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EFFECT OF DIHYDROQUERCETIN ON PERFORMANCE, BACK FAT THICKNESS AND BLOOD BIOCHEMICAL INDICES IN FATTENING PIGS

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Abstract

The study aimed to investigate the effect of gradient levels of 100 mg and 200 mg dihydroquercetin DHQ/kg feed added on performance, back fat thickness, and blood biochemical indices in fattening pigs. An experiment with 30 pigs of the Danube White breed with an initial live weight of 66.3 - 66.5 kg and a final live weight of 100.9 - 102.8 kg, randomly assigned to three treatments – control(C), DHQ1 and DHQ2, was carried out. Pigs were housed individually for 43 days. At the end of the experiment, the thickness of the back fat was measured, and blood samples were taken. Biochemistry indices and fat metabolism indices were studied. Administration of dihydroquercetin did not affect parameters of pig performance in fattening period. The addition of two consecutive levels of DHQ increased MLT, measured in vivo, linearly (P=0.025). The blood glucose content was linearly reduced (P<0.05). A statistically significant effect on high-density lipoproteins (HDL) in animals treated with 200 mg of DHQ (P=0.012), having a high linear dependence (P=0.007) was found, and a trend to reduce the content of triglycerides in the blood of fattening pigs.

Key words*: ADG, back fat thickness, biochemical indices, dihydroquercetin, fat metabolism, FCR, pigs.*

INTRODUCTION

Dihydroquarcetin (DHQ, also known as Taxifolin) is a powerful natural antioxidant and capillary protector related to bioflavonoids with P-vitamin activity. As a substance with a high degree of biological activity, DHQ has a whole range of positive (pleiotropic) effects on metabolic reactions and the dynamics of various pathological processes, which were identified in a number of studies by Russian and foreign scientists, in particular, in terms of antioxidant, radioprotective, membrane-protective, capillary-protective, angioprotective, lipid-lowering, anti-inflammatory, anti-allergic, cardioprotective, hepatoprotective, detoxifying, neuroprotective, gastroprotective, immunomodulatory, retinoprotective, endocrinological properties (Fomichev et al., 2017; Sunil & Xu, 2019; Liu et al., 2023). DHQ is known for preventing stress syndrome and chronic fatigue, restoring and improving the state of the body under high physical and psycho-emotional stress (Plotnikov et al., 2005).

The introduction of DHQ in the feeding of farm animals and poultry has a positive effect on immunodeficiency, broncho-pulmonary diseases and disorders in the functional state of the liver and other organs, which are usually a consequence of the impact of adverse environmental factors and modern breeding technologies, inadequate to the physiology of farm animals. Positive effects as improving productivity, survival, food safety, reducing the incidence of animal diseases, normalizing metabolic processes in the body and the functional state of the liver have been noticed when DHQ take place in animals' diets (Nikanova & Fomichev, 2012; Bogolyubova et al., 2019; Zou et al., 2016b). Dihydroquercetin is interesting for pig breeding as adaptogen positively affecting the antioxidant status of animals (Semenova et al., 2020). It was established that the use of dihydroquercetin in pig nutrition blocks lipid peroxidation processes throughout the growing and fattening period (Fomichev et al., 2017). In another experiment, it was found that quercetin attenuated oxidative stress and reduced intestinal inflammation, while reducing the number of reactive oxygen species and malondialdehyde in the intestine, endotoxins in the blood serum, and increasing

the height of jejunal villi (Zou et al., 2016a). Recently, a series of new 3-monoacylated dihydroquercetin derivatives with enhanced antioxidant properties have been synthesized (Knyazev et al., 2018). Dihydroquercetin can affect lipid metabolism by regulating enzyme activity, reduce hepatic fat synthesis, inhibit intracellular cholesterol synthesis, and inhibit cholesterol esterification, triacylglycerol and phospholipid synthesis (Ren et al., 2021; Wang et al., 2022).

There is no consensus on the effective doses of DHQ for effects on the organism of farm animals. Both insufficient data and differences exist in the literature. We worked with doses of 3.5 mg/kg and 7.5 mg/kg live weight in order to improve the quality of the meat by enriching it with 95% purified biologically active additive DHQ (Ivanova et al., 2021a). Kuzmina et al. (2021) applied DHQ, produced by the Russian company Ametis, according to their recommendation instructions, to broiler chickens, in amounts of 0.50 g, 0.75 g and 1.00 g per 100 kg of feed, with the best results in terms of fattening and slaughtering qualities at the dosage of 1 g of DHQ per 100 g of feed. At the same time, Pirgozliev et al. (2021), applying DHQ to broiler feed diets at doses of 0.5 g, 1.5 g and 4.5 g per 1 kg of feed, concluded that it could be beneficial at levels, greater than 1.5 g/kg feed, due to the improved antioxidant status of the birds.

