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 characteristics and blood parameters 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 DDRP/kg/day respectively. The carcass yield of lambs having consumed dihydroquercetin or DDRP compared to lambs from control group C do not have significant differences ($p > 0.05$). 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. 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 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.

Keywords: phytonutrients, diet supplementation, lambs, average daily gain, pH

Abbreviations: CP, crude protein; DDRP, dry distilled rose petals; FUG, feed unit for growth; GLU, blood glucose content; HCT, haematocrit; HDL-C, HDL-cholesterol; HGL,

haemoglobin; LDL-C, LDL-cholesterol; MCH, mean haemoglobin content in erythrocytes; MCHC, mean haemoglobin concentration in erythrocytes; MCV, mean red blood cell count; MPV, mean platelets volume; PLT, platelets; RBC, erythrocytes; RWD, erythrocyte distribution width according to their volume; T CHOL, total cholesterol; TRIG, triglycerides; WBC, leukocytes

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 flavoring 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 Larix spp. 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 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 DHQ1contains 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 (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 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, carcasses

characteristics and blood parameters 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 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 ruminants (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;
- impossibility to conduct an experiment with more groups of animals due to limitations in project funding;

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 (24h, 65°C) and ground

189 to particle size < 0.4 mm. The 13 glycosides of kaempferol, 10 glycosides of quercetin, 6
190 glycosides of gallic acid and 2 flavonol aglycones were identified in dry rose petals.
191 The daily dose of the supplements was calculated according to previous one and mixed
192 with feed mixture (Table 1) and given to the lambs with the morning feeding.
193 *2.2. Sample preparation*
194 The blood sampling was made three times: on 5 February 2019 (in the beginning of the
195 experiment), on 1 March 2019 (in the middle of the experiment) and on 26 March 2019 (at the
196 end of the experiment). The blood samples were analyzed immediately after sampling.
197 Upon completion of the test period the lambs were identified and transported to the
198 slaughterhouse (Golden Fleece Ltd., Veliki Preslav, 16 Brothers Miladinovi Str., Shumen
199 district). After 24 h of pre-slaughter break, lambs were harvested in accordance with the
200 requirements of Ordinance No 15 of May 8, 2009 following normal industry slaughtering
201 procedures. The lamb carcasses were split, classified and chilled. After 24 h of chilling the
202 carcasses with temperature 4°C were moved to a refrigeration where they were stored at 0 -
203 4°C. Samples of m. Longissimus thoracis et lumborum were removed from each carcass.
204 Chilled muscle samples were ground through 3 mm grinder plates and mixed. The pH values
205 were determined as means after five replicates.
206 Five replicates of the proximate composition analyses of granulated combined feed were
207 made too (Table 1).
208 *2.3. Determination of spent feed for one kg average gain*
209 Feed consumption was monitored daily throughout the experimental period. The amount of
210 feed consumed per 1 kg of growth was determined in feed units for growth (FUG) and crude
211 protein (CP), g relative to the total growth obtained for each group.
212 *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 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).

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 (2020) recommendations.

2.7. Statistical analysis

Statistical analyses were performed using different software packages Microsoft Excel 5.0; JMP v. 7 for Windows (SAS, Inc., Cary, NC, USA). 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

3.1 Effect of phytonutrients supplementation on lamb's growth performance and spent feed for one kg average gain

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. Finally 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).

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

3.2.1. Slaughter weight and yield

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 ($p \leq 0.01$) 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.

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 carcasses) (Table 4). 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). 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) compared three groups of lambs.

Finally the conclusion was made 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.

3.2.3. pH values

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 ($p \leq 0.05$). 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.

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 indicator HGL in the experimental group D whose levels were higher ($p \leq 0.05$) than in control group C and in experimental group R. At the end of the experiment the levels of blood 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

311 6). A small increase ($p \leq 0.05$) at the levels of MCH and MCHC in the experimental group R
312 has been found compared to the other two groups - D and C (Table 6).

