CHEMICAL COMPOSITION OF MUSCLE AFTER RED GRAPE POMACE APPLICATION IN THE NUTRITION OF BROILER CHICKENS

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ABSTRACT
The aim of this work was to evaluate basic chemical composition (% of water, protein, fat and cholesterol) of breast and thigh muscle after supplementation with Alibernet red grape pomace (RGP) into diet of broiler chickens Ross 308. At the beginning, 200 one-day-old 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 group E1 was enriched with 1% RGP per 1 kg of feed mixture (FM), experimental group E2 with 2% RGP per 1 kg of FM and experimental group E3 with 3% RGP per 1 kg of FM. The FM were produced without any antibiotics and coccidiostatics and the fattening period lasted for 42 days. Based on the results, we can state that the application of red grape pomace did not significantly affect the chemical composition of the breast muscle except of higher fat content (P<0.05) in all experimental groups compared to control group (♀, ♂). In the case of thigh muscle, we found significantly higher (P<0.05) water content (♀) in control group (71.26 g.100 g-1) compared to experimental groups E2 (70.04 g.100 g-1) and E3 (69.51 g.100 g-1). We also found significant differences (P<0.05) in crude protein content (♀) between experimental group E3 (21.91 g.100 g-1) compared to experimental groups E1 (21.63 g.100 g-1) and E2 (21.59 g.100 g-1). Overall, it seems like that supplementation with RGP in selected amounts did not have significant beneficial effect on chemical composition of chicken meat compared to control group.

Keywords: grape pomace, broiler chicken, meat, chemical composition

INTRODUCTION
Poultry meat is a very good source of proteins with high biological value and micronutrients, which is provided mainly by meat and eggs. Human body is more capable to absorb these nutrients compared to those of plant origin (Raza, Bashir and Tabassum, 2019). Poultry meat production in the world is about to reach 137 million tons in 2020, which is 2.4% more than in 2019 or half the pace of growth recorded last year. Countries like China, the EU, UK, Brazil and Mexico are expected to rise their production, while in India, Thailand, Turkey and the USA production is currently falling. However, the positive outlook could turn negative if recent price drops and unavailability of workers in the sector are expected to rise their production growth recorded last year. 137 million tons will continue to be produced without any limitations (FAO, 2020). Poultry and its main products, meat and eggs, as well as products made from them are of great importance to humans because they provide an excellent source of proteins, fats, essential amino acids, minerals, vitamins and other nutrients. Big advantage is that they are available worldwide (Shaltout, 2019).

Chicken meat is sensory-neutral and thanks to its high tenderness is found to be used in miscellaneous meat products aimed at various consumers. Chicken meat is widely used in the world cuisines thanks to its undemanding preparation what is an advantage especially nowadays, because people have less time to prepare foods (Petracé et al., 2013). There are many religious and social restrictions for consuming red meats, mainly pork, what is not issue of chicken meat. Moreover, comparing with other meat types, it is one of the cheapest kinds (Haščík et al., 2018a). Chicken meat is known for its considerable protein content – average of 22% (Bobko et al., 2017). Chicken proteins are known also for their good digestibility (94%) (Williams, 2007) and high AAS (0.92), compared to legumes (AAS value = 0.57 – 0.71) and wheat gluten only AAS = 0.25 (Pereira and Vicente, 2013). Čuňoš et al. (2013) describes basic chemical composition for breast and thigh muscle as follows: dry matter (%) 25.8 and 26.0, respectively in breasts and 33.1 and 32.6, respectively in thighs; protein content (g.100 g-1) 22.9 in breasts and 19.5 in thighs; fat content (g.100 g-1) 1.8 in breasts and 12.7 in thighs and Cavanii et al. (2009) add energy value (kcal) 104, resp. 115; cholesterol (mg) 62, resp. 80; iron (mg) 0.72, resp. 1.02 and sodium (mg) 65, resp.

