



Effect of dietary Alibernet red grape pomace application into Ross 308 broiler chickens diet on amino and fatty acids profile of breast and thigh meat

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Abstract

The aim of this study was to determine the effects of Alibernet red grape pomace (ARGP) variety Alibernet addition into broiler chickens Ross 308 diet on the essential amino acid (AA) and fatty acid (FA) composition of their breast and thigh meat. At the beginning, 200 one-day Ross 308 broiler chickens of mixed gender were randomly divided into 4 groups ($n = 50$). The control group (C) did not receive any additional supplementation. The feed of experimental groups was enriched with 1% ARGP per 1 kg of feed mixture (FM) (E1), with 2% ARGP per 1 kg of FM (E2) and with 3% ARGP per 1 kg of FM (E3). The FMs were produced without any antibiotics and coccidiostatics and the fattening period lasted for 42 days. Samples from breast and thigh muscle were obtained and analyzed for the content of AAs and FAs content and results were presented as $\text{g } 100 \text{ g}^{-1}$ of dry matter. Results revealed that AA profile of breast muscle was not significantly affected, with the most present AAs Lys and Leu. In the thigh muscle we observed significant differences in the content of Thr, Val, Met, Cys and His, namely in males. From the results of FAs profile, we can state that ARGP influenced namely monounsaturated oleic acid in breast muscle (without gender difference), which had significantly highest content ($p \leq 0.05$) in all experimental groups (E1–36.05, E2–35.60 and E3–36.79 $\text{g } 100 \text{ g}^{-1}$) compared with the control group (31.88 $\text{g } 100 \text{ g}^{-1}$). Overall, it seems that selected feed supplement did not negatively influence AAs and FAs profile of chicken meat.

Keywords Grape pomace · Broiler chickens · Meat · Amino acids · Fatty acids

Introduction

According to the actual statistics, world meat production in 2021 was expected to expand by 4.2% from year 2020 to 353 mil tons. This rise is mainly caused by recovery from COVID-19 pandemic based on expectations of a strong output rebound in all major producing regions (China, USA, EU, Latin America), except Oceania. Among meat, leading position still belongs to the poultry meat (135.4 mil. t), followed with pork (122 mil. t) and bovine meat (71.8 mil. t) (FAO 2021).

The main differences between red pork and beef and white chicken are lower levels of saturated FAs and low energy value. For this reason, it is recommended to consume chicken meat for individuals who want to reduce their intake of fats and calories without giving up eating meat. However, chicken also contains a higher amount of fat when consumed with the skin (2–3 times fatter than without skin). Therefore, if a quality animal protein intake is required without

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unnecessary calorie and fat intake, chicken should be eaten without skin. Chicken meat does not differ much from other types of meat in cholesterol content, but due to other nutritional benefits, this is negligible. Thanks to its high protein content, chicken meat is suitable for groups of people who need a higher intake of quality protein, such as children, athletes, or seniors. For example, the average daily protein requirement for children and athletes is approximately 2-fold higher than for adults (1.12 versus 0.66 g kg⁻¹ body weight) (EFSA 2017). Chicken muscle also contains significantly less collagen, which is a structural protein with a poorer AA composition, which is found to a greater extent in red meat. Therefore, chicken is considered easier to digest (Marangoni et al. 2015).

Poultry meat is sought after for its favorable nutritional composition, which also applies to the content of essential FAs (Nkukwana et al. 2014). Fat in chicken breasts contains 33.5% saturated, 30.5% unsaturated and 32% polyunsaturated FAs, which is one of the reasons for the popularity of chicken meat compared to red meats, which have a high proportion of saturated FAs and low proportion of unsaturated FAs (Morales-Barrera et al. 2013). Chicken meat is therefore important as an available animal source of long-chain n-3 polyunsaturated FAs, especially alpha-linolenic, eicosapentaenoic and docosahexaenoic acids, and efforts are being made to increase their proportion in chicken meat (Dalziel et al. 2015).

Meat quality has been reported to depend on many factors, but its FA and AA profile is influenced mainly by species, variety within species or breed (Gumulka and Potłowicz 2020).

Essential AAs in chicken meat represent about 40% of the total protein content, which is around 20% in chicken meat (Kim et al. 2017). It contains all the essential AAs, especially Leu, Lys, and non-essential AAs aspartic and glutamic acid (Haščík et al. 2020a). The AA score of chicken meat, which indicates protein digestibility, is 0.92, which is a high number especially compared to plant protein sources (Barron-Hoyos et al. 2013).

The population of developed countries is in increasing demand for quality proteins, and therefore the poultry industry has focused on breeding high-performance hybrids of broiler chickens, which reach a live weight of approximately 2500 g in 42 days (Kralík et al. 2018).

At present, researchers from all over the world are still focusing to look for natural alternatives to the once widely used feed antibiotics. However, these alternatives should be as cost-effective as antibiotics, but without negatively affecting meat quality. On the contrary, they should increase the production and quality of meat and thus increase profits from sales (Erinle et al. 2022).

A common problem in animal production is the high need for protein-rich feed and its production has an even

greater environmental impact than animal husbandry alone (Boggia et al. 2010). On the one hand, the production of protein-rich feeds is critical, but on the other hand, it will continue to be necessary to increase their production in view of the growing population of the planet (Röös et al. 2017).

The processing of grapes into juice, and wine produces a bulky by-product called GP (Muhlack et al. 2018). The wine industry consumes about 60 million tons of grapes a year to produce wine, which varies each year according to the quality and quantity of the harvest. GP, after pressing, makes up about 23% of the weight of the processed grapes (Gowman et al. 2019). It is therefore a bulky agricultural by-product that needs to be disposed of. However, nutrient-rich, and biologically active grape substances are concentrated in GP, especially phenolic compounds, insoluble fiber, proteins, and fat in the seeds (Hogervorst et al. 2017; Heuzé and Trans 2020). Due to their antioxidant effects, phenolic compounds in GP are a suitable alternative to feed antibiotics and can be incorporated into the feed ration of livestock. Incorporating biologically valuable waste from grape processing into the feed ration of broiler chickens could increase their productivity and the quality of the chicken meat, thereby reducing the costs associated with disposing of GP and increasing profits for chicken farmers (Erinle et al. 2022).

