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# Apparent and true metabolizable energy and true digestibility of the essential amino acids of chickpeas (*Cicer arietinum* L.) experimented with adult roosters

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## Abstract

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Using adapted for poultry methods for balance experiments, the content of zero nitrogen corrected apparent (AMEn) and true metabolizable energy (TMEn) and the true digestibility of the essential amino acids (TDEAA) of a stocking lot of Bulgarian varieties of chickpeas (*Cicer arietinum* L.) have been established. The experiments were conducted parallel with intact and randomized full-grown Sussex roosters. In both of the experiments were used 6 tube fed and 6 feed deprived birds (total 2x12).

The following results (in dry matter) have been established: For intact: AMEn – 12418 J/g; TMEn- 13208 J/g DM; average true digestibility of the essential amino acids – 81.93±0.58. Highest digestibility was obtained by arginine 88.35 and lowest – by methionine+cystine – 71.65. For randomized birds the results were: AMEn – 13150 J/g; TMEn- 14101 J/g DM; average TDEAA – 82.86±0.31; highest – 90.05 (arginine) and lowest – 72.03 (methionine+cystine).

*Keywords:* chickpeas; metabolizable energy; roosters; true digestibility of essential amino acids

## Introduction

According to Joshi et al. (2001) chickpea (*Cicer arietinum* L.*)* ranks second in production volume among plant protein foods. In recent years, there has been an increased scientific interest in chickpeas, mainly from the point of view of its nutritional and dietary qualities in human nutrition – high protein nutrition (protein content with balanced amino acids), content of essential fatty acids, optimal ratio of different carbohydrate fractions, content of macro- and trace elements (Newman et al., 1987; Rossi et al., 1984; AAC, 2001; Monsoor & Yusuf, 2002; Wood & Grusak, 2007; Zia-Ul-Haq et al., 2007; Knife et al., 2015; Wallace et al., 2016; Yegrem, 2021; Feedinamics, 2022; Relina et al., 2023).

In commercial poultry nutrition (layers and broilers) it is essential to look for protein sources (alternative to soybean meal) which reduce the cost of combined fodders. In this aspect, the research of many authors is concentrated to chickpeas – Brenes et al. (2008), Chavan et al. (1989), Christodoulou et al. (2006), Miller et al. (1991), Savage et al. (1995).

Although different treatments increase both the energy and protein nutritional value of the forage by inactivating some antinutritional factors (Sigh, 1988; Garcia-Alonso et al., 1998; Mariscal-Landin et al., 2002; Brenes et al., 2008), the raw chickpea is mainly used in the preparation of combined fodders.

The aim of the present study is to establish the apparent (AMEn) and the true (TMEn) metabolizable energy and the true digestibility of the essential amino acids of an average stocking lot of Bulgarian varieties chickpeas *(Cicer arietinum L.)* using balanced experiments with roosters.

## Material and Methods

The chickpea, object of the experiment, is an average stocking lot of the varieties Obraztsov chiflik, Balkan and Dobrudzhanets and covers about 75% of the chickpea rotation in Bulgaria.

The balance experiments were carried out in the experimental base of the Faculty of Agriculture – Thrace University – Stara Zagora, separately with intact and randomized one-year-old roosters of the Sussex breed. The methodology of Sibbald (1986) was used, subsequently refined by Ragland et al. (1999), Ravindran et al. (2004), and Penkov (2008).

After a 48-hour pre-fasting period, 6 birds were force-fed through a funnel directly into the throat with about 60 g of the tested feed, and 6 were fed deprived to collect the excretion of the fasting metabolism (during the entire experimental period drinking water *ad libitum* is provided). The excreta collection period continues for another 48 h. To avoid energy depletion, the birds receive 40 ml of glucose per os, which is 100% resorbable and does not affect digestibility results (Penkov, 2011). Excreta of tube fed and feed deprived analogs were collected individually every 12 h and frozen at -18$^0$C. Samples for nitrogen/crude protein and amino acids are processed in native substances and for energy – by drying for 36 h at 65$^0$C. The principle "excreted from each tube fed analog – excreted from each feed deprived analog" was used for data processing.

All procedures were carried out in compliance with the Bulgarian Animal Protection Act (2008).

The energy contents of fodder and excrements were determined using microprocess calorimeter KL 11 Mikado (Poland). The chemical analyses were measured acc. to AOAC (2007). The amino acid composition of feed and excrements was determined using amino analyzer AAA881 (preliminary acid hydrolysis).

