

## Effect of new livestock feeds' phytonutrients on productivity, carcass composition and meat quality in pigs

Sonya Ivanova<sup>1</sup>, Jivko Nakev<sup>1</sup>, Tanya Nikolova<sup>1</sup>, Desislava Vlahova-Vangelova<sup>2</sup>, Dessislav Balev<sup>2</sup>, Stefan Dragoev<sup>2,6\*</sup>, David Gerrard<sup>3</sup>, Lilia Grozlekova<sup>4</sup> and Desislava Tashkova<sup>5</sup>

<sup>1</sup>*Agricultural Academy, Agricultural Institute Shumen, Department of Animal Sciences, Sofia, Bulgaria*

<sup>2</sup>*University of Food Technologies, Technological Faculty, Department of Meat and Fish Technology, Plovdiv, Bulgaria*

<sup>3</sup>*Virginia Tech, College of Agriculture and Life Sciences, Department of Animal and Poultry Science, 3470 Litton-Reaves Hall, Blacksburg, 24061 Virginia, USA*

<sup>4</sup>*Medical University, Faculty of Medicine, Department of Human Anatomy, Histology and Embryology, Plovdiv, Bulgaria*

<sup>5</sup>*Medical University, Faculty of Medicine, Department of General and Clinical Pathology, Plovdiv, Bulgaria*

<sup>6</sup>*Bulgarian Academy of Sciences, Assembly of Academicians and Corresponding Members, Sofia, Bulgaria*

\*Corresponding author: [logos2000lt@gmail.com](mailto:logos2000lt@gmail.com)

### Abstract

Ivanova, S., Nakev, J., Nikolova, T., Vlahova-Vangelova, D., Balev, D., Dragoev, S., Gerrard, D., Grozlekova, L. & Tashkova, D. (2021): Effect of new livestock feeds' phytonutrients on productivity, carcass composition and meat quality in pigs. *Bulgarian Journal of Agricultural Science*, 27(6), 1178–1186

Improving pig growth performance, nutrient digestibility and pork quality is a main goal of pig breeding. The objective of this study was to determine the impact of *Larix sibirica* dihydroquercetin (DHQ) or dry distilled rose (*Rosa damascena*) petals (DDRP) on growth performance, carcass composition, meat quality, blood parameters and histological changes in ovaries and liver in native Danube White fattening pigs. A total of 120 pigs were used in the experiment lasting 45 days prior to harvest. The pigs were divided into five groups. The control group (C) was fed a basal diet. The other four experimental groups were fed the same diets containing either 3.5 and 7.5 mg DHQ/kg/d or 0.255 and 0.545 g DDRP/kg/d. The supplementations with DHQ or DDRP increased the average body weight by 7.74 – 9.05%, the average daily gain by 27.06 – 30.13%, and feed to gain ratio by 12.53 – 15.99%, while decreasing the feed intake by 5.24 – 13.84% and the average liver weight by 10.53 – 21.12% compared to the control group. Both supplementations did not cause pathological changes in the histological structure of pigs' liver and ovaries; and had no influence on pH values and proximate composition of m. *Longissimus thoracis* and m. *Semimembranosus*. No pH determined stress-induced muscle damage was found and the pork carcasses were classified in classes E and U. The two used supplementations reduced the blood LDL cholesterol by 13.27 – 14.29 % and increased the erythrocytes, platelets, hematocrit, mean red blood cell count, mean hemoglobin concentration in erythrocytes, triglycerides and total cholesterol as well. The addition of 3.5 mg DHQ/kg/d or 0.545 g DDRP/kg/d to pigs' diet can be used as growth promoters of natural origin with a favorable effect on blood metabolites and meat quality, due to their bioactive properties.

**Keywords:** blood parameters; dihydroquercetin; dry distilled rose petals; growth performance; histology

## Introduction

The improvement of pig production performance by including plant-derived compounds in diets and promoting animal health and pork quality has been discussed recent years (Guil-Guerrero et al., 2016; Valenzuela-Grijalva et al., 2017). For this purpose, the inclusion of herbal plants derivatives (Hashemi and Davoodi, 2011) or extracts (Costa et al., 2013) in the diets has been discussed.

Dihydroquercetin (DHQ) is a flavonoid, a good electron donor which is able to inhibit hydroxyl radicals (Weidmann, 2012) with capillaryprotective, lipidreducing, anti-inflammatory, cardioprotective, hepatoprotective, detoxifying and immunomodulatory properties (Artem'eva et al. 2015). A combination of relatively low concentrations of DHQ (10 – 50 mg/kg/d), Dahurian larch pulp (*Larix dahurica* Turez) and probiotics have been successfully applied to increase the productive potential of pigs (Fomichev et al., 2016).

Another new potential beneficial phytonutrient is a by-product derived from rose oil production. Dry distilled rose (*Rosa rugosa*) petals (DDRP) contain a wide range of bioactive compounds that possess strong cytotoxic, antioxidant and antimicrobial properties (Nowak et al., 2014; Vlahova-Vangelova et al., 2020).

Therefore, the objective of this study was to determine the impact of Siberian larch DHQ or DDRP on growth performance, carcass composition, meat quality, blood parameters and histological changes in ovaries and liver in native Danube White fattening pigs.

## Materials and Methods

### Pigs and diets

The experiment was conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, Commission Recommendation 2007/526/EC and Council Regulation (EC) 1099/2009. The experiment was approved of the Bulgarian Scientific Ethics Committee and the requirements of the Council Directive 2010/63/EC were met.