In view of the above, the aim of the study was to examine the effect of Alibernet ARGP on the FA and AA profile in the meat of broiler chickens of the hybrid combination Ross 308.

Materials and methods

Animals and experiment design

The experiment took place in the Slovak university of agriculture, Nitra (Test poultry station, Kolíňany, Slovak republic). The methodology of fattening process was realized according to Haščík et al. (2020a). FMs were prepared according to Bulletin of MARD SR (2005) to meet nutritional needs of Ross 308 broiler chickens following the recommended reference levels. In the first stage (1–21 d), broilers were fed with HYD-01 starter FM following with grower HYD-02 from 22nd day to the end of fattening in 42nd day. The starter and grower FMs did not contain any antibiotics and coccidiostatics and were prepared by Biofeed, Inc. (Kolárovo, Slovakia); their composition is presented in Table 1. The control group (C) received the basal diet without supplementation. The FM of experimental groups were enriched with ARGP in amount 1% 100 kg⁻¹ of FM (E1); 2% 100 kg⁻¹ of FM (E2) and 3% 100 kg⁻¹ of FM (E3).

Table 1 Composition of feed mixtures

Ingredients (%)	Starter (HYD-01) (1st – 21st day of age)	Grower (HYD-02) (22nd – 42nd day of age)
Wheat	34.50	30.00
Maize	28.00	39.00
Soybean meal (48% N)	31.00	26.00
Fodder lime	0.65	0.60
Calcium formate	0.80	0.80
Monocalcium phosphate	0.90	0.55
Fodder salt	0.20	0.20
Sodium bicarbonate	0.20	0.20
Lysine	0.10	0.05
Methionine	0.15	0.15
Soybean oil	3.00	1.95
Premix Euromix BR 0.5%*	0.50	0.50
Nutrient content (g kg ⁻¹)		
Linoleic acid	27.82	24.04
Fibre	28.71	27.84
Crude protein	209.68	189.60
Ash	45.45	39.59
Ca	8.12	7.27
P	6.04	5.13
Na	1.61	1.58
ME _N (MJ kg ⁻¹)	11.92	11.92

*active substances per kilogram of premix: vitamin A 2,500,000 IU; vitamin E 50,000 mg; vitamin D3 800,000 IU; niacin 12,000 mg; D–pantothenic acid 3,000 mg; riboflavin 1,800 mg; pyridoxine 1,200 mg; thiamine 600 mg; methadione 800 mg; ascorbic acid 50,000 mg; folic acid 400 mg; biotin 40 mg; cobalamin 10.0 mg; choline 100,000 mg; betaine 50,000 mg; Mn 20,000 mg; Zn 16,000 mg; Fe 14,000 mg; Cu 2,400 mg; Co 80 mg; I 200 mg; Se 50 mg

Characterization of used feed supplement applied in experiment

Used supplement (Alibernet RGP) was analyzed on the Department of Animal Nutrition (SUA, Nitra). Content of nutrients (g kg⁻¹) was determined as follow: dry matter – 383.40, crude protein – 112.85, ether extract – 105.93, crude fiber – 230.33, ash – 65.50, nitrogen free extracts – 485.76, organic matter – 949.58, sugars – 4.88, acid detergent fiber – 450.83, neutral detergent fiber – 525.48, lignin – 281.27, celluloses – 156.46 and hemicelluloses – 91.81.

Slaughter and measurements

After 42 days of the fattening process, 10 females and 10 males broiler chickens Ross 308 from each experimental group were weighed and slaughtered. The slaughter took place at the slaughterhouse of the Slovak University of Agriculture in Nitra. The chickens were slaughtered by

conventional neck cut, bled out, feathers removed, and eviscerated. The dissected carcasses were kept at a temperature of about 18 °C for one-hour *postmortem*, after which they were weighed and stored for 24 h *postmortem* to reach a carcass temperature of 4 °C.

After 24 h *postmortem*, the breast and thighs were excised from the cooled right half-carcass, from which the bones, skin, subcutaneous fat, and connective tissues were removed. Bone and skin-free muscle was used to determine the composition of AAs and FAs. Until analyses, muscle was vacuum packed and stored in a freezer at -18 °C (2 weeks).

AA composition (essential AAs) of the breast and thigh muscles of broiler chickens Ross 308 was measured using an automatic AA analyzer AAA 400 (INGOS Prague, Czech Republic).

The FA composition of breast and thigh meat was determined by a direct method for FA methyl ester (FAME) synthesis. The FA composition of the FAME was determined using a Gas Chromatograph (Agilent, 7890 A series, USA) equipped with a flame ionization detector and a chiral capillary column (J&W Scientific, USA).

The resulting values of AAs and FAs were re-calculated to 100% dry matter and expressed as g AA and FA content per 100 g muscle (Haščík et al. 2020b).

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) using SAS software (version 9.3, Enterprise Guide 4.2, USA). Tables show the results as the mean with standard deviation (SD). Duncan test was used to determine significant differences between experimental groups, differences were considered significant below the significance level of p-value $p \leq 0.05$ (SAS 2008).

Results and discussion

AAs profile of chicken meat

Essential AAs profile of chicken meat of Ross 308 broiler chickens fed with supplemental ARGP is shown in the Table 2 (breast muscle) and Table 3 (thigh muscle).

Based on the results of the AA profile of the Ross 308 broiler chicken breast muscle, we can state that the chosen feed additive (ARGP) did not significantly affect the observed proportion of essential AAs and numerical differences between experimental groups were also minimal ($p > 0.05$). The results show that the most abundant AAs in the breast muscle are Lys and Leu, and conversely, the sulphur AAs Met and Cys were the least represented.