The metabolizable energy levels were calculated according to the formula of Sibbald (1986):

$$AME = (EI – EO)/FI$$
$$AMEn\text{-}o = AME – 34.4\times ANR/FI$$
$$TME = AME + (FEL/FI)$$
$$TMEn\text{-}o = TME – [(34.4\times ANR/FI) – (34.4\times FNL/FI)],$$

where: AME – apparent metabolizable energy; EI – energy input with the fodder; EO – energy output from tube fed analogues; FI – fodder (in DM) input; FEL – energy losses from feed deprived; ANR – apparent nitrogen retained (nitrogen input with fodder – nitrogen output in excrements of tube fed, in kJ); FNL – nitrogen excretion from fed deprived; n-o – corrected to 0 – nitrogen balance.

$$TDAA = [AAI – (AAETF – AAEFD)]/AAI,$$

where: AAI – amino acid input through the fodder; AAETF – amino acid excreted from the tube fed; AAEFD – amino acid excreted from the feed deprived birds

The mean digestibility of essential amino acids (MDEAA) was calculated using the formula:

$$MDEEA = \sum(AA\times DC)/\sum AA,$$

where: AA – amino acid; DC – coefficient of digestibility for every AA

All results were processed using Descriptive Statistics – Microsoft Excel.

## Results and Discussion

Data on the content of gross energy and the chemical composition of the fodder and the content of essential amino acids are reflected in Table 1.

The chemical composition of feed, cited both in international and Bulgarian literature (Feedinamics,2022; NRC, 1994; WPSA, 1986; Summers & Lesson, 1992; Todorov et al., 2007) varies as follows (in % of dry matter): crude pro-

**Table 1. Chemical composition and essential amino acids in the tested stocking lot of chickpeas (*Cicer arietinum* L.)**

| Substances/Gross energy | Content in DM |
|---|---|
| Crude protein, % | 21.40 |
| Crude fats , % | 5.95 |
| Crude fibers, % | 4.20 |
| Non protein extracts, % | 64.50 |
| Gross energy, kJ/kg | 19350 |
| Amino acid: | Content in DM // Content in 100 g protein |
| Threonine | 0.87//4.08 |
| Arginine | 2.19//10.21 |
| Lysine | 1.60//7.49 |
| Histidine | 0.61//2.85 |
| Phenylalanine | 1.42//6.64 |
| Tyrosine | 0.51//2.38 |
| Leucine | 1.69//7.90 |
| Isoleucine | 1.02//4.77 |
| Valine | 1.06//4.96 |
| Methionine+Cystine | 0.57//2.66 |
| ∑ essential amino acids | 11.56//53.94 |

tein – 20–24 (mean 21.3%); crude fats – 5.8–7 (mean 6.3%); crude fiber – 3.3–4.5 (mean 3.9%) and non-protein extract – 62–68 (mean 65.2%).

On this basis, the content of gross energy content is also comparable to those cited by Feedinamics (2022) and Todorov et al. (2007) – an average of 19.6 MJ/kg DM.

The protein nutritional value of chickpeas is a subject of interest all over the world. The amino acid content varying according to the crude protein content of different cultivars and varieties. In our available literature (Summers & Lesson, 1996; Sánchez-Vioque et al., 1999; AAC, 2001; INRA, 2007; Todorov et al., 2007; Zia-Ul-Haq et al., 2009; Wang et al., 2010; Vegfaqs, 2022; Fit adult, 2023), the content of crude protein in the dry matter of the forage is from 18.8 to 22.3 (mean 21.5%), and the content of essential amino acids in 100 g of protein moves within the following limits: Threonine – 2.8–4.2 (mean 3.6%); Arginine – 6.6–11.3 (8.3); Lysine – 5.8–7.5 (6.8); Histidine – 2-3.1 (2.5); Phenylalanine – 4.8–6.1 (5.5); Tyrosine – 1.4–4.6 (2.3); Leucine – 6.4–8.1 (7.3); Isoleucine – 2.6–4.7 (3.9); Valine – 2.9–5 (4); Methionine+Cystine – 1.5–3.4 (mean 2.4%).

The data from our research, both for content of organic substances (gross energy) and essential amino acids are comparable with those cited in the literature, and this is an indicator, that the Bulgarian varieties of chickpeas have comparable to the international levels chemical fractions.

