Effect of dietary cowpea (Vigna unguiculata [L] Walp) and chickpea (Cicer arietinum L.) seeds on growth performance, blood parameters and breast meat fatty acids in broiler chickens

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\textbf{ABSTRACT}

The effects of dietary replacement of soybean meal (SBM) with two different protein sources (cowpea [CWP] and chickpea [CKP]) on growth performance (GP), blood parameters and breast meat fatty acids (FA) content were evaluated. A total of 780 one-d-old broiler chicks (Cobb 500) were allocated, over 2 growth phases (starter, 0–21 d; finisher, 22–42 d), to 5 dietary treatments as follow: (1) a corn-SBM basal diet as control; (2) 10% raw cowpea; (3) 20% raw cowpea; (4) 10% raw chickpea and (5) 20% raw chickpea. The results indicated that CWP and CKP diets conducted to similar GP (body weight, body weight gain, feed intake and feed conversion ratio) when compared to the SBM diet, but changes in the content of blood parameters were observed. Glucose and total cholesterol decreased significantly in birds fed CWP (\(p < .0001\)) and CKP (\(p < .001\)) diet. Also, the use of CWP and CKP had a significant effect on the content of breast muscle FA, especially for \(\alpha\)-linolenic (\(p < .0001\)), eicosapentaenoic (\(p < .001\)), docosapentaenoic (\(p = .034\)) and docosahexaenoic (\(p = .003\)) acids, which were higher than those fed SBM diet. In addition, there was a significant interaction between the protein source and the inclusion level (S/L) for the majority of the FA’s. Based on the results obtained, it can be concluded that CWP and CKP seeds represent an interesting alternative protein sources, which can improve broilers performance, health status and breast fatty acids composition.

\textbf{HIGHLIGHTS}

- Cowpea (CWP) and chickpea (CKP) can be used as replacement for soybean meal in broiler chickens diets, at inclusion levels up to 200 g/kg.
- Dietary use of CWP and CKP decreased plasma cholesterol and glucose concentration.
- Use of CWP and CKP in broilers diets positively modified breast meat fatty acids, especially \(\alpha\)-linolenic acid, eicosapentaenoic or docosahexaenoic acids with potential benefits to human nutrition.

\textbf{Introduction}

Soybean meal (SBM) comes mostly from genetically modified (GM) crops (ISAAA 2017) and this situation prompts studies for other protein sources that could be alternatively introduced into poultry diets. Particularly, in European Union (EU) countries, ecologic livestock systems are in need of non-GM feedstuffs (Council Regulation (EC) No 834/2007; Council Regulation 2018) and of cheap and locally available protein sources, to expand the farm feed base. Currently, breeding efforts and genetic progress have led to the development of improved legume varieties with a lower content of anti-nutritional factors (ANF), high yield potential and high resistance to diseases and adverse environmental conditions (Torres et al. 2010), thus promoting the production and efficient use of legume seeds for animal feed. Moreover, attention to the alternative protein sources for feeding animals are essential subject nowadays after COVID-19 outbreaks (Hafez and Attia 2020).

Cowpea (CWP; Vigna unguiculata [L] Walp) and chickpea (CKP; Cicer arietinum L.) are the world’s most
important seed legumes (FAO 2019) because they are a valuable source of protein and carbohydrates. They are not only rich in nutrients, but also in nutraceuticals such as dietary fibre, antioxidants, polyunsaturated fatty acids (PUFA) and polyphenols, minerals and vitamins (Trinidad et al. 2010; Ashraduzzaman et al. 2011; Heiras-Palazuelos et al. 2013; Shetty et al. 2013; Baptista et al. 2017; Jayathilake et al. 2018). Because of its high protein content, CWP is named ‘the queen of the semi-arid areas’, representing an alternative source to soy and bean crops, under drought conditions, and is locally grown in Romania (Drăghici et al. 2016a, 2016b). Depending on the variety, these seeds have a low content of ANF (i.e. trypsin and chymotrypsin inhibitor) compared with soybeans, and common beans, thus offering fewer problems in poultry nutrition (Bampidis and Christodoulou 2011; Jukanti et al. 2012).