Table 2 Effect of ARGP on AA composition of broiler chickens Ross 308 breast muscle (g 100 g⁻¹)

AA / Group	Sex	C	E1 EAA	E2	E3	<i>p</i> -value
Thr	Male	0.78 ± 0.04	0.76 ± 0.06	0.82 ± 0.06	0.75 ± 0.07	0.329
	Female	0.83 ± 0.05	0.82 ± 0.08	0.82 ± 0.05	0.83 ± 0.04	0.978
	♂+♀	0.81 ± 0.05	0.79 ± 0.07	0.82 ± 0.05	0.79 ± 0.07	0.753
Val	Male	0.80 ± 0.03	0.78 ± 0.04	0.84 ± 0.04	0.76 ± 0.05	0.060
	Female	0.85 ± 0.04	0.84 ± 0.05	0.83 ± 0.04	0.83 ± 0.02	0.845
	♂+♀	0.82 ± 0.04	0.81 ± 0.05	0.83 ± 0.03	0.79 ± 0.05	0.312
Met	Male	0.57 ± 0.03	0.55 ± 0.04	0.62 ± 0.05	0.55 ± 0.04	0.091
	Female	0.60 ± 0.03	0.61 ± 0.05	0.62 ± 0.04	0.63 ± 0.02	0.748
	♂+♀	0.59 ± 0.03	0.58 ± 0.05	0.62 ± 0.04	0.59 ± 0.05	0.283
Ile	Male	0.70 ± 0.04	0.66 ± 0.06	0.74 ± 0.06	0.65 ± 0.07	0.115
	Female	0.75 ± 0.07	0.73 ± 0.08	0.74 ± 0.05	0.75 ± 0.03	0.921
	♂+♀	0.72 ± 0.06	0.70 ± 0.07	0.74 ± 0.05	0.70 ± 0.07	0.436
Leu	Male	1.42 ± 0.08	1.36 ± 0.11	1.50 ± 0.12	1.34 ± 0.13	0.139
	Female	1.51 ± 0.11	1.49 ± 0.15	1.50 ± 0.09	1.53 ± 0.07	0.943
	♂+♀	1.46 ± 0.10	1.42 ± 0.14	1.50 ± 0.10	1.44 ± 0.14	0.500
Phe	Male	0.74 ± 0.04	0.70 ± 0.06	0.78 ± 0.05	0.70 ± 0.06	0.116
	Female	0.78 ± 0.06	0.77 ± 0.07	0.78 ± 0.05	0.79 ± 0.03	0.972
	♂+♀	0.76 ± 0.05	0.74 ± 0.07	0.78 ± 0.05	0.74 ± 0.06	0.451
Lys	Male	1.52 ± 0.09	1.45 ± 0.12	1.62 ± 0.12	1.44 ± 0.14	0.119
	Female	1.63 ± 0.13	1.60 ± 0.16	1.62 ± 0.10	1.64 ± 0.07	0.946
	♂+♀	1.58 ± 0.12	1.52 ± 0.15	1.62 ± 0.11	1.54 ± 0.15	0.423
Cys	Male	0.24 ± 0.01	0.24 ± 0.02	0.26 ± 0.02	0.24 ± 0.02	0.431
	Female	0.25 ± 0.01	0.26 ± 0.02	0.26 ± 0.02	0.27 ± 0.01	0.511
	♂+♀	0.25 ± 0.01	0.25 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.392
His	Male	0.76 ± 0.05	0.74 ± 0.08	0.84 ± 0.09	0.73 ± 0.08	0.119
	Female	0.82 ± 0.04	0.84 ± 0.07	0.82 ± 0.07	0.85 ± 0.03	0.827
	♂+♀	0.79 ± 0.05	0.79 ± 0.08	0.83 ± 0.08	0.79 ± 0.08	0.494
Arg	Male	1.14 ± 0.07	1.09 ± 0.09	1.21 ± 0.09	1.08 ± 0.11	0.123
	Female	1.22 ± 0.10	1.19 ± 0.12	1.21 ± 0.08	1.23 ± 0.05	0.939
	♂+♀	1.18 ± 0.09	1.14 ± 0.12	1.21 ± 0.08	1.15 ± 0.11	0.429

Amino acids are expressed on a dry matter basis (g 100 g⁻¹). Values are given as mean ± SD (standard deviation); *n* = 80; C = control group; E1 – experimental group of chickens fed with 1% supplemental ARGP; E2 – experimental group of chickens fed with 2% supplemental ARGP; E3 – experimental group of chickens fed with 3% supplemental ARGP; EAA = essential amino acid; Thr = threonine; Val = valine; Met = methionine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine; Lys = lysine; Cys = cysteine; His = histidine; Arg = arginine

Regarding the thigh muscle, we observed significant differences in the content of several AAs, although numerically the differences between the individual experimental groups were minimal. Significant differences were observed in males at threonine content ($p \leq 0.05$), where its content was significantly highest in the control group and in the E1 group (0.94 and 0.93 g 100 g⁻¹, respectively) compared to the E3 group (0.85 g 100 g⁻¹). Significant differences ($p \leq 0.05$) were also observed for Val content in males and without gender difference, when its content was highest in the control and E1 groups compared to the E3 group, but with the minimal differences. We found the highest Met content ($p \leq 0.05$) in group E1 (0.70 g 100 g⁻¹) compared to

group E3 (0.65 g 100 g⁻¹) in males. Cys was found to have the highest content in the control group and the E1 group in males (0.29 g 100 g⁻¹), while in females the Cys content was highest in the E3 group (0.29 g 100 g⁻¹), however differences between the groups were generally minimal. Significantly the highest proportion of histidine ($p \leq 0.05$) was observed in males in the control group and E1 (both 0.99 g 100 g⁻¹) compared to group E3 (0.90 g 100 g⁻¹) and without gender difference between groups E1 (0.98 g 100 g⁻¹) and E2 (0.92 g 100 g⁻¹).

