VETERINARSKI ARHIV 89 (3), 351-366, 2019 DOI: 10.24099/vet.arhiv.0314

Effect of dietary carob wholemeal on blood parameters in weaned pigs

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ŠPOLJARIĆ, D., D. MARENČIĆ, M. BENKOVIĆ, B. ŠPOLJARIĆ, A. BELŠČAK CVITANOVIĆ, G. MRŠIĆ, K. VLAHOVIĆ, M. POPOVIĆ, S. SREČEC, I. STOLIĆ: Effect of dietary carob wholemeal on blood parameters in weaned pigs. Vet. arhiv 89, 351-366, 2019.

ABSTRACT

The aim of this study was to investigate the effect of dietary carob wholemeal (*Ceratonia siliqua* L.) through monitoring the changes in some blood parameters (RBC, LEU, CD45+, CD4+, CD8+, CD21+) on the growth and development of weaned pigs (Swedish Landrace and Yorkshire cross-breeds). Thirty pigs were divided into two groups. In the control group the pigs were fed with a standard feed mixture. In the second, the experimental group (EXP), the feed mixture was enriched daily with carob wholemeal in a dose of 40 g/kg diet. Both groups were fed *ad libitum*. At the end of the 42-day experiment the pigs in the EXP groups were on average 23% heavier. The total sugar content, total phenolic content, flavonoids, anthocyanins, tannins and total antioxidant content of the carob wholemeal was determined. Carob wholemeal contains high carbohydrate content (73%). The total phenolic content value was 9.98 ± 0.18 mg GAE/g. The total flavonoid value was 6.56 ± 0.19 mg GAE/g, or 65% of total polyphenols, from which it can be seen that antioxidant activity is correlated to the total phenolic content. The total anthocyanins content was 34.00 ± 0.07 mg CE/g while the total tannin content was 658.75 ± 18.75 µg TAE/g. The ABTS method values were 89.50 ± 0.17 µmol TE/g sample while antioxidant activity determined by the DPPH method was 56.87 ± 0.66 µmol TE/g sample. Carob

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ISSN 0372-5480
Printed in Croatia

supplementation did not affect the amount of red blood cells and leucocytes, but did affect the proportions of the proliferation rate of CD45+ lymphoid cells, CD4+ and CD8+ T cells and CD21+ B cells in peripheral blood. Between days 14 and 42 an increase in the proportions of CD45+, CD4+, CD8+, CD21+ cells (at $p \leq 0.05$ or $p \leq 0.01$, respectively) was observed. Dietary supplementation of weaned piglets with 4% carob wholemeal showed an advantageous or beneficial effect on the immunity and productivity of the weanlings.

Key words: carob; diarrhoea; phytocompounds; immunology; pigs

Introduction

For centuries, plants have been used in the treatment and prevention of disease, depending on their chemical composition. Plants are rich sources of functional dietary micronutrients, fibers and phytochemicals that, individually or in combination, may be beneficial for animal and human health (OZCAN et al., 2014). Carob (*Ceratonia siliqua* L.*)* shows a wide variety of health benefits, including its ability to boost the immune system, reduce the risk of cancer, improve digestion, slow down aging, prevent cardiovascular diseases, and help prevent and manage diabetes (KAÏS et al., 2017).

Carob consists of two major parts: the pulp (90%) and the seeds (10%). The chemical composition of carob depends on cultivars and horticultural conditions. Carob contains high levels of carbohydrates (48-56%), fibers (30-40%), tannins (16%-20%), protein (6.34%) and a low level of fat (1.99%) (YOUSSEF et al., 2013; GOULAS et al., 2016). In addition, carob is a rich source of fatty acids, minerals, cyclitols, amino acids, polyphenols and vitamins. From a pharmacological point of view the most interesting substances of carob are phenolic compound, phenolic acids, flavonoids and tannins. Phenolics, subdivided into benzoic and cinnamic acids, are the most abundant class of polyphenols in carob fruits (GOULAS et al., 2016). The benzoic group includes benzoic acid, gallic acid and its derivatives, syringic acid, 4-hydrobenzoic acid, and gentisic acid. The cinnamic group includes cinnamic acids, coumaric acid, ferulic acid and chlorogenic acid. Carobs are particularly rich in flavonols, such as quercetin, myricetin, kaempferol and their glucosidic derivatives. Carob tannins are mainly condensed tannins (proanthocyanidins), composed of flavan-3-ol groups and their galloyl esters, gallic acid, ellagic acid, (+)-catechin, (-)-epicatechin gallate, (-)-epigallocatechin gallate, delphinidin, pelargonidin and cyanidins (YOUSSEF et al., 2013; GOULAS et al., 2016).

