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Original Article

Impact of Siberian larch dihydroquercetin or dry distilled rose petals as feed supplements on lamb's growth performance, carcass characteristics and blood count parameters

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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, carcasses quality and blood characteristics of lambs from the Bulgarian Dairy Synthetic population sheep. For the purpose of the study there were used 30 clinically healthy male lambs aged 65 days, levelled by live weight. They were housed in a totally indoor barn and were divided into one control and two experimental groups, each consisting of 10 animals that were 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 either with 7.5 mg dihydroquercetin/kg/day or with 545 mg dry distilled rose petals (DDRP)/kg/day respectively. The experimental group D had 5.45% and 8.78% higher slaughter weight comparing to the control group C and experimental group R, respectively. The carcass yield of lambs supplemented with dihydroquercetin was 1.28% and 2.19% higher compared to control group C or the lambs that had consumed DDRP. The carcass yield of lambs having consumed dihydroquercetin is higher by 1.28% and 2.19% respectively compared to lambs from control group C or those having consumed DDRP. The carcass conformation of C or R groups lambs do not differ (70% - class P, 30% - class O). The 90% of lamb's carcasses from the experimental group D were classified in class P. No significant differences were found in the fatness degree. The dihydroquercetin feeding increases the relative 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 erythrocytes (RBC) and haemoglobin (HGL) in the experimental group D which were higher ($p \leq 0.05$) than these in control group C and experimental group R. The conclusion made was that the use of the dihydroquercetin had a positive effect on the lamb's fattening, slaughter weight and carcass yield. Such an effect was not detected when DDRP was used.

KEYWORDS: Phytonutrients, diet supplementation, lambs, average daily gain, carcass quality, pH

Main text

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KEYWORDS: Phytonutrients, supplementation, ruminants, average daily gain, carcass quality, pH

1. 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 anti-microbial properties have been reported too (Westendarp et al. 2005). 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 (Helander et al. 1998). 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 (Orzechowski et al. 2002). 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 (Bessa et al. 2005; 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 (Mathews et al. 2000; Heber 2004). 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 (Jamilah et al. 2009; 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 flavouring properties of produced or processed meat. A number of approaches have been explored to increase the lamb growth performance and meat quality (Bessa et al. 2005; 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 (Lanza et al. 2001), 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 (Salama et al. 2015), 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 (Chumbalov et al. 1970) with strong antioxidant activity because of its ability to act as an electron donor and to inhibit hydroxyl radicals (Chen et al. 2002). There is information that dihydroquercetin manifests radioprotective, membraneprotective, capillaryprotective, angioprotective, lipidreducing, anti-inflammatory, antiallergic, 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 Siberian larch (*Larix sibirica*). Analysis of stereoisomeric composition demonstrated two bioflavonoids derived from Siberian larch (*Larix sibirica*) (Kolesnik et al. 2011). As a result of its healthy effects Fomichev et al. (2016) supposed that 1-5 g *Larix sibirica* dihydroquercetin powder (DHQ-1)/kg supplementation can be used for the realisation of a productive potential of lambs under an impact of stress-factors.

Another new potential beneficial phytonutrient is a by-product derived from rose oil production. There is evidence (Raymond et al. 2001) the Hybrid Perpetuals roses are rich in anthocyanin, quercetin, variation of flavonol glycosides and kaempferol derivatives.

A new source of bioactive compounds are dry distilled rose (*Rosa damascena* Mill) 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 dry distilled rose petals (DDRP) alter positively poultry meat composition (Balev et al. 2015) but in literature no information could be found on the use of dry distilled rose petals (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 dry distilled rose petals (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 Siberian larch dihydroquercetin and dry distilled rose petals (DDRP) on growth performance, blood characteristics and carcasses quality of lambs from the Bulgarian Dairy Synthetic population sheep.

2. Materials and methods

2.1. Lambs and diets

This experiment was conducted in accordance with Art. 14 of Part V. Breeding and Livestock Units from The European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Council Directive 2010/63/EC, Commission Recommendation 2007/526/EC, Council Regulation (EC) No 1099/2009 and the national legislation of the Republic of Bulgaria (Bulgarian Veterinary Medical Activity Act and Ordinance No 20 of 1 November 2012). 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. They were housed in a totally indoor barn in facilities at the Experimental Farm of 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 (see 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 on the same diet supplemented either with 7.5 mg dihydroquercetin/kg/day or 545 mg dry distilled rose petals (DDRP)/kg/day respectively. 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) and 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 (24h, 65°C) and ground to particle size < 0.4 mm.

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.

2.2. Sample preparation

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.
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).

2.3. Determination of total feed consumption and growth performance

The total feed consumption and growth performance were determined by weight measurements.

The feed conversion ratio (FCR) was calculated as a ratio between the average feed consumption (AFC) and the average daily weight gain (ADG) of the lambs. The FCR was calculated by the formula (1):

FCR = AFC/ADG (1), where:

AFC - average feed consumption, kg;
ADG - average daily weight gain, kg;

2.4. Characterisation and 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} (24h *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.

