

# Meat Quality In Broiler Chickens Fed On Cowpea (*Vigna Unguiculata* [L.] Walp) Seeds

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## Research Article

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# Abstract

The study aimed to evaluate the effects of a diet containing untreated cowpea (CWP; Aura 26 variety) seeds as a protein source on quality parameters (physicochemical properties and fatty acid composition) of chickens' breast and thigh muscles. A total of 240 Ross 308 broiler chickens were randomly allotted to two groups (1 fed with soybean meal (SBM) and 2 fed with CWP), 6 replicate pens per group (20 chicks/pen). At 6 weeks of age, twelve birds/group were slaughtered. Breast and thigh muscles of birds fed CWP diets had significantly higher ( $P < 0.05$ ) levels of n-3 polyunsaturated fatty acids (PUFA; C:18:3n-3, C:20:5n-3) compared with birds fed SBM diet. The n-6/n-3 PUFA ratio of the muscles decreased significantly ( $P < 0.05$ ) in the CWP group. The study revealed that the use of CWP as a substitute for SBM had significant effects on physicochemical components of breast and thigh muscles. The lightness ( $L^*$ ) and redness ( $a^*$ ) of breast and thigh muscles were higher in the CWP treatment. The collagen and protein contents were higher, while the fat content was decreased ( $P < 0.05$ ) in the CWP group. The texture profile analysis in breast and thigh muscles were comparable ( $P > 0.05$ ) in both groups. These results confirm that CWP can be considered a promising protein source for broiler chicken feed.

## Introduction

In recent years, poultry meat has become one of the most popular and favourite protein sources due to its low price and high nutritional value. However, today's consumers have become increasingly concerned about the quality and safety of meat. In this direction, researchers are making efforts to improve the nutritional quality of meat, with the least financial effort and without affecting performance, especially for polyunsaturated fatty acids (PUFA), which is beneficial to human health<sup>1</sup>.

Soybean meal (SBM) is often the major dietary plant protein source in broiler diets, and other protein sources other than SBM are used occasionally at competitive prices. Therefore, it is necessary to find new, non-traditional, low-cost feedstuffs to decrease the overall cost of poultry production. Cowpea (CWP; *Vigna unguiculata* [L.] Walp), whose research interest has grown in the past few years, is a crop with reasonable high protein content, values in the range 203-394 g kg<sup>-1</sup> (23-32%) (reviewed by Gonçalves *et al.*<sup>2</sup>), and which differs with the variety (reviewed by Vasconcelos *et al.*<sup>3</sup>). Additionally, CWP seeds are rich in nutrients and nutraceuticals such as dietary fibre, antioxidants, PUFA and polyphenols, and minerals and vitamins<sup>4-8</sup>. However, CWP seeds possess some undesirable properties common to other legumes, such as methionine and cysteine deficiency, as well as considerable contents of antinutritional factors like protease inhibitors and phytic acid tannins, among others<sup>9,10</sup>.

Nevertheless, in our previous study<sup>11</sup>, we have shown that new CWP cultivars (Ofelia drought-tolerant cv.) were characterized by low content of antinutritional factors (i.e., trypsin inhibitor activity). The authors also concluded that CWP seeds are a good source of protein and can partially replace SBM in broiler chicks' diets; no adverse effect on broiler growth performance and carcass traits were found. Therefore, CWP seeds could be an alternative source to soy and bean crops, especially under drought conditions, and are locally grown in Romania<sup>12,13</sup>. Up to date, we couldn't find any research about the effect of raw

CWP on the quality of broiler meat; therefore, this research was an opportunity to test these diets effects on fatty acids (FA) deposition in breast and thigh muscle. The tested hypothesis is: Various legume seeds in diets affect the quality of meat of broiler chickens.

Table 1  
Physicochemical parameters of breast and thigh muscles from 6-week-old broiler chickens. <sup>1</sup>group 1: feed based on soybean meal; group 2: feed based on raw cowpea seeds (*Vigna unguiculata* [L.] Walp, Aura 26 cv.), <sup>2</sup>SEM, standard error of the mean; <sup>a,b</sup> Means in the same row without same superscript differ significantly ( $P < 0.05$ ).

| Parameters       | Group <sup>1</sup> |                    | SEM <sup>2</sup> | P-value |
|------------------|--------------------|--------------------|------------------|---------|
|                  | 1                  | 2                  |                  |         |
| <b>Breast</b>    |                    |                    |                  |         |
| pH <sub>24</sub> | 5.97               | 5.99               | 0.01             | 0.470   |
| Lightness (L*)   | 55.44 <sup>b</sup> | 62.47 <sup>a</sup> | 0.72             | 0.0001  |
| Redness (a*)     | 10.87 <sup>b</sup> | 16.35 <sup>a</sup> | 0.54             | 0.0001  |
| Yellowness (b*)  | 12.27              | 13.61              | 0.37             | 0.070   |
| Protein (%)      | 21.68 <sup>b</sup> | 22.42 <sup>a</sup> | 0.12             | 0.0001  |
| Fat (%)          | 1.72               | 1.41               | 0.13             | 0.274   |
| Moisture (%)     | 76.60              | 76.17              | 0.14             | 0.117   |
| Collagen (%)     | 0.69 <sup>b</sup>  | 0.81 <sup>a</sup>  | 0.02             | 0.0001  |
| <b>Thigh</b>     |                    |                    |                  |         |
| pH <sub>24</sub> | 6.22               | 6.23               | 0.01             | 0.946   |
| Lightness (L*)   | 55.65 <sup>b</sup> | 61.45 <sup>a</sup> | 0.59             | 0.0001  |
| Redness (a*)     | 11.40 <sup>b</sup> | 13.20 <sup>a</sup> | 0.24             | 0.0001  |
| Yellowness (b*)  | 9.28 <sup>b</sup>  | 12.20 <sup>a</sup> | 0.43             | 0.0001  |
| Protein (%)      | 18.13 <sup>b</sup> | 18.47 <sup>a</sup> | 0.08             | 0.016   |
| Fat (%)          | 5.78 <sup>a</sup>  | 5.20 <sup>b</sup>  | 0.10             | 0.001   |
| Moisture (%)     | 76.09              | 76.33              | 0.10             | 0.238   |
| Collagen (%)     | 1.02 <sup>b</sup>  | 1.08 <sup>a</sup>  | 0.01             | 0.0001  |