The main goal of animal production is to ensure a consistent chemical composition of meat, from which its high nutritional value derives. However, in addition to the basic chemical composition, other parameters such as the content of minerals and biologically active substances have recently been monitored (Al-Yasiry, Kiczerowska and Samolińska, 2017). The final quality of meat products, as well as the quality of the large-scale meat production, is ensured by the appropriate composition of the poultry feed (Ciurescu et al., 2016) as well as by selected feeding method (Kiczerowska et al., 2015). Changes in the chemical composition of poultry meat are also related to the rations and feed nutrients assimilation. Nutrition and various supplements can lead to improved but also deteriorating of meat quality (Morales-Barrera et al., 2013; Dwiloka et al., 2015; Yogesh et al., 2015). Supplementation of chicken nutrition is nowadays aim of many researchers. Many plant products are being tested, such as probiotics, prebiotics, organic acids, enzymes, plant products and other antimicrobials applied via water or feed. Their aim is to improve chickens’ digestion, health and in the end final products – meat and eggs (Trembecká et al., 2017; Pavelková et al., 2020).

The growth of the world’s population is forcing scientists to look for alternative sources of feed for cereals, which are the main components of the human diet. Each year, several researchers focus on the use of agricultural by-products and wastes produced in billions of tons (Nie et al., 2016). Nowadays, the use of plant-origin supplements in animal nutrition has become very popular. Because of this, researchers’ attention concentrates towards natural additives application into animal diets due to their content of different functional components (Ramay
and Yalçın, 2020). Eco-innovations in agriculture are based on the circular economy. The circular economy is an innovative approach that focuses on the “zero waste” economy, where new waste and raw materials are already generated from other waste and raw materials (Mirabella, Castellani and Sala, 2014). In addition, there has recently been a growing interest in obtaining various biologically active substances (polyphenols, flavonoids and simple phenolic substances) from these wastes and using them in animal nutrition for improving their health and performance (Lieber et al., 2017; Leskovec et al., 2018).

Antibiotics are commonly used not only for treatment but also for prevention of bacterial diseases in the industrial breeding of broiler chickens. Antibiotic-resistant bacterial strains as well as their residues in meat and various animal products have reduced the use of feed antibiotics (Zhang et al., 2016; Wang et al., 2017). The global strategy also leads to a reduction in the use of antibiotics and other medicines in livestock, and therefore various products rich in bioactive compounds with antimicrobial, antioxidant and anti-inflammatory properties are being sought. It is these natural feed additives that are promising alternatives to antibiotics (Niewold, 2014; Lillehoj et al., 2018).

The grapevine is one of the most economically important crops in the world. Grapes are mainly used for wine production, but also for consumption and processing of grape pomace (Fortes and Pais, 2010). Grape pomace is a waste originating mainly from the production of wine, where the stems are discarded. Among the known products that are produced from grape pomace are grape seed oil and grappa. Pomace from red grape varieties is used to obtain natural food colorings – anthocyanins (Schreiber, 2019). It is estimated that grape pomace makes up to 20% of the total weight of the grapes, therefore this is a considerable waste disposal problem for the wineries and other grape-processing industries (Llobera and Cañellas, 2007). Due to their nutritional composition (various antioxidants, especially polyphenols), they should not be easily disposed of and should be further processed and used across the food sector (Theagarajan et al., 2019), for example in an animal nutrition (Aditya et al., 2018). The effect of grape pomace enrichment in broiler chicken nutrition was demonstrated in recent experiments (Ebrahimzadeh et al., 2018; Haščík et al., 2020; Turcu et al., 2020). Among the older researches, we can mention that enrichment with source of antioxidants in form of GP at 30 g kg⁻¹ (Brenes et al., 2008) and 60 g kg⁻¹ (Goli et al., 2007) led to lower lipid oxidation in chicken meat. Viveros et al. (2010) found higher amounts of beneficial bacteria in the broilers’ intestines after application of GP. On the other side, dried RGP at levels ranging from 2.5 to 10 g kg⁻¹ feed failed in extending the shelf life and the eating quality of the produced meat (Kasapidou et al., 2016).

The aim of the present study was to evaluate the chemical composition of Ross 308 broiler chickens’ muscle (breasts and thighs) after addition of RGP (variety Alibernet) into their diet.

### MATERIAL AND METHODS

**Animals and experimental design**

The experiment took place in the SUA, Nitra (Test poultry station, Kolíany). The methodology of fattening process was realized according to Haščík et al. (2020).