A total of 120 Danube White fattening pigs of both sexes (60 males and 60 females) were used in the experiment. The age of the animals was 155 days, with a mean body weight of  $72.500 \pm 1.937$  kg. The pigs were housed in a barn consisting of individual pens equipped with feeders and drinkers at the Experimental Farm of Agricultural Institute, Shumen, Bulgaria. The study period was 45 days – the time for fattening the pigs. At the end of this period, the 200-day-old pigs weighed  $110.300 \pm 2.274$  kg. During the trial period, the temperature was between 19 to 27°C. Feed and water were available *ad*

*libitum*. The pigs were divided into five groups (one control and four experimental), each containing 24 animals receiving different supplementations. The control group (C) was fed a basal diet (Table 1). Bio-concentrates BK14 and BK16 included in the formulations of the grower and finisher basal diets were supplied by Vasil Kostov Feed Factory, village Lyuben Karavelovo, Varna District, Bulgaria.

The other four experimental groups were fed the same diet containing either 3.5 mg DHQ/kg/d (D1), 7.5 mg DHQ/kg/d (D2), 0.255 g DDRP/kg/d (R1) or 0.545 g DDRP/kg/d (R2). Residual feed was monitored daily and was weighed and subtracted from of the daily amount of feed consumed.

DHQ was provided by the company Flavitlife Bio JSC (Sofia, Bulgaria) and the DDRP were supplied by Damascena rose oil distillery, village of Skobelevo, municipality of Pavel Banya, Stara Zagora district (Bulattars Production Company Ltd, Sofia, Bulgaria). After pressing, petals were dried and ground to particle size < 0.4 mm. The main polyphenolic compounds of DDRP were determined as follows: gallic acid glycosides  $1.43 \pm 0.04$  mg/g; quercetin-3-O-galactoside  $0.96 \pm 0.18$  mg/g; quercetin-3-O-glucoside  $0.79 \pm 0.10$  mg/g; quercetin glycosides  $1.98 \pm 0.05$  mg/g; kaempferol glycosides  $1.97 \pm 0.05$  mg/g and kaempferol  $0.22 \pm 0.03$  mg/g (Dragoev et al., 2021).

The individual daily doses of the supplements were chosen after a careful analysis of the antioxidant effects as well as the economic efficiency for the application of the supplements. The dihydroquercetin antiradical activity is manifesting in a concentration 0.0001 – 0.00001% (Fomichev et al., 2016). Its concentration was re-calculated taking into account the much higher purity (96%) in the isolate used compared to the significantly lower levels in the preparation “Ekostimul-1” (Fomichev et al., 2016) with an idea comparable concentration of biologically active compounds and observing the economic effect of the application of the preparations due to their very high price (Vlahova-Vangelova et al., 2020). Individual daily doses of the supplements were calculated according to previous weighing of animals, mixed with feed (Table 1) and given with the morning feeding. Animals were weighed every two weeks. At the end of the experiment the age of the pigs was 200 days and the live weight was  $110.300 \pm 2.274$  kg. The growth performance parameters and liver weight were determined at the beginning and the end of the trial period.

### Sample preparation

The blood sampling was done at the end of the trial period. The blood samples were analyzed immediately after sampling. The pigs were transported to a processing plant (Unitemp Ltd, Voyvodinovo village, Plovdiv district, Bulgaria). After 18 h

**Table 1. Ingredients, chemical composition and energy values of basal diet**

Components	Grower	Finisher
<b>Formulations</b>		
Maize, g/kg	150.00	130.00
Barley, g/kg	250.00	100.00
Wheat, g/kg	270.00	500.00
Wheat bran, g/kg	80.00	70.00
Bio-concentrate BC14 <sup>b</sup> , g/kg	250.00	–
Bio-concentrate BC16 <sup>c</sup> , g/kg	–	200.00
Total:	1000.00	1000.00
<b>Calculated chemical compositions</b>		
Moisture, g/kg	171.00	157.00
Dry matter, g/kg	829.00	843.00
Organic substances, g/kg	782.20	799.10
Crude protein, g/kg	157.50	150.20
Crude fats, g/kg	28.10	24.20
Crude ash, g/kg	46.80	43.90
Neutral detergent fibers, g/kg	47.90	38.40
Lysine, g/100 g	0.80	0.72
Calcium, g/100 g	1.31	1.26
Phosphorus, g/100 g	0.85	0.31
Energy intake		
Digestible energy, MJ/kg forage	13.46	13.72
Exchangeable energy, MJ/kg forage	12.92	13.18

**Legend:**

<sup>a</sup> The quantities of Siberian larch dihydroquercetin or dry distilled rose petals added as supplements to the diets were calculated as 3.5 mg dihydroquercetin/kg/d (D1); 7.5 mg dihydroquercetin/kg/d (D2); 0.255 g dry distilled rose petals/kg/d (R1); or 0.545 g dry distilled rose petals/kg/d (R2)

<sup>b</sup> The bio-concentrate BC14 contents: 312.10 g/kg crude protein, 10.70 g/kg crude fat, 153.00 g/kg crude ash, 38.10 g/kg crude fibers, 5.88 g/100 g lysine, 2.79 g/100 g methionine, 7.80 g/100 g calcium, 2.69 g/100 g phosphorus, 268 mg/kg copperas sulphate, 670 mg/kg dl- $\alpha$ -tocopherol, 93800 UI/kg vitamin A, 16080 UI/kg vitamin D3, 1975.845 kcal/kg total energy