Table 2

Textural properties of breast and thigh muscle from 6-week-old broiler chickens <sup>1</sup>group 1: feed based on soybean meal; group 2: feed based on raw cowpea seeds (*Vigna unguiculata* [L.] Walp, Aura 26 cv.), <sup>2</sup>SEM, standard error of the mean; <sup>a,b</sup> Means in the same row without same superscript differ significantly ( $P < 0.05$ ).

| Parameters        | Group <sup>1</sup> |         | SEM <sup>2</sup> | P-value |
|-------------------|--------------------|---------|------------------|---------|
|                   | 1                  | 2       |                  |         |
| <b>Breast</b>     |                    |         |                  |         |
| Hardness (g)      | 2602.50            | 2456.43 | 176.47           | 0.701   |
| Adhesiveness (mJ) | 0.27               | 0.30    | 0.03             | 0.682   |
| Cohesiveness      | 0.21               | 0.25    | 0.01             | 0.097   |
| Springiness (mm)  | 2.20               | 2.22    | 0.06             | 0.917   |
| Gumminess (g)     | 605.86             | 705.43  | 59.15            | 0.422   |
| Chewiness (mJ)    | 13.11              | 15.60   | 1.52             | 0.435   |
| Resilience        | 0.21               | 0.20    | 0.06             | 0.839   |
| <b>Thigh</b>      |                    |         |                  |         |
| Hardness (g)      | 4190.83            | 3508.33 | 845.15           | 0.732   |
| Adhesiveness (mJ) | 0.37               | 0.57    | 0.17             | 0.612   |
| Cohesiveness      | 0.34               | 0.31    | 0.08             | 0.873   |
| Springiness (mm)  | 3.73               | 2.45    | 0.46             | 0.184   |
| Gumminess (g)     | 1969.33            | 1241.67 | 613.08           | 0.611   |
| Chewiness (mJ)    | 85.63              | 29.37   | 28.01            | 0.372   |
| Resilience        | 0.21               | 0.23    | 0.02             | 0.519   |

The study aimed to compare the meat traits, including physicochemical and textural properties as well as fatty acids composition, in broiler chickens fed on diets containing untreated CWP seeds as an alternative to SBM.

## Results

There were no significant differences ( $P > 0.05$ ) in the values of pH<sub>24</sub> for the breast muscles (PM; *Pectoralis major*) obtained from birds in group 2 fed CWP than in group 1 fed SBM diet (Table 1). However, the values of colour parameters for PM, such as lightness (L\*) and redness (a\*) from birds fed

CWP, were significantly different ( $P < 0.0001$  and  $P < 0.0001$ , respectively) than in the SBM group, while the yellowness ( $b^*$ ) tended the increase ( $P = 0.070$ ).

Table 3

Fatty acids composition (% of total fatty acids) in breast muscles from 6-week-old broiler chickens. <sup>1</sup>group 1: feed based on soybean meal; group 2: feed based on raw cowpea seeds (*Vigna unguiculata* [L.] Walp, Aura 26 cv.). <sup>2</sup>SEM, standard error of the mean. <sup>3</sup>Total n-6 PUFA: sum of C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:2n-6 + C22:3n-6 + C22:4n-6. <sup>4</sup>Total n-3 PUFA: sum of C18:3n-3 + C18:4n-3 + C20:2n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3. <sup>a,b</sup> Means in the same row without same superscript differ significantly ( $P < 0.05$ ).