It has been found that various flavonoids show significant pharmacological and biochemical activity that effects the normal functions of immune cells such as B-cells, T-cells, macrophages, neutrophils, basophiles and mast cells. (DURGA et al., 1998; MIDDLETON et al., 2000). Phenolic acids, tannins, and flavonoids also have radical-scavenging capacity, which is involved in their antioxidant properties, and the ability to inhibit biomolecule oxidation, cause the inactivation of carcinogens, the inhibition of enzymes involved in pro-carcinogen activation, the activation of xenobiotic detoxification

enzymes, and neuroprotective abilities (KIM et. al., 1998; OZCAN et al., 2014). Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immune responses (OZCAN et al., 2014).

Post-weaning diarrhea (PWD) caused by *Escherichia coli* is responsible for enormous economic losses in pig production worldwide. Weaning stress causes physiological and metabolic processes that may alter the response of the immune system and lead to dehydration, diarrhea and growth retardation in surviving piglets, right up to death (MOESER et al., 2017).

Today it is known that the proper selection of food, rich in probiotics, antioxidants and antiallergenic compounds, may prevent or inhibit these disorders and improve the health status of piglets (ŠPIRANEC et al., 2013; PASCOAL et al., 2012; THOMAZ, et al., 2009).

The aim of this study was to investigate the effect of dietary carob whole meal (*Ceratonia siliqua*) through monitoring the changes in some blood parameters (leucocytes, RBC, CD45+, CD4+, CD8+, CD21+), on the growth and development of weaned pigs (Swedish Landrace and Yorkshire cross-breeds).

Materials and methods

Carob. Carob pods of the "Komižki rogač" local carob cultivar were collected during September 2015 on the island of Drvenik Mali in Croatia. The whole carob pods with the seeds were ground to a particle size of approximately 400 μm (or precisely 404.77 μm ± 10.42), determined by Laser diffraction particle sizing, using a Malvern Mastersizer 2000 equipped with a Scirocco dry dispersion unit (Malvern Instruments, United Kingdom), using a HPM-250 hammer mill (Ščukanec, Croatia). Dry matter content (dm) in the carob whole meal was approximately 92% (or precisely 92.20% ± 0.17), determined by the Association of Official Analytical Chemists (AOAC method) as detailed earlier (WOLF et al., 2001).

Chemical and anti-oxidative characterization. Determination of the polyphenolic constituents of carob whole meal was undertaken according to the procedure described previously by MAKRIS and KEFALAS (2004), using 70% acetone as the extraction solvent. Values are expressed as the equivalent of a gallic acid (GEA) 132.19 ± 1.15 μg/g sample. The total polyphenol content (TPC) was determined using the Folin-Ciocalteu method (BLAINSKI et al., 2013). To determine the flavonoids, formaldehyde precipitation was used and the flavonoid content was calculated as the difference between total phenol and non-flavonoid contents. Proanthocyanidins (*i.e.* condensed tannins) were analyzed by the procedure described by PORTER et al. (1986). The content of tannins was determined according to SCHNEIDER (1976) and expressed as a percentage of the mass of dry plant

material. Individual polyphenolic compounds were identified and quantified according to the HPLC method (High-performance liquid chromatography) described previously by BELŠČAK-CVITANOVIĆ et al. (2011). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was carried out according to the standard procedure by BRAND-WILLIAMS et al. (1995). All measurements were performed in triplicate. The trolox equivalent antioxidant capacity (TEAC) was estimated by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation decolorization assay (RE et al. 1999). The results, obtained from triplicate analyses, are expressed as trolox equivalents (TE). The method for determination of individual carbohydrates by HPLC analysis was described previously by BELŠČAK-CVITANOVIĆ et al. (2011). Additionally, the sweetness index (OBANDO-ULLOA et al., 2009) was used to estimate the total sweetness perception, expressed as sucrose equivalents (Suceq):

