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Black pepper dietary supplementation increases high-density lipoprotein (HDL) levels in pigs

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ABSTRACT

Background: Black pepper, the briefly cooked and dried unripe fruits of *Piper nigrum* L. has broad culinary applications around the world and has been considered as the "king of spices". In recent years, studies have shown that a diet supplemented with black pepper not only improves digestion but also has anti-oxidant and anti-inflammatory effects as well as the ability to regulate levels of serum lipids.
Purpose: The objective of this study was to investigate the effects of dietary supplementation with milled black pepper on serum lipoproteins, three anti-oxidant status-related parameters (serum vitamin C, polyphenols, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging capacity), average daily growth, and food intake in growing and fattening pigs.
Study design/Methods: Two groups of randomly assigned piglets with a body weight of each 30 kg (17 animals per group, 34 animals overall) of Bulgarian Danube White breed were subject to feeding a diet supplemented with or without black pepper. As feed additive, milled black pepper at a dose of 3 g/kg food weight was mixed into standardized food to continuously feed the animals gaining weight to 100 kg. The changes in lipoproteins and anti-oxidant index were examined both in the growing and fatting periods.
Results: The results of the measured serum profile in the black pepper diet group showed i) a significant increase of high-density lipoprotein (HDL), and ii) a significant increase of vitamin C in comparison with the group fed a normal diet. Supplementation with black pepper also showed a tendency to improve daily food intake and decrease average daily growth of pigs (both without reaching statistical significance) during both the growing period and the fattening period.
Conclusion: Dietary intake of black pepper might lead to positive effects on blood lipid regulation and anti-oxidant capacity. Overall the gained data in our study suggest that black pepper can be used as a natural food additive to support animal health. Moreover, the results obtained from this porcine model might hint for potential beneficial effects on lipid metabolism in humans and thus warrant further exploratory studies.

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; AC, atherogenic coefficient; Apo A1, apolipoprotein A1; BBC, blend bioconcentrate (protein); BPO, black pepper oil; CETP, cholesteryl ester transfer protein; CVD, cardiovascular diseases; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FI, food intake; GAE, gallic acid equivalent; HDL, high-density lipoprotein; HFD, high-fat-diet; LBW, live body weight; LDL, low-density lipoprotein; RCT, reverse cholesterol transport; SR-BI, scavenger receptor class B type I; TC, total cholesterol; TG, triglycerides; PPAR-γ, peroxisome proliferator-activated receptor gamma.

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1. Introduction

Recent accumulation of clinical evidence has yielded increasing evidence that cardiovascular diseases (CVD) are closely associated with lipid metabolism dysregulation, obesity, diabetes and physical inactivity (Jokinen, 2015). Also a large number of epidemiological studies have consistently shown that lipid abnormalities of high-density lipoprotein (HDL) metabolism are associated with a high risk profile for CVD and obesity (Bastien et al., 2014). HDL exhibits a variety of anti-atherogenic effects, including anti-inflammatory and anti-oxidant activity, and promotion of cholesterol efflux, which not only affects foam cell formation but also positively affects the reverse cholesterol transport (RCT) (Navab et al., 2011; Podrez, 2010). Along this line, a recent large scale clinical study supported the role of HDL as an inverse predictor of cardiovascular events and biomarker of residual risk (Khera et al., 2017).

Plants are a reach source of biologically active constituents (Atanasov et al., 2015; Uhrin et al., 2018; Yeung et al., 2018), and dietary supplements and nutraceuticals have in particular proven to be of high value for modulation of CVD risk factors (Banach et al., 2018; Mozos et al., 2018; Waltenberger et al., 2016; Wang et al., 2018). Black pepper (*Piper nigrum* L.), native to south India, is one of the earliest spices used by people. It is often ground into powder and added to food as seasoning (Sen, 2004). Recently, black pepper has attracted increased scientific attention due to the various reported biological activities of its components, in particular alkaloids, oleoresin and oil. In traditional medicine the spice is also used to increase appetite and upregulate caloric intake (Liu and Yin, 2015). Apart from food usage, black pepper has also a tradition to be used as preservative and medicine (Meghwal and Goswami, 2013), e.g., in traditional Chinese and Indian medicine for treating a variety of diseases like common cold, abdominal pain, diarrhea and epilepsy (Gao et al., 2006).

The main bioactive ingredient of black pepper fruits, i.e. piperine, has a wide range of pharmacological effects, including anti-oxidant, antibacterial, anti-proliferative and anti-tumor, and cholesterol-lowering properties (Mair et al., 2015; Samykutty et al., 2013; Srinivasan, 2007; Vijayakumar and Nalini, 2006b). Piperine also enhances energy metabolism in the body; upon oral administration it was shown to markedly decrease the free cholesterol/esterified cholesterol ratio and phospholipid fractions level in rat testis (Malini et al., 1999). Also other physiological activities like anti-obesity and neuroendocrine-modulating effects are reported in the literature (Chonpathompikunlert et al., 2010; Meriga et al., 2017).