Table 2 shows the basic data for calculating the metabolizable energy. Intact and randomized birds received identical amounts of energy and nitrogen – 392487 and 343745 J and 3.072 and 3.196 g respectively. Energy and nitrogen output from feed deprived analogs are also close in value – respectively, 79184 vs. 81791 J and 1.249 vs. 1.225 g.

Higher are the differences in the released energy from tube fed analogs – 631000 J (intact) vs. 519359 J (randomized), as well as in the amounts of released nitrogen. On this basis, the difference in the apparent nitrogen retained is also higher: -1.137 g (intact) vs. -1.594 g (randomized).

When the metabolizable energy was calculated, the following values were obtained (Table 3).

The nitrogen-corrected apparent metabolizable energy was significantly ($P < 0.05$) higher in the randomized birds compared to the intact ones – 13150 vs. 12417 J/g DM. In the true nitrogen corrected true metabolizable energy the differences are significant too – 14101 vs. 13207 J/g DM.

While it is normal to have differences between AMEn and TMEn (in this case, in both types of experiments, the differences were statistically significant ($P < 0.05$) in favor of TMEn), it is more difficult to explain the differences in favor of the randomized birds ($P < 0.05$) in both AMEn and with TMEn. Apparently, the caecectomy has mobilized the digestive system of the birds to better absorb the nutrients of the chickpeas.

In the main available literature (WPSA, 1986; INRA, 1988; NRC, 1994; Feedinamics, 2022; Todorov et al., 2007; Viveros et al., 2001) the levels of AMEn-o of chickpea for poultry (layers, broilers) ranges from 12.44 to 13.00 MJ/kg DM, and those of TMEn-o – from 14 to 15 MJ/kg DM.

Obtained in the present study data from both intact and randomized birds and for both metabolizable energies are consistent with those reported in the cited sources.

In Table 4 the true digestibility coefficients of the essential amino acids are shown.

The average digestibility of amino acids obtained with the intact birds was 81.93, and with the randomized birds –

**Table 2. Dry matter, gross energy and nitrogen input and energy and nitrogen output from tube fed and feed deprived analogs (n = 6 tube fed and 6 feed deprived)**

| Method – Intact/randomized | DM input, g | GE input, J | N input, g | GE output– tube fed, J | N output – tube fed, g | Apparent N retained, g | GE output – feed deprived, J | N output – feed deprived, g |
|---|---|---|---|---|---|---|---|---|
| | x±Sx | x±Sx | x±Sx | x±Sx | x±Sx | x±Sx | x±Sx | x±Sx |
| Intact | 54.54 ±0.01 | 1007390.11 ±170.04 | 1.0865 ±0.001 | 392486.70 ±41173 | 3.072 ±0.19 | -1.063 ± 0.10 | 79183.96 ±2660.44 | 1.249 ±0.103 |
| Randomized | 54.75 ±0.01 | 1017526.40 ±62.02 | 1.0872 ±0.001 | 343745.10 ±22000 | 3.196 ±0.16 | -1.337 ±0.16 | 81790.54 ±1342.70 | 1.225 ±0.075 |

**Table 3. Apparent and true metabolizable energy, zero N-balance corrected in the dry matter of chickpeas (*Cicer arietinum* L.) , J/g**

| Rooster type | AME x±Sx | AME(n-o) **x±Sx** | TME x±Sx | TME (n-o) **x±Sx** |
|---|---|---|---|---|
| Intact* | 11747.60±359 | 12417.98±372ab | 11747.60±359 | 13207.53±396ab |
| Randomized* | 12350.34±161 | 13149.99±165ab | 12521.35±162 | 14101.44±185ab |

*Significant differences (P<0.05) between: a-a – AMEn and TMEn (rows); b-b – both AMEn and both TMEn (columns)

**Table 4. Coefficients of true digestibility of the essential amino acids of chickpeas established with intact and randomized roosters (fecal and ileal true digestibility)***

| Amino acid | Intact birds (fecal) | Randomized birds (ileal) |
|---|---|---|
| | Xmean ± SE | Xmean ± SE |
| Threonine | 74.93±1.76 | 74.63±0.65 |
| Arginine | 88.35±0.40a | 90.05±0.36a |
| Lysine | 86.80±0.62 | 87.42±0.25 |
| Histidine | 77.76±0.78 | 78.92±0.35 |
| Phenylalanine | 83.78±0.57 | 84.86±0.24 |
| Tyrosine | 76.77±1.18 | 77.93±0.47 |
| Leucine | 78.80±0.96 | 79.69±0.32 |
| Isoleucine | 83.21±0.78 | 83,87±0,27 |
| Valine | 78.81±0.10a | 79.74±0.33a |
| Methionine+Cystine | 71.65±1.26 | 72.03±0.51 |
| Avr. digest. of all AA | 81.93±0.58 | 82.86±0.31 |
| Digest. of the crude protein | 80.14±1.24 | 83.62±2.34 |