$$Suceq = 1 \times [Sucrose] + 0.74 \times [Glucose] + 1.73 \times [Fructose]$$

The soluble polysaccharide content of carob extracts was determined by the phenol-sulfuric acid method, using glucose as standard (LAURENTIN and EDWARDS, 2003).

Animals. The experiment was conducted on a commercial pig farm in eastern Croatia. 30 crossbred pigs (Swedish Landrace and Yorkshire), both females and castrates aged 28 days, with a uniform body mass of about 7 kg, were used in the experiment. All procedures used in this research were in compliance with the European guidelines for the care and use of animals in research (ANONYM., 2010) and had approval from the Ethics Committee for Animal Experimentation, Faculty of Veterinary Medicine, University of Zagreb, Croatia (records No.: 640-01/16-17/81; file No.: 251-61-01/430-16-2).

Study design and procedures. The pigs were weaned and randomly divided into two groups: the control (CON/15 piglets) and the experimental (EXP/15 piglets) groups, at 26 day of life according to rearing technology of the farm. The animals were kept in separate pens in the same rearing facility of the farm. The experiment started on the second day of weaning after the pigs had adjusted to the new conditions (28 days of age), and lasted 42 days (70 days of age). The pigs in the control group were fed with a standard feed mix (grain-soybean meal-based diet, which according to the farm owner was composed of: crude proteins 20.0%, crude fat 50.0%, crude fiber 3.7%, calcium 0,6%, phosphorus 0.51%, sodium 0.29%, lysine 1.64%, methionine and cysteine 0.83%; ME/MJ 14.05). The pigs in the experimental group were daily fed with the standard feed mix enriched with 4% (40 g per 1 kg food) of carob whole meal. During the experiment food and water were available *ad libitum*.

Monitoring of health indicators. The animals were weighed on days 0, 14, 28 and 42 of the experiment, and changes in their body mass were recorded as detailed earlier (ŠPOLJARIĆ et al., 2015). The pigs were monitored daily for diarrhea and other clinical signs of health disorders. Fecal consistency was verified twice a day, at 08:00 h and 17:00

h, by means of visual analysis, according to the fecal consistency scoring method, as described previously by VALPOTIĆ et al. (2014).

Blood sampling and analysis. Blood samples for analysis were collected on the 0, 14th, 28th and 42nd days of experiment. Blood samples (3 mL) were drawn from the cranial vein into vitreous glass tubes (Venosafe, Terumo, EU) containing 1mL of disodium salt of ethylenediaminetetraacetic acid (EDTA, Sigma). Blood was collected from seven marked (by numbers 1-7) pigs from each group. The blood parameters examined in the peripheral blood of pigs were: red blood cells (RBC), leukocytes, CD45+ lymphoid cells, CD4+, CD8+ T cells, and CD21+ B cells. The erythrocyte and leucocyte counts were determined by standard methods using a Serono Baker System 9120 (Pennsylvania, SAD) automated counter as detailed earlier (ŽDERIĆ SAVATOVIĆ et al., 2017). The proliferation rates of CD45+ lymphoid cells, CD4+ and CD8+ T cells and CD21+ B cells were quantified using a Coulter EPICS-XL flow cytometer (Beckman Coulter, Miami, FL, USA),) at the Department of Veterinary Biology, Faculty of Veterinary Medicine, University of Zagreb, as previously detailed as previously detailed (VALPOTIĆ et al., 2013)

Statistical analysis. The software package Statistica 8.0 (StatSoft Inc., 2008) was used for statistical analysis. The significance of differences between analytical data, between the two groups of weaned pigs (control and experimental) was checked using the *t*-test for dependent samples (HILL and LEWICKI, 2007). The level of statistical significance was $P<0.05$.

