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Impact of Siberian Larch Dihydroquercetin or Dry Distilled Rose Petals as Feed Supplements on Lamb's Growth Performance, Carcass Characteristics and Blood Count Parameters

Research Article

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ABSTRACT

The objective of this study was to determine the impact of dihydroquercetin from Siberian larch and dry distilled rose petals (DDRP) on growth performance, carcass characteristics and blood parameters of lambs from the Bulgarian dairy synthetic population sheep. For the purpose of the study 30 clinically healthy male lambs aged 65 days, levelled by live weight were used. One control group (C) fed 50 days with ground alfalfa + granulated compound feed and two experimental groups (D) and (R) fed on the same diet supplemented either with 7.5 mg dihydroquercetin/kg/day or with 545 mg DDRP/kg/day respectively, were studied. The dihydroquercetin feeding increases the fat content ($P \leq 0.01$) of lamb carcasses but adversely affects their conformation. No significant differences ($P > 0.05$) were found between 1st h and 24th h post-mortem pH of control group C and experimental group D. Compared to them the pH values of the experimental group R were by 0.14-0.15 pH units lower ($P \leq 0.05$). No significant differences ($P > 0.05$) were found in the blood count of the three studied groups of lambs. Exceptions were made for haemoglobin (HGL) in the experimental group D which were with 6-7 g/L higher ($P \leq 0.05$) than these in control group C and experimental group R and the blood glucose (GLU) in the experimental group R which is with 0.25-0.28 mmol/L lower than determined in control group C and experimental group D.

KEY WORDS average daily gain, diet supplementation, lambs, pH, phytonutrients.

INTRODUCTION

In the last few years the attention of both farmers and researchers worldwide has been focused on the improvement of lamb survival and performance with the aim to enhance the productivity of sheep farming enterprises (McCoard *et al.* 2017). Different innovations have been discussed about the animal production chain (Hassan *et al.* 2018). Several phytogenic compounds or their mixtures with antimicrobial properties have been reported too (Singh and Gaicwad, 2020).

Those authors turned their attention to essential oils for the nutrition of ruminants. It is assumed that certain essential oils have abilities to increase the permeability of bacterial cell membranes (Thakur *et al.* 2020). According to Sabino *et al.* (2018) dietary supplementation with essential oils can be used as a new strategy for animal health improving. Therefore, few selected phytochemicals have been proposed as potential alternatives to antibiotics and growth-promoters. Potential impacts of breed, age and especially pasture rearing have been most commonly discussed as factors influencing the fatty acid composition of meat and

fat in previous studies with lambs (Popova, 2007; Baldi *et al.* 2019; Holman *et al.* 2019).

According to *in vitro* analyses polyphenols have been considered bioactive components of food and feed acting as antioxidants through the scavenging of reactive oxygen species (Sun *et al.* 2018). Oh *et al.* (2016) recommend that long-term *in vivo* experiments are needed to evaluate the true phytonutrients' activity for altering rumen microbial fermentation and enhancing animal growth performance. The growing interest in improving growth performance and carcass quality has drawn researchers' attention to the relationship between ruminant nutrition and their health (De Brito *et al.* 2017). It is maintained that certain phytonutrients have a positive effect on the health of the body due to their active involvement in the regulation of cellular functions (Fomichev *et al.* 2016; Thakur *et al.* 2020). Perhaps for these reasons, it is recommended that further research be carried out before formulating scientifically sound nutritional recommendations (Teodoro, 2019).

In the last few years the number of scientific evidence for use of natural sources of biologically active substances and non-traditional feed additives in livestock has increased. Some publications (Miltko *et al.* 2019) discussed its beneficial effect on the health status and productivity of animals. On the other hand, the phytonutrients are responsible for improving and maintaining the nutritional, technological and flavoring properties of produced or processed meat. A number of approaches have been explored to increase the lamb growth performance and meat quality (De Brito *et al.* 2017; Chikwanha *et al.* 2019). To achieve these objectives various feed supplements have been discussed such as: replacement of cereal grains by orange pulp and carob pulp in faba bean-based diets (Guenaoui *et al.* 2019), fungal enzyme cocktail treatment (Cruywagen and van Zylb, 2008), varying levels of Zizyphus (*Zizyphus mauritiana*) leaf meal inclusion in concentrate diet (Abdu *et al.* 2012), vitamin E (Delosière *et al.* 2020), vegetable oils (El-Sabaawy *et al.* 2015), sugar beet pulp and roasted canola seed in a concentrate diet (Asadollahi *et al.* 2017). Oh *et al.* (2016) are of the opinion that due to their phenolic nature some phytonutrients are less susceptible to degradation in the rumen by microorganisms and may also be active post-ruminally.

The dihydroquercetin is a flavonoid (Sunil and Xu, 2019) with strong antioxidant activity because of its ability to act as an electron donor and to inhibit hydroxyl radicals. These results again confirm the hypothesis that the effect of phytonutrients is highly species specific and depends on dose, metabolism, digestive system and many other factors. There is information that dihydroquercetin manifests radioprotective, membraneprotective, capillaryprotective, angioprotective, lipid reducing, anti-inflammatory, anti-allergic, cardioprotective, hepatoprotective, detoxifying, neuroprotective, gastroprotective, immunomodulatory, retinoprotective and endocrinological properties (Artem'eva *et al.* 2015) and taken as a dietary supplement has beneficial effects on immunodeficiency, bronchopulmonary diseases and liver function.

Fomichev *et al.* (2016) first drew attention to the potential of bioflavonoids obtained from bark of *Larix* spp. Analysis of stereoisomeric composition demonstrated two bioflavonoids derived from Siberian larch (*Larix sibirica*) (Sunil and Xu, 2019). As a result of its healthy effects Fomichev *et al.* (2016) supposed that 1-5 g *Larix dahurica* Turcz dihydroquercetin preparation DHQ1/kg supplementation can be used for the realisation of a productive potential of lambs under an impact of stress-factors. In this study the used preparation DHQ1 contains 1.0-2.0% of pure dihydroquercetin and pulp of Dahurian larch with dry matter not higher than 80%.