<sup>c</sup> The bio-concentrate BC16 contents: 348.00 g/kg crude protein, 17.40 g/kg crude fat, 165.00 g/kg crude ash, 108.30 g/kg crude fibers, 2.26 g/100 g lysine, 0.67 g/100 g methionine, i.e. 1.25 g/100 g methionine + cystine, 1.31 g/100 g threonine, 3.66 g/100 g calcium, 0.95 g/100 g phosphorus, i.e. 0.67 g/100 g absorbable phosphorus, 0.78 g/100 g sodium, 560.00 mg/kg iron, 545.00 mg/kg zinc, 195.00 mg/kg manganese, 100.00 mg/kg copper, 4.10 mg/kg iodine, 1.50 mg/kg selenium, 0.40 g/100g antioxidants, 320.00 mg/kg vitamin E, 32500 UI/kg vitamin A, 6000 UI/kg vitamin D3

of pre-slaughter break pigs were showered and harvested in accordance with the requirements of the EUROP Community scale following normal industry processing procedures. Carcasses were split and chilled to 4 °C. Ovaries from 60 female pigs and 120 livers were collected at harvest. The testicles of the 60 male pigs could not be examined because the animals were usually neutered. Samples are of m. *Longissimus thoracis*. For analysis was used the portion from 10<sup>th</sup> to 12<sup>th</sup> intercostal space and m. *Semimembranosus* were removed from

each carcass. Chilled muscle samples were ground through 3 mm grinder plates and subjected to further analysis. The proximate composition analyses of grower and finisher basal diets, meat proximate composition and pH were determined as means from five replicates (Table 1).

**Blood tests**

At the end of the experimental period the pigs from the five experimental groups were immobilized and 10 mL of blood was extracted from the jugular vein of each pig. The blood samples were taken in vacutainer tubes using a California-type needle and were transferred to the laboratory within the first 3 hrs for further analysing. The blood profile analyses were done by differential hematology analyzer Abacus 5 (Diatron Medical Instruments PLC, Budapest, Hungary). The fat profile and glucose analyses were done by automated Olympus AU640 chemistry analyzer (International Equipment Trading Ltd., Mundelein, Illinois, USA) (Weiser, 2012). The blood estradiol concentration was measured by Roche Elecsys 2010 immunoassay analyzer (Roche Diagnostics, Bellport, New York, USA) using chemiluminescence immunoassay (CLIA) method.

**Histological imaging of ovaries and liver**

For the morphological analysis, ovaries and liver tissue cuts with size 2 × 1 × 1 cm were placed in formalin, dehydrated sequentially in ascending order alcohols, acetone and xylol. The dehydrated samples were included in paraffin at 56°C for 24 h. The paraffin blocks were cut into sections of approximately 5  $\mu$ m and contrasted with hematoxylin-eosin. Microphotographs were performed with Nikon Microphot SA microscope (Nikon, Tokyo, Japan), combined with Camedia-5050Z digital camera (Olympus Corporation, Tokyo, Japan).

**Determination of pH as an indicator of stress-induced muscle damage**

The pH value in the m. *Longissimus thoracis* and m. *Semimembranosus* at 45 min, 24 h and 7 d *post mortem* was measured with a laboratory pH meter MS 2004 (Microstyst, Plovdiv, Bulgaria) equipped with combined pH electrode Sensorex 450 CD (Sensorex, Inc., Garden Grove, USA). The potential stress-related muscular injuries were determined as described by Warriss (2000).

**Determination of lean meat content**

After the slaughter of the animals, warm and cold carcass weights were registered 1 and 24 h *post mortem*. The thickness of the back fat at two points, the thickness of m. *Longissimus thoracis and lumborum* and the percentage of lean

meat were measured using Ultra Fom 200 apparatus (Carometec Food Technology, Harley, Denmark). The device was equipped with a 4 MHz ultrasonic probe (Krautkrämer MB 4 SE). The ultrasonic signal was digitized, stored and processed by a microprocessor (Intel 80 C 32 type).

#### Determination of proximate composition of pork

Moisture was determined by drying samples at 105°C. Ash was determined after sample's mineralisation (AOCS, 2004). The total protein was determined by the Kjeldahl method, using the Automatic Distillation System Model UDK 152 (Foss Tecator AB, Hoganas, Sweden). The intramuscular fat content (IMF) was determined by weight after extraction with a non-polar solvent in the Soxhlet apparatus (AOCS, 2004).

Calcium and phosphorus were determined by the method of Anwar et al. (2016).

#### Statistical analysis

Statistical analyses were performed using JMP v. 7 (SAS, Inc., Cary, NC, USA) The effect of the supplementation with DHQ and DDRP on the performance, blood parameters, carcass measurements, and meat quality was assessed through one way ANOVA. When the effect was significant, the means of the examined traits were compared by the Tukey's Honest Significant Difference test ( $p < 0.05$ ).

## Results

#### Growth performance

The dietary supplements affected significantly ( $p < 0.05$ ) the final average body weight (ABW) leading to its increase by 9.05% in the pigs from D1 group, 7.74% in the pigs from R2, 4.64% in the R1 pigs and 3.81% in the animals from D2 group (Table 2).

The average daily gain (ADG) was also higher in the experimental groups ( $p < 0.01$ ) in comparison to the control group C showing the following increase 30.13% in D1; 27.06% in R2; 15.48% for R1, and 13.11% in D2. The two studied phytonutrients reflected the feed intake and feed to gain ratio as well. The use of the studied supplementations resulted in an increase in the amount of the feed intake between 5.24% and 13.84%. The supplementation with DHQ and DDRP significantly affected the feed to gain ratio ( $p < 0.05$ ). The best feed utilization was found in the D1 and R2 groups when compared to the control group. The feed to gain ratio tended to differ between the two groups receiving DHQ as well as the DDRP. Those results proved the better efficiency of the higher concentration of 545 mg DDRP compared to the lower concentration of 255 mg DDRP, where gaining of 1 kg body weight was realized by 0.297 kg less feed. Similarly, the lower concentration of 3.5 mg DHQ had better efficiency compared to the higher concentration of 7.5

**Table 2. Average body weight, average daily gain, feed consumption and feed to gain ratio in fattening pigs in response to supplementation with DHQ and DDRP**