Results

The measured chemical parameters of the carob whole meal used in this experiment are given in Tables 1 and 2.

The overall mean values for the total sugar and reducing sugar, and their standard deviations are presented in Table 1. The carob whole meal contained 73% carbohydrates, namely 37.00% sucrose, 17.00% fructose and 19.00% glucose, and 29.11% soluble polysaccharides. The calculated sweetness index for the carob whole meal was 0.80% suceg (sucrose has a relative sweetness value of 100%).

Table 1. Content of sucrose, glucose, fructose and soluble polysaccharides in carob extracts of carob whole meal (the results are exposed as mean ± SD).

Sugar content			∑ sugars	Suceq*	Soluble polysaccharides
Sucrose	Fructose	Glucose			
0.37 ± 0.02	0.17 ± 0.01	0.19 ± 0.01	0.73	0.80	29.11 ± 0.70

* - sucrose equivalents

The TPC value in the carob extract was 9.98 ± 0.18 mg GAE/g making it a relatively good source (Table 2). The total flavonoid (TF) values in the whole meal analyzed was 6.56 ± 0.19 mg GAE/g. Correlation analysis of the obtained values showed that around 65% of the polyphenols are flavonoids, suggesting that the antioxidant activity was correlated to TPC content. The total anthocyanin content (TAC) was 34.00 ± 0.07 mg CE/g, while total tannin (TT) content was 658.75 ± 18.75 µg TAE/g (tannic acid equivalent). In the ABTS assay, antioxidant activity was 89.50 ± 0.17 µmol TE/g sample, while in the DPPH assay it was 56.87 ± 0.66 µmol TE/g sample (Table 2).

Table 2. Content of total polyphenols and specific sub-groups of polyphenols, as well as antioxidant capacity of carob extracts in carob whole meal (the results are presented as mean ± SD).

Total polyphenols	Total flavonoids	Procyanidins	Tannins	Antioxidant capacity	
				ABTS	*DPPH*
mg GAE/g	mg GAE/g	mg CE/g	µg TAE/g	µmol TE/g sample	µmol TE/g sample
9.98 ± 0.18	6.56 ± 0.19	34.00 ± 0.07	658.75 ± 18.75	89.50 ± 0.17	56.87 ± 0.66

GEA-gallic acid equivalent; CE-cyanidin chloride equivalents; TAE- tannic acid equivalent; TE -trolox equivalents; ABTS -(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical cation decolorization assay; DPPH-(2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

Table 3. Differences in body mass (kg) between weaned pigs fed by added carob whole meal (EXP group) and animals without added carob whole meal (CON group) in mixture (the results are presented as mean ± SD) during 42 days (the results are presented as mean ± SD)

Group	Day of experiment/ BW (kg)			
	0	14	28	42
CON (n = 15)	7.47 ± 0.36*	10.16 ± 0.58*	14.34 ± 0.24*	20.10 ± 0.38*
EXP (n = 15)	6.98 ± 0.61*	10.01 ± 0.44*	15.11 ± 0.39*	25.23 ± 0.39*.**
Difference	-0.49	-0.15	-0.23	1.33**
Probability	0.096	0.614	0.212	0.000038

CON- control group; EXP- experimental group; *- means P<0.05; **- means P<0.01; ***not significant differences are unmarked in the Table

The changes in body mass in the experimental group of animals (EXP) were calculated on the basis of the differences between either the body mass at the beginning of the experiment (day 0 equals 100% body mass), or the average group body mass of the animals on days 0, 14, 28 and 42 of the experiment, in comparison to the average body

mass of the animals from the control group. Pigs treated with carob had a significant increase in live body mass compared to the control pigs on Day 42 of the experiment. They had higher average body mass at the end of the experiment (~25 kg) than the control pigs (~20 kg), and higher body mass gain in relation to Day 0 (361.46% *vs.* 269.07%), as well as in relation to the control pigs (16.7%) (Table 3).

