultry meat. *Poult. Sci* **2017**, *96*, 2853–2861. [CrossRef] [PubMed]
13. Elsharkawy, M.S.; Chen, Y.; Liu, R.; Tan, X.; Li, W.; El-Wardany, I.; Zhao, D.; Zheng, M.; Wen, J.; Zhao, G. Paternal Dietary Methionine Supplementation Improves Carcass Traits and Meat Quality of Chicken Progeny. *Animals* **2021**, *11*, 325. [CrossRef] [PubMed]
14. Li, Y.; Cheang, B.; Lim, A. Grocery perishables management. *Prod. Oper. Manag.* **2012**, *21*, 504–517. [CrossRef]
15. Wang, Z.; Pan, X.; Peng, Z.; Zhao, R.; Zhou, G. Methionine and selenium yeast supplementation of the maternal diets affects color, water-holding capacity, and oxidative stability of their male offspring meat at the early stage. *Poult. Sci.* **2009**, *88*, 1096–1101. [CrossRef] [PubMed]
16. Fikre, A.; Yami, A.; Kuo, Y.H.; Ahmed, S.; Gheysen, G.; Lambein, F. Effect of methionine supplement on physical responses and neurological symptoms in broiler chicks fed grass pea (*Lathyrus sativus*)-based starter ration. *Food Chem. Toxicol.* **2010**, *48*, 11–17. [CrossRef]

17. Pokoo-Aikins, A.; Timmons, J.R.; Min, B.R.; Lee, W.R.; Mwangi, S.N.; Chen, C. Effects of Feeding Varying Levels of DL-Methionine on Live Performance and Yield of Broiler Chickens. *Animals* **2021**, *11*, 2839. [CrossRef]
18. de Sousa, F.C.B.; Del Vesco, A.P.; Zancanela, V.; Santana, T.P.; de Souza Khatlab, A.; Feitosa, V.E.M.; Brito, C.O.; Barbosa, L.T.; Gasparino, E. Effects of methionine as free amino acid and dipeptide on productive efficiency and meat quality of broilers under acute and chronic heat stress. *Anim. Prod. Sci.* **2022**, *62*, 342–357. [CrossRef]
19. Miao, Z.Q.; Dong, Y.Y.; Qin, X.; Yuan, J.M.; Han, M.M.; Zhang, K.K.; Shi, S.R.; Song, X.Y.; Zhang, J.Z.; Li, J.H. Dietary supplementation of methionine mitigates oxidative stress in broilers under high stocking density. *Poult. Sci.* **2021**, *100*, 101231. [CrossRef]
20. National Research Council. *Nutritional Requirements of Poultry*; National Academy Press: Washington, DC, USA, 1994.
21. USDA. National organic program; amendment to the national list of allowed and prohibited substances (livestock). *Fed. Regist.* **2011**, *76*, 13501–13504.
22. Zhang, W.; Xiao, S.; Lee, E.J.; Ahn, D.U. Consumption of oxidized oil increases oxidative stress in broilers and affects the quality of breast meat. *J. Agric. Food Chem.* **2011**, *59*, 969–974. [CrossRef]
23. Van Laack, R.; Liu, C.H.; Smith, M.; Loveday, H. Characteristics of pale, soft, exudative broiler breast meat. *Poult. Sci.* **2000**, *79*, 1057–1061. [CrossRef] [PubMed]
24. Min, B.; Green, B.W. Use of microbial transglutaminase and nonmeat proteins to improve functional properties of low NaCl, phosphate-free patties made from channel catfish (*Ictalurus punctatus*) belly flap meat. *J. Food Sci.* **2008**, *73*, E218–E226. [CrossRef] [PubMed]
25. Bourne, M.C. Chapter 1—Texture, Viscosity, and Food. In *Food Texture and Viscosity*, 2nd ed.; Bourne, M.C., Ed.; Academic Press: London, UK, 2002; pp. 1–32.