Another new potential beneficial phytonutrient is a by-product derived from rose oil production. There is evidence (Vijayanchali, 2017) the hybrid perpetuals roses are rich in anthocyanin, quercetin, variation of flavonol glycosides and kaempferol derivatives.

A new sources of bioactive compounds are dry distilled rose (*Rosa damascena*) petals (DDRP). They possess a wide range of strong cytotoxic, antioxidant and antimicrobial properties (Nowak *et al.* 2014). It has been shown that dihydroquercetin or DDRP alter positively poultry meat composition (Balev *et al.* 2015) but in literature no information could be found on the use of DDRP as feed additives in lambs' feeding.

Not many studies have been published that deal with the effect of nutritional supplements of phytonutrients with antioxidant properties, such as dihydroquercetin and DDRP on growth efficiency, carcass quality and blood characteristics not only in lambs but also in ruminants in general. Therefore, the objective of this study was to determine the impact of dihydroquercetin from Siberian larch and dry distilled rose petals (DDRP) on growth performance, carcass characteristics and blood parameters of lambs from the Bulgarian Dairy Synthetic population sheep.

MATERIALS AND METHODS

Lambs and diets

This experiment was conducted in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Council Regulation (EC) No 1099/2009, Commission Recommendation 2007/526/EC and Bulgarian Veterinary Medical Activity Act. The experiment was approved by the Bulgarian Scientific Ethics Committee and requirements of the Council Directive 2010/63/EC were met.

A total of 30 clinically healthy male lambs aged 65 days, levelled by live weight were used in the experiment. They were housed indoors in the Experimental Farm of the Agricultural Institute, Shumen, Bulgaria. The lambs were divided into one control and two experimental groups, each containing 10 animals being fed for 50 days. The control group (C) was fed ground alfalfa + granulated compound feed. The experimental groups (D) and (R) were fed on the same diet supplemented with either 7.5 mg dihydroquercetin/kg/day or 545 mg dry distilled rose petals (DDRP)/kg/day, respectively.

Feeding the lambs was *ad libitum* in group boxes, with access to water and salt. Individual daily doses of the supplements were calculated according to previous weighing of the animals, mixed with supplementary feed (Table 1) and given with the morning feeding. The control group (C) was fed ground alfalfa + supplementary feed granules for lambs (Table 1) which were supplied by feed factory Sole trader "Vasil Kostov", Lyuben Karavelovo village, Aksakovo municipality, Varna district, Bulgaria. The experimental groups (D) and (R) were fed the same diet supplemented either with 7.5 mg dihydroquercetin/kg/day or 545 mg DDRP/kg/day, respectively. Irrespective of the scarce information on the use of dihydroquercetin as biologically active substance in the lambs' diet and lack of published data on DDRP supplementation in lambs in the selection of the above shown doses we were guided by the following considerations:

- The dihydroquercetin antiradical activity manifesting in a concentration 0.0001- 0.00001% (Fomichev *et al.* 2016).
- The concentration of natural bioflavonoid dihydroquercetin was recalculated taking into account the much higher purity (96%) in the isolate used by us in this study compared to the significantly lower levels in the preparation used by Fomichev *et al.* (2016). The idea was to be applied comparable concentrations of biologically active compounds.
- The using of higher concentration of natural polyphenolic compounds comparing the experiment with pigs (Vlahova-Vangelova *et al.* 2020) with idea to compare the results between monogastric (pigs) and small ruminant (lambs) animals.
- Seeking the application of the minimum possible concentrations in view of the economic efficiency in the future application of the preparations given their very high price.

Daily control of the amount of combined feed consumption during the experiment was exercised. Residual feed was weighed and subtracted from of the daily amount of feed consumed. Lambs were weighed every two weeks.

The dihydroquercetin was provided by the company Flavitlife Bio JSCo (Sofia, Bulgaria).

Its chemical composition was 96% dihydroquercetin, 3% dihydrokaempferol and traces of naringenin. The distilled rose petals were supplied by Damascena rose oil distillery, village of Skobelevo, municipality of Pavel Banya, Stara Zagora district, part of Bulattars Production Company Ltd (Sofia, Bulgaria). After pressing, the petals were dried (24 h, 65 ˚C) and ground to particle size < 0.4 mm. The 13 glycosides of kaempferol, 10 glycosides of quercetin, 6 glycosides of gallic acid and 2 flavonol aglycones were identified in dry rose petals.

The daily dose of the supplements was calculated according to previous one and mixed with feed mixture (Table 1) and given to the lambs with the morning feeding.

Blood sampling

The blood sampling was made three times: on 5 February 2019 (in the beginning of the experiment), on 1 March 2019 (in the middle of the experiment) and on 26 March 2019 (at the end of the experiment). The blood samples were analyzed immediately after sampling.

Growth Performance measurements

The live weights of the lambs were recorded at the beginning of the experiment and during the experiment period every two weeks interval before morning feeding. Fed intake was measured daily. These parameters were used to calculate the average daily gain as well as the feed intake per kg weight gain.

Feed consumption was monitored daily throughout the experimental period. The amount of feed consumed per 1 kg of growth was determined in feed units for growth (FUG) and crude protein (CP), g relative to the total growth obtained for each group.

Slaughtering procedure

Upon completion of the test period the lambs were identified and transported to the slaughterhouse (Golden Fleece Ltd., Veliki Preslav, 16 Brothers Miladinovi Str., Shumen district). After 24 h of pre-slaughter break, lambs were harvested in accordance with the requirements of Ordinance No 15 of May 8, 2009 following normal industry slaughtering procedures.

The lamb carcasses were split, classified and chilled. After 24 h of chilling the carcasses with temperature 4 ˚C were moved to a refrigeration where they were stored at 0 – 4 ˚C. Samples of m. longissimus thoracis et lumborum were removed from each carcass.