Regarding the incidence and severity of diarrhea, a much lower total diarrhea severity score (DSS) was recorded in the pigs treated with carob (0 or -100%) than in the control pigs (Table 4). A higher average diarrhea severity (ADS) was recorded in the control pigs (0.21), whereas the pigs treated with carob had much lower ADS (0 or -100%) after 42 days of the experiment. None of animals died during the experimental period.

Table 4. Incidence and severity of diarrhea, and mortality in pigs over the 42 day experiment

Group	No. of diarrheic pigs/total no.of pigs (%)	Diarrhea severity score (DSS)		Average diarrhea severity (ADS)		No. of dead pigs/total no. of pigs (%)
		Sum of DSS*	% difference *vs.* control	ADS ratio**	% difference *vs.* control	
CON (n = 15)	5/15	9	/	0.21	/	0/15 (0)
EXP (n = 15)	0/15	0	- 100	/	- 100	0/15 (0)

CON- control group; EXP- experimental group; * - diarrhea severity score (DSS): 0 = normal feces, 1 = soft feces, 2 = fluid feces, 3 = projectile feces as summarized during 42 days of experiment; ** Sum of DSS/42 days

The effect of dietary supplementation of carob whole meal on the selected blood parameters is presented in Tables 5-10. Carob whole meal did not affect the values of red blood cells and leukocytes in the peripheral blood of pigs treated with carob during the observation period of 42 days (Tables 5-6).

Table 5. Differences in leukocyte counts in the peripheral blood of pigs fed by added carob whole meal (EXP group) and pigs without added carob whole meal (CON group) in mixture (the results are presented as mean ± SD)

Group	Day of experiment			
	0	14	28	42
CON(n = 15)	16.4 ± 2.54	18.63 ± 5.23	19.27 ± 3.39	17.81 ± 4.22
EXP(n = 15)	16.3 ± 3.55	17.17 ± 3.37	18.77 ± 5.84	17.94 ± 5.81
Difference	-0.1	-1.46	-0.5	0.13
Probability	0.951	0.547	0.848	0.963

CON- control group; EXP- experimental group; *- means P<0.05; **- means P<0.01; ***not significant differences are unmarked in the Table

Table 6. Differences in RBC counts in the peripheral blood of pigs fed by added carob whole meal (EXP group) and pigs without added carob whole meal (CON group) in mixture (the results are presented as mean ± SD)

	Day of experiment			
Group	0	14	28	42
CON (n = 15)	6.71 ± 0.33	6.49 ± 0.37	6.73 ± 0.59	6.32 ± 0.50
EXP n = 15)	6.88 ± 0.47	6.92 ± 1.05	6.04 ± 0.44	6.88 ± 0.47
Difference	0.17	0.43	-0.69	-0.17
Probability	0.433	0.34	0.512	0.514

CON- control group; EXP- experimental group; *- means P<0.05; **- means P<0.01; ***not significant differences are unmarked in the Table

The proportions of CD45+ lymphoid cells, CD4+ T cell, CD8+ T cells and CD21+ B cells in the peripheral blood of pigs treated with carob whole meal were much higher than in the control pigs during the last four weeks of the experiment. During that period we recorded a significantly increased proportion of CD45+ lymphoid cells (at P<0.05 or P<0.01, respectively) on Days 14, 28 and 42 of the experiment, as well as of CD4+ T cells, CD8+ T cells and CD21+ B cells on Days 28 and 42 of the experiment (P<0.01, respectively) (Tables 7-10).

Table 7. Differences in proportion of CD45+ lymphoid cells in the peripheral blood of pigs fed by added carob whole meal (EXP group) and pigs without added carob whole meal (control group) in mixture (the results are presented as mean ± SD)

	Day of experiment			
Group	0	14	28	42
CON (n = 15)	51.01 ± 1.25	55.90 ± 1.23	57.93 ± 0.49	61.01 ± 0.63
EXP (n = 15)	50.46 ± 1.34	59.63 ± 0.44	68.15 ± 3.53	72.07 ± 0.42
Difference	-0.55	3.73*	10.22**	11.06**
Probability	0.438	0.024	0.00013	0.00011

CON- control group; EXP- experimental group; *- means P<0.05; **- means P<0.01; ***not significant differences are unmarked in the Table