313 **4. Discussion**

314 *4.1. Effect of phytonutrients supplementation on lamb's growth performance and spent feed for*
315 *1 kg average gain*

316 One possible explanation of the determined effect of phytonutrients supplementation on
317 lamb's growth performance and nutrient digestibility is the relationship between the ruminant
318 nutrition and their health (Bessa et al., 2005; De Brito et al., 2017). Acting as a strong
319 antioxidant the dihydroquercetin probably possessed considerable protective activity from
320 oxidative DNA damage (Liang et al., 2013). It is likely that the increased level of anabolic
321 processes and body antioxidant protection reduced the morbidity and mortality in reared lambs.
322 So the dihydroquercetin supplemented lambs have increased vitality and longevity (Molyanova
323 et al., 2019). Additionally, it can positively affect the activity of antioxidant enzymes, it may
324 suppress the process of lipid peroxidation (Molyanova et al., 2019) and consequently raise the
325 lamb's appetite. Therefore, the dihydroquercetin can be considered a natural immunostimulant.
326 There evidence that low concentrations of dihydroquercetin as food supplement increase the
327 immune status of gilthead seabream by stimulation of both cellular and humoral immune
328 parameters (Awad et al., 2015).

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

Similarly of our findings about lower spent feed for 1 kg average 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.

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

4.2.1. Slaughter weight and yield

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 (Carter and Gallo, 2008; Lambe et al., 2009). 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).

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.

4.2.2. Lamb's carcasses classification

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) 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.

4.2.3. pH values

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 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. 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

385 is 0.18 pH units. They speculated that this difference can be a result of fat content or
386 physiological differences. Finally McGeehin et al. (2001) concluded that the variations in the
387 postmortem pH of the lamb have an inherent variable nature dependent on numerous factors
388 which are not the subject of consideration in this study.

389 *4.3. Effect of supplemented phytonutrients on lamb's blood count*

390 The determined increasing of RBC and HGL support the hypothesis that the addition of
391 7.5 mg dihydroquercetin to the lamb's combined feed stimulates hematopoiesis and thus
392 improves haemoglobin synthesis and prolongs the erythrocyte life in the blood. This is probably
393 due to the ability of the dihydroquercetin to contribute the reduction of the lipid peroxidation
394 products in blood erythrocytes, reducing carbohydrate metabolism, and delaying the adrenal
395 cortex function and a slower response to stress (Molyanova et al., 2019).

396 A small increase of the MCH, MCHC and PLT in the experimental group R are probably
397 caused by antioxidant and antimicrobial properties of DDRP (Nowak et al., 2014). They are
398 due to the specific composition of DDRP containing twenty two major compounds including
399 kaempferol and quercetin glycosides, quercetin 3-O-galactoside and quercetin 3-O-xyloside
400 (Schieber et al., 2005) well correlated with their free radical scavenging potential (Pal et al.,
401 2018).

402 **5. Conclusions**

403 The supplementation of lambs with 7.5 mg dihydroquercetin/kg/day or 545 mg
404 DDRP/kg/day cannot be used for significant beneficial effect on slaughter weight, carcass yield
405 and conformation. The 7.5 mg dihydroquercetin/kg/day supplementation increases the

haemoglobin concentration with 6 -7 g/l but 545 mg DDRP/kg/day contributes to reducing with 0.25 - 0.28 mmol/l the blood glucose comparing to control group C and experimental group D.

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References

Abdu, S.B., Ehoche, O.W., Adamu, A.M., Bawa, G.S., Hassan, M.R., Yashim, S.M., Adamu, H.Y., 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_e7384d8bc0bdccd90b23845f29846fec.pdf

Artem’eva, O.A., Pereselkova, D.A., Fomichev, Y.P., 2015. Dihydroquercetin, the bioactive substance, to be used against pathogenic microorganisms as an alternative to antibiotics. Sel’skokhozyaistvennaya Biologiya [Agric. Biol.], 50, 513-519. Available at http://www.agrobiology.ru/articles/4-2015artemeva.pdf

Asadollahi, S., Sari, M., Erafanimajd, N., Kiani, A., Ponnampalam, E.N., 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

Awad, E., Awaad, A.S., Esteban, M.A., 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

Baldi, G., Chauhan, S.S., Linden, N., Dunshea, F.R., Hopkins, D.L., Sgoifo Rossi, C.A., Dell'Orto, V., Ponnampalam, E.N., 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

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. Available at http://rimsa.eu/images/stockbreeding_18-2_2015.pdf

Bessa, R.J.B., Portugal, P.V., Mendes, I.A., 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

Borisov, A.Y., 2014. 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. PhD Thesis, Samara State Agricultural Academy, Izhevsk. (In Russian)

Bulgarian Veterinary Medical Activity Act, in force since 02 May 2006. Published in the State Gazette, issue 87 of November 1, 2005, last amendment in the State Gazette, issue 24 of March 22, 2019. (In Bulgarian)