The aim of the present study was to test the effect of two gradient levels of dihydroquercetin on weight development, back fat thickness and biochemical parameters in fattening pigs.

MATERIALS AND METHODS

One trial with total 30 pigs of the Danube White breed was carried out in the Experimental Unit of the Agricultural Institute - Shumen, randomly assigned into three group, as follows:

1. Control group (C) - 10 pigs, without added biologically active component to the feed;
2. Experimental group 1 (DHQ1) - 10 pigs, with added 100 mg dihydroquercetin/kg feed;
3. Experimental group 2 (DHQ2) - 10 pigs, with added 200 mg dihydroquercetin/kg feed.

Increasing doses of DHQ formulated as gradient levels were used, with two values, to determine whether there was a linear relationship on weight and biochemical parameters.

Table 1. Component composition and content of energy and nutrients in 1 kg of compound feed

Components	Persentage (%)
Maize	13.00
Barley	10.00
Wheat	50.00
Wheat brains	7.00
Bioconcentrate BC14*	25.00
Total:	100%
1 kg of compound feed contents:	
Digestible energy, MJ	13.72
Crude protein, %	15.70
Lysine, %	0.72
Calcium, %	0.86
Phosphorous, %	0.60

*Bioconcentrate BC14 contents: 312.10 g/kg crude protein, 10.70 g/kg crude fats, 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 phosphorous, 2680 mg/kg Cu sulphate, 670 mg/kg dl-α-tocopherol, 93800 UI/kg vitamin A, 16080 UI/kg vitamin D3, 1975,845 kcal/kg total energy.

The pigs for the experiment were selected and equalized by origin, age, live weight, sex, immediately after weaning. After reaching an average weight of 66 kg, beginning of finishing phase, all pigs were weighed and moved to a room with individual partly slatted pens. Pigs have a total area of 2.50 m^2 with a solid concrete part (150 x 100 cm) and slatted part (100x100 cm). All pens were equipped by an individual feeder (100 x 50 cm) and an individual nipple drinker. During the fattening period, the animals were raised according to the requirements of Council Directive 2008/120/EC laying down minimum standards for the protection of pigs. They were fed a special diet, which was analysed in the Forage Laboratory of the Agricultural Institute – Shumen. The feed of the animals was weighed individually, twice a day. Pigs from the two experimental groups received DHQ, according to the amount of feed consumed for the day, in two concentrations described above. A trial of the studying effect of dihydroquercetin started from 66 kg live weight until the fattening pigs reached about 100 kg and lasted for 43 days.

The following indicators were controlled: initial and final live weight, daily diet, feed consumption - daily, individual, total and average daily gain for the period, feed utilization, health status.

A chemical analysis was performed on diet based on corn, barley, wheat bran and Bioconcentrate. The chemical composition of the feed

samples was determined according to the methods adopted in Agricultural Institute - Shumen.

Each pig was fed individually, with a daily check for the presence of residues. During the experiment, none were found. The supplement was also stretched individually for each pig according to the daily feed intake. Each daily dose of the supplement was mixed with a small amount of feed immediately after weighing and given at the same time as the animals' morning feed.

The weight development of the animals was measured in the beginning and at the end of the fattening period. The total gain, the average daily gain (ADG) and the feed consumption per kilogram of gain were calculated for each pig separately.

Back fat thickness and lean meat percentage *in vivo* were determined using a "Piglog 105" apparatus (Carometec Food Technology A/S, Kolding, Denmark). The following regression model was used:

LM=63.8662-0.4465x1-0.5096x2+0.1281x3

where:

LM - percentage of lean meat in the carcass;

X_1 - back fat thickness measured between the 3-4 lumbar ribs at 7 cm laterally (mm);

X_2 - back fat thickness measured between the 3-4 lumbar ribs at 7 cm laterally (mm);

X_3 - thickness of *m. Longissimus thoracis* (MLT) between the 3-4 lumbar ribs at 7 cm laterally (mm).

In vivo lean meat content was measured on the day of termination of the experiment when animals reached 100 kg live weight.

The health status of the pigs was monitored. Blood sampling was performed at the end of the experimental period, with vacuum containers from the *sinus ophtalmicus* in the medial corner of the eye. Immediately after blood collection, the containers were transferred to the laboratory and centrifuged to separate the serum at 3000 rpm for 15 min at 4°C. After separation of the serum, it was frozen at -20˚C. Analyses of biochemical parameters were performed in a laboratory with special porcine kits with an Olympus AU640 apparatus (Beckman/Olympus counter) according to methods approved by the International Federation for Clinical Chemistry (IFCC). Biochemical indicators of liver enzymes Alanine aminotransferase (ALAT) and Aspartate aminotransferase (ASAT) were analysed by UV kinetic method; the total protein in the blood, by photometric colorimetric method. The indicators responsible for carbohydrate and protein metabolism were also investigated: glucose content by the hexokinase glucose-6-phosphate dehydrogenase method, creatinine - by the Jaffe kinetic method and urea by the enzymatic method (Urease/GLDH).