2.5. pH determination

The pH value of the samples at 45 min and 24 h in m. *Longissimus thoracis et lumborum* was measured electropotentiometrically (Korceala et al. 1986) 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) (Young et al. 2004).

2.6. 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 (2019) recommendations.

2.7. Statistical analysis

Statistical analyses were performed using different software packages Microsoft Excel 5.0; JMP, v.7 JMP v. 7 for Windows (SAS, Inc., Cary, NC, USA) (Leman et al. 2013). All data was tested for normal distribution. The statistical analysis was performed by ANOVA: Single Factor Regression. The Fisher F-test for significant differences ($p \leq 0.05$ or $p \leq 0.01$) was used.

3. Results and discussion

3.1. Effect of phytonutrients supplementation on lamb's growth performance and nutrient digestibility

At the end of the experiment lambs from the experimental group D supplemented by 7.5 mg dihydroquercetin/kg/d had the highest average body weight (Table 2). They were superior compared to their analogues from control group C by 2.86% and by 4.15% from the experimental group R (lambs consumed the granulated combined feed supplemented with DDRP). The results for the average daily weight gains were again in favor of the lambs from experimental group D - 0.336 kg/lamb/d compared to 0.319 kg/lamb/d in control group C and 0.318 kg/lamb/d in experimental group R which means by 5.33% more than control group C. In terms of average adopted feed experimental group D was inferior to control group C by 2.02% and by 2.49% to the compared experimental group R. The observed trends in the experimental group D are at the expense of the reduced by 5.33% consumption of granulated combined feed per 1 kg growth and increased by 4.98% consumed ground alfalfa hay. The results from the statistical analysis of the compared data in each row show no significant differences ($P > 0.05$)(Table 2).

One possible explanation is the relationship between the ruminant nutrition and their health (Bessa et al. 2005; De Brito et al. 2017). Being a well-known strong antioxidant the dihydroquercetin probably possessed considerable protective activity from oxidative DNA damage (Liang et al. 2013). 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 (experimental group D) 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 evidence 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 (Table 6) 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. According to Fomichev et al. (2016) 1-5 g *Larix sibirica* dihydroquercetin/kg supplementation can be successfully used for increasing the lamb's productivity as an effective protection against the stress-factors.

It was found that the feed conversion ratio in experimental group D was by 3.24% lower in comparison to the control samples C and by 3.09% lower in comparison to the experimental samples R.

Similarly, 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. According to Borisov (2014) the use 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.

3.2. Effect of phytonutrients supplementation on lamb's carcass characteristics

3.2.1. Slaughter weight and yield

The main 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 (Carter and Gallo 2008; Lambe et al. 2009). The results (Table 3) show that although dihydroquercetin supplementation (experimental group D) had a very weak beneficial effect on slaughter weight and yield compared to the other two groups (control C and experimental R). The slaughter weight and the weight of warm and chilled carcasses of experimental group D was 5.45%, 5.43% and 4.18% higher respectively compared to the control group C and 8.78%, 8.64% and 8.30% higher respectively compared to the experimental group R but it is not significantly different ($p > 0.05$). No effect ($p > 0.05$) of DDRP granulated combined feed supplementation (experimental group R) was found too.

However, the fat content is an important factor connected with the price of the carcass (Díaz et al. 2002). 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 (Díaz et al. 2002; Carrasco et al. 2009). 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 ($p \leq 0.01$) respectively (Table 3).

Similar tendencies were found comparing the yields of experimental group D and the other two groups (control C and experimental R) (Table 3). The carcass yield of lambs having consumed finisher combined feed with dihydroquercetin supplementation was 1.28% and 2.19% higher compared to lambs that had consumed finisher combined feed without phytonutrients or those that had used DDRP supplementation respectively. Analogous results were obtained with respect to: yield of warm carcasses which in experimental group D was higher by 1.24% and 1.89%; yield of chilled carcasses which in experimental group D was higher by 0.65% and 1.87%; and the total slaughter yield in experimental group D which was higher by 1.03% and 2.35% compared to control group C and experimental group R respectively. Simultaneously with the higher carcass yield of D lambs the yield of heads and by-products was found to be slightly lower (Table 3). Unfortunately, those results were also not significantly different ($p > 0.05$).

The two studied phytonutrient supplementations were not effect to the yield of the by-products (Table 3). 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.

3.2.2. Lamb's carcasses classification

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 carcases) (Table 4). It was established that lamb's carcasses from control group C and experimental group R do not differ in their carcass conformation (70% - class P 30% - class O). In the experimental group D 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).

Although the differences between the three groups are in most cases statistically insignificant it can be summarized that the use of the biologically active supplementation of 7.5 mg dihydroquercetin has a slightly pronounced negative effect on the lambs' fattening capacity while DDRP supplementation had no impact. The addition of 7.5 mg dihydroquercetin/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 unlike the addition of 545 mg DDRP/kg/day.

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 (Mathews et al. 2000; Heber 2004). 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.

3.2.3. pH values and meat quality

The 1^{st} h and 24^{th} h postmortem pH values in controls C and samples D were not significantly different ($p > 0.05$). Compared to them, the 1^{st} h and 24^{th} h postmortem pH in samples R were with 0.14 - 0.15 pH units lower ($p \leq 0.05$). An explanation of this 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.