Chilled muscle samples were ground through 3 mm grinder plates and mixed. The pH values were determined as means after five replicates. Five replicates of the proximate composition analyses of granulated combined feed were made too (Table 1).

Table 1 Chemical composition of ground alfalfa hay and combined feed granules and their ingredients*

Components of lamb's feed	**Complementary feed for lambs after 2 months**
Ground alfalfa hay *ad libitum*	
Calculated chemical compositions, g/kg	
Crude protein	164.00
Crude fats	30.21
Dietary fibers	90.05
Minerals, mg/100g	
Calcium	1500.00
Phosphorus	671.00
Formulation (No 36-16) of granulated compound feed, g/kg	
Maize/corn	117.76
Low-cellulose sunflower pomace	189.98
Wheat bran	300.00
Peas	100.00
Lucerne meal	50.00
Corn germ	200.00
Chalk	34.26
Salt	6.00
Vitamin-mineral premix ME + B OB - E	2.00
Calculated chemical compositions of granulated combined feed, g/kg	
Moisture	95.42
Crude protein	165.00
Crude fats	33.00
Including linoleic acid C18:2	1.74
Crude ash	79.00
Dietary fibers	93.00
Starch	330.02
Amino acids, %	
Lysine	0.842
Methionine	0.321
Methionine + cystine	0.591
Tryptophan	0.224
Arginine	1.218
Threonine	0.747
Minerals, mg/100g	
Calcium	1500.00
Phosphorus	671.00
digestible phosphorus	395.00
Chlorides	734.00
Chlorine	441.00
Sodium	494.00
Manganese	131.00
Zinc	120.00
Iron	180.00
Cupper	9.50
Iodine	1.85
Selenium	0.64
Cobalt	0.40
Vitamins	
Vitamin A, UI/kg	10000
Vitamin D_3, UI/kg	2000
Vitamin E, mg/kg	100.00

* The quantities of Siberian larch dihydroquercetin and dry distilled rose petals added as supplements to the diets were calculated as D: 7.5 mg dihydroquercetin/kg live weight per day R: 545 mg dry distilled rose petals/kg live weight per day.

The quantities of Siberian larch dihydroquercetin or dry distilled rose petals added as supplements to the diets were calculated as D1: 3.5 mg dihydroquercetin/kg/d; D2: 7.5 mg dihydroquercetin/kg/d; R1: 0.255 g dry distilled rose petals/kg/d and R2: 0.545 g dry distilled rose petals/kg/d.

The bio-concentrate BC14 contents: Crude protein: 312.10 g/kg; Crude fat: 10.70 g/kg; Crude ash: 153.00 g/kg; Crude fibers: 38.10 g/kg; Lysine: 5.88 g/100 g; Methionine: 2.79 g/100 g; Calcium: 7.80 g/100 g; Phosphorus: 2.69 g/100 g; Copperas sulphate: 268 mg/kg; DL-α-tocopherol: 670 mg/kg; vitamin A: 93800 UI/kg; vitamin D_3: 16080 UI/kg and Total energy: 1975.845 kcal/kg.

The bio-concentrate BC16 contents: crude protein: 348.00 g/kg; Crude fat: 17.40 g/kg; Crude ash: 165.00 g/kg; Crude fibers: 108.30 g/kg; Lysine: 2.26 g/100; Methionine: 0.67 g/100; Methionine + cystine: 1.25 g/100; Threonine: 1.31 g/100; Calcium 3.66 g/100; Phosphorus: 0.95 g/100 g; Absorbable phosphorus: 0.67 g/100; Sodium: 0.78 g/100; Iron 560.00 mg/kg; Zinc: 545.00 mg/kg; Manganese: 195.00 mg/kg; Copper: 100.00 mg/kg; Iodine: 4.10 mg/kg; Selenium: 1.50 mg/kg; Antioxidants: 0.40 g/100g; vitamin E: 320.00 mg/kg; vitamin A: 32500 UI/kg and vitamin D_3: 6000 UI/kg.

Vitamin-mineral premix for lambs including phosphate, lysine, methionine, enzymes, coccidiostatic, antitoxic substances contents: vitamin A: 8000 IU/kg; vitamin E: 40.00 mg/kg; vitamin D_3: 2000 IU/kg; Iron: 170.26 mg/kg; Iodine: 1.10 mg/kg; Cobalt: 0.30 mg/kg; Copper: 5.65 mg/kg; Manganese: 95.61 mg/kg; Zinc: 108.86 mg/kg and Selenium: 0.40 mg/kg.

Classification of lamb carcasses

After the carcasses production the m. longissimus thoracis et lumborum was removed and pH values hot and cold carcass, pH_1 (45min post mortem) and pH_{24} (24 h post mortem) were measured. The stress that influenced the post mortem quality of lamb meat was discussed depending on the values of pH_1 and pH_{24}.

The classification of lamb's carcasses was made following recommendations of Article 30 of Commission Regulation (EC) No 1249/2008 and the classes of conformation and fat cover, carcasses weight and colour of meat were determined according Article 29 and Annex VII of Commission Regulation (EC) No 1249/2008.

pH determination

The pH value of the samples at 45 min and 24 h in m. longissimus thoracis et lumborum was measured electropotentiometrically with a laboratory pH Meter Hanna HI98107 (Hanna Instruments, Villafranca Padovana, PD, Italy) equipped with a temperature and combined pH electrode Sensorex 450 CD (Sensorex, Inc., Garden Grove, USA).