Previous studies indicate that these seeds, in raw form, are valuable sources of dietary protein used to feed chickens (Christodoulou et al. 2006; Brenes et al. 2008; Garsen et al. 2008; Abdelgani et al. 2013; Embaye et al. 2018) and turkeys (Ciurescu et al. 2020). Because meat quality is important for producers and, above all, for consumers, an important meat quality trait is the content of PUFA, which is beneficial to human health (Korakas et al. 2018). Up to date we couldn’t find any research about the effect of raw CWP and CKP on the quality of broiler meat, therefor this research was an opportunity to test these diets effect on fatty acids (FA) deposition in breast muscle.

Thus, the objective of this study was to investigate an appropriate inclusion level of raw CWP and CKP in broilers’ diet as an alternative to SMB and to assess the effects on growth performance, blood parameters and breast meat FA content.

Materials and methods

Birds, housing and diets

A total of 780 one-d-old healthy broiler chicks (Cobb 500; mixed sexes) were bought from a local hatchery (Prahova, Romania). Upon arrival, chicks were individual weighed (initial BW 43.0 ± 3.2 g) and allocated to 5 dietary treatments in a complete randomised experimental design. Each treatment contained 6 replicates (pens) with 26 chickens each. One pen of broilers represented the experimental unit. Birds were kept on floors covered with wood shavings as litter. The room temperature was maintained at 33 °C for the first 3 d, and then was reduced by 3 °C weekly until reaching 20 °C by using thermostatically controlled heaters, fans, and adjustable sidewall inlets. Relative humidity was about 50–70%. Light was provided in 20-h light (5–10 Lux) and 4-h dark cycles.

The CWP (cv. Ofelia, drought-tolerant) and CKP (cv. Burnas) seeds were obtained from two plant breeding stations (SCDA Draganesti-Vlasca and SCDCPN Dabuleni, Romania). The dietary treatments were formulated as follow: (1) a corn-SBM basal diet as control (SBM); (2) 10% raw cowpea (10CWP); (3) 20% raw cowpea (20CWP); (4) 10% raw chickpea (10CKP) and (5) 20% raw chickpea (20CKP). Raw CWP and CKP replaced part of the SBM and corn. In order to meet or exceed the nutrient requirements of breeder guide (Cobb-Vantress 2018), for each feeding phase (starter, 0–21 d; finisher, 22–42 d), diets were formulated to be isocaloric, isonitrogenous and with similar content of total lysine, total sulphur amino acids (TSAA; methionine + cysteine), calcium and available phosphorous as it is summarised in Table 1. Diets in mash form and water were provided ad libitum.

Feeds chemical analysis

Ingredients and diets were analysed in duplicate for DM, CP, EE, and ash content, using standard procedures in accordance with the methods of the Commission Regulation (EC) no. 152 (OJEU.2009). Carbohydrate content was estimated as nitrogen-free extract (NFE). Apparent metabolisable energy (AME) content of the diets was calculated on the basis of the energy content of individual feed ingredients using European tables of energy values for poultry feedstuffs equation (WPSA.1989). Amino acids (AA; excluding tryptophan) were analysed using a HPLC System (Surveyor Plus, Thermo Fisher Scientific Inc., San Jose, CA, USA), according to the conditions described by Ciurescu et al. (2018). All composition data are on a DM basis. The CWP and CKP cultivars were also analysed for urease activity (UA). The UA was used as a ‘marker’ to indirectly reflect the presence of ANF, mainly trypsin inhibitors activity (TIA). The UA assay was done as described by AOCS (2006), and the TIA assay was done according to Valdebeuze et al. (1980).

Growth performances

Body weight (BW) and feed intake (FI) were determined on a pen basis on d 21 and d 42, and mortality was recorded daily to calculate body weight gain (BWG) and feed conversion ratio (FCR).
Plasma analyses

At 42 d of age, 30 birds with an average live weight within the treatments were selected (6 from each treatment; one bird/replicate). Blood samples were collected from the wing vein into 5 mL heparinised tubes for plasma biochemistry assay. Glucose (Glu), total cholesterol (T-Cho), triglycerides (TG), total protein (T-Pro), albumin (Alb), total bilirubin (T-Bil), creatinine (Cre), urea, Ca, P, Mg, Fe, alanine aminotransferase (ALT/GPT), aspartate aminotransferase (AST/GOT), alkaline phosphatase (AP), gamma-glutamyl-transferase (GGT), lactate dehydrogenase (LDH), and creatine kinase (CK) were determined by routine methods as previously described by Ciurescu et al. (2020).