Table 8. Differences in proportion of CD4+ lymphoid cells in the peripheral blood of pigs fed by added carob whole meal (EXP group) and pigs without added carob whole meal (control group) in mixture (the results are presented as mean ± SD)

	Day of experiment			
Group	0	14	28	42
CON (n = 15)	17.48 ± 0.68	18.83 ± 0.48	20.18 ± 0.26	21.36 ± 0.44
EXP (n = 15)	17.85 ± 0.46	21.44 ± 0.53	27.19 ± 0.25	27.42 ± 0.34
Difference	0.37	2.61	7.01**	6.06**
Probability	0.262	0.848	0.001	0.0001

CON- control group; EXP- experimental group; *- means P<0.05; **- means P<0.01; *** not significant differences are unmarked in the Table

Table 9. Differences in proportion of CD8+ lymphoid cells in the peripheral blood of pigs fed by added carob whole meal (EXP group) and pigs without added carob whole meal (control group) in mixture (the results are presented as mean ± SD)

	Day of experiment			
Group	0	14	28	42
CON (n = 15)	9.67 ± 0.55	11.09 ± 0.60	13.99 ± 0.22	12.17 ± 0.29
EXP (n = 15)	9.99 ± 0.85	12.03 ± 0.43	15.22 ± 0.51	14.82 ± 0.27
Difference	0.32	0.94	1.23**	2.65**
Probability	0.413	0.443	0.001	0.0001

CON- control group; EXP- experimental group; *- means P<0.05; **- means P<0.01; ***not significant differences are unmarked in the Table

Table 10. Differences in proportion of CD21+ lymphoid cells in the peripheral blood of pigs fed by added carob wholemeal (EXP group) and pigs without added carob whole meal (control group) in mixture (the results are presemted as mean ± SD)

	Day of experiment			
Group	0	14	28	42
CON (n = 15)	20.22 ± 0.47	22.11 ± 0.49	23.97 ± 0.66	24.08 ± 0.35
EXP (n = 15)	20.20 ± 0.64	24.30 ± 0.68	26.13 ± 0.45	29.19 ± 0.29
Difference	-0.02	2.19**	2.16**	5.11**
Probability	0.937	0.000017	0.000011	0.000011

CON- control group; EXP- experimental group; *- means P<0.05; **- means P<0.01; ***not significant differences are unmarked in the Table

Discussion

Over recent decades, antibiotics have been used as feed additives to improve animal growth and to prevent diseases caused by pathogenic microorganisms. However, after the discovery that antibiotic use in food animals can result in antibiotic-resistant infections in humans, their use in the EU has been forbidden, while their use outside the EU is restricted (WINDISCH et al., 2008). Accordingly, considerable effort has been dedicated to identify alternatives to antibiotics as growth promoters in the animal industry (HONG et al., 2002). Of these, natural compounds are being investigated as alternatives to antimicrobial usage in food-producing animals (ŠPOLJARIĆ et. al., 2011; LIU, 2013).

For piglets, weaning is a most stressful event associated with gastrointestinal disorders and increased probability of disease. In this period, piglets often have diarrhea which in some cases leads to death. In this study, a significant difference was observed in the number of diarrhea cases between the Con and EXP groups. Unlike the EXP group, in which no case of diarrhea was noted, in the CON group one third of the piglets had diarrhea. KOTROTSIOS et al. (2012) published an article describing the influence of phenol from carobs on the prevention of diarrhea in weaned piglets. In this study piglets in the EXP group were fed daily with 4% carob whole meal in which the total polyphenol content (TPC) was 9.98 ± 0.18 mg GAE/g. Therefore, it can be assumed that the TPC value determined is sufficient to prevent diarrhea in weaned piglets. Also, it was found that along with flavonoids, tannins are a second class of compounds responsible for an antidiarrheal effect (PALOMBO, 2006). The mode of action of both classes includes increasing colonic water and electrolyte reabsorption. The carob whole meal used in the study contained 658.75 ± 18.75 µg TAE/g of tannins.