Studies have indicated that supplementation of black pepper to daily diet can promote absorption of nutrients and modulate lipid profile and obesogenic marker expression in high-fat-diet (HFD)-treated rats (Vijayakumar and Nalini, 2006b). In the same animal model, it was found that animals given a diet supplemented with the black pepper constituent piperonal displayed elevated plasma levels of HDL and apolipoprotein A1 (apoA1), and decreased plasma levels of total cholesterol (TC), triglyceride (TG), and low-density lipoproteins (LDL) cholesterol (Meriga et al., 2017). In human THP-1-derived macrophages piperine increased ATP-binding cassette transporter A1 (ABCA1) expression and promoted cholesterol efflux by inhibiting ABCA1 degradation (Wang et al., 2017). Although the beneficial role of black pepper-supplemented diet on HFD-induced hyperlipidemia rat model has been demonstrated, the effects of a diet containing black pepper on weight gain and lipoprotein regulation in lifestock animals deserves more attention in view of putative economic impacts and animal health care during breeding. Recent studies found that normal rabbits which were fed with diet including black pepper oil (BPO) have decreased levels of serum TG, TC and an increased live body weight (LBW) at some growing periods. Furthermore, both immune parameters and the antioxidant index were also improved in the rabbits fed BPO-enriched diets (Abd El-Hack and Alagawany, 2019; Abdelnour et al., 2018). Our present study aimed to address the potential influence of milled black pepper as a health-promoting natural food additive acting on blood lipid levels, antioxidant status and growth performance using a porcine model that not only has economic relevance but is also more similar to human physiology than the rodent models so far investigated.

2. Material and methods

2.1. Animals and experimental design

The current experiments were carried out in the Experimental farm of Agricultural Institute – Shumen, Bulgaria. All experimental procedures were carried out according to standards defined by the Local Experimental Animal Care Committee, and according to the ethics of the institutional committee. Animals were housed and fed individually and had access to food and water ad libitum.

To test the influence of black pepper (*Piper nigrum* L.) supplementation on the productivity of growing piglets, we used 34 randomly selected growing piglets (17 animals of mixed gender per group) of Bulgarian Danube White breed (*Sus scrofa domesticus*), each with the starting weight of about 27 kg. Our investigation was conducted until 100 kg of LBW was reached, and the supplied basic diet varied in the growing period (period 1) ending at about 60 kg LBW, and the fattening period (period 2) starting at 60 kg LBW and ending at about 100 kg LBW (Table 1). The animals were randomly divided into two groups. Feed intake and LBW were continuously measured through the study course.

The control group (group 1) of pigs was fed with standardized feed according to Table 1. The feed of the second group (group 2) was supplemented with milled black pepper in a concentration of 3 g/kg food weight throughout the experiment. Black pepper with a quantified amount of 6.03% ± 0.01% piperine was purchased from a local commercial supplier at Shumen, Bulgaria. A voucher specimen is deposited at the Department of Pharmacognosy, University of Vienna, Austria. The dried fruits were freshly milled daily and blended into the feed of group 2. The only difference in the feed of the two groups was the black pepper additive, which was freshly milled daily and blended into the feed of group 2. The animals of group 1 reached 100 kg LBW in average for 96.4 days, and the animals of group 2 for 97.6 days. The animals of both groups were with mixed gender and we did not observe gender-specific difference in the black pepper supplementation-induced effects. All piglets were raised with the same diet before the experiment started. Recipe blends for both groups were developed on the basis of a preliminary chemical analysis of the components for water, dry matter, crude protein, crude fats, crude fiber, calcium, phosphorus and nitrogen-free extract. The composition of the basic diet is presented in Table 1.

2.2. Blood biochemistry

Blood samples from jugular vein from 10 pigs per group (chosen randomly) were collected by an authorized skilled veterinarian. Lipid profiles were measured with the following commercially available chemical tests kits: HDL-Cholesterol plus 3rd generation, LDL-Cholesterol plus 2nd

Table 1
Composition of the basic pig feed mixtures for period 1 and period 2.

Period 1 (30 to 60 kg)		Period 2 (60 to 100 kg)	
Components	Percentage	Components	Percentage
BBC - 14 *	22.00	BBC - 16 *	18.00
Wheat	58.00	Wheat	58.00
Barley	10.00	Barley	14.00
Wheat bran	10.00	Wheat bran	10.00
Total	100.00	Total	100.00
Metabolizable energy: 12.25 MJ		Metabolizable energy: 12.32 MJ	
Nutrients content [%]		Nutrients content [%]	
Crude protein	16.95	Crude protein	15.06
Crude fiber	5.36	Crude fiber	5.13
Crude fats	1.84	Crude fats	1.87
Lysin	0.76	Lysin	0.69
Methionine + cystine	0.62	Methionine + cystine	0.62
Calcium	0.77	Calcium	0.66
Phosphorus	0.66	Phosphorus	0.63

* BBC - a protein bioconcentrate blend. The protein bioconcentrate was purchased from „ФЗ Васил Костов"ООД ("FZ Vasil Kostov" Ltd., Bulgaria).