Muscle sampling and fatty acids analyses

The same birds were then slaughtered and the left side of the breast muscles (Pectoralis major) was manually removed, cleaned and packed into polyethylene bags, then stored at −20°C until analysis. The muscle content of FA’s was analysed as previously described by Ciurescu et al. (2018) using a gas chromatograph (Perkin-Elmer Clarus 500, Massachusetts, United States). The chromatograph has flame ionisation detector (FID) and capillary separation column with a high polar stationary phase TRACE TR-Fame, (Thermo Electron, Massachusetts, USA), with dimensions of 60 m × 0.25 mm × 0.25 μm. The results were expressed for each FA as % of total Fatty Acid Methyl Esters (FAME).

Statistical analyses

The statistical processing of the results was done using general linear model (GLM) of SPSS, version 20.0 (SPSS Inc., Chicago, IL, USA). When comparing treatments means, Post hoc Tukey’s multiple range test was carried out to assess any significant differences for the measured parameters. Data were also analysed
by excluding the SBM dietary treatment. In this case, the experimental variable included 2 protein sources (S; CKP and CWP, respectively), each at 2 inclusion levels (L; 10 and 20%). Differences were considered significant at $p<.05$ and tendency was set at $p<.10$. Replicate-pen was used as the experimental units for the analysis of GP (BW, BWG, FI, and FCR). For blood and breast meat FA’s data, the sampling unit was individual broilers.

**Results and discussion**

**Nutrient composition of CKP and CWP seeds**

As it can be observed from Table 2, the CWP seeds had a higher CP content in comparison with CKP (295.4 vs. 233.9 g/kg DM) and a lower content of EE (by 29.5%). AME value and AA concentrations were similar in both evaluated seeds. As with the characteristics of legume, the CWP and CKP seeds were high in lysine (6.7–7% of the protein), but low in the TSAA (methionine and cysteine, 2%), compared to the requirements of broilers in the starter phase. Present findings regarding proximate composition are in line with values described in previous literature (Tshovhote et al. 2003; Bampidis and Christodoulou 2011; Jukanti et al. 2012; Anjos et al. 2016). TIA concentration was higher in CKP compared to CWP seeds (10.2 vs. 5.4 TIU/mg). Previous reports (Anjos et al. 2016) describing a local Mozambican CWP (Vigna unguiculata, var. nhemba) show a TIA concentration of 6,700 TIU/g. Muzquiz et al. (2012) observed that the content of trypsin inhibitors in different Spanish CKP ranged from 1.22 to 1.02 TIU/mg (Cicer arietinum L., var. Duratón). On the other hand, Garsen et al. (2008) reported that the content of Greek variety chickpea ‘Amorgos’ in ANFs trypsin and chymotripsin inhibitors (TIU and HTIU) is very low (1.6 and 3.0/mg of extracted sample, respectively). These different results could be due to differences among cultivars or growing conditions and also moisture levels.

When evaluating the FA’s content, linoleic acid (LA, C18:2n-6) was the predominant one in both CKP and CWP seeds (54.97 vs. 32.04% of total FAME). Nevertheless, in comparison with CKP, the CWP seeds contain almost ten times more alpha-linolenic acid (ALA, C18:3n-3; 20.74 vs. 2.71%) and more than twice as much palmitic acid (C16:0; 24.14 vs. 10.34%). Furthermore, CWP had the highest proportion of total n-3 PUFA, thus the n-6/n-3 ratio was lower in CWP compared with CKP (1.17 vs. 20.38%). These results are in close agreement with ones reported in the literature by Zia-Ul-Haq et al. (2010), Antova et al. (2014), Gonçalves et al. (2016), Baptista et al. (2017).

**Growth performance**

There was no effect of the dietary treatments on the GP (BW, BWG, FI and FCR; Table 3). The results show that broilers fed diets containing CWP and CKP had comparable GP to those fed SBM-based diet throughout the study period (d 1 to d 42; $p > .05$). Neither