The lipid profile was studied - contents of total cholesterol, LDL, HDL cholesterol and triglycerides in the blood serum. The content of total cholesterol and triglycerides was determined by an enzymatic colorimetric method, and LDL and HDL - by a direct method.

Statistical analysis of the results

The experiment was based on a 2 x 2 factorial design (sex of pigs x dose of dihydroquercetin). Data were analyzed using the statistical software package Genstat (21st edition) (IACR Rothamstead, Hertfordshire, UK). Comparisons between study variables were performed by one-way ANOVA analysis (with spatial blocks) followed by Duncan's multiple range test. All data were checked for homogeneity of variances and normality before conducting ANOVA. An orthogonal polynomial test was used to check if there is a potential linear relationship between the increase in DHQ levels and the increase in the values of the investigated parameters. In all cases, differences between groups were reported as significant at $P<0.05$

RESULTS AND DISCUSSIONS

During the experimental period, no difference in feed consumption was found both between groups and within groups between individuals (Table 2). In total, for the research period of 43 days from the end of October to the beginning of December 2021, a feed intake of 3.186 kg/pig/day was recorded. Similar feed consumption was recorded in our previous study (Ivanova et al., 2021a) - 3.095 kg/pig/day in the group consuming 7.5 mg DHQ per kg live weight per day. No statistically significant differences were found in the final live weight of the fattening pigs between the groups with different levels of DHQ tested and the control group, indicating that the intake of DHQ had no effect on the weight development of the animals. No differences were found between male and female animals and in ADG as well ($P>0.05$). In

contrast to our study, growth-stimulating effect of DHQ was found in Russia when the preparation "Ekostimul-1" (containing about 80% DHQ) was administered to 4 groups of weaned pigs of the Large White breed after weaning at the age of 60 days for 30 days (Fomichev et al., 2016). In two of the experimental groups, there was an increase in ADG with a mean value of 540 g, which was 97 g or 21.8% higher than in the control group (P<0.001). Moreover, the combined use of DHQ with probiotics increased the average daily gain of suckling piglets by 21.5% (P<0.001), while reducing feed consumption per 1 kg of gain by 17.7%, reducing the number of disorders in the digestive system for the entire experimental period by an average of 45.6%, and increases the economic effect by 16.5% (Fomochev et al., 2017). The inclusion of another feed supplement with DHQ (Ekostimul-2, containing an extract of Daurian larch with 80% DHQ and 20% other antioxidants) in the diet of pigs in the post-weaning period in a dosage of 50 mg/head/day significantly weakened the effect of stress factors of the environment and increased the adaptive capacity of animals (Nikanova & Fomichev, 2012). As a result, ADG in the post-weaning period was 20.6% higher than in control individuals.

Regarding feed utilization, no statistically significant differences were found in this study. The application of DHQ resulted in a reduction of feed required to form 1 kg body mass by 4.80% in DHQ1 and by 4.22% in DHQ2 (Table 2). The values of the indicator itself were relatively high, due to the temperature in the room in the autumn-winter period. Different levels of DHQ generally had an effect on back fat thickness in this study. Small differences were observed in the thickness of the fat at point X_2, where it was the thinnest in DHQ2 - by 5.67% compared to the C and by 1.42% compared to the DHQ1. In another study carried out by us (Ivanova et al., 2021a), with the addition of DHQ to the feed of fattening pigs in an amount of 7.5 g/kg live weight, a reduction of back fat by 15.49% was found at point X_1 (P<0.05). Similarly, in a study of Yordanova et al. (2022) in fattening pigs, a 12.02% reduction in back fat at point X2 was found, when adding 7.5 g per head per day apple pectin (containing polyphenols). An effect of supplementation with active components polyphenols was also reported in our study with entire male pigs (Ivanova et al., 2021b). A 33.5% (P<0.0001) and 18.32% reduction in the point X_2 (n.s.) of the back fat were reported. This effect of dihydroquercetin is likely due to its composition. Numerous human nutrition studies have found that polyphenols have the property to affect adipose tissue, decreasing its content (Hu et al., 2020; Singh et al., 2020; Aloo et al., 2023).