Determination of blood count

During the experiment blood from each lamb was sampled three times in the beginning, in the middle (25 days) and at the end (50 days). Samples (10 mL of blood) were taken in the morning on an empty stomach from v. jugularis in vacuum containers with closed system. In consequence, they were transferred to the laboratory within the first three hours for further analysing. Analytical procedures for blood counting were performed with an automatic hematology analyzer with 5-type differential counting SYSMEX XS 500i (Sysmex Europe GmbH, Norderstedt, Germany) and an automatic biochemical analyzer Selectra Pro XL (ELITech Group, Puteaux, France) in accordance with the manufacturer's instructions. They included determination of leukocytes (WBC) by conductometric and visual optical method, erythrocytes (RBC) by conductometric method, haemoglobin (HGL) by cian-methaemoglobin method, haematocrit (HCT) by indirect based on conductometric analyses method, mean red blood cell count (MCV) by conductometric method, mean haemoglobin content in erythrocytes (MCH), mean haemoglobin concentration in erythrocytes (MCHC) and erythrocyte distribution width according to their volume (RWD) - by calculations, platelets (PLT) and mean platelets volume (MPV) - conductometric method following flotation of erythrocytes. The fat profile and glucose analyses were done by fully automated Olympus AU640 chemistry analyzer (International Equipment Trading Ltd., Mundelein, Illinois, USA) as follows: blood glucose content (GLU) using GOD/PAP; Hexokinase/G-6-PDH method, total cholesterol (T CHOL) by enzymatic colorimetric CHOD-PAP method, triglycerides (TRIG) by enzyme colorimetric-GPO-PAP method, LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) through direct method. Analytical procedures of blood tests were made following the Heatley and Russell (2020) recommendations.

Statistical analysis

Statistical analyses were performed using different software packages Microsoft Excel 5.0; JMP v. 7 for Windows (SAS, 2004). All data was tested for normal distribution. The effect of the supplements on the examined traits was assessed though one way ANOVA. Whenever significant effect existed the mains were compared through Fisher Least Significant Difference test ($P \leq 0.05$ and $P \leq 0.01$).

RESULTS AND DISCUSSION

Table 2 presents the data on the fattening capacity of the lambs and the feed consumption per 1 kg of growth. At the end of the experiment, the highest average live weight was achieved by lambs from experimental group D – 37.380 kg, in which the biologically active additive dihydroquercetin was applied. They are superior to their analogues from control group C by 1.040 kg and 1.490 kg from those from experimental group R, using DDPR. The results for the average daily gain and the total growth are again in favor of the lambs from experimental group D compared to those for control group C and experimental group R. Unfortunately, due to the large deviations of SEM in biological systems, the differences we found in terms of growth intensity were not statistically significant ($P>0.05$) at these levels of feed supplements used. The consumption of net energy (FUG) and crude protein (CP) per 1 kg of growth was equalized in lambs from control group C and experimental group R and slightly lower for their D analogues (Table 2).

One possible explanation of the determined effect of phytonutrients supplementation on lamb's growth performance and nutrient digestibility is the relationship between the ruminant nutrition and their health (De Brito *et al.* 2017). Acting as a strong antioxidant the dihydroquercetin probably possessed considerable protective activity from oxidative DNA damage (Fomichev *et al.* 2016). It is likely that the increased level of anabolic processes and body antioxidant protection reduced the morbidity and mortality in reared lambs. So, the dihydroquercetin supplemented lambs have increased vitality and longevity (Molyanova *et al.* 2019). Additionally, it can positively affect the activity of antioxidant enzymes, it may suppress the process of lipid peroxidation (Molyanova *et al.* 2019) and consequently raise the lamb's appetite. Therefore, the dihydroquercetin can be considered a natural immunostimulant. There is evi-

dence that low concentrations of dihydroquercetin as food supplement increase the immune status of gilthead seabream by stimulation of both cellular and humoral immune parameters (Awad *et al.* 2015).

The reduced level of blood glucose in experimental group D results in a weakened function of the adrenal cortex and in this way restricts a response to stress (Molyanova *et al.* 2019) and directly influences the nutrient digestibility and the lamb's growth performance. Similarly of us Fomichev *et al.* (2016) reported that 1-5 g dihydroquercetin from *Larix dahurica* Turcz/kg supplementation can be successfully used for increasing the lamb's productivity as an effective protection against the stress-factors.

Similar to our findings about feed intake per kg weight gain Pavlova (2017) has established the positive effect of the feed additive "Laricarvit" on fattening lambs from Romanov breed which contains: β-carotene - ≥ 1700 mg/kg, dihydroquercetin - ≥ 700 mg/kg, chlorophyll - ≥ 500 mg/kg, and silica as a filler - up to 1 kg. The addition of dihydroquercetin in the diets for feeding of heifers and the subsequent milk production from cows of Holstein Friesian cattle breed contributes to significantly higher growth rates too (Borisov, 2014).

The differences we found in terms of growth intensity are not statistically significant at these levels of supplements used. However, we can conclude that the use of the biologically active supplement dihydroquercetin has a positive effect on the fattening ability of lambs until such is established when using DDRP. Specific recommendations for practice require further research aimed at refining dosages and the possible inclusion of additional active substances in the fattening of lambs. It is quite possible that the effect of the applied additives will be more pronounced in the fattening of lambs weaned at a younger age and with a lower live weight, which also requires additional experiments.

The results (Table 3) show that although dihydroquercetin or DDRP supplementations had not significant (P>0.05) beneficial effect on slaughter weight, yield, and the weight of warm or chilled carcasses compared to the control group C. The proportion of internal fat in experimental group D was by 0.300% and 0.386% higher than in control group C and experimental group R (P≤0.01) respectively (Table 3). Similar tendencies were found comparing the yields of heads of experimental group D and the other two groups (control C and experimental R) (Table 3). It was determined the two studied phytonutrient supplementations in the conditions of the experiment were not effect (P>0.05) to the yield of the by-products and skin (Table 3) too.

The main economic criterion used for evaluation of the carcass quality is its slaughter weight. It affects other important parameters such as: Fat content and meat marbling, conformation of the carcasses and weight of different cuts (Szendrő *et al.* 2016). However, the fat content is an important factor connected with the price of the carcass. Some of the measurements for this criterion are the back fat thickness, the weight of fats around the kidney, the weight of the pelvic fat and the visual assessment of the carcass fat content (Zhang *et al.* 2019).