| Fatty acid                    | Group <sup>1</sup> |                    | SEM <sup>2</sup> | P-value |
|-------------------------------|--------------------|--------------------|------------------|---------|
|                               | 1                  | 2                  |                  |         |
| Caprylic (C8:0)               | 0.03               | 0.02               | 0.01             | 0.374   |
| Capric (C10:0)                | 0.05               | 0.03               | 0.01             | 0.145   |
| Lauric (C12:0)                | 0.01               | 0.04               | 0.01             | 0.140   |
| Myristic (C14:0)              | 0.53 <sup>b</sup>  | 0.58 <sup>a</sup>  | 0.01             | 0.003   |
| Myristoleic (C14:1)           | 0.12               | 0.12               | 0.01             | 0.998   |
| Pentadecanoic (C15:0)         | 0.54               | 0.47               | 0.02             | 0.110   |
| Pentadecenoic (C15:1)         | 0.06               | 0.09               | 0.01             | 0.060   |
| Palmitic (C16:0)              | 21.21 <sup>b</sup> | 22.30 <sup>a</sup> | 0.25             | 0.002   |
| Palmitoleic (C16:1)           | 4.34               | 4.40               | 0.03             | 0.255   |
| Heptadecanoic (C17:0)         | 0.12               | 0.12               | 0.01             | 0.795   |
| Heptadecenoic (C17:1)         | 0.11               | 0.10               | 0.01             | 0.101   |
| Stearic (C18:0)               | 7.44 <sup>a</sup>  | 7.09 <sup>b</sup>  | 0.10             | 0.002   |
| Oleic (C18:1n-9cis)           | 34.89              | 35.14              | 0.22             | 0.641   |
| Linoleic (C18:2n-6)           | 22.74              | 22.38              | 0.11             | 0.081   |
| Arachidic (C20:0)             | 0.06               | 0.01               | 0.02             | 0.117   |
| Gamma-linolenic (C18:3n-6)    | 0.27               | 0.25               | 0.01             | 0.158   |
| Alpha-linolenic (C18:3n-3)    | 0.47 <sup>b</sup>  | 0.61 <sup>a</sup>  | 0.03             | 0.005   |
| Linoleic conjugate (C18:2)    | 0.16               | 0.07               | 0.06             | 0.514   |
| Octadecatetraenoic (C18:4n-3) | 0.51               | 0.53               | 0.03             | 0.774   |
| Eicosadienoic (C20:2n-6)      | 0.23               | 0.20               | 0.03             | 0.692   |
| Eicosatrienoic (C20:3n-6)     | 0.58               | 0.50               | 0.04             | 0.366   |

| Fatty acid                  | Group <sup>1</sup> |                    | SEM <sup>2</sup> | P-value |
|-----------------------------|--------------------|--------------------|------------------|---------|
|                             | 1                  | 2                  |                  |         |
| Erucic (C22:1n-9)           | 0.05               | 0.09               | 0.03             | 0.510   |
| Eicosadienoic (C20:2n-3)    | 0.61               | 0.63               | 0.01             | 0.559   |
| Arachidonic (C20:4n-6)      | 2.55               | 2.09               | 0.14             | 0.108   |
| Docosadienoic (C22:2n-6)    | 0.16               | 0.11               | 0.02             | 0.383   |
| Docosatrienoic (C22:3n-6)   | 0.08               | 0.09               | 0.01             | 0.116   |
| Eicosapentaenoic (C20:5n-3) | 0.15 <sup>b</sup>  | 0.18 <sup>a</sup>  | 0.01             | 0.016   |
| Lignoceric (C24:0)          | 0.16 <sup>b</sup>  | 0.21 <sup>a</sup>  | 0.01             | 0.006   |
| Nervonic (C24:1n9)          | 0.75               | 0.64               | 0.03             | 0.063   |
| Docosatetraenoic (C22:4n-6) | 0.20               | 0.18               | 0.01             | 0.184   |
| Docosapentaenoic (C22:5n-3) | 0.14               | 0.14               | 0.01             | 0.998   |
| Docosahexaenoic (C22:6n-3)  | 0.10               | 0.11               | 0.01             | 0.643   |
| Other                       | 0.56               | 0.46               | 0.05             | 0.439   |
| Total n-6 PUFA <sup>3</sup> | 26.81 <sup>a</sup> | 25.82 <sup>b</sup> | 0.26             | 0.033   |
| Total n-3 PUFA <sup>4</sup> | 2.00               | 2.20               | 0.06             | 0.142   |
| n-6/n-3 PUFA ratio          | 13.47 <sup>a</sup> | 11.72 <sup>b</sup> | 0.47             | 0.045   |

Specifically, muscles from group 2 were characterized by higher L\*, a\* and b\* (CWP: 62.47, 16.35 and 13.61; SBM: 55.44, 10.87 and 12.27, respectively). The analysis of the chemical composition of PM also showed significant differences in the content of protein (P < 0.0001) as well as collagen (P < 0.0001). No differences (P > 0.05; Table 1) were found in PM's moisture, and the fat level of broilers fed the SBM and CWP diets.

The thigh muscles (BF; *Biceps femoris*) obtained from birds fed with CWP were characterized by comparable pH<sub>24</sub> after slaughter than the SBM (Table 1). All colour values of BF from birds fed CWP were significantly higher (P < 0.0001) compared to birds fed SBM. However, the lightness (L\*) redness (a\*) and the yellowness (b\*) of BF differed depending on the source of protein in the feed. Moreover, dietary protein sources influenced the BF protein, fat, and collagen content (Table 1).

Muscles collected from broilers fed CWP diet registered a significantly higher (P = 0.016) content of protein as well as collagen (P < 0.0001), while the fat level was lower (P < 0.001) compared with birds fed SBM diet.

No differences ( $P > 0.05$ ) were found in the moisture content of the BF of birds fed SBM and that fed CWP diet.