To our knowledge, no data have been published on the effect of supplemental GP or other grape by-products on the AA composition of chicken meat, as thanks to its

Table 3 Effect of ARGP on AA composition of broiler chickens Ross 308 thigh muscle (g 100 g⁻¹)

AA / Group	Sex	C	E1 EEA	E2	E3	p-value
Thr	Male	0.94 ± 0.04 ^a	0.93 ± 0.04 ^a	0.89 ± 0.05 ^{ab}	0.85 ± 0.04 ^b	0.016
	Female	0.88 ± 0.06	0.91 ± 0.03	0.85 ± 0.04	0.91 ± 0.04	0.106
	♂+♀	0.91 ± 0.06	0.92 ± 0.04	0.87 ± 0.04	0.88 ± 0.05	0.060
Val	Male	0.93 ± 0.04 ^a	0.92 ± 0.03 ^a	0.89 ± 0.04 ^{ab}	0.86 ± 0.03 ^b	0.016
	Female	0.90 ± 0.04	0.91 ± 0.03	0.86 ± 0.04	0.90 ± 0.03	0.151
	♂+♀	0.91 ± 0.04 ^a	0.91 ± 0.03 ^a	0.87 ± 0.04 ^b	0.88 ± 0.03 ^b	0.016
Met	Male	0.69 ± 0.03 ^a	0.70 ± 0.03 ^a	0.66 ± 0.04 ^{ab}	0.65 ± 0.03 ^b	0.032
	Female	0.65 ± 0.04	0.68 ± 0.02	0.65 ± 0.03	0.69 ± 0.02	0.141
	♂+♀	0.67 ± 0.04	0.69 ± 0.03	0.66 ± 0.03	0.67 ± 0.04	0.213
Ile	Male	0.87 ± 0.05	0.88 ± 0.04	0.83 ± 0.05	0.80 ± 0.05	0.087
	Female	0.82 ± 0.06	0.86 ± 0.04	0.80 ± 0.04	0.86 ± 0.05	0.200
	♂+♀	0.85 ± 0.06	0.87 ± 0.04	0.82 ± 0.05	0.83 ± 0.05	0.154
Leu	Male	1.74 ± 0.09	1.75 ± 0.08	1.65 ± 0.10	1.60 ± 0.09	0.057
	Female	1.63 ± 0.11	1.72 ± 0.07	1.61 ± 0.08	1.71 ± 0.09	0.167
	♂+♀	1.69 ± 0.11	1.73 ± 0.07	1.63 ± 0.09	1.66 ± 0.10	0.134
Phe	Male	0.90 ± 0.05	0.90 ± 0.04	0.85 ± 0.05	0.83 ± 0.04	0.071
	Female	0.85 ± 0.06	0.88 ± 0.03	0.83 ± 0.05	0.88 ± 0.05	0.199
	♂+♀	0.87 ± 0.06	0.89 ± 0.04	0.84 ± 0.05	0.86 ± 0.05	0.128
Lys	Male	1.88 ± 0.11	1.88 ± 0.10	1.79 ± 0.12	1.73 ± 0.10	0.087
	Female	1.76 ± 0.13	1.86 ± 0.08	1.73 ± 0.10	1.85 ± 0.11	0.196
	♂+♀	1.82 ± 0.13	1.87 ± 0.08	1.76 ± 0.11	1.79 ± 0.12	0.156
Cys	Male	0.29 ± 0.01 ^a	0.29 ± 0.01 ^a	0.28 ± 0.01 ^{ab}	0.27 ± 0.02 ^b	0.013
	Female	0.26 ± 0.02 ^c	0.28 ± 0.01 ^{ab}	0.27 ± 0.02 ^{bc}	0.29 ± 0.01 ^a	0.011
	♂+♀	0.28 ± 0.02	0.29 ± 0.01	0.28 ± 0.01	0.28 ± 0.02	0.328
His	Male	0.99 ± 0.03 ^a	0.99 ± 0.03 ^a	0.93 ± 0.04 ^b	0.90 ± 0.04 ^b	0.002
	Female	0.93 ± 0.06	0.96 ± 0.03	0.91 ± 0.07	0.97 ± 0.02	0.246
	♂+♀	0.96 ± 0.05 ^{ab}	0.98 ± 0.03 ^a	0.92 ± 0.05 ^b	0.94 ± 0.04 ^{ab}	0.045
Arg	Male	1.41 ± 0.08	1.41 ± 0.07	1.34 ± 0.09	1.29 ± 0.08	0.091
	Female	1.32 ± 0.10	1.39 ± 0.06	1.30 ± 0.07	1.39 ± 0.08	0.203
	♂+♀	1.36 ± 0.10	1.40 ± 0.06	1.32 ± 0.08	1.34 ± 0.09	0.157

Amino acids are expressed on a dry matter basis (g 100 g⁻¹). Values are given as mean ± SD (standard deviation); n = 80; C = control group; E1 – experimental group of chickens fed with 1% supplemental ARGP; E2 – experimental group of chickens fed with 2% supplemental ARGP; E3 – experimental group of chickens fed with 3% supplemental ARGP; EAA = essential amino acid; Thr = threonine; Val = valine; Met = methionine; Ile = isoleucine; Leu = leucine; Phe = phenylalanine; Lys = lysine; Cys = cysteine; His = histidine; Arg = arginine. a–c = means within the same row with different superscripts differ significantly (p ≤ 0.05)

composition it is used to upgrade meat FAs composition (as discussed below) or to improve oxidative stability (Jurčaga et al. 2021). Among the other feed supplements, our results are comparable with Haščík et al. (2020a, b), who examined the effect of supplemental bee pollen, propolis and probiotics into Ross 308 broiler chickens and also found the highest proportion of Leu and Lys in both breast and thigh muscle and the least present were sulphur AAs Cys and Met. Yu et al. (2021) revealed that dietary inclusion of 600 mg kg⁻¹ of trans-anethole increased the concentration of Met, Thr, Asp, Ser, and Glu in breast muscle, tended to increase the Lys concentration. Among the physical ways to improve the

AA content of chicken meat, Kim et al. (2013) revealed, that among 6 used sources of monochromatic light, light-emitting diode light colours as white light improved the concentration of the most essential and nonessential AAs.