26. Prior, R.L.; Hoang, H.; Gu, L.; Wu, X.; Bacchiocca, M.; Howard, L.; Hampsch-Woodill, M.; Huang, D.; Ou, B.; Jacob, R. Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL)) of plasma and other biological and food samples. *J. Agric. Food Chem.* **2003**, *51*, 3273–3279. [CrossRef] [PubMed]
27. Min, B.; McClung, A.M.; Chen, M.H. Phytochemicals and antioxidant capacities in rice brans of different color. *J. Food Sci.* **2011**, *76*, C117–C126. [CrossRef] [PubMed]
28. Khan, I.A.; Parker, N.B.; Löhr, C.V.; Cherian, G. Docosahexaenoic acid (22: 6 n-3)-rich microalgae along with methionine supplementation in broiler chickens: Effects on production performance, breast muscle quality attributes, lipid profile, and incidence of white striping and myopathy. *Poult. Sci.* **2021**, *100*, 865–874. [CrossRef] [PubMed]
29. Bezerra de Carvalho, G. *Níveis e Fontes de Metionina na Nutrição de Frangos de Corte*; Universidade Federal de Goiás: Goiânia, Brazil, 2017.
30. de Freitas Dionizio, A.; de Souza Khatlab, A.; Alcalde, C.R.; Gasparino, E.; Feihrmann, A.C. Supplementation with free methionine or methionine dipeptide improves meat quality in broilers exposed to heat stress. *J. Food Sci. Tech.* **2021**, *58*, 205–215. [CrossRef] [PubMed]
31. Hunt, M.C.; King, A.; Barbut, S.; Clause, J.; Cornforth, D.; Hanson, D.; Lindahl, G. *Meat Color Measurement Guidelines*; American Meat Science Association: Champaign, IL, USA, 2012; pp. 1–135.
32. Qamar, A.; Mohyuddin, S.G.; Hamza, A.; Lartey, K.A.; Shi, C.Q.; Yang, F.; Lu, Z.; Yang, J.; Chen, J.J. Physical and Chemical Factors Affecting Chicken Meat Color. *Pak. J. Sci.* **2019**, *71*, 82.
33. Faustman, C.; Suman, S.P. *Chapter 11—The Eating Quality of Meat: I—Color; In Lawrie's Meat Science*, 8th ed.; Toldra, F., Ed.; Woodhead Publishing: Sawston, UK, 2017; pp. 329–356.
34. Fouad, A.; El-Senousey, H. Nutritional factors affecting abdominal fat deposition in poultry: A review. *Asian-Australas J. Anim. Sci.* **2014**, *27*, 1057. [CrossRef] [PubMed]
35. Bowker, B.; Zhuang, H. Relationship between muscle exudate protein composition and broiler breast meat quality. *Poul. Sci.* **2013**, *92*, 1385–1392. [CrossRef] [PubMed]
36. Li, J.; Yang, C.; Peng, H.; Yin, H.; Wang, Y.; Hu, Y.; Yu, C.; Jiang, X.; Du, H.; Li, Q. Effects of slaughter age on muscle characteristics and meat quality traits of Da-Heng meat type birds. *Animals* **2020**, *10*, 69. [CrossRef] [PubMed]
37. Petracci, M.; Mudalal, S.; Soglia, F.; Cavani, C. Meat quality in fast-growing broiler chickens. *Worlds Poult. Sci. J.* **2015**, *71*, 363–374. [CrossRef]
38. Baéza, E.; Guillier, L.; Petracci, M. Review: Production factors affecting poultry carcass and meat quality attributes. *Animals* **2022**, *16*, 100331. [CrossRef]
39. Guan, R.-F.; Lyu, F.; Chen, X.-Q.; Ma, J.-Q.; Jiang, H.; Xiao, C.-G. Meat quality traits of four Chinese indigenous chicken breeds and one commercial broiler stock. *J. Zhejiang Univ. Sci. B* **2013**, *14*, 896–902. [CrossRef] [PubMed]
40. Zaboli, G.; Huang, X.; Feng, X.; Ahn, D.U. How can heat stress affect chicken meat quality—A review. *Poult. Sci.* **2019**, *98*, 1551–1556. [CrossRef]