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

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., (In Spanish) 40, 259-266. https://doi.org/10.4067/S0301-732X2008000300006

Chen, J.W., Zhu, Z.Q., Hu, T.X., Zhu, D.Y., 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

Chikwanha, O.C., Moelich, E., Gouws, P., Muchenje, V., Nolte, J.V., Dugan, M.E.R., 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

Chumbalov, T.K., Pashinina, L.T., Leiman, Z.A., 1970. Flavonoids of the bark of Larix sibirica. Chem. Natur. Comp., 6, 776-776. https://doi.org/10.1007/BF00565363

Cruywagen, C.W., van Zylb, W.H., 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

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. Official Journal of the European Union, L 197, 1-89.

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

481 carcases and the reporting of prices thereof. Official Journal of the European Union, L 337,
482 3-30.
483 Council Directive 2010/63/EC of 22 September 2010 on the protection of animals used for
484 scientific purposes. Official Journal of the European Union, L 276, 33-79.
485 Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at
486 the time of killing. Official Journal of the European Union L 303, 1-30.
487 De Brito, F.G., Ponnampalam, N.E., Hopkins, L.D., 2017. The effect of extensive feeding
488 systems on growth rate, carcass traits, and meat quality of finishing lambs. Comp. Rev. Food
489 Sci. Safety, 16: 23-38. https://doi.org/10.1111/1541-4337.12230
490 Díaz, M., Velasco, S., Cañeque, V., Lauzurica, S., Ruiz de Huidobro, F., Pérez, C., González,
491 J., Manzanares, C., 2002. Use of concentrate or pasture for fattening lambs and its effect on
492 carcass and meat quality. Small Rum. Res., 43: 257-268. https://doi.org/10.1016/S0921-
493 4488(02)00016-0
494 El-Sabaawy, E.H., Gad, S.M., El- Bedawy, T.M., Ali, H.M., Abd El-Gawad, A.M., 2015.
495 Growth performance and conjugated linoleic acid (CLA) content on meat of growing lambs
496 fed diets containing vegetable oils. Adv. Environ. Biol., 9, p. 8+. Available at
497 https://go.gale.com/ps/anonymous?id=GALE%7CA440716303&sid=googleScholar&v=2.1
498 &it=r&linkaccess=abs&issn=19950756&p=AONE&sw=w
499 Fomichev, Y., Nikanova, L., Lashin, A., 2016. The effectiveness of using dihydroquercetin
500 (taxifolin) in animal husbandry, poultry and apiculture for prevention of metabolic disorders,
501 higher antioxidative capacity, better resistance and realisation of a productive potential of
502 organism. Agric. Food., 4, 140-159. Available at http://en.ametis.ru/files/ametis/files/doklad-
503 bolgariyaporosyata-28.02.16_fomichev_yu.p.pdf.

Heber, D., 2004. Vegetables, fruits and phytoestrogens in the prevention of diseases. J. Postgrad. Med., 50, 145-149. Available at http://www.jpgmonline.com/text.asp?2004/50/2/145/8259

Heatley, J.J., Russell, K.E., 2020. Exotic Animal Laboratory Diagnosis, first ed. Wiley-Blackwell, Hoboken, USA.

Helander, I.M., Alakomi, H.L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E.J., Gorris, L.G., 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

Hassan, Y.I., Lahaye, L., Gong, M.M., Peng, J., Gong, J., Liu, S., Gay, C.G., 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

Holman, B.W.B., Baldi, G., Chauhan, S.S., Hopkins, D.L., Seymour, G.R., Dunshea, F.R., Collins, D., Ponnampalam, E.N., 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

Jamilah, B., Mohamed, A., Abbas, K., Abdul, R.R., 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

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

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

Lanza, M., Priolo, A., Biondi, L., Bella, M., Salem, H.B., 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

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

Matthews, K.R., Homer, D.B., Thies, F., Calder, P.C., 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

McCoard, S.A., Sales, F.A., Sciascia, Q.L., 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

McGeehin, B., Sheridan, J.J., 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

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

Molyanova, G., Ermakov, V., Bistrova, I., 2019. Correcting physiological and biochemical status of service dogs with dihydroquercetin. In: Proceedings of XII International Scientific