In study of Gheorghe et al. (2021), the use of dietary sorghum-peas and sorghum alone as partial substitutes of corn and soybean meal did not affect the AAs concentrations of breast or thigh muscle. A significant effect of dietary treatments was found for some valine and phenylalanine, that were higher. Although Cys and Met levels were slightly lower in breast and thigh muscle compared to the control group, their amounts were more-less similar, what

Table 4 Effect of ARGP supplements on FA composition of broiler chickens Ross 308 breast muscle (g 100 g⁻¹)

FA / Group	Sex	C	E1	E2	E3	p-value
Lauric (C12:0)	Male	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.430
	Female	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.101
	♂+♀	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.066
Myristic (C14:0)	Male	1.38 ± 0.04	1.36 ± 0.03	1.37 ± 0.04	1.36 ± 0.02	0.887
	Female	1.39 ± 0.03	1.34 ± 0.03	1.36 ± 0.04	1.35 ± 0.02	0.081
	♂+♀	1.38 ± 0.03	1.35 ± 0.03	1.36 ± 0.04	1.36 ± 0.04	0.108
Palmitic (C16:0)	Male	24.51 ± 0.33	24.40 ± 0.17	24.46 ± 0.24	24.46 ± 0.15	0.891
	Female	24.39 ± 0.17	24.35 ± 0.19	24.48 ± 0.17	24.49 ± 0.14	0.513
	♂+♀	24.45 ± 0.25	24.37 ± 0.17	24.47 ± 0.19	24.47 ± 0.14	0.618
Heptadecanoic (C17:0)	Male	0.29 ± 0.03	0.33 ± 0.03	0.28 ± 0.04	0.32 ± 0.03	0.147
	Female	0.29 ± 0.04	0.32 ± 0.02	0.32 ± 0.04	0.32 ± 0.02	0.306
	♂+♀	0.29 ± 0.04	0.32 ± 0.03	0.30 ± 0.04	0.32 ± 0.03	0.078
Stearic (C18:0)	Male	10.73 ± 0.30	10.69 ± 0.17	10.92 ± 0.29	10.82 ± 0.23	0.504
	Female	10.55 ± 0.27	10.79 ± 0.22	10.71 ± 0.23	10.93 ± 0.14	0.090
	♂+♀	10.64 ± 0.28	10.74 ± 0.19	10.81 ± 0.27	10.87 ± 0.19	0.161
Oleic (C18:1 cis)	Male	32.45 ± 6.06	36.34 ± 1.41	34.95 ± 2.93	37.02 ± 0.75	0.207
	Female	31.31 ± 7.14	35.77 ± 1.64	36.23 ± 2.13	36.56 ± 0.70	0.145
	♂+♀	31.88 ± 6.27 ^b	36.05 ± 1.47 ^a	35.60 ± 2.51 ^a	36.79 ± 0.78 ^a	0.015
Vaccenic (C18:1 trans-11)	Male	4.88 ± 0.15	4.87 ± 0.10	4.78 ± 0.14	4.83 ± 0.10	0.607
	Female	4.91 ± 0.10 ^a	4.80 ± 0.11 ^{ab}	4.83 ± 0.12 ^{ab}	4.70 ± 0.07 ^b	0.026
	♂+♀	4.89 ± 0.12	4.84 ± 0.11	4.81 ± 0.13	4.76 ± 0.11	0.083
Linoleic (C18:2 cis)	Male	5.72 ± 0.62	5.54 ± 0.74	6.37 ± 0.52	6.09 ± 0.52	0.189
	Female	5.17 ± 0.86	5.88 ± 0.26	5.80 ± 0.84	6.20 ± 0.16	0.103
	♂+♀	5.48 ± 0.78	5.71 ± 0.55	6.09 ± 0.73	6.15 ± 0.37	0.071
Conjugated Linoleic (C18:2 n-6)	Male	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.762
	Female	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.757
	♂+♀	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.915
α-Linolenic (C18:3 n-3)	Male	0.17 ± 0.02	0.18 ± 0.01	0.16 ± 0.01	0.16 ± 0.03	0.522
	Female	0.16 ± 0.01	0.17 ± 0.03	0.15 ± 0.01	0.16 ± 0.02	0.563
	♂+♀	0.16 ± 0.02	0.17 ± 0.02	0.16 ± 0.01	0.16 ± 0.02	0.244
Arachidonic (C20:4 n-6)	Male	1.84 ± 0.30	1.78 ± 0.44	1.71 ± 0.17	1.74 ± 0.21	0.906
	Female	1.69 ± 0.12	1.77 ± 0.39	1.72 ± 0.15	1.73 ± 0.18	0.951
	♂+♀	1.77 ± 0.23	1.78 ± 0.39	1.71 ± 0.15	1.73 ± 0.18	0.935
Eicosapentaenoic (C20:5 n-3)	Male	0.10 ± 0.01 ^a	0.10 ± 0.01 ^a	0.09 ± 0.01 ^b	0.10 ± 0.01 ^a	0.045
	Female	0.10 ± 0.02	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.928
	♂+♀	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.285
Docosapentaenoic (C22:5 n-3)	Male	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.927
	Female	0.13 ± 0.02	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	0.904
	♂+♀	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.908
Docosahexaenoic (C22:6 n-3)	Male	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.777
	Female	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.430
	♂+♀	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.317
Omega 3	Male	0.46 ± 0.05	0.47 ± 0.02	0.52 ± 0.02	0.49 ± 0.05	0.122
	Female	0.48 ± 0.04	0.49 ± 0.06	0.51 ± 0.04	0.51 ± 0.04	0.612
	♂+♀	0.47 ± 0.04	0.48 ± 0.04	0.48 ± 0.03	0.50 ± 0.04	0.074
Omega 6	Male	8.50 ± 0.65	8.28 ± 0.44	9.12 ± 0.51	9.00 ± 0.50	0.071
	Female	8.37 ± 0.46	8.82 ± 0.21	8.95 ± 0.43	8.96 ± 0.21	0.051
	♂+♀	8.43 ± 0.54 ^b	8.55 ± 0.43 ^b	9.04 ± 0.45 ^a	8.98 ± 0.36 ^a	0.009
∑ SFA	Male	34.53 ± 0.79	35.34 ± 0.83	35.28 ± 0.83	35.13 ± 0.95	0.444
	Female	34.77 ± 1.13	35.46 ± 0.92	34.70 ± 1.50	35.17 ± 0.88	0.693
	♂+♀	34.65 ± 0.93	35.40 ± 0.83	34.99 ± 1.18	35.15 ± 0.87	0.373