Our results are similar to those reported by Pavlova (2017) that the use of "Laricarvit" feed additive in fattening lambs increased the live weight by 5.06% and the carcass weight by 12.35%. The persistent differences in the values of some slaughter indicators found by us and Pavlova (2017) indicate the need for additional studies aimed at refining the dosage of used feed supplementation of dihydroquercetin and other biological active substances such as phytonutrients in lamb fattening. Similar results were presented by Balev *et al.* (2015) in an experiment with broiler chickens.

Another variable used as a general indicator of the carcass quality is its conformation. It includes a visual evaluation of the forms and profiles of the musculature together with the intramuscular and subcutaneous fat related to the size of the skeleton and the degree of fatness (representing the deposited subcutaneous fat related to the size of the carcasses) (Table 4). One potential effect of DHQ supplementation in broiler feed is contributed to weight gain due to intensive muscle growth (Fomichev *et al.* 2016). This observation was not confirmed in our lamb experiment. It was established that lamb's carcasses from control group C and experimental group R are equal in their carcass conformation (70% - class P 30% - class O). Compared to C and R in lambs supplemented with DHQ the 90% of the carcasses were classified in class P and 10% in class O. No significant differences (P>0.01) were found in the degree of fatness (Table 4) compared three groups of lambs. In conclusion, the addition of 7.5 mg dihydroquercetin/kg/day or 545 mg DDRP/kg/day to the daily ration of lambs does not affect significantly either the degree of fatness (P>0.01) or the conformation of the lamb carcasses.

Perhaps the concentrations of biologically active components used in the experiment are very low, considering the fermentation processes occurring in the rumen of ruminants independently of their positive effect on health (Thakur *et al.* 2020) for significant effect on carcass conformation. On the other hand, the experiment was conducted with 65-day old lambs from the synthetic population of a Bulgarian milk sheep breed which is probably the reason for the observed classification in the lower classes of carcass conformation. Finally, in future research it is recommended higher doses of dihydroquercetin or DDRP or combination of natural phytonutrients to be examined (Teodoro, 2019) and if it is possible the experiments to be held with lambs of breeds used for production of wool and meat.

Table 2 Average daily gain, feed intake per kg weight gain of studied groups of lambs

Parameter	Treatments			P-value
	Control (C) (0 mg phytonutrients/ kg/day)	Experimental (D) (7.5 mg dihydroquercetin/ kg/day)	Experimental (R) (545 mg dry distilled rose petals/kg/day)	
Initial live weight, kg	20.700±0.238	20.900±0.446	20.300±0.390	0.511
Average live weight on the end of experiment, kg	36.340±0.934	37.380±1.045	35.890±0.765	0.512
Average daily gains, kg/lamb/d	0.319±0.016	0.336±0.015	0.318±0.013	0.612
Total gain, kg	15.640±0.766	15.480±0.727	15.590±0.622	0.613
Feed units for growth, kg/kg	6.969	6.706	6.969	-
Crude protein, g/kg	1188.478	1151.796	1186.549	-

Control (C): lambs received a basal diet (granulated combined feed+ground alfalfa hay) only without addition of phytonutrients; Experimental group (D): fed with a basal diet with addition of 7.5 mg dihydroquercetin/kg/d; Experimental group (R) and fed with a basal diet with addition of 545 mg dry distilled rose petals/kg/d.
SEM: standard error of the means.

Table 3 Carcass characteristics of studied groups of lambs on the end of the experiment

Data were collected on	Treatments			P-value
26 March 2019	Control (C) (0 mg phytonutrients/ kg/day)	Experimental (D) (7.5 mg dihydroquercetin/ kg/day)	Experimental (R) (545 mg dry distilled rose petals/kg/day)	
Slaughter weight, kg	17.122±0.565	18.055±0.489	16.597±0.560	0.173
Weight of warm carcasses, kg	16.600±0.560	17.502±0.460	16.110±0.540	0.180
Weight of chilled carcasses, kg	16.275±0.530	16.955±0.440	15.655±0.530	0.205
Weight of internal fat, % of body weight	1.440±0.046^{b}	1.470±0.074	1.354±0.127^{b}	0.638
Carcass yield, % of pre-slaughter body weight	47.063±0.624	48.338±0.617	46.150±0.736	0.081
Yield of warm carcasses, % of pre-slaughter body weight	45.624±0.640	46.868±0.600	44.796±0.690	0.091
Yield of chilled carcasses, % of pre-slaughter body weight	44.745±0.590	45.399±0.520	43.530±0.690	0.104
Yield of heads, % of pre-slaughter body weight	3.309±0.122^{c}	3.284±0.074^{a}	3.361±0.035^{ac}	0.808
Yield of by-products, % of pre-slaughter body weight	10.145±0.142	9.927±0.189	9.690±0.178	0.189
Total slaughter yield, % of pre-slaughter body weight	60.517±0.671	61.548±0.659	59.200±0.675	0.061
Yield of skins, % of pre-slaughter body weight	10.782±0.299	11.256±0.294	11.835±0.383	0.094

Control (C): lambs received a basal diet (granulated combined feed+ground alfalfa hay) only without addition of phytonutrients; Experimental group (D): fed with a basal diet with addition of 7.5 mg dihydroquercetin/kg/d; Experimental group (R) and fed with a basal diet with addition of 545 mg dry distilled rose petals/kg/d.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Classification of the lamb carcasses

Classifications	Treatments		
	Control (C) (0 mg phytonutrients/ kg/day)	Experimental (D) (7.5 mg dihydroquercetin/ kg/day)	Experimental (R) (545 mg dry distilled rose petals/kg/day)
Class by conformation			
S	-	-	-
E	-	-	-
U	-	-	-
R	-	-	-
O	30%	10%	30%
P	70%	90%	70%
Class by degree of fatness			
1	-	-	-
2	-	-	-
3	30%	30%	20%
4	70%	70%	70%
5	-	-	10%

Control (C): lambs received a basal diet (granulated combined feed+ground alfalfa hay) only without addition of phytonutrients; Experimental group (D): fed with a basal diet with addition of 7.5 mg dihydroquercetin/kg/d; Experimental group (R) and fed with a basal diet with addition of 545 mg dry distilled rose petals/kg/d.