As a whole, the pH values measured in 1^{st} h postmortem in every one of the three studied groups showed levels slightly lower than the neutral area (7.00) which is normal for warm meat. The results of the 24^{th} 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 24^{th} 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. 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. 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. Finally 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.

3.3. Effect of supplemented phytonutrients on lamb's blood count

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/in indicators such as erythrocytes (RBC) and haemoglobin (HGL) in the experimental group D whose levels were higher ($p \leq 0.05$) than in control group C and in experimental group R. The determined results support the hypothesis that the addition of 7.5 mg dihydroquercetin to the lamb's combined feed stimulates haematopoiesis and thus improves haemoglobin synthesis and prolongs the erythrocyte life in the blood. This is probably due to the ability of the dihydroquercetin to exhibit antioxidant properties and it contributes to the reduction of the concentration of lipid peroxidation products in blood erythrocytes (Molyanova et al. 2019).

At the end of the experiment the levels of blood glucose (GLU) in the experimental group D were with 0.44 mmol/L lower ($p \leq 0.05$) compared to control group C and experimental group R (Table 6). This indicates a reduced carbohydrate metabolism, a delayed adrenal cortex function and a slower response to stress (Molyanova et al. 2019).

A small increase has been found($p \leq 0.05$) at the levels of the mean haemoglobin content in erythrocytes (MCH), the mean haemoglobin concentration in erythrocytes (MCHC) and platelets (PLT) in the experimental group R compared to the other two groups - C and D (Table 6). These changes are probably caused by antioxidant and antimicrobial properties of dry distilled rose (*Rosa damascena* Mill) petals (DDRP) (Nowak et al. 2014). They are due to the specific composition of dry distilled rose petals 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).

4. Conclusions

The use of the dihydroquercetin as a biologically active additive has a positive effect on the lamb's fattening, slaughter weight and carcass yield. Such effect is not established when dry distilled rose petals were used. Dihydroquercetin increases the relative fat content ($P \leq 0.01$) but adversely affects the conformation of the carcass. Further research is needed in the future with higher concentrations of added phytonutrients to provide clarity on the issues studied.

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Disclosure statement

No potential conflict of interest was reported by the authors. That is way the authors declare no conflict of interests in this research.

References

1. Abdu SB, Ehoche OW, Adamu AM, Bawa GS, Hassan MR, Yashim SM, Adamu HY. 2012. Effect of varying levels of Zizyphus (*Zizyphus mauritiana*) leaf meal inclusion in concentrate diet on performance of growing Yankasa ram lambs fed maize stover basal diet. Iran. J. Appl. Anim. Sci., 2: 323-330. Available at http://ijas.iaurasht.ac.ir/article_514282.html [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

2. Artem'eva OA, Pereselkova DA, Fomichev YP. 2015. Dihydroquercetin, the bioactive substance, to be used against pathogenic microorganisms as an alternative to antibiotics. Sel'skokhozyaistvennaya Biologiya [Agric. Biol.], 50: 513-519. http://www.agrobiology.ru/articles/4-2015artemeva.pdf [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

3. Asadollahi S, Sari M, Erafanimajd N, Kiani A, Ponnampalam EN. 2017. Supplementation of sugar beet pulp and roasted canola seed in a concentrate diet altered carcass traits, muscle (longissimus dorsi) composition and meat sensory properties of Arabian fattening lambs. Small Rum. Res., 153: 95-102. https://doi.org/10.1016/j.smallrumres.2017.05.012 [Crossref], [Scopus®], [Google Scholar], [Web of Science®]

4. Awad E, Awaad AS, Esteban MA. 2015. Effects of dihydroquercetin obtained from deodar (*Cedrus deodara*) on immune status of gilthead seabream (*Sparus aurata* L.). Fish Shellfish Immunol., 43: 43-50. https://doi.org/10.1016/j.fsi.2014.12.009 [Crossref], [Scopus®], [Google Scholar], [Web of Science®]

5. Baldi G, Chauhan SS, Linden N, Dunshea FR, Hopkins DL, Sgoifo Rossi CA, Dell'Orto V, Ponnampalam EN. 2019. Comparison of a grain-based diet supplemented with synthetic vitamin E versus a lucerne (alfalfa) hay-based diet fed to lambs in terms of carcass traits, muscle vitamin E, fatty acid content, lipid oxidation, and retail colour of meat. Meat Sci., 148: 105-112. https://doi.org/10.1016/j.meatsci.2018.10.013 [Crossref], [PubMed], [Web of Science®], [Google Scholar], [Scopus®]

6. Balev D, Vlahova-Vangelova D, Mihalev K, Shikov V, Dragoev S, Nikolov V. 2015. Application of natural dietary antioxidants in broiler feeds. J. Mt. Agric. Balk., 18: 224-232. http://rimsa.eu/images/stockbreeding_18-2_2015.pdf [Google Scholar]