41. Hughes, J.; Oiseth, S.; Purslow, P.; Warner, R. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Sci.* **2014**, *98*, 520–532. [CrossRef] [PubMed]
42. Betti, M.; Schneider, B.; Wismer, W.; Carney, V.; Zuidhof, M.; Renema, R. Omega-3-enriched broiler meat: 2. Functional properties, oxidative stability, and consumer acceptance. *Poult. Sci.* **2009**, *88*, 1085–1095. [CrossRef]
43. Zhai, W.; Peebles, E.; Schilling, M.; Mercier, Y. Effects of dietary lysine and methionine supplementation on Ross 708 male broilers from 21 to 42 d of age (I): Growth performance, meat yield, and cost effectiveness. *J. Appl. Poult. Res.* **2016**, *25*, 197–211. [CrossRef]

44. Lepetit, J. Collagen contribution to meat toughness: Theoretical aspects. *Meat Sci.* **2008**, *80*, 960–967. [CrossRef]
45. Belloir, P.; Méda, B.; Lambert, W.; Corrent, E.; Juin, H.; Lessire, M.; Tesseraud, S. Reducing the CP content in broiler feeds: Impact on animal performance, meat quality and nitrogen utilization. *Animals* **2017**, *11*, 1881–1889. [CrossRef] [PubMed]
46. Jiang, S.G.; Pan, N.X.; Chen, M.J.; Wang, X.Q.; Yan, H.C.; Gao, C.Q. Effects of Dietary Supplementation with dl-Methionine and dl-Methionyl-dl-Methionine in Breeding Pigeons on the Carcass Characteristics, Meat Quality and Antioxidant Activity of Squabs. *Antioxidants* **2019**, *8*, 435. [CrossRef] [PubMed]
47. Berri, C.; Métayer-Coustard, S.; Geraert, A.; Mercier, Y.; Tesseraud, S. Effect of 3 methionine sources and levels on broiler meat quality. In Proceedings of the World's Poultry Congress, Salvador, Brazil, 5–9 August 2012.
48. Fagundes, N.S.; Milfort, M.C.; Williams, S.M.; Da Costa, M.J.; Fuller, A.L.; Menten, J.F.; Rekaya, R.; Aggrey, S.E. Dietary methionine level alters growth, digestibility, and gene expression of amino acid transporters in meat-type chickens. *Poult. Sci.* **2020**, *99*, 67–75. [CrossRef] [PubMed]
49. Moghadam, M.H.B.; Shehab, A.; Cherian, G. Methionine supplementation augments tissue n-3 fatty acid and tocopherol content in broiler birds fed flaxseed. *Anim. Feed Sci. Tech.* **2017**, *228*, 149–158. [CrossRef]
50. Sahebi-Ala, F.; Hassanabadi, A.; Golian, A.; Rajaei-Sharifabadi, H. Effects of replacement different levels and sources of methionine with betaine on jejunal morphology, duodenal mitochondrial respiration, and lipid peroxidation in heat-stressed broiler chickens. *Ital. J. Anim. Sci.* **2021**, *20*, 1601–1611. [CrossRef]
51. Andreo, A.I.; Doval, M.M.; Romero, A.M.; Judis, M.A. Influence of heating time and oxygen availability on lipid oxidation in meat emulsions. *Euro. J. Lipid. Sci. Tech.* **2003**, *105*, 207–213. [CrossRef]
52. NOSB Chair: Jean Richardson National Organic Standards Board Livestock Subcommittee Synthetic Methionine (MET) in Organic Poultry Feed Proposal Revised 31 January 2015. Available online: https://www.ams.usda.gov/sites/default/files/media/LS%20MET%20Final%20Rec.pdf (accessed on 1 November 2021).
53. Burley, H.K.; Patterson, P.H.; Anderson, K.E. Alternative feeding strategies and genetics for providing adequate methionine in organic poultry diets with limited use of synthetic amino acids. *Worlds Poult. Sci. J.* **2016**, *72*, 168–177. [CrossRef]