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The experiment took place in the SUA, Nitra (Test poultry station, Kolíany). The methodology of fattening process was realized according to Haščík et al. (2020). FMFs 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 FMFs were not fed without any antibiotics and coccidiosis and were prepared by Biofeed, Inc. (Kolárovo, Slovakia). The control group (C) received the basal diet without supplementation. The FM of experimental groups were enriched with RGP in amount 1%·100 kg⁻¹ of FM (E1); 2%·100 kg⁻¹ of FM (E2) and 3%·100 kg⁻¹ of FM (E3). Composition of starter and grower FMFs are designed according to study of Haščík et al. (2020).

**Characterization of grape pomace 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 follows: dry matter – 383.50, crude protein – 112.80, ether extract – 105.91, crude fiber – 230.31, ash – 65.55, nitrogen free extracts – 485.74, organic matter – 949.56, sugars – 4.89, acetic acid – 450.81, neutral detergent fiber – 525.46, lignin – 281.29, celluloses – 156.48 and hemicelluloses – 91.82.

**Slaughter and measurements**

At 42nd day of age, 10 females and 10 males from each group were selected. After weighing, they were slaughtered at the experimental slaughterhouse of the Department of Technology and Quality of Animal Products (SUA, Nitra). Slaughter was executed by conventional neck cut, with following bleeding, feathers removing and eviscerating.

Skinless breast (musculus pectoralis major) and thigh muscle (musculus biceps femoris) from each group were taken for evaluation of basic chemical composition (water, crude protein fat and cholesterol content in g·100 g⁻¹) performed with INFRATEC 1265 device (Germany).

**Statistical analysis**

Acquired data were processed with SAS software, Enterprise Guide 4.2 application (version 9.3, SAS Institute Inc., USA, 2008), using analysis of variance (ANOVA). Results were reported as mean ± standard deviation. Statistical significances between groups were calculated using the t-test and considered significant at P<0.05.

**RESULTS AND DISCUSSION**

The results of experiment with Ross 308 broiler chickens after addition of Alibernet RGP, which was aimed to analyze and evaluate chemical parameters, are presented as follows: the results of water content, crude protein, fat, and cholesterol content in breast and thigh muscle are given Table 1 and 2, respectively.

### Table 1 Chemical composition of breast muscle of broiler chickens regarding sex (g·100 g⁻¹)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>sex</th>
<th>C E1</th>
<th>E2</th>
<th>E3</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content</td>
<td>Male</td>
<td>70.56 ± 0.95</td>
<td>70.77 ± 0.79</td>
<td>70.77 ± 1.01</td>
<td>70.35 ± 0.83</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>70.83 ± 0.78</td>
<td>71.16 ± 0.63</td>
<td>70.72 ± 0.75</td>
<td>70.78 ± 0.36</td>
</tr>
<tr>
<td>Crude protein</td>
<td>Male</td>
<td>24.02 ± 0.13</td>
<td>23.88 ± 0.22</td>
<td>23.65 ± 0.41</td>
<td>24.27 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>23.72 ± 0.18</td>
<td>23.97 ± 0.34</td>
<td>23.75 ± 0.47</td>
<td>0.162</td>
</tr>
<tr>
<td>Fat</td>
<td>Male</td>
<td>0.90 ± 0.24</td>
<td>1.19 ± 0.04</td>
<td>1.32 ± 0.42</td>
<td>1.12 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1.03 ± 0.25</td>
<td>1.18 ± 0.21</td>
<td>1.22 ± 0.21</td>
<td>1.40 ± 0.08</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Male</td>
<td>0.040 ± 0.004</td>
<td>0.040 ± 0.007</td>
<td>0.041 ± 0.002</td>
<td>0.041 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.041 ± 0.006</td>
<td>0.041 ± 0.005</td>
<td>0.044 ± 0.006</td>
<td>0.048 ± 0.004</td>
</tr>
</tbody>
</table>

**Notes:** mean ± S.D. (standard deviation); C = control group; E1, E2, E3 = experimental groups; a, b, c = means significant differences between groups (P<0.05) determined with t-test.