7. Bessa RJB, Portugal PV, Mendes IA, Santos-Silva J. 2005. Effect of lipid supplementation on growth performance, carcass and meat quality and fatty acid composition of intramuscular lipids of lambs fed dehydrated lucerne or concentrate. Livest. Prod. Sci., 96: 185-194. https://doi.org/10.1016/j.livprodsci.2005.01.017 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

8. Borisov AY. 2014. Влияние дигидрокверцетина и ионола на рост, развитие ремонтных телок и последующую молочную продуктивность коров-первотелок черно-пестрой породы. [The effect of dihydroquercetin and ionol on the growth, development of repair heifers and the subsequent milk production of cow after first calving from breed Holstein Friesian cattle]. Higher Attestation Commission of the Russian Federation 06.02.10, PhD Thesis, Samara State Agricultural Academy, Izhevsk, p. 126. [in Russian] [Google Scholar]

9. Bulgarian Veterinary Medical Activity Act, in force since 02 May 2006. Published in the State Gazette, issue 87 of November 1, 2005, amended in the State Gazette, issue 30 of April 11, 2006, amended in the State Gazette, issue 31 of April 14, 2006, amended in the State Gazette, issue 55 of 7 July 2006, amended in the State Gazette, issue 88 of 31 October 2006, amended in the State Gazette, issue 51 of 26 June 2007, amended in the State Gazette, issue 84 of 19 October 2007, amended in the State Gazette, issue 13 of February 8, 2008, amended in the State Gazette, issue 36 of April 4, 2008, amended in the State Gazette, issue 100 of November 21, 2008, amended in the State Gazette, issue 27 of April 10, 2009, amended in the State Gazette, issue 35 of May 12, 2009, amended in the State Gazette, issue 74 of September 15, 2009, amended in the State Gazette, issue 95 of December 1, 2009, amended in the State Gazette, issue 102 of December 22, 2009, amended in the State Gazette, issue 25 of March 30, 2010, amended in the State Gazette, issue 41 of June 1, 2010, amended in the State Gazette, issue 8 of January 25, 2011, amended in the State Gazette, issue 92 of November 22, 2011, amended in the State Gazette, issue 77 of October 9, 2012, amended and supplemented in the State Gazette, issue 82 of October 26, 2012, amended in the State Gazette, issue 97 of December 7, 2012, amended in the State Gazette, issue and supplement. DV. Issue 7 of January 25, 2013, amended in the State Gazette, issue 15 of February 15, 2013, amended in the State Gazette, issue and supplement. DV. No. 66 of July 26, 2013, amended in the State Gazette, issue 68 of August 2, 2013, amended and supplemented in the State Gazette, issue 83 of September 24, 2013, amended and supplemented in the State Gazette, issue99 of 15 November 2013, amended in the State Gazette, issue 98 of November 28, 2014, amended in the State Gazette, issue 14 of February 20, 2015, amended and supplemented in the State Gazette, issue14 of February 19, 2016, amended in the State Gazette, issue 34 of May 3, 2016, amended in the State Gazette, issue 58 of July 26, 2016, amended in the State Gazette, issue 58 of July 18, 2017, amended in the State Gazette, issue 85 of October 24, 2016 2017, amended and supplemented in the State Gazette, issue 17 of February 23, 2018, amended in the State Gazette, issue 98 of November 27, 2018, amended in the State Gazette, issue 24 of March 22, 2019. [in Bulgarian][Google Scholar]

10. Carrasco S, Ripoll G, Sanz A, Álvarez-Rodríguez J, Panea B, Revilla R, Joy M. 2009. Effect of feeding system on growth and carcass characteristics of *Churra tensina* light lambs. Livestock Sci., 121: 56-63. https://doi.org/10.1016/j.livsci.2008.05.017 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

11. Carter L, Gallo C. 2008. Efectos del transporte exencionado por vía terrestre y cruce marítimo en transbordador sobre pérdidas de peso vivo y características de la canal en corderos. [Impact of transport extended by land and sea with ferry on the loss of live weight and carcass characteristics in lambs.] Arch. Med. Vet., 40: 259-266. [In Spanish] https://doi.org/10.4067/S0301-732X2008000300006 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

12. Chen JW, Zhu ZQ, Hu TX, Zhu DY. 2002. Structure-activity relationship of natural flavonoids in hydroxyl radical-scavenging effects. Acta Pharmacol. Sin., 23: 667-672. http://www.chinaphar.com/article/view/7716/8168 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

13. Chikwanha OC, Moelich E, Gouws P, Muchenje V, Nolte JV, Dugan MER, Mapiye C. 2019. Effects of feeding increasing levels of grape (*Vitis vinifera* cv. *Pinotage*) pomace on lamb shelf-life and eating quality. Meat Sci., 157: 107887. https://doi.org/10.1016/j.meatsci.2019.107887 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

14. Chumbalov TK, Pashinina LT, Leiman ZA. 1970. Flavonoids of the bark of Larix sibirica. Chem. Natur. Comp., 6: 776-776. https://doi.org/10.1007/BF00565363 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

15. Cruywagen CW, van Zylb WH. 2008. Effects of a fungal enzyme cocktail treatment of high and low forage diets on lamb growth. Anim. Feed Sci. Technol., 145: 151-158. https://doi.org/10.1016/j.anifeedsci.2007.05.038 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