In addition, weaning is also associated with low and variable feed intake, resulting in a decrease in the piglets' development, making make them highly sensitive to viral, bacterial and other diseases (MOESER et al., 2007). In this study it was noticed that at the end of the experiment (Day 24) piglets in the EXP group gained on average approximately 55% more body mass than piglets in the CON group. These results may be explained by the findings of ANDRES-ELIAS et al. (2007) which showed that diets containing carob modulated the changes in the intestinal ecology in treated weaned pigs. Further, BADIA et al. (2012) reported that the mannose-rich components of dietary carob mimic the host cell receptor to which the pathogen adheres instead of attaching to the intestinal mucosa cells. Although we did not test the mannose content, its influence on growth performance in weaned piglets cannot be excluded.

Maintaining a good immune status helps the animal to protect itself against numerous pathogens. Weaned piglets in general have low passive immunity while active immunity has not yet fully developed. In commercial farming conditions along with pathogens, the good immune status of weaned piglets has often been reduced by the increased frequency of diarrhea, gut infections and stressful life conditions. The immune system in piglets

can be modified by diet, pharmacological agents, environmental pollutants, and naturally occurring food chemicals (MOESER et al., 2017).

The results obtained in this study indicate that treatment of pigs with carob whole meal for 6 weeks did not cause any harmful side effects on the monitored blood parameters, nor on the general health status of the animals. It was noticed that carob whole meal did not affect the distribution of red blood cells and leukocytes. Namely, the values of red blood cells and leukocytes values in both groups were within physiological parameters in accordance with their age (FELDMAN et al., 2000).

STEPANOVA et al. (2007), reported that weaning affects (decreases) the amount of T cell subsets, especially in lymphatic nodes or the *lamina propria* of the intestinal mucosa.

The significant increase in the proportion of CD45+ lymphoid cells, CD4+ T cell, CD8+ T cells and CD21+ B cells in the peripheral blood of pigs treated with carob whole meal during the last four weeks of the experiment could be to promote more powerful, accelerated and supportable protection of weaned pigs from antigenic challenges. In addition, the increased proportions of these cells certainly contributed to the reduction in the incidence or severity of diarrhea and maintenance of the gut health of the weanlings, and thus could be of importance in promoting the resistance of weaned pigs to enteric infections. It is known that flavonoids, along with their anti-inflammatory, antioxidant, and anti-allergic effects, have immune-modulating effects (TANAKA and TAKAHASHI, 2013). Thus, the flavonoid myricetin isolated from carob in biochemical testing on rats showed excellent antioxidative activity in the protection of liver and kidney cells (HSOUNA et al., 2011). PAPADOPOULOU et al. (2005) demonstrated that polyphenols influenced intestinal microflora, decreasing the number of *Bactericides* and *Clostridia,* and increasing *Lactobacilli* numbers. The exact mechanism by which polyphenols exhibit antimicrobial activity is not clear. It is assumed that their bacteriostatic and/or bactericidal actions are associated with inhibition of the adhesion of infection-causing bacteria within the cells of the intestinal tract. The total polyphenols content (TPC) added to the daily diet was 9.98 ± 0.18 mg GAE/g making carob an average phenol source. Among the polyphenol classes, the highest amount, approximately 65%, were flavonoids (estimated value 6.56 ± 0.19 mg GAE/g) then anthocyanins 34.00 ± 0.07 mg CE/g and tannins 658.75 ± 18.75 µg TAE/g. It seems that the daily proportion of polyphenol content applied is sufficient to improve the immune system of weanling piglets.

Flavonoids are powerful antioxidants, since they stabilize the reactive oxygen species by reacting with the reactive compound of the radicals, scavenge nitric oxide and inhibit xanthine oxidase activity (TANAKA and TAKAHASHI, 2013). Therefore, from the obtained results it may be assumed that flavonoids, as the dominant polyphenols in carob whole meal, increased the proportion of CD45+ lymphoid cells, CD4+ T cell, CD8+ T cells and CD21+ B cells in the peripheral blood of pigs treated with carob

whole meal, compared to the control pigs. The aryl hydrocarbon receptor (AhR) is a ligand-activated transcriptional factor that mediates the toxic and biological actions of many aromatic environmental pollutants, such as dioxins. It has recently been shown that the aryl hydrocarbon receptor (AhR is a ligand-activated transcriptional factor that mediates the toxic and biological actions of many aromatic environmental pollutants) is a regulator of differentiation of naïve CD4+ T cells into effector T cell subsets (TANAKA and TAKAHASHI, 2013), which suggests that flavonoids modulate immune functions through their binding to AhR.