The 1st h and 24th h postmortem pH values in controls C and samples D were not significantly different (P>0.05). Compared to them, the 1st h and 24th h postmortem pH in samples R were with 0.14-0.15 pH units lower (P≤0.05). As a whole, the pH values measured in 1st h postmortem in every one of the three studied groups showed levels slightly lower than the neutral area (7:00). Those results (Table 5) were in good agreement with data about lamb's carcass conformation and degree of fatness (Table 4). Three of the factors that can have effect on the lamb's early postmortem pH such as age, ambient temperature and season were put at a constant level according to the used ANOVA single factor regression. The slaughter weight of the lamb carcasses (Table 3) was a resulting value for every one of the studied groups of lambs. That is why we speculate that sex is a factor responsible for the observed pH changes at 24th h postmortem.

An explanation of pH values phenomenon can be found in the specific composition (Schieber *et al.* 2005) and the physiological action of DDRP (Pal *et al.* 2018) as free radical scavengers. The results of the 24th h postmortem are interesting for discussion. The expected decrease in pH values to levels between 5.40-5.70 (McGeehin *et al.* 2001; Stahlke *et al.* 2019) was not observed. Those results demonstrate either that the postmortem rigor mortis process proceeded very quickly and to 24th h postmortem the pH values started to rise or vice versa-the glycogen content of the musculature was very low and the so-called DFD meat was detected. This assumption of ours is based on the results reported by McGeehin *et al.* (2001) namely that the pH decline in female lambs proceeds at a faster rate than in male lambs and the difference is 0.18 pH units. They speculated that this difference can be a result of fat content or physiological differences. McGeehin *et al.* (2001) concluded that the variations in the postmortem pH of the lamb have an inherent variable nature dependent on numerous factors which are not the subject of consideration in this study.

No significant differences (P>0.05) were found between the blood count indicators in the beginning, in the middle stage and at the end of the experiment in all three studied groups of lambs (Table 6). Exceptions to these findings were made by indicator HGL in the experimental group D whose levels were higher (P≤0.05) than in control group C and in experimental group R. These observations suggest that dihydroquercetin treatment have positive effect on erythropoiesis while the near-in structure quercetin induce anemia in rats (Riuz *et al.* 2005). At the end of the experiment the levels of blood GLU in the experimental group D were with 0.44 mmol/L lower (P≤0.05) compared to control group C and experimental group R (Table 6). A small increase (P≤0.05) at the levels of MCH and MCHC in the experimental group R has been found compared to the other two groups - D and C (Table 6). The determined increasing of RBC and HGL support the hypothesis that the addition of 7.5 mg dihydroquercetin to the lamb's combined feed stimulates hematopoiesis and thus improves haemoglobin synthesis and prolongs the erythrocyte life in the blood. This is probably due to the ability of the dihydroquercetin to contribute the reduction of the lipid peroxidation products in blood erythrocytes, reducing carbohydrate metabolism, and delaying the adrenal cortex function and a slower response to stress (Molyanova *et al.* 2019).

A small increase of the MCH, MCHC and PLT in the experimental group R are probably caused by antioxidant and antimicrobial properties of DDRP (Nowak *et al.* 2014). They are due to the specific composition of DDRP containing twenty-two major compounds including kaempferol and quercetin glycosides, quercetin 3-O-galactoside and quercetin 3-O-xyloside (Schieber *et al.* 2005) well correlated with their free radical scavenging potential (Pal *et al.* 2018).

Although the widely described reducing in blood lipid levels by preventing HMG-CoA reductase or influencing the apolipoprotein ratio (Fomichev *et al.* 2016; Sunil and Xu, 2019) in our research these observations have not been proven. These results again confirm the hypothesis that the effect of phytonutrients is highly species specific and depends on dose, metabolism, digestive system and many other factors.

Table 5 Lamb's m. longissimus thoracis et lumborum pH values on 45 min and 24 h post-mortem

Item	Treatments			P-value
	Control (C) (0 mg phytonutrients/ kg/day)	Experimental (D) (7.5 mg dihydroquercetin/ kg/day)	Experimental (R) (545 mg dry distilled rose petals/kg/day)	
pH_1 (45 min)	6.689±0.030	6.679±0.050	6.540±0.030^a	0.023
pH_2 (24 h)	6.553±0.041^b	6.569±0.035^b	6.494±0.035^a	0.016

Control (C): lambs received a basal diet (granulated combined feed+ground alfalfa hay) only without addition of phytonutrients; Experimental group (D): fed with a basal diet with addition of 7.5 mg dihydroquercetin/kg/d; Experimental group (R) and fed with a basal diet with addition of 545 mg dry distilled rose petals/kg/d.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Blood count of lambs in the beginning, in middle stage and at the end of the experiment