16. Commission Recommendation 2007/526/EC of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (notified under document number C(2007) 2525). Official Journal of the European Union, L 197: 1-89. [Google Scholar]

17. Commission Regulation (EC) No 1249/2008 of 10 December 2008 laying down detailed rules on the implementation of the Community scales for the classification of beef, pig and sheep carcases and the reporting of prices thereof. Official Journal of the European Union, L 337: 3-30. [Google Scholar]

18. Council Directive 2010/63/EC of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union, L 276: 33-79. [Google Scholar]

19. Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. Official Journal of the European Union L 303: 1-30. [Google Scholar]

20. De Brito FG, Ponnampalam NE, Hopkins LD. 2017. The effect of extensive feeding systems on growth rate, carcass traits, and meat quality of finishing lambs. Comp. Rev. Food Sci. Safety, 16: 23-38. https://doi.org/10.1111/1541-4337.12230 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

21. Díaz M, Velasco S, Cañeque V, Lauzurica S, Ruiz de Huidobro F, Pérez C, González J, Manzanares C. 2002. Use of concentrate or pasture for fattening lambs and its effect on carcass and meat quality. Small Rum. Res., 43: 257-268. https://doi.org/10.1016/S0921-4488(02)00016-0 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

22. El-Sabaawy EH, Gad SM, El- Bedawy TM, Ali HM, Abd El-Gawad AM. 2015. Growth performance and conjugated linoleic acid (CLA) content on meat of growing lambs fed diets containing vegetable oils. Adv. Environ. Biol., 9: p. 8+. Gale Academic Onefile, Accessed 20 Jan. 2020. Available at https://go.gale.com/ps/anonymous?id=GALE%7CA440716303&sid=googleScholar&v=2.1&it=r&linkaccess=abs&issn=19950756&p=AONE&sw=w [Web of Science®], [Scopus®], [Crossref], [Google Scholar]

23. Fomichev Y, Nikanova L, Lashin A. 2016. The effectiveness of using dihydroquercetin (taxifolin) in animal husbandry, poultry and apiculture for prevention of metabolic disorders, higher antioxidative capacity, better resistance and realisation of a productive potential of organism. Agric. Food., 4: 140-159. http://en.ametis.ru/files/ametis/files/doklad-bolgariyaporosyata-28.02.16_fomichev_yu.p.pdf. [Google Scholar]

24. Heber D. 2004. Vegetables, fruits and phytoestrogens in the prevention of diseases. J. Postgrad. Med., 50:145-149. Available from: http://www.jpgmonline.com/text.asp?2004/50/2/145/8259 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

25. Helander IM, Alakomi HL, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris LG, von Wright A. 1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. J. Agric. Food Chem., 46: 3590-3595. https://doi.org/10.1021/jf980154m [Crossref], [PubMed], [Web of Science®], [Google Scholar]

26. Hassan YI, Lahaye L, Gong MM, Peng J, Gong J, Liu S, Gay CG, Yang Ch. 2018. Innovative drugs, chemicals, and enzymes within the animal production chain. Vet. Res., 49: 1-17. https://doi.org/10.1186/s13567-018-0559-1 [Crossref], [PubMed], [Web of Science®], [Google Scholar], [Scopus®]

27. Holman BWB, Baldi G, Chauhan SS, Hopkins DL, Seymour GR, Dunshea FR, Collins D, Ponnampalam EN. 2019. Comparison of grain-based diet supplemented with synthetic vitamin E and lucerne hay-based diet on blood oxidative stress biomarkers and lamb meat quality. Small Rum. Res., 177: 146-152. https://doi.org/10.1016/j.smallrumres.2019.05.016 [Crossref], [PubMed], [Web of Science®], [Google Scholar], [Scopus®]

28. Jamilah B, Mohamed A, Abbas K, Abdul RR, Karim R. 2009. A review on the effect of animal diets and presence of selected natural antioxidants on lipid oxidation of meat. J. Food Agric. Environ., 7, 76-81. https://doi.org/10.1234/4.2009.1542 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

29. Kolesnik Y, Titova E, Chertkov V, Tashlitsky V, Tikhonov V, Shmatkov D. 2011. Stereoisomeric composition of two bioflavonoids from *Larix sibirica*. Planta Med., 77: PA17. https://doi.org/10.1055/s-0031-1282213 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

30. Lambe N, Navajas E, Bünger L, Fisher A, Roehe R, Simm G. 2009. Prediction of lamb carcass composition and meat quality using combinations of postmortem measurements. Meat Sci., 81: 711-719. https://doi.org/10.1016/j.meatsci.2008.10.025 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

31. Lanza M, Priolo A, Biondi L, Bella M, Salem HB. 2001. Replacement of cereal grains by orange pulp and carob pulp in faba bean-based diets fed to lambs: effects on growth performance and meat quality. Anim. Res., 50: 21-30. https://doi.org/10.1051/animres:2001101 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

32. Liang L, Gao Ch, Luo M, Wang W, Zhao Ch, Zu Y, Efferth Th, Fu Y. 2013. Dihydroquercetin (DHQ) induced HO-1 and NQO1 expression against oxidative stress through the Nrf2-dependent antioxidant pathway. J. Agric. Food Chem., 61: 2755-2761. https://doi.org/10.1021/jf304768p [Web of Science®], [Scopus®], [Crossref], [Google Scholar]

33. Matthews KR, Homer DB, Thies F, Calder PC. 2000. Effect of whole linseed (*Linum usitatissimum*) in the diet of finishing pigs on growth performance and on the quality and fatty acid composition of various tissues. Br. J. Nutr., 83: 637-643. https://doi.org/10.1017/s0007114500000817 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

34. McCoard SA, Sales FA, Sciascia QL. 2017. Invited review: impact of specific nutrient interventions during mid-to-late gestation on physiological traits important for survival of multiple-born lambs.