Table 4 (continued)

FA / Group	Sex	C	E1	E2	E3	<i>p</i> -value
∑ MUFA	Male	49.07 ± 1.54	48.65 ± 1.04	49.49 ± 0.72	48.51 ± 1.01	0.521
	Female	47.70 ± 1.01 ^b	49.42 ± 0.38 ^a	49.34 ± 0.50 ^a	48.92 ± 0.21 ^a	0.001
	♂+♀	48.39 ± 1.42	49.03 ± 0.84	49.42 ± 0.59	48.72 ± 0.72	0.112
∑ PUFA	Male	11.27 ± 0.86	10.67 ± 0.40	11.41 ± 0.27	11.24 ± 0.27	0.145
	Female	11.54 ± 0.85	10.99 ± 0.11	11.54 ± 0.39	11.44 ± 0.37	0.304
	♂+♀	11.41 ± 0.82 ^a	10.83 ± 0.33 ^b	11.48 ± 0.32 ^a	11.34 ± 0.33 ^a	0.026

FAs are expressed on a dry matter basis (g 100 g⁻¹). Values are given as mean ± SD (standard deviation); *n* = 80; C = control group; E1 – experimental group of chickens fed with 1% supplemental ARG; E2 – experimental group of chickens fed with 2% supplemental ARG; E3 – experimental group of chickens fed with 3% supplemental ARG; a–b = means within the same row with different superscripts differ significantly (*p* ≤ 0.05)

is coherent also with our results. The experiment of Gao et al. (2022) was the first to verify the effect sodium butyrate and/or vitamin D3 supplementation on the AA composition of broiler breast muscle, and the results indicated that too high levels of vitamin D3 should be avoided as it tends to decrease the AA content of the chicken breast muscle. Results on the AAs proportions (% of total AAs) in study of Dalle Zotte et al. (2020) revealed that wooden breast and white stripping myopathies, which nowadays often occur in the intensive broiler chickens breeding, negatively affect the meat proximate composition and the AA content, although in their study they confirmed that Lys, Arg, Leu, Glu, Asp, Ala, and Ser were the most present essential and nonessential AAs in all 3 examined meat-types chickens. Overall, it is important to mention, that any feed supplement that improve AAs content of meat is desirable as there has been increasing evidence highlighting the association of the proportional composition of the FAs in meat to human health (Mir et al. 2020).

FAs profile of chicken meat

FAs profile of Ross 308 broiler chickens’ meat fed with supplemental ARG is shown in the Table 4 (breast muscle) and Table 5 (thigh muscle).

Analysis of the FA profile in the breast muscle of Ross 308 chickens after the application of ARG revealed several significant changes in their composition between the experimental groups, but most of the FAs analysed were not significantly affected by the selected feed supplement. Significant differences were observed for monounsaturated oleic acid without gender difference, which was significantly higher content (*p* ≤ 0.05) in all experimental groups (E1–36.05, E2–35.60 and E3–36.79 g 100 g⁻¹) compared to the control group (31.88 g 100 g⁻¹). We found the highest content of vaccenic acid (*p* ≤ 0.05) in the control group (4.90 g 100 g⁻¹) compared to the E3 group (4.70 g 100 g⁻¹), but these differences were indistinct. Although we observed

significant differences (*p* ≤ 0.05) in the content of eicosapentaenoic acid in males, its content in the experimental groups was almost the same. Regardless of the gender difference, we recorded the highest content of omega-6 FAs (*p* ≤ 0.05) in experimental groups E2 (9.04 g 100 g⁻¹) and E3 (8.98 g 100 g⁻¹). The total MUFA content was significantly higher in the experimental groups in females after the any addition of ARG to the chicken diet (*p* ≤ 0.05), which corresponds to the significantly higher oleic acid content in these groups. The significantly highest PUFA content (*p* ≤ 0.05) was recorded without gender difference in experimental group E2 after application of 2% ARG to the chicken diet (11.48 g 100 g⁻¹).

In the thigh muscle, after the application of ARG to the nutrition of Ross 308 broiler chickens, significant differences were observed in the content of linoleic acid (*p* ≤ 0.05), the highest content of which was observed in group E3 (4.91 g 100 g⁻¹) without gender difference in acid α -linolenic in males (*p* ≤ 0.05) in the control group (0.18 g 100 g⁻¹). Significantly higher eicosenic acid content was observed in males and without gender difference (*p* ≤ 0.05) in the control group (average 0.35 g 100 g⁻¹) and groups E2 (average 0.36 g 100 g⁻¹) and E3 (average 0.34 g 100 g⁻¹) compared to group E1 (0.26 g 100 g⁻¹). In contrast to breast muscle, the thigh muscle had the highest content of omega-6 FAs in the control group (*p* ≤ 0.05) in males (7.03 g 100 g⁻¹) and without gender difference (7.15 g 100 g⁻¹), however, the MUFA content was significantly the lowest in the control group (*p* ≤ 0.05) as in breast muscle (49.28 g 100 g⁻¹ in females and 49.71 g 100 g⁻¹ without gender difference). Although the total PUFA content was significantly the highest in the control group without gender difference (*p* ≤ 0.05) – 9.97 g 100 g⁻¹, but relatively balanced in all experimental groups.