Parameters	Control group(C)	Experimental group (D1)	Experimental group (D2)	Experimental group (R1)	Experimental group (R2)	SEM	Significance
Initial average body weight (ABW), kg. Pigs at 155 days of age	72.750	72.500	72.500	72.625	72.125	0.96	NS
Final average body weight (ABW), kg. Pigs at 200 days of age	105.000 <sup>a</sup> (100.00%)	114.500 <sup>b</sup> (109.05%)	109.000 <sup>ab</sup> (103.81%)	109.875 <sup>ab</sup> (104.64%)	113.125 <sup>b</sup> (107.74%)	1.24	*
Average daily gain (ADG) on the end of the experiment, kg/pig/day	0.717 <sup>a</sup> 100.00%	0.933 <sup>c</sup> 130.13%	0.811 <sup>ac</sup> 113.11%	0.828 <sup>ac</sup> 115.48%	0.911 <sup>c</sup> 127.06%	0.02	**
Feed intake, kg/pig/day	2.941 <sup>a</sup> 100.00%	3.348 <sup>b</sup> 113.84%	3.095 <sup>ab</sup> 105.24%	3.099 <sup>ab</sup> 105.37%	3.139 <sup>ab</sup> 106.73%	0.03	*
Feed to gain ratio, for the whole experimental period, kg	4.102 <sup>b</sup> 100.00%	3.588 <sup>a</sup> 87.47%	3.816 <sup>ab</sup> 93.03%	3.743 <sup>ab</sup> 91.29%	3.446 <sup>a</sup> 84.01%	0.07	*

#### Legend:

Control group (C) pigs received a typical commercial diet;

Experimental group (D1) fed with a commercial diet with addition of 3.5 mg dihydroquercetin/kg live weight per day;

Experimental group (D2) fed with a commercial diet with addition of 7.5 mg dihydroquercetin/kg live weight per day;

Experimental group (R1) fed with a commercial diet with addition of 0.255 g dry distilled rose petals/kg live weight per day;

Experimental group (R2) fed with a commercial diet with addition of 0.545 g dry distilled rose petals/kg live weight per day

Results are presented as mean values and standard error of mean (SEM), significance of factor: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; NS-non significant, means connect with different superscripts are significantly different ( $p < 0.05$ )

mg DHQ where gaining of 1 kg body weight was realized by 0.228 kg less feed. The difference in the parameter feed to gain ratio between the control and experimental groups was most pronounced for R2 (15.99%) followed by D1 (12.53%), R1 (8.71%) and D2 (6.97%) groups, respectively (Table 2).

### Blood parameters

The dietary supplementation with DHQ or DDRP did not affect the amount of WBC, HGL, MCH, MPV, GLU and HDLs in the blood of pigs (Table 3). The pigs from group D2 did not display significant differences, compared to the pigs from the control group C in regard to these blood parameters. It was found that the supplementation of 0.545 g DDRP/kg body weight (group R2) reduced RBC by 1.13% and PLT by 20.34% ( $p < 0.05$ ) in comparison to control group C. An even more pronounced significant ( $p < 0.001$ ) reduction was found for the HCT – by 12.19%, MCV – by 10.95% and MCHC – by 3.02%. In R1 group significant increase ( $p < 0.05$ ) in RBC by 1.61%, PLT by 2.58%, T CHOL by 10.85%, HCT by 4.88%, MCV by 2.74%, RWD by 10.72%, TRIG by 10.81%, and MCHC by 6.06% ( $p < 0.001$ ), compared to control group C was observed. At the same time, the LDLs was reduced by 13.27% ( $p < 0.01$ ). The most pronounced changes were established in the blood samples from D1 group. Compared to control group C a significant increase

( $p < 0.05$ ) in RBC (by 4.35%), PLT (by 15.61%), T CHOL (by 13.57%) and a significant increase ( $p < 0.01$ ) in HCT (by 7.32%), MCV (by 3.80%), MCHC (by 11.45%), RWD (by 25.60%) and TRIG (by 18.02%) were observed. In the same time LDLs was reduced by 14.29% ( $p < 0.01$ ).

A dramatic decrease of the hormone estradiol in the blood samples between the control and experimental groups was observed (Table 3). In R2 group the estradiol level was reduced approximately 8 times and in groups D2, D1 and R1 the decrease was 2 – 2.5 times.

### Liver weight and histology of ovaries and liver

The lowest average liver weigh ( $p < 0.01$ ) was measured in pigs from group R1, followed by group D1. The ratio of average liver weight (ALW)/final ABW  $\times$  100 in pigs from group R1 was 21.12% lower and from group D1 – 10.53% lower in comparison to the control group C. No significant differences were found in ALW and ALW/ABW ratio in pigs from groups D2, R2 and C (Table 4). The lower levels of the two studied supplements contributed to the reduction of the liver weight. A well-preserved liver structure of classical hepatic lobule with centrally located vein (v. centralis) was observed. Preserved cytoarchitectonics, radially located hepatocytes on hepatocyte lamellae were found.