Table 4

Fatty acids composition (% of total fatty acids) in thigh muscles from 6-week-old broiler chickens. <sup>1</sup>group 1: feed based on soybean meal; group 2: feed based on raw cowpea seeds (*Vigna unguiculata* [L.] Walp, Aura 26 cv.). <sup>2</sup>SEM, standard error of the mean. <sup>3</sup>Total n-6 PUFA: sum of C18:2n-6 + C18:3n-6 + C20:2n-6 + C20:3n-6 + C20:4n-6 + C22:2n-6 + C22:3n-6 + C22:4n-6. <sup>4</sup>Total n-3 PUFA: sum of C18:3n-3 + C18:4n-3 + C20:2n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3; <sup>a,b</sup> Means in the same row without same superscript differ significantly ( $P < 0.05$ ).

| Fatty acid                    | Group <sup>1</sup> |                    | SEM <sup>2</sup> | P-value |
|-------------------------------|--------------------|--------------------|------------------|---------|
|                               | 1                  | 2                  |                  |         |
| Caprylic (C8:0)               | 0.07 <sup>b</sup>  | 0.12 <sup>a</sup>  | 0.01             | 0.042   |
| Capric (C10:0)                | 0.04               | 0.10               | 0.02             | 0.176   |
| Lauric (C12:0)                | 0.04               | 0.13               | 0.03             | 0.121   |
| Myristic (C14:0)              | 0.71 <sup>b</sup>  | 0.90 <sup>a</sup>  | 0.05             | 0.041   |
| Myristoleic (C14:1)           | 0.15               | 0.18               | 0.01             | 0.161   |
| Pentadecanoic (C15:0)         | 0.35               | 0.43               | 0.02             | 0.064   |
| Pentadecenoic (C15:1)         | 0.18 <sup>b</sup>  | 0.67 <sup>a</sup>  | 0.12             | 0.015   |
| Palmitic (C16:0)              | 27.00 <sup>a</sup> | 25.12 <sup>b</sup> | 0.44             | 0.005   |
| Palmitoleic (C16:1)           | 6.23 <sup>a</sup>  | 5.36 <sup>b</sup>  | 0.20             | 0.0001  |
| Heptadecanoic (C17:0)         | 0.08 <sup>b</sup>  | 0.15 <sup>a</sup>  | 0.02             | 0.024   |
| Heptadecenoic (C17:1)         | 0.19               | 0.17               | 0.05             | 0.901   |
| Stearic (C18:0)               | 6.13               | 6.66               | 0.28             | 0.399   |
| Oleic (C18:1n-9cis)           | 38.87              | 37.72              | 0.42             | 0.192   |
| Linoleic (C18:2n-6)           | 15.07 <sup>b</sup> | 15.82 <sup>a</sup> | 0.18             | 0.009   |
| Arachidic (C20:0)             | 0.02               | 0.08               | 0.02             | 0.203   |
| Gamma-linolenic (C18:3n-6)    | 0.09               | 0.11               | 0.01             | 0.479   |
| Alpha-linolenic (C18:3n-3)    | 0.17 <sup>b</sup>  | 0.37 <sup>a</sup>  | 0.05             | 0.018   |
| Linoleic conjugate (C18:2)    | 0.19 <sup>b</sup>  | 0.35 <sup>a</sup>  | 0.04             | 0.044   |
| Octadecatetraenoic (C18:4n-3) | 0.67 <sup>b</sup>  | 1.10 <sup>a</sup>  | 0.10             | 0.001   |
| Eicosadienoic (C20:2n-6)      | 0.79 <sup>a</sup>  | 0.48 <sup>b</sup>  | 0.07             | 0.0001  |

| Fatty acid                  | Group <sup>1</sup> |                    | SEM <sup>2</sup> | P-value |
|-----------------------------|--------------------|--------------------|------------------|---------|
|                             | 1                  | 2                  |                  |         |
| Eicosatrienoic (C20:3n-6)   | 0.14 <sup>b</sup>  | 0.19 <sup>a</sup>  | 0.01             | 0.006   |
| Erucic (C22:1n-9)           | 0.01               | 0.02               | 0.01             | 0.132   |
| Eicosadienoic (C20:2n-3)    | 0.12 <sup>b</sup>  | 0.16 <sup>a</sup>  | 0.01             | 0.008   |
| Arachidonic (C20:4n-6)      | 0.50               | 0.55               | 0.02             | 0.233   |
| Docosadienoic (C22:2n-6)    | 0.11 <sup>b</sup>  | 0.24 <sup>a</sup>  | 0.03             | 0.049   |
| Docosatrienoic (C22:3n-6)   | 0.17 <sup>b</sup>  | 0.22 <sup>a</sup>  | 0.01             | 0.039   |
| Eicosapentaenoic (C20:5n-3) | 0.24 <sup>b</sup>  | 0.41 <sup>a</sup>  | 0.04             | 0.006   |
| Lignoceric (C24:0)          | 0.23 <sup>b</sup>  | 0.37 <sup>a</sup>  | 0.03             | 0.014   |
| Nervonic (C24:1n9)          | 0.12               | 0.14               | 0.01             | 0.158   |
| Docosatetraenoic (C22:4n-6) | 0.02 <sup>b</sup>  | 0.08 <sup>a</sup>  | 0.02             | 0.035   |
| Docosapentaenoic (C22:5n-3) | 0.04               | 0.23               | 0.07             | 0.154   |
| Docosahexaenoic (C22:6n-3)  | 0.10               | 0.31               | 0.08             | 0.206   |
| Other                       | 0.83               | 1.08               | 0.06             | 0.025   |
| Total n-6 PUFA <sup>3</sup> | 16.89 <sup>b</sup> | 17.69 <sup>a</sup> | 0.19             | 0.013   |
| Total n-3 PUFA <sup>4</sup> | 1.34 <sup>b</sup>  | 2.58 <sup>a</sup>  | 0.30             | 0.011   |
| n-6/n-3 PUFA ratio          | 12.63 <sup>a</sup> | 7.00 <sup>b</sup>  | 1.29             | 0.002   |

The effect of different protein sources on the texture profile analysis (TPA) of PM and BF is shown in Table 2. Overall, the dietary inclusion of CWP into broilers' diets did not negatively impact the TPA attributes of PM and BF, which showed similar values in both groups. Hardness, adhesiveness, cohesiveness, springiness, gumminess, chewiness, and resilience values were not different ( $P > 0.05$ ) among groups in PM and BF samples.