Table 2. Performance and back fat thickness measurements *in vivo* (PigLog 105) at two levels of DHQ in fattening pigs

Variables	Parameters								
	Initial live weight (kg)	Final live weight (kg)	Total gain (kg)	Av. daily gain (kg)	FCR[a] (kg)	X_1[b], (mm)	X_2[b] (mm)	X_3[c] (MLT) (mm)	LM[d] (%)
Sex									
Female (F)	64.9	102.7	36.40	0.847	3.736	17.3	12.60	47.3	55.79
Male (M)	68.0	101.3	34.75	0.808	3.900	19.4	14.93	44.0	53.01
SEM	2.32	2.23	1.065	0.025	0.128	1.44	0.856	1.70	0.964
DHQ (dihydroquercetin)									
Level 0[e]	66.5	100.9	34.43	0.801	3.936	18.5	14.10	41.6	53.92
Level 1[e]	66.3	102.8	33.50	0.848	3.747	18.2	13.90	47.3	54.72
Level 2[e]	66.5	102.3	35.85	0.834	3.770	18.3	13.30	48.2	54.36
SEM	2.85	2.73	1.304	0.030	0.157	1.77	1.048	2.08	1.180
Sex.DHQ									
Fx0	68.2	102.2	34.00	0.791	4.019	17.4	12.80	43.4	55.10
Fx1	65.4	104.8	39.40	0.916	3.409	16.4	12.40	47.8	56.36
Fx2	61.0	96.8	35.80	0.833	3.779	18.0	12.60	50.8	55.92
Mx0	64.7	99.6	34.86	0.811	3.854	19.6	15.40	39.7	52.74
Mx1	67.3	100.8	33.50	0.779	4.086	20.0	15.40	46.8	53.08
Mx2	71.9	107.8	35.90	0.835	3.761	18.6	14.00	45.6	53.20
SEM	4.03	3.85	1.844	0.043	0.222	2.50	1.482	2.94	1.669

CV%	13.6	8.5	11.6	11.6	13.0	30.5	24.1	14.2	6.9
Probabilities									
Sex	0.285	0.645	0.285	0.285	0.373	0.307	0.066	0.158	0.052
DHQ	0.540	0.878	0.540	0.540	0.654	0.993	0.855	0.051	0.880
LIN[g]	0.449	0.720	0.449	0.449	0.461	0.937	0.594	0.025	0.705
Sex.DHQ	0.155	0.121	0.155	0.155	0.151	0.836	0.855	0.746	0.962
Interaction	0.838	0.090	0.838	0.838	0.743	0.752	0.689	0.781	0.915

Legend: a - FCR - Feed conversion ratio;
b - thickness of back fat in points X_1 and X_2;
c - X_3 (MLT) - Thickness of *m. Longissimus Thoracis* (MLT);
d - LM - lean meat percentage;
e - Level 0 - control group, without DHQ; level 1-100 g DHQ/kg feed; level 2-200 g DHQ/kg feed;
f - probability trough F criterion of Fisher;
g - LIN - linearity - linear raising of parameters at linear increase of amount of DHQ added to feed.

In this study, a trend for the effect of dihydroquercetin on the thickness of MLT, measured *in vivo* was found. Muscle thickness was higher in both DHQ-fed groups, by 12.05% and 13.69%, resp. than in the C group. It was very close to significance (P = 0.051), obeying a linear relationship (P = 0.025), and indicating that the addition of two successive levels of DHQ increased the value of the thickness of MLT in a gradient manner. This is an interesting result and has not been reported before. It indicating that, despite minimal differences in back fat thickness, and lean meat percentage, there is an effect of DHQ on MLT thickness. This important characteristic has a favourable effect on consumer expectations for a quality product. A similar result was found in our previous study with DHQ in fattening pigs, but with an inverse relationship regarding the increase in the amount of supplement in the diet. The dosage of 3.5 mg/kg live weight significantly increased MLT by 13.37% (P<0.05), and the dosage of 7.5 mg/kg by 6.63% (n.s.). In addition, dihydroquercetin may have a beneficial effect in the prevention of myopathic changes in the structure and proportion of muscle fibres in the MLT (Semenova et al., 2020). This result was found in a study of fattening pigs fed with a DHQ supplement at a dose of 40 mg/kg, given as an adaptogen to test the effect of a modelled technological stress on the state of muscle tissue.

The percentage of lean meat measured *in vivo* did not follow the trend found in MLT, i.e. no statistically significant differences were observed between the experimental and control groups. A trend was found for the effect of sex on the percentage of lean meat in female animals in the experiment (P = 0.052). Greater MLT thickness of 6.97% was observed in female animals. The percentage of lean meat was also higher in females compared to males - 2.78% in absolute terms. A statistically significant higher percentage of lean meat (*in vivo*) was found in our previous study using polyphenols (dry residue of distilled rose petal) in male entire pigs, and the differences with the castrated control group were significant (P = 0.006), but the trial ended at a higher live weight of the animals (Ivanova et al., 2021b).