**Table 3. Blood tests parameters in fattening pigs according to the supplementation with DHQ and DDRP**

Indicator	Control group (C)	Experimental group (D1)	Experimental group (D2)	Experimental group (R1)	Experimental group (R2)	SEM	Significance
WBC leukocytes	20.41	20.17	21.65	19.18	19.89	3.35	NS
RBC erythrocytes	6.20 <sup>ab</sup>	6.47 <sup>b</sup>	6.15 <sup>ab</sup>	6.30 <sup>ab</sup>	6.13 <sup>a</sup>	0.39	*
HGL hemoglobin	113.50	115.37	114.16	118.08	112.75	7.07	NS
HCT hematocrit	0.41 <sup>abc</sup>	0.44 <sup>a</sup>	0.38 <sup>bc</sup>	0.43 <sup>ab</sup>	0.36 <sup>c</sup>	0.40	***
MCV mean red blood cell count	66.06 <sup>a</sup>	68.57 <sup>a</sup>	63.11 <sup>ab</sup>	67.87 <sup>a</sup>	58.82 <sup>b</sup>	8.24	***
MCH mean hemoglobin content in erythrocytes	18.30	18.31	18.64	18.26	18.31	0.57	NS
MCHC mean hemoglobin concentration in erythrocytes	281.50 <sup>bc</sup>	313.95 <sup>a</sup>	275.58 <sup>bc</sup>	300.08 <sup>ab</sup>	273.00 <sup>c</sup>	32.58	***
RWD erythrocyte distribution width according to their volume	19.22 <sup>b</sup>	24.14 <sup>a</sup>	18.20 <sup>b</sup>	21.28 <sup>ab</sup>	18.04 <sup>b</sup>	5.15	***
PLT platelets	418.87 <sup>ab</sup>	484.29 <sup>a</sup>	427.58 <sup>ab</sup>	429.66 <sup>ab</sup>	333.66 <sup>b</sup>	153.48	*
MPV mean platelets volume	7.01	7.48	7.20	7.15	6.12	2.81	NS
GLU glucose	4.56	4.41	4.13	4.42	4.56	0.59	NS
T CHOL total cholesterol	2.21 <sup>b</sup>	2.51 <sup>a</sup>	2.38 <sup>ab</sup>	2.45 <sup>a</sup>	2.35 <sup>ab</sup>	0.30	*
TRIG triglycerides	1.11 <sup>c</sup>	1.31 <sup>a</sup>	1.16 <sup>bc</sup>	1.23 <sup>ab</sup>	1.12 <sup>bc</sup>	0.15	***
LDL-cholesterol	0.98 <sup>a</sup>	0.84 <sup>b</sup>	0.98 <sup>a</sup>	0.85 <sup>b</sup>	0.92 <sup>ab</sup>	0.18	**
HDL-cholesterol	0.45	0.48	0.48	0.44	0.53	0.13	NS
Estradiol, pg/ml	67.225 <sup>d</sup>	26.900 <sup>bc</sup>	32.615 <sup>c</sup>	27.485 <sup>bc</sup>	8.650 <sup>a</sup>	4.027	NS

Legend:

Results are presented as mean values and standard error of mean (SEM), significance of factor: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ; NS: non-significant, means connected with different superscripts are significantly different ( $p < 0.05$ )

**Table 4. Lean meat content in the carcasses and pigs' liver weight as affected by the supplementation with DHQ and DDRP**

Parameters	Control group (C)	Experimental group (D1)	Experimental group (D2)	Experimental group (R1)	Experimental group (R2)	SEM	Significance
Percentage of lean meat in the carcasses							
X <sub>1</sub> , mm	23.37 <sup>ab</sup>	24.25 <sup>b</sup>	19.75 <sup>a</sup>	22.37 <sup>ab</sup>	17.50 <sup>a</sup>	0.84	*
X <sub>2</sub> , mm	18.37	19.37	17.12	17.62	17.35	0.96	NS
X <sub>3</sub> , mm	51.00 <sup>a</sup>	58.87 <sup>b</sup>	54.62 <sup>ab</sup>	53.37 <sup>ab</sup>	53.00 <sup>ab</sup>	0.93	*
LM, %	53.39	52.83	55.07	54.03	55.01	0.54	NS
Liver weight, g							
Male pigs	1831.500 <sup>c</sup>	1671.330 <sup>b</sup>	1814.500 <sup>c</sup>	1408.500 <sup>a</sup>	1805.200 <sup>c</sup>	49.49	*
Female pigs	1681.330 <sup>b</sup>	1695.000 <sup>c</sup>	1694.000 <sup>c</sup>	1440.500 <sup>a</sup>	1714.500 <sup>d</sup>	44.17	*
Average between males and females	1725.500 <sup>c</sup>	1683.165 <sup>b</sup>	1754.250 <sup>c</sup>	1424.500 <sup>a</sup>	1759.850 <sup>c</sup>	48.00	**
Ratio average liver weight (ALW)/ final ABW x 100, %	1.643% <sup>b</sup>	1.470% <sup>ab</sup>	1.609% <sup>b</sup>	1.296% <sup>a</sup>	1.554% <sup>b</sup>	0.02	**

Legend:

LM = 67.13 - 0.3284 X<sub>1</sub> - 0.3725 X<sub>2</sub> + 0.01515 X<sub>3</sub> where:

LM - percentage of lean meat, %;

X<sub>1</sub> - thickness of the fat and skin measured between the 3<sup>rd</sup> and 4<sup>th</sup> lumbar vertebrae at 7 cm laterally, mm;

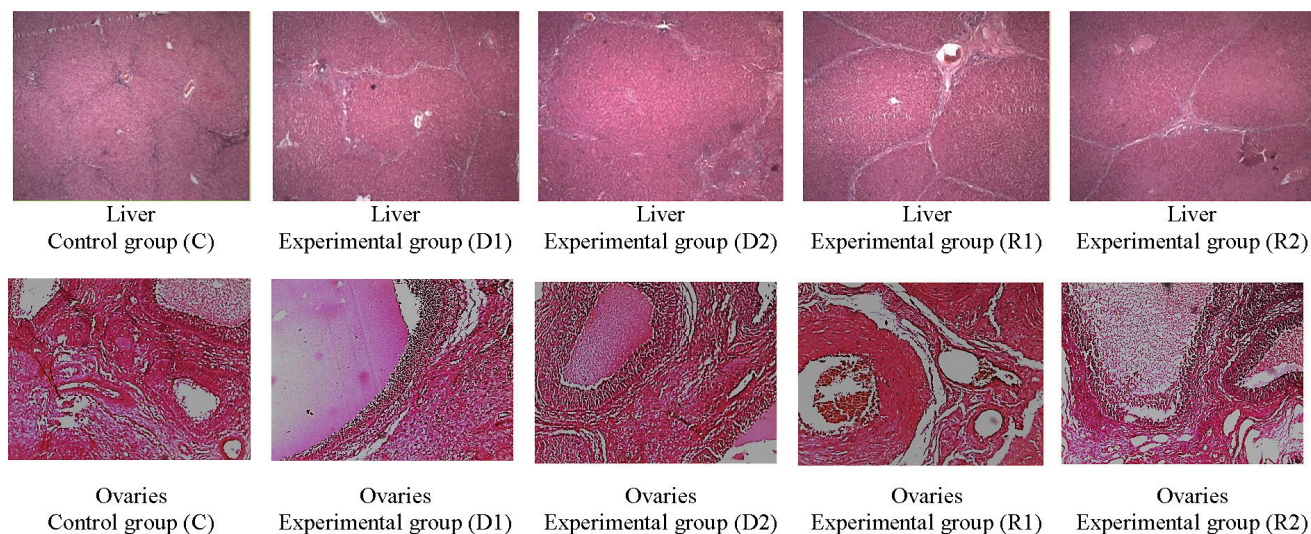
X<sub>2</sub> - thickness of the fat and skin measured between the 3<sup>rd</sup> and 4<sup>th</sup> rib at 7 cm laterally, mm;