Data in Tables 3 and 4 indicate that the use of different protein sources in the diet of broiler chickens can influence the FA content in PM and BF samples. As expected, the predominant FA in the PM and BF of the broiler chickens fed both the SBM and CWP diets was C18:1n9cis (PM: 34.89 vs 35.14% of total FA, respectively; BF: 38.87 vs 37.72%, respectively), followed by C16:0 (PM: 21.21 vs 22.30% of total FA, respectively; BF: 27.00 vs 25.12%, respectively) and C18:2n-6 (PM: 22.74 vs 22.38% total FA; BF: 15.07 vs 15.82% total FA). Alpha-linolenic acid (ALA, C18:3n-3) is the precursor of long-chain n-3 PUFA such as

eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3), and docosahexaenoic acid (DHA, C22:6n-3), which are commonly referred to as n-3 or omega-3 PUFA. In this study, PM from birds fed CWP diets were characterized by a significantly higher content of ALA ( $P = 0.005$ ) and EPA ( $P = 0.016$ ) compared to the SBM diet (Table 3). Moreover, the proportion of total n-3 PUFA was slightly higher in chickens fed CWP (2.20) than in chickens fed SBM (2.00), but the difference was not significant ( $P > 0.05$ ). The n-6/n-3 PUFA ratio was significantly lower ( $P = 0.045$ ) in breast muscles from group 2 (11.72) compared to group 1 (13.47). Likewise, the use of different protein sources in the diet of broiler chickens influenced the FA compositions in BF samples (Table 4). The significantly higher ( $P = 0.018$ ) content of ALA and C18:4n-3 ( $P < 0.0001$ ) as well as EPA ( $P = 0.006$ ) and total n-3 PUFA content ( $P = 0.011$ ) was observed in chickens' thigh muscle fed CWP compared with the SBM. Also, the analysis of BF revealed a significantly lower ( $P = 0.002$ ) n-6 to n-3 PUFA ratio in chickens fed CWP-included diet (1.30) compared to SBM (7.00).

## Discussion

The present study provides new insights into the use of CWP in the diet of broiler chickens. For both groups, breast pH<sub>24</sub> fell in the range of standard poultry meat<sup>14</sup>. Meat colour is an essential quality parameter since it is directly perceived by the consumer. In our study, the change of protein source in the diet significantly influenced the colour of breast and thigh muscle. This has been confirmed by Laudadio *et al.*<sup>15</sup>, who reported differences in the values of redness ( $a^*$ ) and the yellowness ( $b^*$ ) of breast and leg muscles, which were higher in chickens fed a diet with the inclusion of other legume plants (i.e., faba bean) compared to broiler fed SBM. The authors suggested that higher values of yellowness ( $b^*$ ) in leg muscles could be attributed to different FA contents and the saturated FA/ PUFA ratio in these muscles. Another study<sup>16,17</sup> on pea or lupin as a substitute for SBM into broilers' diets also reported that the yellowness ( $b^*$ ) of leg muscles was significantly higher, suggesting a lower level of lipids in the leg muscles. Our study found significantly higher values of lightness ( $L^*$ ) for PM and BF from chickens fed a diet based on CWP. A trend towards higher lightness ( $L^*$ ) of leg muscles was also reported by Kuzniacka *et al.*<sup>18</sup>, who investigated the effect of faba bean (*Vicia faba* var. *minor*) based diet.

In our study, the use of CWP as a substitute for SBM had a significant effect on most of the chemical parameters (the content of protein, fat and collagen) of breast and thigh muscles from 6-weeks-old broiler chickens. Muscles collected from broilers fed CWP diet registered higher protein and collagen contents. Consistently with our study, Laudadio *et al.*<sup>15</sup> reported significantly higher content of collagen in breast and leg muscles from broilers fed a diet containing dehulled-micronized fava beans (310 g/kg). On the other hand, Dal Basco *et al.*<sup>19</sup> reported significantly lower fat content in leg muscles from organic slow-growing chickens fed a diet containing faba bean, which was also confirmed in our study. There is a lack of relative information regarding the texture properties of CWP, and indeed, of legume plants. Therefore, more study is needed to determine the effects of these seeds on textural properties in broiler breasts and meat. The TPA breast meat traits observed in the present research were overall satisfactory and in line with values reported for another legume, i.e., chickpea<sup>20</sup>.