Indicator	Date of blood sampling	n	Treatments: Control (C) (0 mg phytonutrients/ kg/day)			Experimental (D) (7.5 mg dihydroquercetin/ kg/day)			Experimental (R) (545 mg dry distilled rose petals/kg/day)			P-value
			Mean	Variance	SEM	Mean	Variance	SEM	Mean	Variance	SEM	
Leukocytes (WBC) ($\times 10^9$/L)	At the beginning of the experiment on 5 February 2019	10	12.47	7.08	0.84	11.85	12.55	1.12	11.51	4.05	0.64	0.744
	In the middle of the experiment on 1 March 2019	10	11.80	1.59	0.40	10.92	5.04	0.71	11.50	6.73	0.82	0.648
	At the end of the experiment on 26 March 2019	10	11.16	2.99	0.55	11.32	5.73	0.76	11.34	10.32	1.02	0.984
	For whole experimental period	**30**	**11.81**	**3.91**	**0.36**	**11.36**	**7.38**	**0.50**	**11.45**	**6.55**	**0.47**	**0.759**
Erythrocytes (RBC) ($\times 10^{12}$/ L)	At the beginning of the experiment on 5 February 2019	10	6.59	2.98	0.55	6.92	4.66	0.68	5.67	4.66	0.68	0.304
	In the middle of the experiment on 1 March 2019	10	7.38	4.67	0.68	7.92	3.28	0.57	6.75	5.30	0.73	0.475
	At the end of the experiment on 26 March 2019	10	7.46	4.33	0.66	7.54	4.52	0.67	6.43	4.26	0.65	0.428
	For whole experimental period	**30**	**7.14**	**3.88**	**0.36**	**7.46**	**4.04**	**0.37**	**6.29**	**3.92**	**0.36**	**0.066**
Haemoglobin (HGB) (g/L)	At the beginning of the experiment on 5 February 2019	10	113.50	80.72	2.84	119.10	25.43	1.59	115.10	62.32	2.50	0.245
	In the middle of the experiment on 1 March 2019	10	112.60	47.82	2.19	119.70	34.01	1.84	108.20	68.84	2.62	0.004
	At the end of the experiment on 26 March 2019	10	112.70	74.90	2.74	118.10	36.54	1.91	110.10	165.21	4.06	0.184
	For whole experimental period	**30**	**112.93^{a}**	**63.31**	**1.45**	**118.97^{ac}**	**1.00**	**30.20**	**111.13^{c}**	**100.74**	**1.83**	**0.001**
Hematocrit blood test (HCT) (%)	At the beginning of the experiment on 5 February 2019	10	0.24	0.00	0.02	0.25	0.00	0.02	0.21	0.00	0.02	0.208
	In the middle of the experiment on 1 March 2019	10	0.26	0.00	0.02	0.28	0.00	0.02	0.24	0.01	0.02	0.339
	At the end of the experiment on 26 March 2019	10	0.26	0.00	0.02	0.27	0.00	0.02	0.23	0.00	0.02	0.299
	For whole experimental period	**30**	**0.26**	**0.00**	**0.01**	**0.27**	**0.00**	**0.01**	**0.23**	**0.00**	**0.01**	**0.021**
Mean red blood cell count (MCV)	At the beginning of the experiment on 5 February 2019	10	36.58	7.05	0.84	37.53	8.43	0.92	37.68	7.65	0.86	0.635
	In the middle of the experiment on 1 March 2019	10	36.26	6.92	0.83	35.87	6.76	0.82	36.28	9.76	0.99	0.940
	At the end of the experiment on 26 March 2019	10	35.86	6.09	0.78	36.52	6.56	0.81	36.46	10.84	1.04	0.844
	For whole experimental period	**30**	**36.22**	**6.31**	**0.46**	**36.64**	**7.23**	**0.49**	**36.80**	**9.17**	**0.55**	**0.703**
Mean haemoglobin content in erythrocytes (MCH)	At the beginning of the experiment on 5 February 2019	10	18.60	34.84	1.87	18.75	30.63	1.75	21.84	42.97	2.07	0.409
	In the middle of the experiment on 1 March 2019	10	16.88	39.60	1.99	15.92^{a}	16.63	1.29	18,60^{a}	74.74	2.73	0.660
	At the end of the experiment on 26 March 2019	10	16.27	23.25	1.52	16.73^{a}	18.32	1.35	19.07^{a}	58.46	2.42	0.517
	For whole experimental period	**30**	**17.25**	**31.33**	**1.02**	**17.13^{b}**	**21.82**	**0.85**	**19.84^{b}**	**56.79**	**1.38**	**0.154**
Mean haemoglobin concentration in erythrocytes (MCHC)	At the beginning of the experiment on 5 February 2019	10	502.40	18415.60	42.91	492.50	12818.10	35.80	575.40	23256.93	48.23	0.338
	In the middle of the experiment on 1 March 2019	10	458.00	19265.11	43.89	440.40	7323.38	27.10	499.30	36073.79	60.06	0.650
	At the end of the experiment on 26 March 2019	10	449.00	12079.78	34.76	453.20	7714.40	27.80	512.80	27513.29	52.45	0.456
	For whole experimental period	**30**	**469.80**	**16006.58**	**23.10**	**462.03^{b}**	**9153.27**	**17.50**	**529.17^{b}**	**28088.63**	**30.60**	**0.108**
Platelets (PLT) ($\times 10^9$/L)	At the beginning of the experiment on 5 February 2019	10	462.67	25019.75	52.73	633.10	17357.20	41.70	657.30	42497.79	65.19	0.039
	In the middle of the experiment on 1 March 2019	10	545.20	24710.40	49.71	599.50	33577.60	58.00	645.90	31244.54	55.90	0.438
	At the end of the experiment on 26 March 2019	10	560.60	25893.16	50.86	539.60	12892.90	35.90	588.40	37055.37	60.87	0.791
	For whole experimental period	**30**	**524.89**	**25261.17**	**29.51**	**590.73**	**21355.70**	**26.70**	**630.53**	**35326.12**	**34.32**	**0.051**

Continuation of Table 6 Blood count of lambs in the beginning, in middle stage and at the end of the experiment