X<sub>3</sub> - thickness of m. *Longissimus thoracis* between 3<sup>rd</sup> and 4<sup>th</sup> rib at 7 cm laterally, mm.

Results are presented as mean values and standard error of the mean (SEM), significance of factor: \* p < 0.05; \*\* p < 0.01; NS: non-significant, means connected with different superscripts are significantly different (p < 0.05)

The portal spaces formed by an arterial vessel, a venous vessel and a bile duct were normally located in the periphery of the lobules. No pathological changes in liver structure, no fibrinoid accumulation and no fatty degeneration (hepatic steatosis) were observed. There were no visible intracytoplasmic accumulations of triglycerides. No defective remodelling of the ductal plate or abnormal branching of the intrahepatic portal veins, no regressive fibrosis of

portal tracts Anastomosing biliary channels in regular, hepatocyte nodules with central veins and normal architecture were detected. No marked proliferation of bile ductless, no angulated bile ducts, no inflammation, and no regenerative nodules were observed (Figure 1). A preserved thick connective tissue capsule - the tunica albuginea of ovaries, covered by a simple squamous mesothelium - germinal epithelium, was found.



**Fig. 1. Histological structure of pigs' liver and ovaries (contrasted with hematoxylin- eosin; magnification × 100)**

There was found a cortex, with the ovarian follicles, and a highly vascular medulla with coiled arteries called heli-crane arteries. The oocytes were surrounded by epithelial cells and formed follicles. It contained many primordial follicles which were mostly found around the edges of the cortex. There were fewer follicles with histological appearance in different stages of development (Figure 1).

#### The lean meat content

The values of the variables involved in the models for determining the relative proportion of lean meat in the carcass indicated that the pigs consuming higher doses of the two studied supplements (groups R2 and D2) were characterized by a thinner fat at the two measurement points (Table 4). The R2 and D2 pigs were classified in class E and those from the other three groups in class U according to the SEUROP system. On the other hand, the deviations of lean meat content were not significant and ranged between 52.83 – 55.07% (Table 4).

#### pH determined stress-induced muscle damage

No significant differences between pH values of the two muscles of the five examined groups 45 min *post mortem*, and after 7 days of storage at 0 – 4°C were found. Conversely, 24 h *post mortem* the supplements affected the pH of m. *Longissimus thoracis* ( $p < 0.01$ ) as lowest values were measured in R2, D1 and R1 groups. The highest pH value ( $p < 0.05$ ) was determined in the control group (C). The difference in the mean values between the lowest and the highest pH level was about 2.5% or 0.15 pH units. Similar pH values were found in m. *Semimembranosus*. Significant differences ( $p < 0.05$ ) in the pH values were found 24 h *post mortem*. The deviation of pH levels was only 1.92% or 0.11 pH units (Table 5). According to Warriss (2000) our results did not indicate the stress-induced muscle damage. The pork from all studied groups was classified as normal.

**Table 5. pH na of pork m. *Longissimus thoracis* and m. *Semimembranosus* during 7 days storage at 0–4°C according to supplementation with DHQ and DDRP**

Parameters	Control group (C)	Experimental group (D1)	Experimental group (D2)	Experimental group (R1)	Experimental group (R2)	SEM	Significance
<i>m. Longissimus thoracis</i>							
pH <sub>1</sub> (45 min)	6.25	6.21	6.19	6.15	6.28	0.03	NS
pH <sub>2</sub> (24 h)	6.00 <sup>b</sup>	5.88 <sup>a</sup>	5.92 <sup>ab</sup>	5.89 <sup>ab</sup>	5.85 <sup>a</sup>	0.02	**
pH <sub>3</sub> (7 d)	6.11	6.09	6.12	6.13	6.07	0.03	NS
<i>m. Semimembranosus</i>							
pH <sub>1</sub> (45 min)	6.21	6.14	6.13	6.10	6.20	0.04	NS
pH <sub>2</sub> (24 h)	5.70 <sup>b</sup>	5.60 <sup>a</sup>	5.69 <sup>ab</sup>	5.71 <sup>b</sup>	5.61 <sup>a</sup>	0.03	*
pH <sub>3</sub> (7 d)	6.01	5.98	6.00	6.03	5.99	0.02	NS

*Legend:* Results are presented as mean values and standard error of the mean (SEM), significance of factor: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; NS: non-significant, means connected with different superscripts are significantly different ( $p < 0.05$ )

**Table 6. Proximate composition of m. *Longissimus thoracis* and m. *Semimembranosus* 24 h *post mortem* (0–4°C) as affected by supplementation with DHQ and DDRP**