However, whether the beneficial effect of dietary carob observed in this study was due to flavonoids remains unknown. But, in any case, the advantageous effect of the carob on both the immunity and productivity of weanlings seems to be interactively connected with improved gut health and physiology. Therefore, it is possible that carob can be used as a feed additive for improving porcine health and productivity.

Conclusion

Dietary supplementation of weaned piglets with 4% carob whole meal showed advantageous effects on the immunity and productivity of weanlings. Since modern pig production forbids the use of antibiotic growth promoters, scientific recommendations for natural alternative strategies for control and prevention of disease in piglets in intensive breeding are of great interest. Future studies must be carried out to determine the effects on health parameters of flavonoids and tannins from carob in diets for weaning pigs. Based on results obtained we have concluded that dietary carob whole meal supplementation, at the dose applied in our study, was shown to be effective as an immunomodulator in stimulating non-specific recruitment of circulating immune cell subsets.

Acknowledgements

The research was funded by the Croatian Science Foundation, grant number: IP-11-2013-3304-TEUCLIC.

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Received: 6 April 2018
Accepted: 14 August 2018

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SAŽETAK

Svrha ovih istraživanja bila je utvrditi učinak hranidbe rogačevom prekrupom na rast i razvoj svinja (švedski landras × jorkšir) nakon odbića, prateći promjene nekih krvnih parametara (RBC, LEU, CD45+, CD4+, CD8+, CD21+). Trideset svinja podijeljeno je u dvije skupine. U kontrolnoj skupini svinje su hranjene standardnom krmnom smjesom, dok je u drugoj, eksperimentalnoj skupini, u krmnu smjesu dodana rogačeva prekrupa u količini od 40 g/kg obroka. Obje su skupine hranjene ad libitum. Na kraju 42-dnevnog pokusa svinje u eksperimentalnoj skupini bile su 23 % teže od svinja iz kontrolne skupine. U prekrupi rogača određena je količina ukupnih šećera, ukupnih polifenola, flavonoida, antocijana, tanina te količina ukupnih antioksidansa. Utvrđen je velik udio ukupnih ugljikohidrata je (73 %). Količina ukupnih polifenola iznosila je 9,98 ± 0,18 mg GAE/g, a količina ukupnih flavonoida 6,65 ± 0,19 mg GAE/g ili 65 % od ukupnih polifenola, što je vidljivo iz veze između antioksidacijske aktivnosti i količine ukupnih polifenola. Količina ukupnih antocijana iznosila je 34,00 ± 0,07 mg CE/g, dok je količina ukupnih tanina iznosila 658,75 ± 18,75 µg TAE/g. Antioksidacijska aktivnost određena metodom ABTS iznosila je 89,50 ± 0,17 µmol TE/g uzorka, dok je antioksidacijska aktivnost određena metodom DPPH iznosila 56,87 ± 0,66 µmol TE/g uzorka. Dodatak rogača u obroku nije uzrokovao promjenu broja crvenih krvnih stanica ni leukocita, ali je djelovao na promjene u proliferacijskom odnosu CD45+ limfoidnih stanica, kao i CD4+ and CD8+ T stanica i CD21+ B stanica u perifernoj krvi. Između 14. i 42. dana hranjenja u eksperimentalnoj skupini životinja ustanovljeno je znakovito povećanje udjela CD45+, CD4+, CD8+, CD21+ stanica (uz vjerojatnost pogreške P≤0,05 odnosno P≤0,01). Dodatak 4 % rogača po obroku za hranidbu svinja nakon odbića pokazao se korisnim za imunost i proizvodnost svinja nakon odbića.

Ključne riječi: rogač; proljevi; biljni spojevi; imunologija; svinje