Indicator	Date of blood sampling	n	Treatments: Control (C) (0 mg phytonutrients/ kg/day)			Experimental (D) (7.5 mg dihydroquercetin/ kg/day)			Experimental (R) (545 mg dry distilled rose petals/kg/day)			P-value
			Mean	Variance	SEM	Mean	Variance	SEM	Mean	Variance	SEM	
Red blood cell distribution width (RWD) (%)	At the beginning of the experiment on 5 February 2019	10	28.72	4.37	0.66	29.35	0.98	0.31	28.50	1.90	0.44	0.458
	In the middle of the experiment on 1 March 2019	10	28.91	3.46	0.59	30.41	3.07	0.55	29.19	2.92	0.54	0.144
	At the end of the experiment on 26 March 2019	10	28.73	5.24	0,72	29.17	3,89	0.62	28,65	2,21	0,47	0,814
	For whole experimental period	**30**	**28.79**	**4.06**	**0,37**	**29.64**	**2.78**	**0.30**	**28.76**	**2.26**	**0.27**	**0.088**
Glucose (GLU) (mmol/L)	At the beginning of the experiment on 5 February 2019	10	4.24	0.13	0.11	4.63	0.27	0.16	4.64	0.23	0.15	0.100
	In the middle of the experiment on 1 March 2019	10	4.32	0.21	0.14	4.29	0.51	0.23	4.70	0.24	0.16	0.217
	At the end of the experiment on 26 March 2019	10	3.91	0.48	0.22	3.47	0.19	0.14	3.90	0.26	0,16	0.149
	For whole experimental period	**30**	**4.16^{a}**	**0.28**	**0.10**	**4.13^{a}**	**0.54**	**0.13**	**4.41**	**0.36**	**0.11**	**0.158**
Total cholesterol (T CHOL) (mmol/L)	At the beginning of the experiment on 5 February 2019	10	1.15	0.05	0.07	1.00	0.08	0.09	1.06	0.08	0.09	0.456
	In the middle of the experiment on 1 March 2019	10	0.87	0.08	0.09	0.79	0.16	0.13	0.69	0.04	0.06	0.436
	At the end of the experiment on 26 March 2019	10	0.98^{c}	0.12	0.11	1.25^{c}	1.44	0.38	0.94^{c}	0.08	0.09	0.606
	For whole experimental period	**30**	**1.00**	**0.09**	**0.06**	**1.01^{c}**	**0.55**	**0.14**	**0.90^{c}**	**0.09**	**0.05**	**0.628**
High-density lipoprotein-cholesterol (HDL-C) (mmol/L)	At the beginning of the experiment on 5 February 2019	10	0.80	0.02	0.04	0.71	0.02	0.05	0.73	0.03	0.05	0.365
	In the middle of the experiment on 1 March 2019	10	0.67	0.03	0.06	0.62	0.06	0.08	0.54	0.02	0.04	0.354
	At the end of the experiment on 26 March 2019	10	0.72	0.05	0.07	0.69	0.05	0.07	0.69	0.03	0.06	0.943
	For whole experimental period	**30**	**0.73**	**0.04**	**0.03**	**0.67**	**0.04**	**0.04**	**0.65**	**0.03**	**0.03**	**0.296**
Triglycerides (TRIG) (mmol/L)	At the beginning of the experiment on 5 February 2019	10	0.18	0.01	0.03	0.15	0.00	0.02	0.17	0.01	0.03	0.650
	In the middle of the experiment on 1 March 2019	10	0.24	0.01	0.02	0.22	0.01	0.02	0.19	0.00	0.02	0.334
	At the end of the experiment on 26 March 2019	10	0.29	0.01	0.03	0.27	0.01	0.03	0.29	0.02	0.04	0.870
	For whole experimental period	**30**	**0.24**	**0.01**	**0.02**	**0.21**	**0.01**	**0.02**	**0.22**	**0.01**	**0.02**	**0.590**
Low-density lipoprotein (LDH) (U/L)	At the beginning of the experiment on 5 February 2019	10	613.98	12689.61	35.87	606.93	31460.35	56.09	576.52	18210.42	42.64	0.828
	In the middle of the experiment on 1 March 2019	10	623.21^{bc}	29139.81	53.98	554.34^{bc}	4124.62	20.31	798.48^{c}	317307.70	178.13	0.274
	At the end of the experiment on 26 March 2019	10	649.44^{b}	23284.25	48.25	669.35	69609.45	83.46	791.96^{b}	180569.73	134.38	0.528
	For whole experimental period	**30**	**628.88^{c}**	**20497.02**	**26.14**	**610.2^{c}**	**34932.83**	**34.12**	**722.32^{c}**	**171167.70**	**75.54**	**0.244**
Low-density lipoprotein-cholesterol (LDL-C) (mmol/l)	At the beginning of the experiment on 5 February 2019	10	0.29	0.01	0.03	0.22	0.01	0.03	0.28	0.01	0.03	0.152
	In the middle of the experiment on 1 March 2019	10	0.25	0.01	0.03	0.19	0.01	0.04	0.20	0.01	0.03	0.458
	At the end of the experiment on 26 March 2019	10	0.29	0.02	0.04	0.28	0.03	0.05	0.29	0.01	0.03	0.972
	For whole experimental period	**30**	**0.27**	**0.01**	**0.02**	**0.23**	**0.02**	**0.02**	**0.26**	**0.01**	**0.02**	**0.298**

Control (C): lambs received a basal diet (granulated combined feed+ground alfalfa hay) only without addition of phytonutrients; Experimental group (D): fed with a basal diet with addition of 7.5 mg dihydroquercetin/kg/d; Experimental group (R) and fed with a basal diet with addition of 545 mg dry distilled rose petals/kg/d.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).

CONCLUSION

The results showed that supplementation of lamb feed with 7.5 mg dihydroquercetin/kg/d or 545 mg DDRP/kg/d did not significantly affect on slaughter weight and carcass yield. DHQ supplementation increases the carcass quality. The supplementation of lamb feed with 7.5 mg dihydroquercetin/kg/d increased the haemoglobin concentration. The supplementation of lambs with 545 mg DDRP/kg/d contributed to reduction of the blood glucose. The other studied blood parameters are in physiological norms after DHQ and DDRP supplementation. Finally, the future research for supplemented dose of dihydroquercetin, DDRP or combination is recommended. The provided analysis will show is a positive effect on the quality and safety of the meat obtained exist.

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