Parameters	Control group (C)	Experimental group (D1)	Experimental group (D2)	Experimental group (R1)	Experimental group (R2)	SEM	Significance
<i>m. Longissimus thoracis</i>							
Moisture, g/100 g	71.18	71.72	72.29	71.37	72.07	0.44	NS
Dry mater, g/100 g	28.81	28.27	27.71	28.62	27.92	0.44	NS
Proteins, g/100 g	22.30	21.94	20.91	21.89	22.04	0.34	NS
Fats, g/100 g	5.43	5.27	5.76	5.68	5.12	0.24	NS
Ash, g/100 g	1.09	1.06	1.04	1.06	1.06	0.04	NS
<i>m. Semimembranosus</i>							
Moisture, g/100 g	69.97	70.36	71.22	70.05	71.01	0.47	NS
Dry mater, g/100 g	30.03	29.64	28.78	29.95	28.99	0.50	NS
Proteins, g/100 g	23.89	23.67	22.79	23.72	22.99	0.38	NS
Fats, g/100 g	5.16	5.02	5.06	5.28	5.01	0.28	NS
Ash, g/100 g	0.98	0.95	0.93	1.00	0.99	0.03	NS

*Legend:* Results are presented as mean values and standard error of the mean (SEM); NS: non-significant

### Proximate composition of pork muscles

The DHQ or DDRP supplementations did not affect the content of water, protein, IMF or mineral substances in the studied pork muscles (Table 6).

## Discussion

One of the possible reasons for the observed ABW, ADG and feed intake and the lower feed to gain ratio in experimental groups compared to the control one is the proven positive effect of polyphenols from the applied phytonutrients to stabilize brain health, blood vessels, muscles, and intestines (Kawabata et al., 2015). Another reason for the positive influence of DHQ and DDRP on the growth performance of pigs is probably due to their properties to stimulate the appetite of the animals and to accelerate the metabolic processes (Valenzuela-Grijalva et al., 2017). On the other hand, the DHQ has strong antioxidant activity and capillary protection (Vlahova-Vangelova et al., 2020). It may intensify the anabolic processes and increase the ADG in pigs (Bogolyubova et al., 2019). The DHQ has good solubility in the intestinal tract due to a generation of chylomicrons in the intestinal mucosa (Lesser et al., 2006) and can effectively suppress facultative pathogens being without negative effects on intestinal probiotic microflora (Artem'eva et al., 2015). Our results from the effect of the DHQ supplementation on pigs' ADG are similar to those reported by Fomichev et al. (2016).

Furthermore, the DDRP contains wide range of flavonoids (Schieber et al., 2005) with strong antioxidant properties. Unlike the DHQ, DDRP are not purified substances. In order to exhibit their biological activity, the dose taken should be relatively higher than this of DHQ. It was determined that the DDRP better stimulate feed utilization in one later stage after the beginning of the experiment. It is likely that the pigs' organism needs some time to accumulate the DDRP biologically active components. Those phenomena may explain the best feed utilization in group R2. The observed reduction of LDLs in the blood in groups R1 and D1 by 13 – 14% is probably due to the maintenance of the blood redox state obtained from the synergistic action of the antioxidant compounds of DHQ and DDRP and vitamins C, and E in the blood stream. As a result, the sensitivity of LDL to oxidation is reduced (Kawabata et al., 2015).

The TRIG and T CHOL increasing in groups R1 and D1 suggested that effects of DHQ and DDRP were dose-dependent. Probably the DHQ and DDRP polyphenols positively modified lipoprotein profile, didn't change the fattiness of liver and ameliorate an antioxidant status in blood circulation (Guil-Guerrero et al., 2016).

The potent hepatoprotective activity of DHQ and DDRP could be assumed due to the lower relative liver weight in groups R1 and D1. It could be explained with inhibition of lipids free-radical oxidation which caused an increasing of antioxidant protection of cell membrane phospholipids of liver tissue cells during the pigs fattening period (Bogolyubova et al., 2019). The flavonoids such as DHQ improved lipid accumulation, inflammatory response and the levels of cellular antioxidants in liver (Kawabata et al., 2015). They had ability to influence various enzymes and their antioxidant properties (Manach et al., 2005).

An explanation for the large deviations of estradiol in the blood can be given for the following reasons. The hormone estradiol is the biologically most active estrogen mainly formed by the ovarian follicles of the yellow body (Reed and Carr, 2018). Estradiol levels increase during the follicular phase and depend on the growth and development of the ovarian follicle (Kevenaar et al., 2007). They increase by the time of ovulation, and again when the yellow body is formed and decrease at the end of the menstrual cycle (Reed and Carr, 2018).

The determined  $\text{pH}_{45\text{min}}$  and  $\text{pH}_{24\text{h}}$  values of two studied muscles didn't indicate stress-induced muscle damage. The meat quality and nutritional value depended on the ratio of its chemical constituents which was an evidence that the used phytonutrients had no effect on the proximate composition of pork m. *Longissimus thoracis* and m. *Semimembranosus*. We suppose this was due to intramuscular fats in studied Danube white pigs were less than 1%.

## Conclusions

The present findings demonstrate that supplementation of pigs' diet with *Larix sibirica* dihydroquercetin or dry distilled rose (*Rosa damascena*) petals can improve the growth performance and have a positive effect on the feed to gain ratio without adversely affecting the blood parameters, carcass composition and muscle pH. It seems that lower dose (3.5 mg) of DHQ/kg/d and higher dose (545 mg) of DDRP/kg/d are more effective compared to higher dose (7.5 mg) DHQ/kg/d and lower dose (252 mg) of DDRP/kg/d. Absence of histological changes in the liver and ovary shows that both phytonutrients are not toxic for the organism of the pigs. Finally, the indicated doses of supplementation with the two phytonutrients show potential to be used as growth promoters of natural origin and with a favorable effect on other variables such as blood metabolites and meat quality due to their bioactive properties.