| Item                  | Cowpea (cv. Ofelia) | Chickpea (cv. Burnas) |
|-----------------------|---------------------|-----------------------|
| Nutrient, g/kg DM     |                     |                       |
| Dry matter            | 899.70              | 895.90                |
| AME* (MJ/kg)          | 12.80               | 12.70                 |
| Crude protein         | 295.40              | 233.90                |
| Ether extract         | 13.10               | 44.40                 |
| Crude fibre           | 51.30               | 53.80                 |
| Ash                   | 43.50               | 60.10                 |
| NFE                   | 640.2               | 667.90                |
| Calcium               | 23.30               | 23.10                 |
| Phosphorous, total    | 62.80               | 62.60                 |
| Antinutrients         |                     |                       |
| TIA, TIU/mg           | 5.40                | 10.20                 |
| UA, pH change         | 0.29                | 0.34                  |
| Amino acids, g/100g   |                     |                       |
| Lysine                | 1.84                | 1.88                  |
| TSAA                  | 0.68                | 0.79                  |
| Threonine             | 1.22                | 1.02                  |
| Arginine              | 1.78                | 2.01                  |
| Leucine               | 1.83                | 1.93                  |
| Isoleucine            | 1.20                | 0.91                  |
| Tyrosine              | 0.71                | 0.63                  |
| Phenylalanine         | 1.39                | 1.25                  |
| Valine                | 1.15                | 0.96                  |
| Aspartic acid         | 2.59                | 2.90                  |
| Glutamic acid         | 5.67                | 4.97                  |
| Serine                | 0.97                | 1.25                  |
| Glycine               | 0.73                | 0.92                  |
| Alanine               | 1.06                | 1.41                  |
| Fatty acids, % of total FAME |       |                      |
| Lauric (C12:0)        | 0.19                | 0.09                  |
| Myristic (C14:0)      | 0.37                | 0.25                  |
| Pentadecanoic (C15:0) | 0.25                | 0.12                  |
| Palmitic (C16:0)      | 24.14               | 10.34                 |
| Palmitoleic (C16:1)   | 0.18                | 0.27                  |
| Heptadecanoic (C17:0) | 0.34                | ND                    |
| Stearic (C18:0)       | 5.11                | 1.41                  |
| Oleic (C18:1n-9)      | 9.17                | 29.23                 |
| Linoleic (C18:2n-6)   | 32.04               | 54.97                 |
| Alfa-linolenic (C18:3n-3) | 20.74            | 2.71                  |
| Heneicosenoic (C21:0) | 0.90                | 0.35                  |
| Eicosadienoic (C20:2n-6) | ND            | 0.26                  |
| Docosahexanoic (C22:6n-3) | 6.58            | ND                    |
| Total n-6 PUFA*       | 32.04               | 55.23                 |
| Total n-3 PUFA*       | 27.32               | 2.71                  |
| n-6/n-3 PUFA ratio    | 1.17                | 20.38                 |

NFE: nitrogen-free extract; TIA: trypsin inhibitor activity; TIU: trypsin international units; UA: urease assay; FAME: fatty acid methyl esters; ND: measured but not detected; AME: apparent metabolizable energy; TSAA: total sulphur amino acids (methionine + cysteine); PUFAs: polyunsaturated fatty acids.

*Calculated value: European Table of Energy Values for Poultry Feedstuffs (WPSA. 1989).

Total n-6 PUFA: sum of C18:2n-6 + C20:2n-6.

Total n-3 PUFA: sum of C18:3n-3 + C22:6n-3.
protein sources nor inclusion level affected GP in starter or finisher phase. Furthermore, no significant interaction was observed between protein source and inclusion level (S x L) for all GP measured variables. Abdelgani et al. (2013) and Embaye et al. (2018), noted that feeding graded levels of raw CWP (at 0–20%) did not affect broilers’ performance, indicating that CWP can replace SBM. On the contrary, Gumaa-Balaiel (2014) recommended up to 10% inclusion of untreated cowpea seeds in broiler chickens diets. Akanji et al. (2016) attributed the depression in growth to the presence of ANF (i.e. protease inhibitors) as they interfere with the digestion of protein and utilisation of minerals. In our study the good performances of chickens suggest that the tested CWP (cv. Ofelia) contains a low level of ANF, being less harmful. Garsen et al. (2008) also found that partial replacement of SBM with raw CKP resulted in similar performance of broiler chickens when chickpeas were supplemented to their diet in gradually increasing levels up to 480 g/kg with respect to the birds’ age (i.e. 160 g/kg for 1–14 d of age, 240 g/kg for 15–28 d, and 480 g/kg for 29–42 d). Similarly, Brenes et al. (2008) noted that increasing chickpea content in the diet (up to 300 g/kg) of broilers (Cobb) from 1 to 21 d of age did not affect birds’ performance. They also reported no differences between raw and extruded chickpeas (var. Kabuli). Likewise, in previous study with turkeys (Ciurescu et al. 2020) the inclusion of 24% raw CKP did not have a negative effect on GP and carcase traits. Conversely, Christodoulou et al. (2006) showed that partial replacement of SBM with raw CKP (i.e. 120 g/kg of diet) resulted in similar performance of broiler chickens (i.e. Cobb 500) compared to the SBM diet, while a higher inclusion level of 240 g/kg of diet adversely affected productive performance. Some differences between our study and cited authors could be caused by the other genotype of chickens that was used in experiments, or even various species (chickens, turkeys). The maintenance conditions could be different, as well as the method of feed preparing, including cultivar of CWP or CKP seeds.