The normal functioning of cells, organs and the body as a whole is maintained by homeostasis. The content of glucose in the blood is regulated by a multicomponent neuroendocrine complex, in which the sugar-lowering factor is insulin, and the sugar-raising factor is adrenaline, glucagon and glucocorticoids. In our study, as it is showed in Table 3, a dependence on DHQ intake was found to reduce blood glucose by 9.73% in the group of pigs receiving 100 mg/kg feed DHQ and by 11.09% in the group of pigs receiving 200 mg/kg feed DHQ (P<0.05). With this indicator, a linear dependence (P = 0.022) was established, which shows that the blood glucose content decreases linearly with an increase in the DHQ dose. The administration of dihydroquercetin in diets of the experimental groups played a role of protected liver function. Aa a result, a reduced glucose synthesis was observed within the physiological norm and an increased glucose content in the animals of the control group, demonstrating the hypoglycemic effect of DHQ. Our results were in line with those of Bule et al. (2019), who in a recent systematic review of the available literature in different animal species, using the method of meta-analyses, showed that quercetin reduced serum glucose levels at doses of 10, 25 and 50

mg/kg per kg body weight in fattening pigs. Studies by Fomichev et al. (2017) showed that supplementation of DHQ in diets of young pigs maintained blood glucose content within the physiological range, while in control pigs it was higher and may suggest increased function of the adrenal cortex. In relation to this, a presence of gluconeogenesis may appear. The trend we found also coincided with the action of flavonoids found in humans, which could regulate glucose metabolism, liver enzyme activity and lipid profile (Al-Ishaq et al., 2019), thus ameliorating the pathogenesis of diabetes and its complications. Vessal et al. (2003) also found that quercetin supplementation for two weeks reliably lowered blood glucose levels, increased the expression of genes involved in cell survival and proliferation in the liver, and enhanced serum insulin in STZ-induced diabetic mice.

Table 3. Biochemical indices in fattening pigs fed two levels of dihydroquercetin

Variables	Parameters									
	GLU	TRI	CHOL	HDL	LDL	ALT	ASAT	TotProt	CRE	UREA
Sex										
Female (F)	4.95	0.321	2.527	1.149	1.213	58.7	36.0	65.79	119.8	6.61
Male (M)	4.61	0.314	2.465	1.113	1.207	54.9	29.4	66.77	113.9	5.56
SEM	0.134	0.016	0.064	0.033	0.048	2.07	4.20	1.004	2.46	0.257
DHQ (dihydroquercetin)*										
Level 0	5.14[b]	0.334	2.400	1.070[a]	1.187	57.7	32.9	67.13	113.1	5.93
Level 1	4.64[a]	0.339	2.447	1.084[a]	1.188	53.5	29.6	64.71	121.1	5.90
Level 2	4.57[a]	0.280	2.640	1.239[b]	1.254	59.2	35.6	67.01	116.3	6.42
SEM	0.164	0.020	0.078	0.040	0.058	2.54	5.15	1.230	3.02	0.314
Fx0	5.30	0.318	2.340	1.066	1.144	60.8	41.8	67.32[ab]	115.8	6.32
Fx1	4.72	0.346	2.520	1.116	1.212	52.8	28.4	62.00[a]	122.2	6.68
Fx2	4.84	0.300	2.720	1.266	1.282	62.4	37.8	68.06[b]	121.4	6.82
Mx0	4.98	0.350	2.460	1.074	1.230	54.6	24.0	66.94[ab]	110.4	5.54
Mx1	4.56	0.333	2.375	1.052	1.165	54.2	30.8	67.42[b]	120.0	5.12
Mx2	4.30	0.260	2.560	1.212	1.226	56.0	33.4	65.96[ab]	111.2	6.02
SEM	0.232	0.028	0.110	0.057	0.082	3.59	7.28	1.739	4.27	0.444
CV%	10.8	19.9	9.9	11.3	15.2	14.1	49.8	5.9	8.2	16.3
Probabilities[c]										
Sex	0.085	0.759	0.500	0.441	0.934	0.215	0.278	0.497	0.102	0.008
DHQ	0.043	0.089	0.092	0.012	0.655	0.277	0.714	0.310	0.190	0.434
LIN[d]	0.022	0.069	0.040	0.007	0.425	0.124	0.714	0.946	0.096	0.281
Sex.DHQ	0.716	0.449	0.377	0.795	0.534	0.475	0.384	0.098	0.646	0.612
Interaction	0.508	0.848	0.519	0.686	0.668	0.227	0.295	0.037	0.456	0.327

Legend: * - 0 – control group, without DHQ; level 1 – 100 g DHQ/kg feed; level 2 – 200 g DHQ/kg feed;
Statistically significant differences are marked with different letters, as follows: a, b – probability at $P < 0,05$; c – probability trough F criterion of Fisher; d – LIN – linearity – linear raising of parameters at linear increase of amount of DHQ added to feed.