Manipulating broiler chickens' diets by adding different protein sources resulted in significant changes in the FA composition of breast and thigh muscle. No research appears to have been reported on the effect of raw CWP seeds on meat FA composition in broiler chickens. Therefore, this subject should be considered a new investigation. The use of CWP influenced markedly the breast and thigh muscle FA content. The highest total n-3 PUFA content was observed in the chickens' breast (2.20 vs 2.00) and thigh muscle (2.58 vs 1.34) fed CWP compared to SBM. This result was associated with a significant increase in most of the n-3 FA's in PM and BF samples, especially for those that may have potential benefits to human nutrition, i.e., ALA and EPA. Similar results were noted in other experiments where broiler chickens were fed diets with different protein sources such as faba bean [15] and raw lentil seeds<sup>21</sup> as replacements for SBM. The authors of the cited studies reported higher total content of n-3 PUFA in the breast and leg muscles from chickens fed with these legumes than SBM; they also observed a reduction in the n-6/n-3 ratio. Recently, Paszkiewicz *et al.*<sup>22</sup> also found that the substitution of SBM by chickpea seeds can affect the FA proportions in the subcutaneous fat tissue of chickens; they also noted that chickpea inclusion decreased the content of main saturated acid (palmitic) and increased the content of main monounsaturated (oleic) and PUFA (linolenic). Desirable changes in the lipid profile of meat, including an increase in total n-3 PUFA content, and a decrease in the n-6/n-3 PUFA ratio, were also observed when SBM was replaced with camelina meal<sup>23</sup> or oil<sup>24</sup> and camelina oil or seeds<sup>25</sup> in diets for broiler chickens. On the other hand, Sirri *et al.*<sup>26</sup> reported that partial replacement of SBM with faba beans affected the proportions of some FA but had no effect on lipid fractions that are believed to be essential for human health.

Table 5

Nutritional composition of cowpea (*Vigna unguiculata* [L.] Walp, Aura 26 variety) seeds added to broiler diets. DM, dry matter; EE, ether extract; CF, crude fibre; Ca, calcium; P, phosphorus; TIA, trypsin inhibitor activity; TIU, trypsin international units; UA, urease assay; TSAA, total sulphur amino acids <sup>1</sup>AME, calculated value European Table of Energy Values for Poultry Feedstuffs (WPSA, 1989). <sup>2</sup>Total n-6 PUFA, a sum of C18:2n-6; C20:2n-6. <sup>3</sup>Total n-3 PUFA, sum of C18:3n-3; C22:6n-3.

| Nutrients                |       | Nutrients                          |       |
|--------------------------|-------|------------------------------------|-------|
| DM                       | 910.0 | <i>Fatty acid, % of total FAME</i> |       |
| CP, g/kg DM              | 288.4 | Lauric (C12:0)                     | 0.25  |
| EE, g/kg DM              | 11.6  | Myristic (C14:0)                   | 0.37  |
| CF, g/kg DM              | 51.1  | Pentadecanoic (C15:0)              | 0.24  |
| Ash, g/kg DM             | 45.5  | Palmitic (C16:0)                   | 24.11 |
| Ca, g/kg DM              | 23.3  | Palmitoleic (C16:1)                | 0.12  |
| P, g/kg DM               | 62.8  | Heptadecaenoic (C17:0)             | 0.33  |
| AME <sup>1</sup> , MJ/kg | 12.8  | Stearic (C18:0)                    | 5.66  |
| <i>Antinutrients</i>     |       | Oleic (C18:1n-9)                   | 9.47  |
| TIA, TIU/mg              | 10.8  | Linoleic (C18:2n-6)                | 34.53 |
| UA, pH change            | 0.29  | Alpha-linolenic (C18:3n-3)         | 21.85 |
| <i>Amino acids (%)</i>   |       | Octadecatetraenoic (C18:4n-3)      | 0.23  |
| Arginine                 | 1.78  | Eicosadienoic (C20:2n-6)           | 0.0   |
| Histidine                | 0.71  | Eicosatrienoic (C20:3n-6)          | 0.0   |
| Isoleucine               | 1.20  | Arachidonic (C20:4n-6)             | 1.24  |
| Leucine                  | 1.84  | Heneicosenoic (C21:0)              | 1.17  |
| Lysine                   | 1.86  | Docosatetraenoic (C22:4n-6)        | 0.43  |
| TSAA                     | 0.68  | Total n-6 PUFA <sup>2</sup>        | 36.20 |
| Phenylalanine            | 1.39  | Total n-3 PUFA <sup>3</sup>        | 22.08 |
| Threonine                | 1.22  | n-6/n-3 PUFA ratio                 | 1.64  |
| Valine                   | 1.15  |                                    |       |

The reasons for the above contradiction could be different combinations of feed ingredients or level of inclusion used for formulating diets in each experiment, or even various genotypes of chickens.

Overall, the use of CWP as an alternative to SBM in the diet of broiler chickens had positive effects on meat quality. It is important because where CWP can be grown locally, low-input farming systems would benefit from using this ingredient for broiler chickens feed.

## Methods

The Animal Ethics Committee approved the study protocol of the National Research and Development Institute for Animal Biology and Nutrition (INCDBNA-IBNA) Balotești, Romania, under the EU Directive 2010/63/EU and Romanian Law on Animal Protection. The slaughter of birds was carried out following the applicable rules on handling animals at the time of slaughter, including humane treatment. Also, the methods used in the meat quality tests were carried out by the current and commonly used methodology described in the Material and methods section. The study was conducted in compliance with the ARRIVE guidelines.

The cowpea seed Aura 26 variety used in this study is part of the collection of genotypes from the Research-Development Station for Plant Culture on Sands, Dăbuleni, Romania, and the National Commission approved registration for horticultural plants, Decision No. 92841/2012.

## Birds And Diets

The study was conducted on 240 one-d-old healthy broiler chickens (Ross 308) divided into two groups [group 1 (control) feed a diet with SBM as a source of protein, and group 2 (treatment) feed a diet with untreated CWP], with similar initial weights ( $46.5 \pm 0.23$  g) into 6 replications with 20 birds each. The nutritional composition of CWP of the Aura 26 variety is described in Table 5.