### Plasma parameters

Blood biochemistry is a labile biochemical system that can reflect the condition of the organism and the changes happening to it under influence of internal and external factors. Dietary treatments influenced the plasma energy parameters in broiler chickens (Table 4). Compared with the SBM-based diet, the CWP or CKP diets decreased (p<.0001) plasma Glu and T-Chol (p<.001) and increased (p<.0001) Ca, P (p<.000) and Fe (p=.006) concentration. Among the two sources of protein, the CKP diets, level independent, decreased (p=.034) the TG concentration and increased (p<.0001) Ca and Mg (p=.068) content. There was no effect of dietary treatments on the plasma protein profile.

Viveros et al. (2007) and Ciurescu et al. (2017) came to similar conclusions and found that compared with SBM other legumes based diets (i.e. lupin, lentil seeds) caused blood parameter changes in chickens such as decreases in serum Glu, TG, cholesterol concentrations. Moreover, in human studies, Abeysekara et al. (2012) suggested that the consumption of pulse-based diet, decreased serum cholesterol (known as a risk factor for cardio vascular disease) and increased the saturation levels of cholesterol in the bile. They also reported that the intake of pulse-based diet (included

### Table 3. Effects of dietary treatments on growth performance of broiler chickens.

| Items                    | Dietary treatments | SEM | p-Valuea treatments | p-Valueb | Source (S) | Level (L) | S x L |
|--------------------------|-------------------|-----|---------------------|----------|------------|-----------|-------|
| IBW, g/bird              | SBM               | 43.2|                     |          | .023       | .587      | .804  |
| BWG, g/bird              | 1,710             |     |                     |          | .963       | .903      | .746  |
| Fl, g/bird               | 3,445             |     |                     |          | .597       | .504      | .611  |
| FCR, g               | 2.01              |     |                     |          | .526       | .595      | .505  |
| Overall (d 0–42)         |                   |     |                     |          |            |           |       |
| IBW, g/bird              | 43.2              |     |                     |          | .023       | .587      | .804  |
| BWG, g/bird              | 1,710             |     |                     |          | .963       | .903      | .746  |
| Fl, g/bird               | 3,445             |     |                     |          | .597       | .504      | .611  |
| FCR, g               | 2.01              |     |                     |          | .526       | .595      | .505  |

SBM: soybean meal; CWP: cowpea; CKP: chickpea; IBW: initial body weight; FBW: final body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; SEM: standard error of the mean of all treatments.

|          |                |     |                      |          |            |           |       |
|-----------|----------------|-----|----------------------|----------|------------|-----------|-------|
|          | Source (S)     |     |                      |          |            |           |       |
|          | Level (L)      |     |                      |          |            |           |       |
|          | S x L          |     |                      |          |            |           |       |

*aData were analysed as a monofactorial arrangement, including SBM dietary treatment.

*bData were analysed excluding SBM dietary treatment.
Table 4. Effects of dietary treatments on blood biochemical parameters of broiler chickens.