Animal. 11: 1727-1736. https://doi.org/10.1017/S1751731117000313 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

35. McGeehin B, Sheridan JJ, Butler F. 2001. Factors affecting the pH decline in lamb after slaughter. Meat Sci., 58: 79-84. https://doi.org/10.1016/S0309-1740(00)00134-0[Web of Science®], [Scopus®], [Crossref], [Google Scholar]

36. Miltko R, Majewska M, Bełżecki G, Kula K, Kowalik B. 2019. Growth performance, carcass and meat quality of lambs supplemented different vegetable oils. Asian-Australas J Anim. Sci., 32, 767-775. https://doi.org/10.5713/ ajas.18.0482 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

37. Mohamed JB, Abbas A, Abdul Rahman KA, Karim R. 2009. A review on the effect of animal diets and presence of selected natural antioxidants on lipid oxidation of meat. J. Food Agric. Environ., 7: 76-81. [Crossref], [Agris], [Google Scholar], [Scopus®]

38. Molyanova G., Ermakov V., Bistrova I. (2019). Correcting physiological and biochemical status of service dogs with dihydroquercetin. XII International Scientific Conference on Agricultural Machinery Industry. IOP Conf. Series: Earth and Environmental Science 403, 012042: 1-5. https://doi.org/10.1088/1755-1315/403/1/012042 [Google Scholar]

39. Nair MPN, Kandaswami C, Mahajan S, Chadha KC, Chawda R, Nair H, Kumar N, Nair RE, Schwartz SA. 2002. The flavonoid, quercetin, differentially regulates Th-1 (IFNγ) and Th-2 (IL4) cytokine gene expression by normal peripheral blood mononuclear cells. Biochem. Biophys. Acta, 1593: 29-36. https://core.ac.uk/download/pdf/82568694.pdf [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

40. Nowak R, Olech M, Pecio Ł, Oleszek W. 2014. Cytotoxic, antioxidant, antimicrobial properties and chemical composition of rose petals. J. Sci. Food Agric., 94: 560-567. https://doi.org/10.1002/jsfa.6294 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

41. Oh J., Wall EH, Bravo DM, Hristov AN. 2016. 1036 Phytonutrients as additives in ruminants: the unexpected target organ. J. Anim. Sci., 94: 496-497. https://doi.org/10.2527/jam2016-1036 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

42. Ordinance No. 15 of 8 May 2009 on the grading and classification of carcasses of bovine, porcine and ovine animals on the Community scale EUROP, reporting of findings and verification in slaughterhouses. Published in the State Gazette, issue 37 of 19 May 2009, amended in the State Gazette, issue 48 of 25 June 2010, amended and supplemented in the State Gazette, issue 23 of March 14, 2014, amended in the State Gazette, issue 16 of 22 February 2019. [in Bulgarian][Google Scholar]

43. Ordinance No 20 of 1 November 2012 on the minimal requirements for protection and human attitude to the experimental animals and the requirements for the establishments of use, farming and/or their delivery. Published in the State Gazette, issue 87 of 9 November 2012, amended and supplemented in the State Gazette, issue 86 of 17 October 2014, amended in the State Gazette, issue 9 of 2 February 2016. [in Bulgarian][Google Scholar]

44. Orzechowski A, Ostaszewski P, Jank M, Berwid SJ. 2002. Bioactive substances of plant origin in food-impact on genomics. Repr. Nutr. Dev. 42: 461-477. https://doi.org/10.1051/rnd:2002037 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

45. Pal A, Bhushan B, Narwal RK, Saharan V. 2018. Extraction and evaluation of antioxidant and free radical scavenging potential correlated with biochemical components of red rose petals. Ir. J. Sci.