The addition of dihydroquercetin to the feed of fattening pigs in our experiment greatly had effect on their fat metabolism, with almost all indicators being affected in the direction of reduction. The results of the study (table 3) show that the intake of 200 mg of DHQ in fattening pigs had a significant effect on the content of triglycerides in the blood. A trend was found for their reduction by 16.17% ($P<0.01$), and it was more pronounced in male animals, which values were lowered by 25.71% ($P<0.01$). Total cholesterol values, however, showed a linear increase ($P=0.040$) with increasing DHQ dose, with a more pronounced effect at the higher DHQ dose (200 mg/kg feed). The blood cholesterol content of all pigs consuming the supplement at this dose was higher than that of control pigs by 9.09% (n.s.). The effect of gender was shown, with the difference being higher in females (13.97%) compared to males (3.90%). Interestingly, the trend shown for the recorded increase in blood cholesterol content was at the expense of the so-called "good" or high-density cholesterol (HDL). This increase of 13.64% in DHQ2 animals showed high probability ($P = 0.012$) and high linear

dependence (P = 0.007). This means that the gradient levels of dihydroquercetin raised the content of "good" cholesterol. Differences in this indicator by gender between the treated and control groups were in favour of female animals as well (15.80% vs. 11.39% in males).

It is evident from the literature that very few studies have been conducted regarding the effects of quercetin and especially dihydroquercetin on lipid metabolism in pigs. Furthermore, the interpretation of the results of the present study suggests that dihydroquercetin lowers blood lipids to levels similar to the effects of other flavonoids, and that the results for blood serum cholesterol content are different from organ cholesterol content. A much more detailed study showed that quercetin reduced triglyceride content via the PPAR signalling pathway in primary hepatocytes of broiler chickens (Wang et al, 2019). Moreover, flavonoids from sea buckthorn fruit had a quadratic effect on the content of triglycerides and VLDL in the liver of broiler chickens (Han et al., 2009). In a study of Tang et al. (2013), quercetin treatment significantly reduced cholesterol content in the liver, heart, kidney and small intestine of rats. Quesada et al. (2009) reported that effect of treatment with grape seed procyanidin extract caused slightly reduction in triglyceride and cholesterol content in the liver of rats. Zhai et al. (2016) found that quercetin reduced serum triglyceride content in tilapia (freshwater fish), which may be useful to avoid pathological changes in fatty liver. Kuipers et al. (2018) also reported that quercetin reduced plasma triglyceride level in 9-week-old mice. Additionally, a flavonoid from hawthorn leaf extract significantly reduced serum cholesterol, triglycerides, and very high-density lipoprotein (VHDL) cholesterol levels in mice.

In our study, no statistically significant differences were found between the control and experimental groups, as well as between the two groups with different levels of DHQ supplementation, regarding the values of the liver enzymes AST and ALT (table 3), which give an idea of the production of enzymes in organism. Under physiological conditions, these enzymes are present in small amounts in the peripheral blood. ALT is a specific marker of the functional state of the liver, and an increase in AST activity is characteristic of disorders of the functions of the cardiovascular system. In our study, dihydroquercetin did not affect the levels of these enzymes in the blood, but in another study with pigs, it was found that after administration of the larch bioflavonoid complex, the activity of alanine aminotransferase decreased by 12.8%, of aspartate aminotransferase by 23, 5% (Fomichev et al., 2017).

A statistically significant difference between the groups as well as an interaction (P=0.037) was found for total blood protein content, with female animals in DHQ1 group having a lower total protein by 8.90% of the other experimental group DHQ2, but both groups had no statistically significant differences with the control group. No differences were found due to the administration of dihydroquercetin in the indicators of protein metabolism, creatinine and urea content in the blood, as well. According to the last indicator, a statistically significant difference of 15.88% was found between male and female animals in favour of females (P=0.008), but such a difference is a gender feature that is also characteristic of humans (Liu et al., 2021).

During the entire study, for the entire experimental period of 43 days, no cases of animal disease were recorded, i.e. Throughout the study, clinically healthy animals were used, which is evident in the values of the biochemical indicators, all of them being within the reference limits. This may be the reason why the activity-stimulating action of dihydroquercetin in the direction of stabilization and improvement of the work of all organs and systems was not manifested. The biologically active substance DHQ was used in animal husbandry, especially when the farming of animals was carried out in areas contaminated with anthropogenic heavy metals (Pb, Cd, As, Hg and others) and radionuclides (90Sr, 137Cs) or they were exposed to pollution from industrial enterprises from the chemical, metallurgical, petrochemical and other industries (Fomichev et al., 2016).