*a-a Statistical significance by rows (P < 0.05)

82.86. The crude protein digestibility was 80.14 and 83.62, respectively. There is no statistical significance for the differences in the two indicators (P > 0.05).

The lowest true digestibility in intact birds was calculated by methionine + cystine – 71.65, and the highest – by arginine – 88.35. Higher digestibility showed lysine – 86.80, phenylalanine – 83.78, and isoleucine – 83.21, and lower – threonine – 74.93, histidine – 77.76, tyrosine – 76.77, leucine – 78.80 and valine – 78.81.

Modern trends in protein nutrition require consideration of the ileal digestibility of amino acids. The highest true digestibility in randomized birds was recorded for arginine – 90.05% and the lowest – for methionine+cystine – 72.03%. Higher digestibility than 80% was found for lysine – 87.42, phenylalanine – 84.86%, isoleucine – 83.87%, and lower – for threonine – 74.63%, histidine – 78.92%, tyrosine – 77.93%, leucine – 79.69% and valine – 79.74%.

It should be mentioned that the preliminary hydrochloric acid hydrolysis during the processing of the samples completely destroys the amino acid tryptophan and partially (up to about 30%) the sulfur-containing amino acids. We believe that the destruction is approximately the same in feed and excreta and the data obtained with methionine+cystine are sufficiently representative.

The study method (intact and randomized birds) showed relatively identical results in digestibility coefficients. The differences are statistically significant (P < 0.05) only for arginine (88.35 vs. 90.05) and for valine (78.81 vs. 79.74%).

Of significant interest is the comparison of the data obtained by us with those cited in other literature sources. In our available literature, we find those obtained in experiments with hens – NRC (1994), Bryden et al. (2000), Todorov et al. (2007), Feedinamics (2022). The average data from the cited sources for the digestibility of essential amino acids are, as follows: Threonine – 72%; Arginine – 85%; Lysine – 77%; Histidine – 78%; Phenylalanine – 76%; Tyrosine – 74%; Leucine – 72%; Isoleucine – 70%; Valine – 73%; Methionine+Cystine – 78%.

Higher true digestibility in our research was reported for lysine (+9%), valine (+8%), phenylalanine and leucine (+6%), and lower – for methionine+cystine (-6%). For the other amino acids, the differences are from 0 to +3%. In the average true digestibility of all amino acids, the difference is relatively low (+3%).

Despite the relative higher digestibility coefficients obtained by us, comparing them with those for soybean meal (NRC, 1994; Sibbald, 1986; Todorov et al., 2007; Penkov, 2008; Summers & Lesson, 1992), it's confirmed that the amino acids of chickpea are less available (in the range of -3 to -8%).

## Conclusions

The 0-nitrogen corrected metabolizable energy of the of chickpeas (*Cicer arietinum* L.) – Bulgarian varieties was as follow:

AMEn-o: Intact roosters – 12418; Randomized roosters – 13150; average – 12784 J/g dry matter.

TMEn-o: Intact roosters – 13208; Randomized roosters – 14101; average – 13625 J/g dry matter.

Significant differences (P < 0.5) between AMEn and TMEn both in intact and in caecectomized birds so as between both of AMEn and both of TMEn (randomized and intact) have been established.

The average true digestibility of the essential amino acids, established with intact and randomized Sussex roosters was 81.93 and 82.86% respectively. There were no statistical differences between both coefficients.

Highest digestibility coefficients were obtained by lysine (88.35 by intact vs. 90.05 by randomized birds), phenylalanine (83.78 vs. 84.86) and isoleucine (83.21 vs. 83.87) and lowest – by methionine+cystine (71.65 vs. 72.03).

The research method (intact – randomized) does not have a significant impact on digestibility – significant differences (P < 0.05) were recorded only for arginine and valine, but in absolute values they ranged from 1 to 1.5%.

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