The FAs composition of the meat is considered an important index for meat quality. In the study of Turcu et al. (2019), the PUFAs content in breast meat was significantly higher in experimental group fed with supplemental grape

Table 5 Effect of ARGP supplements on FA composition of broiler chickens Ross 308 thigh muscle (g 100 g⁻¹)

FA / Group	Sex	C	E1	E2	E3	p-value
Lauric (C12:0)	Male	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.095
	Female	0.13 ± 0.02	0.13 ± 0.05	0.13 ± 0.05	0.13 ± 0.01	0.752
	♂+♀	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.436
Myristic (C14:0)	Male	1.34 ± 0.02	1.33 ± 0.03	1.38 ± 0.04	1.35 ± 0.01	0.125
	Female	1.37 ± 0.02	1.37 ± 0.03	1.34 ± 0.04	1.35 ± 0.01	0.361
	♂+♀	1.35 ± 0.03	1.35 ± 0.04	1.36 ± 0.04	1.35 ± 0.01	0.842
Palmitic (C16:0)	Male	24.34 ± 0.22	24.39 ± 0.19	24.31 ± 0.19	24.38 ± 0.24	0.931
	Female	24.48 ± 0.15	24.25 ± 0.21	24.31 ± 0.14	24.29 ± 0.27	0.320
	♂+♀	24.41 ± 0.19	24.32 ± 0.20	24.31 ± 0.16	24.34 ± 0.24	0.704
Heptadecanoic (C17:0)	Male	0.26 ± 0.02	0.28 ± 0.02	0.25 ± 0.02	0.27 ± 0.03	0.317
	Female	0.29 ± 0.04	0.28 ± 0.04	0.28 ± 0.03	0.25 ± 0.03	0.368
	♂+♀	0.27 ± 0.04	0.28 ± 0.03	0.27 ± 0.03	0.26 ± 0.03	0.685
Stearic (C18:0)	Male	10.79 ± 0.11	10.80 ± 0.15	10.71 ± 0.24	10.82 ± 0.21	0.790
	Female	10.78 ± 0.25	10.66 ± 0.15	10.88 ± 0.16	10.88 ± 0.20	0.264
	♂+♀	10.78 ± 0.18	10.73 ± 0.16	10.80 ± 0.21	10.85 ± 0.20	0.583
Oleic (C18:1 cis)	Male	37.71 ± 3.48	38.96 ± 1.37	36.64 ± 3.80	37.51 ± 3.02	0.697
	Female	35.47 ± 3.59	36.11 ± 2.09	38.71 ± 2.08	39.15 ± 2.67	0.110
	♂+♀	36.59 ± 3.54	37.54 ± 2.24	37.68 ± 3.09	38.33 ± 2.82	0.626
Vaccenic (C18:1 trans-11)	Male	4.77 ± 0.05	4.72 ± 0.12	4.85 ± 0.09	4.79 ± 0.07	0.140
	Female	4.81 ± 0.07	4.83 ± 0.05	4.79 ± 0.10	4.73 ± 0.06	0.197
	♂+♀	4.79 ± 0.06	4.78 ± 0.11	4.82 ± 0.10	4.76 ± 0.07	0.460
Linoleic (C18:2 cis)	Male	4.53 ± 0.53	4.86 ± 0.20	4.32 ± 0.25	4.84 ± 0.42	0.107
	Female	4.44 ± 0.55	4.74 ± 0.24	4.50 ± 0.35	4.97 ± 0.28	0.133
	♂+♀	4.49 ± 0.51 ^{bc}	4.80 ± 0.22 ^{ab}	4.41 ± 0.30 ^c	4.91 ± 0.34 ^a	0.009
Conjugated Linoleic (C18:2 n-6)	Male	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.598
	Female	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.03	0.12 ± 0.01	0.948
	♂+♀	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.890
α-Linolenic (C18:3 n-3)	Male	0.18 ± 0.02 ^a	0.15 ± 0.01 ^b	0.15 ± 0.01 ^b	0.13 ± 0.02 ^b	0.004
	Female	0.14 ± 0.01	0.15 ± 0.01	0.13 ± 0.02	0.16 ± 0.03	0.186
	♂+♀	0.16 ± 0.02	0.15 ± 0.01	0.14 ± 0.02	0.15 ± 0.02	0.265
Arachidonic (C20:4 n-6)	Male	0.35 ± 0.01 ^a	0.25 ± 0.04 ^b	0.36 ± 0.09 ^a	0.35 ± 0.03 ^a	0.023
	Female	0.33 ± 0.08	0.26 ± 0.08	0.36 ± 0.12	0.32 ± 0.07	0.330
	♂+♀	0.34 ± 0.06 ^a	0.26 ± 0.06 ^b	0.36 ± 0.10 ^a	0.33 ± 0.05 ^a	0.011
Eicosapentaenoic (C20:5 n-3)	Male	1.45 ± 0.31	1.53 ± 0.17	1.57 ± 0.11	1.70 ± 0.16	0.569
	Female	1.73 ± 0.16	1.52 ± 0.24	1.59 ± 0.28	1.52 ± 0.29	0.498
	♂+♀	1.59 ± 0.28	1.52 ± 0.20	1.58 ± 0.20	1.57 ± 0.23	0.917
Docosapentaenoic (C22:5 n-3)	Male	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.759
	Female	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.487
	♂+♀	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.893
Docosahexaenoic (C22:6 n-3)	Male	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.542
	Female	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.785
	♂+♀	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.986
Omega 3	Male	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.503
	Female	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.383
	♂+♀	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.840
Omega 6	Male	0.54 ± 0.02	0.55 ± 0.04	0.52 ± 0.04	0.50 ± 0.03	0.257
	Female	0.52 ± 0.06	0.54 ± 0.02	0.54 ± 0.02	0.54 ± 0.03	0.833
	♂+♀	0.53 ± 0.04	0.54 ± 0.03	0.53 ± 0.03	0.52 ± 0.04	0.613
Σ SFA	Male	7.03 ± 0.28 ^a	7.03 ± 0.19 ^a	6.22 ± 0.28 ^b	6.71 ± 0.48 ^a	0.004
	Female	7.26 ± 0.60	6.80 ± 0.29	6.31 ± 0.45	7.10 ± 0.71	0.060
	♂+♀	7.15 ± 0.46 ^a	6.92 ± 0.26 ^a	6.27 ± 0.36 ^b	6.91 ± 0.61 ^a	0.001