| Items                    | Dietary treatments | p Value<sup>d</sup> | p Value<sup>e</sup> |
|--------------------------|-------------------|-------------------|-------------------|
|                          | SBM   | CWP10  | CWP20  | CKP10  | CKP20  | SEM   | Source (S) | Level (L) | S × L   |
| Plasma energy profile    |       |        |        |        |        |       |            |           |         |
| Glucose, mg/dL           | 290.54<sup>a</sup> | 256.73<sup>b</sup> | 265.40<sup>b</sup> | 255.38<sup>ab</sup> | 256.82<sup>b</sup> | 4.91   | .0001       | .354      | .346    | .497   |
| Cholesterol, mg/dL       | 114.99<sup>a</sup> | 84.03<sup>b</sup> | 91.22<sup>b</sup> | 86.75<sup>b</sup> | 83.60<sup>b</sup> | 5.01   | .001        | .638      | .698    | .327   |
| Triglycerides, mg/dL     | 73.43  | 72.68  | 78.27  | 65.92  | 70.52  | 3.70   | .247        | .034      | .122    | .875   |
| Plasma protein profile   |       |        |        |        |        |       |            |           |         |        |
| Total protein, g/dL      | 4.94   | 4.77   | 4.82   | 4.85   | 4.75   | 0.13   | .871        | .938      | .860    | .609   |
| Albumin, g/dL            | 2.82   | 2.62   | 2.60   | 2.75   | 2.68   | 0.07   | .243        | .147      | .955    | .292   |
| Total bilirubin, mg/dL   | 0.22   | 0.23   | 0.22   | 0.23   | 0.22   | 0.02   | .975        | .950      | .572    | .950   |
| Creatinine, mg/dL        | 0.39   | 0.40   | 0.39   | 0.41   | 0.37   | 0.02   | .821        | .830      | .255    | .637   |
| Urea, mg/dL              | 6.00   | 6.21   | 6.62   | 6.94   | 6.03   | 0.39   | .377        | .846      | .472    | .068   |
| Plasma mineral profile   |       |        |        |        |        |       |            |           |         |        |
| Calcium, mg/dL           | 5.27<sup>c</sup> | 6.69<sup>b</sup> | 7.25<sup>b</sup> | 8.39<sup>a</sup> | 8.51<sup>a</sup> | 0.25   | .0001       | .0001     | .238    | .438   |
| Phosphorus, mg/dL        | 4.23<sup>b</sup> | 7.01<sup>a</sup> | 6.25<sup>a</sup> | 6.28<sup>b</sup> | 6.96<sup>b</sup> | 0.38   | .0001       | .976      | .927    | .108   |
| Magnesium, mg/dL         | 1.79   | 1.80   | 2.12   | 2.25   | 2.69   | 0.26   | .114        | .068      | .164    | .827   |
| Iron, µg/dL              | 68.53<sup>b</sup> | 95.82<sup>a</sup> | 89.02<sup>ab</sup> | 103.92<sup>a</sup> | 90.13<sup>ab</sup> | 5.84   | .006        | .459      | .109    | .572   |
| Plasma enzyme profile    |       |        |        |        |        |       |            |           |         |        |
| ALT/GPT, U/L             | 50.22  | 47.84  | 50.58  | 47.40  | 47.12  | 3.20   | .899        | .514      | .680    | .613   |
| AST/GOT, U/L             | 60.47  | 57.37  | 63.75  | 57.21  | 61.21  | 3.08   | .540        | .665      | .108    | .702   |
| AP, U/L                  | 69.16  | 71.96  | 57.97  | 53.39  | 58.63  | 5.66   | .139        | .138      | .457    | .113   |
| GGT, U/L                 | 17.44  | 18.34  | 15.68  | 17.76  | 15.72  | 1.47   | .608        | .846      | .106    | .824   |
| LDH, U/L                 | 614.04 | 560.19 | 576.62 | 668.66 | 636.76 | 70.71  | .791        | .227      | .865    | .784   |
| CK, U/L                  | 1016.11 | 980.18 | 869.56 | 918.67 | 843.24 | 50.34  | .121        | .382      | .075    | .723   |

SBM: soybean meal; CWP: cowpea; CKP: chickpea; ALT/GPT: alanine aminotransferase; AST/GOT: aspartate aminotransferase; AP: alkaline phosphatase; GGT: gamma-glutamyl-transferase; LDH: lactate dehydrogenase; CK: creatinkinaza; SEM: standard error of the mean of all treatments.

<sup>a</sup>b<sup>c</sup><sup>d</sup>e<sup>f</sup>Means within a row lacking a common superscript differ (<sup>p</sup><0.05).

<sup>d</sup>eData were analysed as a monofactorial arrangement, including SBM dietary treatment.

<sup>e</sup>dData were analysed excluding SBM dietary treatment.

lentils, chickpeas, beans and peas), because of their low glycaemic index and mineral content, has favourable effects on blood pressure and glycaemic regulation. It seems that plasma cholesterol concentration is influenced by the FA composition of the dietary fat with high levels of long chain SFA increasing plasma cholesterol level, compared to high levels of MUFA and PUFA.