### Acknowledgements

*Funding acknowledgement statement:* This study was supported by the Bulgarian National Science Fund (BNSF), Ministry of Education and Science of Republic of Bulgaria of state contract No DN 06/8 of December 17, 2016 “Study of the mechanism of biological active compounds of plant origin accumulation in the organism of Bulgarian breed agricultural animals and their impact on the meat quality as a natural functional food”.

We thank to Mr. Hristo Nestorov for his help and dihydroquercetin donation. The authors are grateful to Flavitlife Bio JSCo (Sofia, Bulgaria) and Damascena rose oil distillery, village of Skobelevo, municipality of Pavel Banya, Stara Zagora district subdivision of Bulattars Production Company Ltd (Sofia, Bulgaria) for their support and help.

### References

- Anwar, M.N., Ravindran, V., Morel, P.C.H., Ravindran, G. & Cowieson, A.J. (2016). Measurement of true ileal calcium digestibility in meat and bone meal for broiler chickens using the direct method. *Poult Sci.*, 95(1), 70-76.
- AOCS (American Oil Chemists' Society) (2004). Official methods and recommended practices of the American Oil Chemists' Society, 5th ed. Champaign, IL, USA: American Oil Chemists Society.
- Artem'eva, O.A., Pereselkova, D.A. & Fomichev, Yu.P. (2015). Dihydroquercetin, the bioactive substance, to be used against pathogenic microorganisms as an alternative to antibiotics. *Sel'skokhoz Biol [Agric Biol]*; 50(4), 513-519.
- Bogolyubova, N.V., Chabaev, M.G., Fomichev, Yu.P., Tsis, E.Yu., Semenova, A.A. & Nekrasov, R.V. (2019). Ways to reduce adverse effects of stress in pigs using nutritional factors. *Ukr J Ecol.*, 9(2), 239-245.
- Costa, L.B., Luciano, F.B., Miyada, V.S. & Gois, F.D. (2013). Herbal extracts and organic acids as natural feed additives in pig diets. *S Afr J Anim Sci.*, 43(2), 181-93.
- Dragoev, S., Vlahova-Vangelova, D., Balev, D., Bozhilov, D. & Dagnon, S. (2021). Valorization of waste by-products of rose oil production as feedstuff phytonutrients. *Bulg J Agric Sci.*, 27(1), 209-219.
- 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(1), 140-159.
- Guil-Guerrero, J.L., Ramos, L., Moreno, C., Zúñiga-Paredes, J.C., Carlosama-Yépez, M. & Rualesa, P. (2016). Plant-food by-products to improve farm-animal health. *Anim Feed Sci Technol.*, 220(10), 121-135.
- Hashemi, S.R. & Davoodi, H. (2011). Herbal plants and their derivatives as growth and health promoters in animal nutrition. *Vet Res Commun.*, 35(3), 169-180.
- Kawabata, K., Mukai, R. & Ishisaka, A. (2015). Quercetin and related polyphenols: new insights and implications for their bioactivity and bioavailability. *Food Funct.*, 6(5), 1399-1417.
- Kevenaar, M.E., Themmen, A.P.N., Laven, J.S.E., Sonntag, B., Fong, S.L., Uitterlinden, A.G., de Jong, F.H., Pols, H.A.P., Simoni, M. & Visser, J.A. (2007). Anti-Müllerian hormone and anti-Müllerian hormone type II receptor polymorphisms are associated with follicular phase estradiol levels in normo-ovulatory women. *Hum Reprod.*, 22(6), 1547-1554.
- Lesser, S., Cermak, R. & Wolffram, S. (2006). The fatty acid pattern of dietary fat influences the oral bioavailability of the flavonol quercetin in pigs. *Br J Nutr.*, 96(6), 1047-1052.
- Manach, C., Williamson, G., Morand, C., Scalbert, A. & Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr.*, 81(1), 230S-242S.
- Nowak, R., Olech, M., Pecio, L. & Oleszek, W. (2014). Cytotoxic, antioxidant, antimicrobial properties and chemical composition of rose petals. *J Sci Food Agric.*, 94(3), 560-567.
- Reed, B.G. & Carr, B.R. (2018). The normal menstrual cycle and the control of ovulation. In: Feingold, K.R., Anawalt, B., Boyce, A., Chrousos, G., Dungan, K., Grossman, A., Hershman, J.M., Kaltsas, G., Koch, C., Kopp, P., Korbonits, M., McLachlan, R., Morley, J.E., New, M., Perreault, L., Purnell, J., Rebar, R., Singer, F., Trencé, D.L., Vinik, A., Wilson, D.P. (eds). Endotext, USA: MDText.com, Inc., 1-33.
- Schieber, A., Mihalev K., Berardini N. & Mollov P. (2005). Flavonol glycosides from distilled petals of *Rosa damascena* Mill. *Z Naturforsch C J Biosci.*, 60(5-6), 379-384.
- Valenzuela-Grijalva, N.V., Pinelli-Saavedra, A., Muhlia-Almazan, A., Domínguez-Díaz, D. & González-Ríos, H. (2017). Dietary inclusion effects of phytochemicals as growth promoters in animal production. *J Anim Sci Technol.*, 59(8), 1-17.
- 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(3), 5624-5633.
- Warriss P.D. (2010). Animal welfare. In: Warriss, P.D. (ed). Meat science: An introductory text. USA: CABI, 151-162.
- Weidmann A.E. (2012). Dihydroquercetin: More than just an impurity? *Eur J Pharmacol.*, 684(1-3), 19-26.
- Weiser G. (2012). Laboratory technology for veterinary medicine. In: Thrall, M.A., Weiser, G., Allison, R., Campbell, T. (eds). *Veterinary hematology and clinical chemistry*, 2nd ed. USA: John Wiley & Sons Inc., 3-33.

*Received:* September, 14, 2020; *Accepted:* February, 11, 2021; *Published:* December, 2021