CONCLUSIONS

Administration of dihydroquercetin did not affect parameters of pig performance in fattening period from 66 to 100 kg live weight.

The addition of two consecutive levels of DHQ increased MLT, measured *in vivo*, linearly (P=0.025).

Dihydroquercetin had a positive effect on carbohydrate metabolism in fattening pigs in the direction of reducing blood glucose content, with linear reliability at both levels used 100 mg/kg and 200 mg/kg feed (P<0.05).

The intake of DHQ affected the fat metabolism of pigs. The addition of DHQ at a dose of 200 mg/kg feed to the diets of fattening pigs showed a trend to reduce the content of triglycerides and linearly increase total cholesterol (P<0.05), and this increase was at the expense of high-density cholesterol HDL (P<0.05).

REFERENCES

AL-Ishaq, R., Abotaleb, M., Kubatka, P., Kajo, K. & Büsselberg, D. (2019). Flavonoids and their anti-diabetic effects: cellular mechanisms and effects to improve blood sugar levels. *Biomolecules*, *9*, 430. https://doi.org/10.3390/biom9090430

Aloo, S.O., Ofosu, F., Kim, N.H., Kilonzi, S.M. & Oh, D.H. (2023). Insights on Dietary Polyphenols as Agents against Metabolic Disorders: Obesity as a Target Disease. *Antioxidants, 12*, 416. https://doi.org/10.3390/antiox12020416

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.

Bule, M., Abdurahman, A., Nikfar, S., Abdollahi, M. & Amini, M. (2019). Antidiabetic effect of quercetin: A systematic review and meta-analysis of animal studies. *Food and Chemical Toxicology*, *125*, 494-502.

Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs, https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32008L0120, last accessed 18.04.2024.

David, A., Arulmoli, R. & Parasuraman, S. (2016). Overviews of Biological Importance of Quercetin: A Bioactive Flavonoid. *Pharmacogn Rev.*, *10*(20), 84-89.

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. *Journal of International Scientific Publications: Agriculture & Food*, *4*, 140-159.

Fomichev, Y.P., Nikanova, L.A., Dorozhkin, V.I., Torshkov, A., Romanenko, A., Eskov, E., Semenova, A., Gonotsky, V., Dunaev, A., Yarosevich, G., Lashin, S. & Stolnaya, N. (2017). *Dihydroquercetin and arabinogalactan - natural bioregulators in human and animal life, application in agriculture and food industry*. Moscow, RU: Scientific Library Publishing House, p. 701.

Hu, J., Zhenyu, W. Bee, T. & Mark. C. (2020). Dietary polyphenols turn fat "brown": A narrative review of the possible mechanisms. *Trends in Food Science & Technology*, *97*, 221-232.

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

Ivanova, S., Stoyanchev, T., Nikolova, T. &Penchev, I. (2021b), Effect of addition of dry distilled rose petals in the diet on the meat quality in entire male pigs, *Journal of Central European Agriculture*, *22*(4), 678–691.

Knyazev V., Rogovskii, V., Sveshnikova E., Semeikin A., Matyushin, A., Fedotcheva T., Shimanovskii, N., Pozdeev, A., Koroteev, A. &Koroteev. M. (2018). Synthesis and Antioxidant and Cytotoxic Activity of New Dihydroquercetin Derivatives. *Pharmaceutical Chemistry Journal*, *52*(9) 205–208.

Kuipers, E.N, Dam, A.D.V., Held, N.M., Mol, I.M., Houtkooper, R.H., Rensen, P.C.N., & Boon, M.R. (2018). Quercetin Lowers Plasma Triglycerides Accompanied by White Adipose Tissue Browning in Diet-Induced Obese Mice. *Int. J. Mol. Sci.*, *16*, 19(6), 1786. doi: 10.3390/ijms19061786.

Kuzmina, N., Petrov, O., Karynbayev, A., & Alentayev, A. (2021). The efficiency of dihydroquercetin in the feeding of broiler chickens. *REPORTS, 2*, 13-17.

Liu, X, Liu, W., Ding, C., Zhao, Y., Chen, X., Ling, D., Zheng, Y., & Cheng, Z. (2021). Taxifolin, extracted from waste *Larix olgensis* Roots, attenuates CCl4-induced liver fibrosis by regulating the PI3K/AKT/mTOR and TGF-β1/Smads Signaling Pathways. *Drug Des Devel Ther*, *15*, 871-887.

Liu, Y., Shi, X., Tian, Y., Zhai, S., Liu, Y., Xiong, Z., & Chu, S. (2023). An insight into novel therapeutic potentials of taxifolin. *Front Pharmacol*, *12*, 14, 1173855. doi: 10.3389/fphar.2023.1173855.