Table 5 (continued)

FA / Group	Sex	C	E1	E2	E3	<i>p</i> -value
∑ MUFA	Male	33.19 ± 0.67	33.91 ± 1.07	33.54 ± 1.02	33.19 ± 1.32	0.661
	Female	34.00 ± 0.82	33.82 ± 0.63	33.52 ± 1.05	33.41 ± 0.57	0.626
	♂+♀	33.59 ± 0.83	33.86 ± 0.83	33.53 ± 0.97	33.30 ± 0.97	0.581
∑ PUFA	Male	50.14 ± 0.58	50.12 ± 1.29	50.31 ± 0.32	50.95 ± 0.85	0.387
	Female	49.28 ± 0.79 ^c	50.16 ± 0.32 ^{bc}	50.30 ± 0.85 ^b	51.28 ± 0.63 ^a	0.003
	♂+♀	49.71 ± 0.80 ^b	50.14 ± 0.89 ^b	50.31 ± 0.61 ^b	51.12 ± 0.73 ^a	0.002

FAs are expressed on a dry matter basis (g 100 g⁻¹). Values are given as mean ± SD (standard deviation); *n* = 80; C = control group; E1 – experimental group of chickens fed with 1% supplemental ARGP; E2 – experimental group of chickens fed with 2% supplemental ARGP; E3 – experimental group of chickens fed with 3% supplemental ARGP; a–c = means within the same row with different superscripts differ significantly (*p* ≤ 0.05)

seed meal (29.25 g 100 g⁻¹) than in control group (27.73 g 100 g⁻¹), what is in contradiction with our results as we did not find such as relationship. Same was for the content of omega-6 PUFAs in this study, which was also significantly higher in experimental group fed with grape seed meal compared with control group. The breast meat content of alfa-linoleic acid in this study was 1.82 g 100 g⁻¹ in control group and 1.97 g 100 g⁻¹ of total FAs in experimental group. These authors also confirmed higher proportion of omega-3, omega-6 PUFAs and total PUFAs in experimental group in thigh muscle, but with none significance. The results for breast and thigh meat FAs content of Ross 308 broiler chickens can be also compared with Olteanu et al. (2017) who used 2% flaxseed meal and 3% grapeseed meal as natural antioxidant in Cobb 500 broiler diets and found PUFAs content at level 32.46 g 100 g⁻¹ in breast muscle and for thigh meat it was 37.68 g 100 g⁻¹ of total FAs. On the other side Chamorro et al. (2015) studied the effect of including different levels of GP phenolic compounds (0, 5, and 10%) and the addition of hydrolysing enzymes (carbohydrase enzyme complex and tannase at 500 ppm, individually or combined with GP) into broiler chickens’ diets and found decreased MUFA content and increased PUFA concentration in the thigh muscle.

The evaluation of the chicken meat FA profile in study of Bennato et al. (2020) showed only a significant increase of linoleic acid in in experimental groups fed with the 5% (25.7 g 100 g⁻¹ of all FAs) and 7% (25.8 g 100 g⁻¹ of all FAs) supplemental GP. Supplementary GP also caused the significant reduction in SFA, for same experimental groups samples (5% GP – 35.8 g 100 g⁻¹ and 7% GP – 37.1 g 100 g⁻¹ of all FAs) compared with the control group (40.4 g 100 g⁻¹ of all FAs). Besides C18:2, the only PUFA identified by these authors was C18:3, which content did not change by using supplemental GP. However, the increased amount of C18:2 in 5% and 7% addition of GP was effective in increasing total PUFA (5% GP – 27.2 g 100 g⁻¹ and 7% GP

– 27.4 g 100 g⁻¹ of all FAs). Any significant changes in this experiment were reported in the content of MUFAs, what is in contradiction with our findings, as we found significant increase after using supplemental ARGP. We observed increase in linoleic acid and overall increase in MUFAs and PUFAs in breast and thigh muscle obtained from chickens that received the ARGP supplementation. This finding can be fully justified by the fact that linoleic acid has been reported to be the most represented FA in GP (Tsiplakou and Zervas 2008; Manso et al. 2016; Ianni and Martino 2020).

Conclusion

This experiment focused on the use of a bulk agricultural by-product (ARGP) as a feed supplement in the Ross 308 hybrid combination of broiler chickens. The analyses examined how this polyphenol-rich by-product affects the AAs and FAs profile. The AAs profile in the breast muscle was not significantly affected by this addition, while in the thigh muscle the highest proportion of selected AAs was observed in the E1 groups and in the control group (namely in males). However, the differences in the content of the monitored AAs were minimal between the experimental groups. GP consists largely of seeds rich in unsaturated FAs and therefore a change in their composition in chicken meat was expected. This was demonstrated especially in the MUFA content in experimental group E3 with a 3% addition of ARGP to the feed. Overall, however, the differences between the groups were not marked enough and it appears that the selected percentage of ARGP supplement did not significantly affect the profile of AAs and FAs in Ross 308 broiler chicken. There is also a lack of similar literature to compare with, so we recommend experimenting with this polyphenol-rich agricultural by-product, whether in a different form or in a different amount.

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Authors' contributions Peter Haščík: Research concept and design, final approval of article. Matej Čech: Writing the article. Miroslava Kačániová: Collection and/or assembly of data. Peter Herc: Critical revision of the article. Lukáš Jurčaga: Data analysis and interpretation. Ondřej Bučko: Collection and/or assembly of data.

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Data availability Not applicable for that section.

Code availability SAS software (version 9.3, by application Enterprise Guide 4.2).

Declarations

Ethics approval This study was performed in a school breeding station and animals were handled following the legislation on animal welfare (DL n. 126, 07/07/2011, EC Directive 2008/119/EC). Chickens were slaughtered in compliance with Regulation 1099/2009 of the European Union on the protection of animals at the time of killing.

Consent to participate All authors participated voluntarily on this project.

Consent for publication All authors agree to publish this article in *Biologia* Journal.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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