### Table 2 Chemical composition of thigh muscle of broiler chickens regarding sex (g·100 g⁻¹)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>sex</th>
<th>C E1</th>
<th>E2</th>
<th>E3</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content</td>
<td>Male</td>
<td>71.26 ± 0.51</td>
<td>70.65 ± 0.31</td>
<td>70.04 ± 0.48</td>
<td>69.51 ± 0.77</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>70.44 ± 0.46</td>
<td>70.12 ± 0.42</td>
<td>69.58 ± 0.60</td>
<td>70.29 ± 0.36</td>
</tr>
<tr>
<td>Crude protein</td>
<td>Male</td>
<td>21.78 ± 0.18</td>
<td>21.63 ± 0.14</td>
<td>21.59 ± 0.18</td>
<td>21.91 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>21.80 ± 0.11</td>
<td>21.69 ± 0.04</td>
<td>21.75 ± 0.28</td>
<td>21.79 ± 0.27</td>
</tr>
<tr>
<td>Fat</td>
<td>Male</td>
<td>2.51 ± 0.24</td>
<td>2.67 ± 0.26</td>
<td>2.67 ± 0.49</td>
<td>2.45 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1.99 ± 0.19</td>
<td>2.31 ± 0.20</td>
<td>2.82 ± 0.35</td>
<td>2.83 ± 0.26</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Male</td>
<td>0.061± 0.002</td>
<td>0.065 ± 0.004</td>
<td>0.065 ± 0.005</td>
<td>0.062 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.054 ± 0.005</td>
<td>0.060 ± 0.001</td>
<td>0.069 ± 0.003</td>
<td>0.064 ± 0.004</td>
</tr>
</tbody>
</table>

**Notes:** mean ± S.D. (standard deviation); C = control group; E1, E2, E3 = experimental groups; a, b, c = means significant differences between groups (P<0.05) determined with t-test.
Water content

The highest water content in breast muscle was recorded in experimental groups E1 and E2 – 70.77 g.100 g⁻¹ in males, while in females it was E1 group 71.16 g.100 g⁻¹, but these differences between groups were not statistically significant (P≥0.05). However, in thigh muscle we found significantly lower (P<0.05) water content in experimental group E2 – 70.04 g.100 g⁻¹ and E3 – 69.51 g.100 g⁻¹ compared to control group – 71.26 g.100 g⁻¹). In females we did not find significant differences (P≥0.05) between groups with the highest water content in comparison E1 70.04 g.100 g⁻¹ and E3 70.04 g.100 g⁻¹ (♂). In comparison, significantly lower (P<0.05) water content revealed Nardoia (2016) in broilers breast muscle after feed-addition of 4% grape skin (74.38 g.100 g⁻¹) and 4% of grape pomace (74.61 g.100 g⁻¹) compared to control group (75.35 g.100 g⁻¹). Any significant differences (P<0.05) were described after enrichment with supplemental pine needles supplementation of 2.5%, 5% and 7% by Zaj, J et al., 2013; Mironeasa et al., 2016). This might be a reason why we found generally higher fat content (P<0.05) in experimental groups in comparison with control group. The highest fat content in breast muscle was in experimental group E2 3.32 g.100 g⁻¹ and 2.67 g.100 g⁻¹ (♀). In thigh muscle fat content was the highest in experimental groups E1 and E2 – 2.67 g.100 g⁻¹ (♂) and E3 – 2.83 g.100 g⁻¹ (♀).

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Fat

Grape seeds as a major part of grape pomace are a rich source of lipids, ranging between 14 – 17% (Gill et al., 2013; Mironoasa et al., 2016). This might be a reason why we found generally higher fat content (P<0.05) in experimental groups in comparison with control group. The highest fat content in breast muscle was in experimental group E2 3.32 g.100 g⁻¹ and 2.67 g.100 g⁻¹ (♀). In thigh muscle fat content was the highest in experimental groups E1 and E2 – 2.67 g.100 g⁻¹ (♂) and E3 – 2.83 g.100 g⁻¹ (♀).