Plasma ALT/GPT, AST/GOT, AP, and LDH are parameters for liver damage assessment, and diagnosis of these enzymes is frequently used for hepatic function evaluation. In the present study the replacement of SBM with CWP or CKP did not affect the activity of these plasma enzymes, indicating that the protein source or level had no effect on liver health. In addition, the CK, a marker for heart or skeletal muscle damage, was found in normal physiological parameters for broiler chickens. As presented in Table 4, there was no significant differences (p<0.05) on the interaction between protein source and inclusion level (S × L) on blood biochemical characteristics.

**Meat fatty acids profile**

Data shown in Table 5 indicate that the use of different sources of protein in the diet of broiler chickens can influence the FA content in breast meat. Across dietary treatments, oleic acid (C18:1n9cis, 34.21–32.51%; p<0.001), followed by palmitic acid (C16:0, 27.50–28.68%; p<0.0001) and LA (17.25–14.35%; p<0.0001) were the main fatty acids found in the breast muscle. No research appears to have been reported on the effect of raw CWP or CKP seeds on meat FA composition in broiler chickens. Therefore, this subject should be considered a new investigation.

Alpha-linolenic acid (ALA) 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, breast muscles from birds fed CWP or CKP diets was characterised by a significantly higher (p<0.001) ALA content compared to the SBM diet. Moreover, a significant increase (p<0.001) of EPA, DPA (p=0.034) and DHA (p=0.003) content was observed. Also, the proportion of total n-3 PUFA increased significantly (p<0.001) compared with the SBM diet.

The dietary level (20% vs. 10%) of CWP and CKP influenced markedly the breast meat FA profile. The highest total n-3 PUFA content (2.97 vs. 2.36) was observed in chickens’ breast muscle fed 20% CWP or CKP in comparison with lower level (2.40 vs. 2.28). Simultaneously, there was a significant interaction (p<0.001) between protein source and inclusion level (S × L) for the majority of the FA’s, especially for those which may have potential benefits to human nutrition i.e. LA (p<0.0001), ALA (p=0.006), and EPA (p<0.0001). Similar results were noted in other experiments where
The analysis of breast muscles revealed a significantly lower (p<.0001) n-6 to n-3 PUFA ratio in chickens fed CWP-included diets (from 6.76 to 5.97) and CKP (from 8.04 to 8.11, respectively), compared to SBM (10.08). Recently, Paszkiewicz et al. (2020) also showed that the substitution of SBM by raw CKP seeds, can affect the FA proportions in the subcutaneous fat tissue of broiler chickens; they also, noted that raw CKP inclusion decreased the content of main saturated acid (palmitic acid) and increased the content of main monounsaturated (oleic) and polyunsaturated (linolenic) acids. 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 camellina meal (Aziza et al. 2010) or oil (Jaśkiewicz et al. 2014) and camellina oil or seeds (Ciurescu et al. 2016) in diets for broiler chickens. On the other hand, Sirri et al. (2010) 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 important for human health. The reasons for the above contradiction could be different combinations of feed ingredients used for formulating diets in each experiment, and the inclusion of raw or processed (dehulled, micronized) legume seeds. The results obtained in this study reveals that the broilers fed raw CWP or CKP-containing diets had higher proportion of total n-3 PUFA in breast meat, which may have potential benefits to human nutrition.

Conclusions

CWP (Ofelia cv.) and CKP (Burnas cv.) seeds are good sources of essential nutrients, including amino acids like lysine, and are characterised by a low content of antinutrients. Both can be used as an alternative to replace part of SBM and corn in chicken diets, at inclusion levels up to 200 g/kg, to support growth performance, without any detrimental effects on broilers’
health. In addition, these legumes can improve breast meat fatty acids content of ALA, EPA, DPA and DHA. The results obtained in this study indicate that where cowpea or chickpea can be grown locally, low-input farming systems would benefit from the use of this ingredients for broiler chickens feed.

**Ethical approval**

Broiler chickens were treated in accordance with the Romanian legislation for handling and protection of animals used for experimental purposes. The study protocol was approved by the Ethical Committee for Animal Experiments of the National Research & Development Institute for Biology and Animal Nutrition (IBNA). Testing was done in the Experimental Bio-base located in Balotesti, Romania.

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No potential conflict of interest was reported by the author(s).

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