Table 6

Ingredient and chemical composition of diets (as-fed basis) SBM, soybean meal; CWP, cowpea; ME, metabolizable energy; TSAA, total sulphur amino acids. <sup>1</sup>Supplied per kg diet: 12000 IU vitamin A, 5000 IU vitamin D<sub>3</sub>, 75 mg vitamin E, 3 mg vitamin K<sub>3</sub>, 3 mg vitamin B<sub>1</sub>, 8 mg vitamin B<sub>2</sub>, 5 mg vitamin B<sub>6</sub>, 0.016 mg vitamin B<sub>12</sub>, 13 mg pantothenic acid, 55 mg nicotinic acid, 2 mg folic acid, 0.2 mg biotin, 120 mg Mn, 100 mg Zn, 40 mg Fe, 16 mg Cu, 1.25 mg I and 0.3 mg Se, 70 mg Monteban G100, 0.2 g Aextra PHY 5000 L (1000 FTU).

| Items                                | Starter (d 1–24) |       | Finisher (d 25–42) |       |
|--------------------------------------|------------------|-------|--------------------|-------|
|                                      | SBM              | CWP   | SBM                | CWP   |
| <b>Ingredient (%)</b>                |                  |       |                    |       |
| Corn                                 | 57.98            | 45.71 | 64.09              | 51.13 |
| Soybean meal                         | 31.00            | 23.00 | 25.20              | 17.30 |
| Corn gluten                          | 4.00             | 4.00  | 3.50               | 3.50  |
| Cowpea, cv. Aura 26                  | -                | 20.00 | -                  | 20.00 |
| Soybean oil                          | 2.00             | 2.30  | 2.60               | 3.57  |
| Monocalcium phosphate                | 1.67             | 1.61  | 1.45               | 1.36  |
| Calcium carbonate                    | 1.45             | 1.52  | 1.27               | 1.32  |
| Salt (NaCl)                          | 0.28             | 0.28  | 0.28               | 0.28  |
| L-Lysine HCl                         | 0.29             | 0.21  | 0.28               | 0.18  |
| DL-Methionine                        | 0.25             | 0.29  | 0.25               | 0.28  |
| Choline-chloride (50%)               | 0.08             | 0.08  | 0.08               | 0.08  |
| Vitamin-mineral mixture <sup>1</sup> | 1.00             | 1.00  | 1.00               | 1.00  |
| <b>Calculated composition</b>        |                  |       |                    |       |
| ME (MJ/kg)                           | 12.70            | 12.69 | 13.23              | 13.24 |
| CP                                   | 22.0             | 22.0  | 19.50              | 19.50 |
| Lysine, total                        | 1.34             | 1.34  | 1.15               | 1.15  |
| Lysine, digestible                   | 1.20             | 1.22  | 1.04               | 1.03  |
| TSAA, total                          | 0.98             | 0.98  | 0.90               | 0.90  |
| TSAA, digestible                     | 0.89             | 0.90  | 0.83               | 0.83  |
| Ca                                   | 0.90             | 0.91  | 0.78               | 0.79  |
| Available P                          | 0.45             | 0.45  | 0.39               | 0.39  |

| Items       | Starter (d 1–24) |      | Finisher (d 25–42) |      |
|-------------|------------------|------|--------------------|------|
|             | SBM              | CWP  | SBM                | CWP  |
| Crude fat   | 4.92             | 5.10 | 5.65               | 6.77 |
| Crude fibre | 2.80             | 3.34 | 2.62               | 3.16 |

The feeding program was divided into two feeding phases: starter (days 1-24) and finisher (days 25-42). Diets for each feeding phase were formulated to be isocaloric, isonitrogenous, with similar total lysine, total sulphur amino acids (TSAA; Table 6), calcium and available phosphorous, and to meet or exceed breeder guidelines (Ross 308, Aviagen Ltd., Midlothian, UK). Diets were manufactured in mash form, without the inclusion of growth promoters or antibiotics. However, narasin as a coccidiostat (Monteban G100, Elanco GmbH) and phytase (Aextra PHY 5,000 L, Danisco Animal Nutrition, Marlborough, UK) as exogenous enzymes were included in premixes of all two experimental diets.

Feed and water were provided *ad libitum*. Chickens were kept in pens on shavings litter in a temperature-controlled room with pens of identical size (1.75 × 1.55 m). Room temperature was maintained at 34°C for the first 5 d and then gradually reduced according to standard management practices until a temperature of 22°C by using thermostatically controlled heaters, fans, and adjustable sidewall inlets. Lighting was provided for 23 h/d from 1D to 7D, and from 8D, the light decreased by 1 h a day until 20 h, according to EU legislation (EU Council Directive 2007/43/EC). Broilers were vaccinated at the hatch for Marek's, Newcastle, and Infectious Bronchitis Disease.

## Slaughtering Procedures And Muscle Sampling

At 42 days of age, twelve birds (two birds/pen) from each feeding group (chosen based on pen average final live weight) were individually identified and weighed. The birds were electrically stunned before slaughter, exsanguinated by neck cut, scalded, and eviscerated. Chickens live weight was registered before slaughtering. A total of twelve breasts and twelve thighs were collected on their right and left sides, individually vacuum-sealed and refrigerated (4±1°C). Meat quality parameters (pH<sub>24</sub>, and color) were assessed on the *Pectoralis major* muscle (PM) on the right breast and the *Biceps femoris* muscle (BF) on the right thigh, while the left breast and thigh meat were frozen at -20°C until further analysis (instrumental TPA, proximate chemical composition and FA composition).