Also, Nardoia (2016) did not find significant differences (P≥0.05) in fat content in broilers breast muscle after feed-addition of 4% grape seeds (1.97%), 4% grape skin (1.59%) and 4% of grape pomace (1.74%) compared to control group (1.71%). Bennato et al. (2020) found similar reliance in increasing fat content after enrichment with grape pomace supplementation of 2.5% (1.16 g.100 g⁻¹), 5% (1.22 g.100 g⁻¹) and 7% (1.25 g.100 g⁻¹) as we did, however these differences were not significant (P≥0.05). On the other hand, Reyes et al. (2020) found significant differences in breast muscle after 20% addition of white grape pomace (1.6%) and 20% addition of red grape pomace (2.8%) compared to control group (0.9%). In contrast with our results may be feed enrichment with camellina, flax and sunflower described by Zaj, J et al., 2020, who found significantly lower (P<0.05) fat content (in g.100 g⁻¹) in all experimental groups – 1.07 (camellina), 1.04 (flax) and 1.08 (sunflower) compared to control group (1.34) in breast muscle and 5.10 (camellina), 5.06 (flax) and 4.61 (sunflower) compared to control group (7.50) in thigh muscle. Haščič et al. (2018b) found significantly lowest fat content after enrichment with 1% humic acid in breast muscle (0.84 g.100 g⁻¹) and in control group in thigh muscle (7.15 g.100 g⁻¹). Any significant changes (P≥0.05) were also observed after dietary supplementation with raw and fermented sour cherry kernel (1, 2, and 4%) in breast and thigh fat content (Gungor and Erener, 2020) or supplemental pine needles powder (0.25, 0.50, 0.75 and 1%) in breast fat content (Ramay and Yalçın, 2020).

Cholesterol

Cholesterol content was not markedly affected (P≥0.05) by selected dietary supplementation. In breast muscle its content varied between 0.040 – 0.048 g.100 g⁻¹ in all observed groups (♂, ♀) and slightly higher in thigh muscle 0.063 g.100 g⁻¹ with the lowest concentrations in control group – 0.061 g.100 g⁻¹ (♂) and 0.054 g.100 g⁻¹ (♀). However, this may be due to overall higher fat content in thigh muscle. Our results are similar with results of Turcu et al. (2019), who observed that after addition of grape seed meal the cholesterol level in the fat from the breast meat samples was 8.13% lower (P<0.05) in the experimental group (40.68 mg.100 g⁻¹) compared to the control group (44.28 mg.100 g⁻¹) and 23.85% lower (P<0.05) in the experimental group (46.38 mg.100 g⁻¹) compared to the control group (60.91 mg.100 g⁻¹) in thigh muscle. Haščič et al. (2016) discovered higher (P<0.05) cholesterol content in thigh muscle in experimental groups – 113.08 mg.100 g⁻¹ (probiotics addition), 118.68 mg.100 g⁻¹ (propolis addition) in comparison with control group 121.25 mg.100 g⁻¹. However similar results were described by Haščič et al. (2018b) who also did not find any differences to the cholesterol content in chicken breasts tested after enrichment with grape pomace supplementation of 2.5% (P>0.05), ranging from 0.033 g.100 g⁻¹ (experimental group P1 with addition humic acids) to 0.039 g.100 g⁻¹ (experimental group P2 with addition humic acids and probiotic). In thigh muscle, the cholesterol content was significantly lowest (P<0.05) in control group – 0.070 g.100 g⁻¹ compared to 0.086 g.100 g⁻¹ in experimental groups P1 and P2. Cholesterol lowering in broiler meat (breasts and thighs) is important for human nutrition and health, in reducing the risks associated with cardiovascular diseases (Pavlović et al., 2018).

CONCLUSION

The results of our study have shown that the application of the red grape pomace in used concentrations did not markedly influence on the chemical composition of breast muscle. The results have shown that dietary supplementation with red grape pomace in chicken nutrition slightly improved the cholesterol protein and decreased the water content in the thigh muscle (P≥0.05). However, selected parameters were not significantly affected (P≥0.05) in females. Chosen dietary supplementation increased (P<0.05) fat content in experimental groups (♂, ♀) without significant increase (P≥0.05) of cholesterol content. The conclusion is that any feed supplement should not have a negative impact on the final quality of broiler chicken meat, but also on the health of the food consumer. From this point of view, red grape pomace as a voluminous agricultural waste seems to be a suitable candidate in broiler chicken’s nutrition.

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