552 Conference on Agricultural Machinery Industry. IOP Conf. Series: Earth and Environmental
553 Science 403, e012042. https://doi.org/10.1088/1755-1315/403/1/012042
554 Nowak, R., Olech, M., Pecio, Ł., Oleszek, W., 2014. Cytotoxic, antioxidant, antimicrobial
555 properties and chemical composition of rose petals. J. Sci. Food Agric., 94, 560-567.
556 https://doi.org/10.1002/jsfa.6294
557 Oh, J., Wall, E.H., Bravo, D.M., Hristov, A.N., 2016. 1036 Phytonutrients as additives in
558 ruminants: the unexpected target organ. J. Anim. Sci., 94, 496-497.
559 https://doi.org/10.2527/jam2016-1036
560 Orzechowski, A., Ostaszewski, P., Jank, M., Berwid, S.J., 2002. Bioactive substances of plant
561 origin in food-impact on genomics. Repr. Nutr. Dev. 42, 461-477.
562 https://doi.org/10.1051/rnd:2002037
563 Pal, A., Bhushan, B., Narwal, R.K., Saharan, V., 2018. Extraction and evaluation of antioxidant
564 and free radical scavenging potential correlated with biochemical components of red rose
565 petals. Ir. J. Sci. Technol., Transac. A: Sci., 42, 1027-1036. https://doi.org/10.1007/s40995-
566 016-0071-2
567 Pavlova, M.V., 2017. Hygiene of growing lambs with the use of feed additives Laricarvit and
568 Bacell. PhD Thesis, Chuvash State Agricultural Academy, Cheboksary. (in Russian)
569 Popova, T., 2007. Effect of the rearing system on the fatty acid composition and oxidative
570 stability in m. longissimus dorsi and m. semimembranosus in lambs. Small Rum. Res., 71,
571 150-157. https://doi.org/10.1016/j.smallrumres.2006.06.001
572 Raymond, O., Fiasson, J.-L., Jay, M., 2001. The domestication syndrome within Hybrid
573 Perpetuals roses: the effect of unconscious selection on flavonoids. Biochem. Syst. Ecol. 29,
574 513-529. https://doi.org/10.1016/S0305-1978(00)00085-5
575 Sabino, M., Carmelo, V.A.O., Mazzoni, G., Cappelli, K., Capomaccio, S., Ajmone-Marsan, P.,
576 Verini-Supplizi, A., Trabalza-Marinucci, M., Kadarmideen, H.N., 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

Salama, R., Boraei, M.A., Fouda, S.M., El-Sysy, M.A.I., 2015. Effect of vitamin E supplementation on the productive performance of Rahmani sheep. Egypt. J. Nutr. Feeds, 18, 65-75.

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

Stahlke, E.R., Rossa, L.S., Silva, G.M., Sotomaior, C.S., Pereira, A.J., Luciano, F.B., Borges, T.D., de Macedo, R.E.F., 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

Teodoro, A.J., 2019. Bioactive compounds of food: Their role in the prevention and treatment of diseases oxidative. Med. Cell. Long. 2019, e3765986. https://doi.org/10.1155/2019/3765986

Vlahova-Vangelova, D., Balev, D., Ivanova, S., Nakev, J., Nikolova, T., Dragoev, S., Gerrard, D., 2020. Improving the oxidative stability of pork by antioxidant type phytonutrients. Biointerface Res. Appl. Chem., 10, 5624-5633. https://doi.org/10.33263/BRIAC103.624633

Westendarp, H., 2005. Essential oils for the nutrition of poultry, swine and ruminants. DTW. Deut. Tierarztl. Woch., (in German) 112, 375-380.

607 Table 1

608 Chemical composition of ground alfalfa hay and combined feed granules and their ingredients

609

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 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

Average daily gain, feed intake and feed consumption per kg growth 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 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	

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);

No significantly ($p > 0.05$) differences between means in this table were found

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 ($p \leq 0.05$).

671 Table 4

672 Classification of the lamb carcasses

673

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%

674

675 **Legend:**

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

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

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

680 [d] Results are presented as %;

681

682

683

684 Table 5

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

686

	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	
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

687

688 **Legend:**

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

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

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

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

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

695

696 Table 6

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

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	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	10	0.26	0.00	0.02	0.28	0.00	0.02	0.24	0.01	0.02	0.339

Hematocrit blood test (the number of red blood cells) (%)	on 1 March 2019											
	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	12818.10	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**
PLT Platelets ($x10^9$/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**

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}**	**171167.70**	**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 ($p \leq 0.05$).