## Meat Quality Traits

### pH value and instrumental colour measurements

After 24 h cold storage at 2°C, the pH value was measured in triplicate using a Hanna portable pH-meter (model HI 99163, Hanna Instruments, Romania), fitted with a spear-type electrode (FC 099 stainless steel

blade tip) and an automatic temperature compensation probe. The colour of the muscles was determined using a portable colourimeter (model CR 410, Konica Minolta Inc., Osaka, Japan) calibrated with a white ceramic tile on D65 illuminate. The results were expressed in the CIE Lab colour space<sup>27</sup>. The lightness component is represented by  $L^*$ , which ranges between 0 to 100, redness by  $a^*$  and yellowness by  $b^*$ , both of which have a range of -120 to +120. For instrumental colour determination, three measurements were performed on a fat-free surface area in different locations of each muscle (PM, BF).

## Texture profile analysis

The texture measurements of raw chicken muscle samples were analyzed individually by a double cycle compression using a texture analyzer (Model CT3 BROOKFIELD Engineering Laboratories, Inc. MA, USA). To texture analysis, each muscle (PM and BF) was cut into 3 cylinder shapes with a diameter of 20 mm and a height of 15 mm. For an increased accuracy of the parameters reading, there were avoided any large areas of fat. The texture analyzer was equipped with a 50 kg load cell, a cylinder probe of  $76.2 \times 10$  mm to compress the samples and a fixture base table. The probe moved towards the sample at a constant speed of  $2.0 \text{ mm s}^{-1}$  (pre-test),  $1.0 \text{ mm s}^{-1}$  (test), and  $2.0 \text{ mm s}^{-1}$  (post-test). The data was collected using Texture Pro CT Software.

## Proximate chemical composition

PM and BF samples were minced, homogenized, and divided into two parts. A portion was used to perform NIR (near-infrared reflectance) spectroscopy analysis. The remaining part was frozen and afterwards analyzed for total lipid extracts and FA composition. NIR data were acquired using a DA6200 meat analyzer (PerkinElmer, Inc. MA, USA), with transmission spectroscopy that uses diode array detectors in the wavelength range of 850 to 1050 nm. Raw minced and homogenized samples from the PM and BF muscles were loaded into a magnetically coupled plastic sample dish of 14 mm height and a volume of 170mL and analyzed for moisture, protein, fat and collagen contents. To minimize sampling error, we set two duplicates, and each replication was measured twice. Before the sample measurements, a polystyrene check sample was used to verify the optical performance. The averaged spectrum was then used in subsequent analysis.

## Fatty acid composition

The FA content was assessed via fatty acid methyl ester (FAME) gas chromatography. The method used for FA composition were done in the same way as previously described by Ciurescu *et al.*<sup>28</sup>. In brief, FA from the total lipid extracts was converted to methyl esters by transesterification (in methanol containing 3% concentrated  $\text{H}_2\text{SO}_4$ , for 4 h at  $80^\circ\text{C}$ ). Methyl esters of FA were evaluated in a Perkin Elmer-Clarus 500 chromatograph equipped with a flame ionization detector (FID) fitted with a BPX70 capillary column ( $60 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$  film thickness). The column temperature was programmed at  $5^\circ\text{C}/\text{min}$  from  $180^\circ\text{C}$  to  $220^\circ\text{C}$ . The carrier gas was hydrogen ( $35 \text{ cm/s}$  linear velocities at  $180^\circ\text{C}$ ), and the splitting ratio was 1:100. The injector and detector temperatures used were  $250^\circ\text{C}$  and  $260^\circ\text{C}$ , respectively. Peaks were identified by injecting pure FAME standards; quantification was assessed using tridecanoic acid (C13:0)

as an internal standard. The results were expressed as the percentage of the total detected FA. The ratio of n-6 PUFA to n-3 PUFA (n-6/n-3 PUFA ratio) was calculated.

## Statistical analysis

The data were processed with SPSS Statistics software, v.20.0 for Windows (IBM SPSS Statistics, Armonk, NY, USA). A one-way analysis of variance (ANOVA) was used. Results are reported as means and standard error of the mean (SEM). The effect of the diet on the meat quality parameters was analyzed using Student's *t*-tests for independent samples. Significance was declared at  $P < 0.05$ . A statistical trend was considered for  $0.05 < P \leq 0.10$ .

## Ethics

The Animal Ethics Committee approved the bird's care and used protocol at the National Research-Development Institute for Biology and Animal Nutrition (INCDBNA-IBNA), Balotești, Romania, following the principles of EU Directive 2010/63/EU as transposed to Romanian legislation on Animal Protection used for Scientific Purposes (Law no. 199/2018). Also, the methods used in the meat quality tests were carried out in accordance with the current and commonly used methodology described in the Material and methods section.

## Declarations

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### Author contributions

All authors took part in meat quality analysis. G.C., R.D.—designed the experiment, G.C., L.I., A.G analyzed physicochemical and textural traits of meat, M.R. analyzed the fatty acid composition, G.C., A.G. analyzed data, G.C., L.I., A.G. wrote the paper in cooperation with all of the authors. All authors approved the final manuscript.

### Competing interests

The authors declare no competing interests.

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