Technol., Transac. A: Sci., 42: 1027–1036. https://doi.org/10.1007/s40995-016-0071-2 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

46. Pavlova MV. 2017. Гигиена выращивания ягнят с применением кормовых добавок Ларикарвит и Бацелл. [Hygiene of growing lambs with the use of feed additives Laricarvit and Bacell]. Higher Attestation Commission of the Russian Federation 06.02.05, PhD Thesis, Chuvash State Agricultural Academy, Cheboksary, p. 155. [in Russian] [Google Scholar]

47. Popova T. 2007. Effect of the rearing system on the fatty acid composition and oxidative stability in m. longissimus dorsi and m. semimembranosus in lambs. Small Rum. Res., 71: 150-157. https://doi.org/10.1016/j.smallrumres.2006.06.001 [Crossref], [PubMed], [Web of Science®], [Google Scholar], [Scopus®]

48. Raymond O, Fiasson J-L, Jay M. 2001. The domestication syndrome within Hybrid Perpetuals roses: the effect of unconscious selection on flavonoids. Biochem. Syst. Ecol. 29, 513-529. https://doi.org/10.1016/S0305-1978(00)00085-5 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

49. Sabino M, Carmelo VAO, Mazzoni G, Cappelli K, Capomaccio S, Ajmone-Marsan P, Verini-Supplizi A, Trabalza-Marinucci M, Kadarmideen HN. 2018. Gene co-expression networks in liver and muscle transcriptome reveal sex-specific gene expression in lambs fed with a mix of essential oils. BMC Genom., 19: 23601-23615. https://doi.org/10.1186/s12864-018-4632-y [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

50. Salama R, Boraei MA, Fouda SM, El-Sysy MAI. 2015. Effect of vitamin E supplementation on the productive performance of Rahmani sheep. Egypt. J. Nutr. Feeds, 18: 65-75. [Google Scholar]

51. Schieber A, Mihalev K, Berardini N, Mollov P, Carlea R. 2005. Flavonol glycosides from distilled petals of Rosa damascena Mill. Zeitschr. Naturforsch. C – J. Biosci., 60: 379-384. https://doi.org/10.1515/znc-2005-5-602 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

52. Stahlke ER, Rossa LS, Silva GM, Sotomaior CS, Pereira AJ,Luciano FB,Borges TD, de Macedo REF. 2019. Effects of modified atmosphere packaging (MAP) and slaughter age on the shelf life of lamb meat. Food Sci. Technol. Campinas, 39: 328-335. https://doi.org/10.1590/fst.29617 [Crossref], [Web of Science®], [Google Scholar], [Scopus®]

53. Teodoro AJ. 2019. Bioactive compounds of food: Their role in the prevention and treatment of diseases oxidative. Med. Cell. Long. Volume 2019: 3765986, 4 pages. https://doi.org/10.1155/2019/3765986 [Crossref], [Google Scholar], [Web of Science®], [Scopus®]

54. Westendarp H. 2005. Essential oils for the nutrition of poultry, swine and ruminants. DTW. Deut. Tierarztl. Woch., 112: 375-380. [in German] [PubMed], [Google Scholar]

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
Barley	-
Peas	100.00
Lucerne meal	50.00
Corn germ	200.00
Soybean meal 43%	-
Chalk	34.26
Salt	6.00
Vitamin-mineral premix ME+B OB-E	2.00
Sodium bicarbonate ($NaHCO_3$)	-
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 D3, UI/kg	2000
Vitamin E, mg/kg	100.00

Legend:

[a] The quantities of Siberian larch dihydroquercetin and dry distilled rose petals added as supplements to the diets were calculated as 7.5 mg dihydroquercetin/kg live weight per day (D) or 0.545 g dry distilled rose petals/kg live weight per day (R).

Table 2. Comparisons of growth performance and nutrient digestibility of studied groups of lambs

	Treatments			P-value
	Control (C) (0 mg phytonutrients/ kg/day) Mean ± SEM	Experimental (D) (7.5 mg dihydroquercetin/ kg/day) Mean ± SEM	Experimental (R) (545 mg dry distilled rose petals/kg/day) Mean ± SEM	
Initial live weight, kg	20.700 ± 0.238	20.900 ±0.446	20.300 ± 0.390	0.511
Average live weight of lambs on the end of the experiment, kg	36.340 ± 0.934	37.380 ± 1.045	35.890 ± 0.765	0.512
Total live weight gain of one lamb for the experimental period, kg	15.640 ± 0.766	16.480 ± 0.728	15.590 ± 0.622	0.613
Average daily gains of the lambs for the experimental period, kg/lamb/d	0.319 ± 0.016	0.336 ± 0.015	0.318 ± 0.013	0.612
Average adopted feed for the experimental period, kg/lamb/d	2.182 ± 0.044	2.226 ± 0.053	2.172 ± 0.057	0.029
Consumed granulated combined feed for the experimental period, kg	62.800 ± 0.027	62.800 ± 0.032	62.800 ± 0.072	0.013
Consumption of granulated combined feed per 1 kg growth	4.015 ± 0.156	3.811 ± 0.141	4.028 ± 0.163	0.054
Consumed ground alfalfa hay for the experimental period , kg	44.200 ± 0.528	46.400 ± 0.397	43.700 ± 0.473	0.461
Consumption of ground alfalfa hay per 1 kg growth	2.826 ± 0.111	2.816 ± 0.123	2.803 ± 0.130	0.038
Feed conversion ratio (FCR)	6.841 ± 0.372	6.626 ± 0.311	6.831 ± 0.355	0.193

Legend:

[a] Control group (C) lambs received a basal diet (granulated combined feed + ground alfalfa hay) only without addition of phytonutrients.