Nikanova, L., & Fomichev, Y. (2012). The role of feed additives in mitigating environmental temperature stress in pigs, *Russian Journal of Problems of Veterinary Sanitation, Hygiene and Ecology*, *1*(7), 81-86 (Russian).

Pirgozliev, V., Mansbridge, S., Whiting, I., Arthur, C., Rose, S., & Atanasov, A. (2021) Antioxidant status and growth performance of broiler chickens fed diets containing graded levels of supplementary dihydroquercetin. *Res Vet Sci, 141*, 63-65.

Plotnikov, M., Tyukavkina, N., & Plotnikova, T. (2005). *Medicines based on dikvertin*. Tomsk, BG: Tomsk University Press Publishing House, 228.

Quesada, H., del Bas, J., Pajuelo, D., Díaz, S., Fernandez-Larrea, J., Pinent, M., Arola, L., Salvadó, M., & Bladé, C. (2009). Grape seed proanthocyanidins correct dyslipidemia associated with a high-fat diet in rats and repress genes controlling lipogenesis and VLDL assembling in liver. *Int J Obes*, *33*, 1007–1012.

Ren, L., Hao-Nan, G., Jun, Y., Xiao-Ying, G., Ye-Sheng, W., & Zhao, Y. (2021). Dissecting efficacy and metabolic characteristic mechanism of taxifolin on renal fibrosis by multivariate approach and ultra-performance liquid chromatography coupled with mass spectrometry-based metabolomics strategy. *Frontiers in Pharmacology, Renal Pharmacology*, 11. https://doi.org/10.3389/fphar.2020.608511

Semenova, A., Kuznetsova, T., Nasonova, V., Nekrasov, R., & Bogolubova, N. (2020). Effect of modelled stress and adaptogens on microstructural characteristics of pork from fast-growing hybrid animals. *Slovak Journal of Food Sciences*, *14*, 656–663.

Singh, M., Thrimawithana, T., Shukla, R., & Adhikari, B. (2020). Managing obesity through natural polyphenols: A review, *Future Foods*, *1–2*, 100002.

Sunil, C., & Xu, B. (2019). An insight into the health-promoting effects of taxifolin (dihydroquercetin). *Phytochemistry*, *166*, 112066. doi:10.1016/j.phytochem.2019.112066.

Tang, Y., Tian, H., Shi, Y., Gao, Ch., Xing, M., Yang, W., Bao, W., Di Wang, Liu, L., & Yao, P. (2013). Quercetin suppressed CYP2E1-dependent ethanol hepatotoxicity via depleting heme pool and releasing CO. *Phytomedicine*, *20*(8–9), 699-704.

Vessal, M., Hemmati, M., & Vasei, M. (2003). Antidiabetic effects of quercetin in streptozocin-induced diabetic rats. *Comp. Biochem. Physiol. Toxicol. Pharmacol.*, 135C(3), 357-364.

Wang, M., Xiao, F. L., Mao, Y. J., Ying, L. L., Zhou, B., & Li., Y. (2019) Quercetin decreases the triglyceride content through the PPAR signalling pathway in primary hepatocytes of broiler chickens, *Biotechnology & Biotechnological Equipment*, *33*(1), 1000-1010.

Wang, M., Han, H., Wan, F., Zhong, R., Do, Y. J., Oh, S.I., Lu, X., Liu, L., Yi, B., & Zhang, H. (2022). Dihydroquercetin Supplementation Improved Hepatic Lipid Dysmetabolism Mediated by Gut Microbiota in High-Fat Diet (HFD)-Fed Mice. *Nutrients*, *14*(24), 5214. https://doi.org/10.3390/nu14245214

Yordanova, G. (2022). Effect of Apple Pectin Supplementation on Productivity and Some Blood Parameters in Fattening Pigs. *Zhivotnovadni Nauki*, *59*(6), 57-62 (Bg)

Zhai, S.W., Shu-Lan, L., & Xue-Hao, C. (2015). Effects of quercetin on alleviating dietary lead (Pb)-induced growth retardation and oxidative stress in juvenile tilapia (*Oreochromis niloticus*). *The Israeli journal of aquaculture-Bamidgeh*., *67*, 1-7.

Zou, Y., Wei, H. K., Xiang, Q.-H., Wang, J., Zhou, Y.F., & Peng, J. (2016a). Protective effect of quercetin on pig intestinal integrity after transport stress is associated with regulation oxidative status and inflammation. *Journal of Veterinary Medical Science*, *78*(9), 1487-1494.

Zou, Y., Xiang, Q., Wang, J., Wei, H., & Prng, J. (2016b). Effects of oregano essential oil or quercetin supplementation on body weight loss, carcass characteristics, meat quality, and antioxidant status in finishing pigs under transport stress. *Livestock Science*, *192*, 33-38.