[b] Experimental group (D) fed with a basal diet with addition of 7.5 mg dihydroquercetin/kg/d;

[c] Experimental group (R) fed with a basal diet with addition of 0.545 g dry distilled rose petals/kg/d;

[d] Results are presented as Means ± Standard error of the means (SEM);

[f a- b] Means with different superscripts in each row differ significantly ($p \leq 0.05$).

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

Data were collected on 26 March 2019	Treatments			P-value
	Control (C) (0 mg phytonutrients/ kg/day) Mean ± SEM	Experimental (D) (7.5 mg dihydroquercetin/ kg/day) Mean ± SEM	Experimental (R) (545 mg dry distilled rose petals/kg/day) Mean ± SEM	
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[b] ± 0.046	1.470 ± 0.074	1.354[b] ± 0.127	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[c] ± 0.122	3.284[a] ± 0.074	3.361[ac] ± 0.035	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

Legend:

[a] Control group (C) lambs received a basal diet (granulated combined feed + ground alfalfa hay) only without addition of phytonutrients.

[b] Experimental group (D) fed with a basal diet with addition of 7.5 mg dihydroquercetin/kg/d;

[c] Experimental group (R) fed with a basal diet with addition of 0.545 g dry distilled rose petals/kg/d;

[d] Results are presented as Means ± Standard error of the means (SEM);

[f a- b] Means with different superscripts in each row differ significantly ($p \leq 0.05$).

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%

Legend:

[a] Control group (C) lambs received a basal diet (granulated combined feed + ground alfalfa hay) only without addition of phytonutrients.

[b] Experimental group (D) fed with a basal diet with addition of 7.5 mg dihydroquercetin/kg/d;

[c] Experimental group (R) fed with a basal diet with addition of 0.545 g dry distilled rose petals/kg/d;

[d] Results are presented as %;

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

	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)	
	Mean ± SEM	Mean ± SEM	Mean ± SEM	
pH_1 (45 min)	6.689 ± 0.030	6.679 ± 0.050	6.540[a] ± 0.030	0.023
pH_2 (24 h)	6.553[b] ± 0.041	6.569[b] ± 0.035	6.494[a] ± 0.035	0.016

Legend:

[a] Control group (C) lambs received a basal diet (granulated combined feed + ground alfalfa hay) only without addition of phytonutrients.

[b] Experimental group (D) fed with a basal diet with addition of 7.5 mg dihydroquercetin/kg/d;

[c] Experimental group (R) fed with a basal diet with addition of 0.545 g dry distilled rose petals/kg/d;

[d] Results are presented as Means ± Standard error of the means (SEM);

[f a- b] Means with different superscripts in each row differ significantly ($p \leq 0.05$).

Table 6. Blood count of lambs in the beginning, in middle stage and at the end of the experiment (5 February 2019, 1 March 2019 and 26 March 2019)

Indicator	Date of blood sampling	n	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)			
			Mean	Variance	SEM	Mean	Variance	SEM	Mean	Variance	SEM	
WBC leukocytes (x10^9/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**
RBC erythrocytes (x10^{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**
HGB haemoglobin (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**
HCT Hematocrit blood test (the number of red blood cells) (%)	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**
MCV mean red blood cell count	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**
MCH mean haemoglobin content in erythrocytes	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**
MCHC mean haemoglobin concentration in erythrocytes	At the beginning of the experiment on 5 February 2019	10	502,40	18415,60	42,91	492,50	12818,1	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	27,1	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	27,8	512,80	27513,29	52,45	0,456
	For whole experimental period	**30**	**469,80**	**16006,58**	**23,10**	**462,03[b]**	**9153,27**	**17,5**	**529,17[b]**	**28088,63**	**30,60**	**0,108**
PLT platelets (x10^9/L)	At the beginning of the experiment on 5 February 2019	10	462,67	25019,75	52,73	633,1	17357,2	41,7	657,3	42497,79	65,19	0,039
	In the middle of the experiment on 1 March 2019	10	545,20	24710,40	49,71	599,5	33577,6	58	645,9	31244,54	55,90	0,438
	At the end of the experiment on 26 March 2019	10	560,60	25893,16	50,86	539,6	12892,9	35,9	588,4	37055,37	60,87	0,791
	For whole experimental period	**30**	**524,89**	**25261,17**	**29,51**	**590,73**	**21355,7**	**26,7**	**630,53**	**35326,12**	**34,32**	**0,051**
RWD erythrocyte distribution width according to their volume **-CV (%)**	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**
GLU glucose (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**
T CHOL total cholesterol (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**
HDL- cholesterol (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**
TRIG triglycerides (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**
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]**	**171167,7**	**75,54**	**0,244**
LDL- cholesterol (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**

Legend:

[a] Control group (C) lambs received a basal diet (granulated combined feed + ground alfalfa hay) only without addition of phytonutrients.

[b] Experimental group (D) fed with a basal diet with addition of 7.5 mg dihydroquercetin/kg/d;

[c] Experimental group (R) fed with a basal diet with addition of 0.545 g dry distilled rose petals/kg/d;

[d] Results are presented as Means, Variance and Standard error of the means (SEM);

[f a- b] Means with different superscripts in each row differ significantly ($p \leq 0.05$).

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