generation (Roche Diagnostics Ltd., Rotkreuz, Switzerland). The quantifications were performed by COBAS INTEGRA® 400 plus system (Roche Diagnostics Ltd., Rotkreuz, Switzerland). The level of vitamin C in serum was determined using a LambdaBio-20 spectrophotometer (Perkin Elmer, Waltham, USA) (Jóźwik et al., 2012). The determination of the content of total phenols was performed as previously described (Škerget et al., 2005). Measurements for radical scavenging activity were performed with a routine assay procedure (Brand-Williams et al., 1995) using a synthetic DPPH radical (1,1-diphenyl-2-picrylhydrazyl) (Lipińska et al., 2019). Folin-Ciocalteu reagent was used as an oxidizing reagent and all the chemicals were purchased from Sigma-Aldrich Chemie Gmbh (Munich, Germany) in the highest available purity (Jóźwik et al., 2012).

2.3. Statistical analysis

The results were processed using the variation statistics method and are shown as mean ± SD. For determination of differences between the two groups, Student's *t*-test was applied. For multiple comparisons, data were analyzed by one-way analysis of variance (ANOVA). Significance was determined as $p < 0.05$.

3. Results

3.1. Black pepper elevates the concentration of HDL in serum

The concentrations of HDL in the blood of group 2 (supplemented with black pepper) is significantly elevated (*$P < 0.05$) by 18% in comparison with group 1 fed with standard diet (Fig. 1A). LDL levels between the two groups are similar, although there is a slight trend towards decreased LDL levels in group 2, without reaching statistical significance (Fig. 1B). The level of atherogenic coefficient (AC = (TC-HDL)/HDL), which is related to low risk of some chronic diseases like CVD and atherosclerosis, is lower by 18.6% in group 2 compared to group 1, but without showing statistical significance (Fig. 1C).

3.2. Black pepper increases the level of vitamin C in serum

Considering that black pepper exhibits significant anti-oxidant effects reported in the literature (Srinivasan, 2007), three indicators of anti-oxidant status (vitamin C, total polyphenol levels, and DPPH scavenging potential) in serum were quantified. Vitamin C was tested as an important physiological anti-oxidant which has been shown to regenerate other anti-oxidants within the body. Polyphenols are micronutrients with anti-oxidant activity (Croft, 2016), which are also described in literature as inhibitors of LDL oxidation (Castaner et al., 2012). Moreover, a DPPH anti-oxidant assay was used to test general radical scavenging activity of the serum.

As shown in Fig. 2A, vitamin C levels in blood were significantly (**$P < 0.01$) enhanced in group 2 by 22% compared to group 1. However, no significant differences in the content of polyphenols and the scavenging capacity of DPPH (Fig. 2 B and C, respectively) were observed between the two groups.

3.3. Black pepper has no effects on the growth rate and feed utilization of growing pigs

Feed intake and LBW of the animals from the two groups were continuously measured through the study course. The collected feeding data are shown in Table 2 and did not yield any significant differences between group 1 and group 2 for any of the two periods.

In terms of growth, only minimal and statistically insignificant differences between groups were observed. For feed consumption per kg increment, there was also no significant difference between the two test groups. For the whole trial period (period 1 + 2), the pigs of the two groups consumed almost the same amount of feed (0.4%), metabolizable energy (0.4%) and nutrients (protein 1.7%). The results for the average daily growth for the entire study period are also almost indifferent with 1.4% difference (777 and 766 g for the first and second group, respectively, with low coefficients of variation C = 10.43 and C = 9.05, respectively; besides, the pigs in the black pepper-supplemented group consumed on average 49 g more feed to obtain a 1 kg gain (49 g per kg LBW gain) compared to the pigs of the control group in the whole experimental time, but there is no statistical significance). In general, the group which received the diet supplemented with milled black pepper fruits consumed more feed than the control group, but the difference is not statistically significant.

4. Discussion

Epidemiological research has accumulated strongly convincing data that low plasma levels of HDL are associated with an increased risk of CVD (Rye and Barter, 2014). Indeed, patients with chronic inflammatory diseases such as diabetes and rheumatoid arthritis have been shown to have varying degrees of HDL reduction and vascular damage and it is widely insinuated that the progression of these chronic inflammatory diseases has a certain potential link to the risk of CVD. Therefore, scientists have been paying more attention to identify natural products with anti-inflammatory and lipid regulating functions, especially elevating HDL concentration in blood serum, which would be a potentially promising strategy for protecting the cardiovascular system (Kardassis et al., 2014).

In our study, milled black pepper influences serum lipoprotein profile, elevating the concentration of HDL significantly. The observed outcome of our study in a porcine model is consistent with previous studies using rabbit and rat models (Abdelnour et al., 2018; Malini et al., 1999; Meriga et al., 2017; Tu et al., 2014; Vijayakumar and Nalini, 2006b). It is worth mentioning that atherogenic coefficient (AC), which is a ratio calculated as (TC-HDL)/HDL is relying on the significance of HDL for predicting the risk of CAD (Brehm et al., 2004). We also attempted to calculate other lipoprotein ratios or “atherogenic indices”. Although black pepper slightly reduced the AC level, including Castelli's risk index I (TC/HDL) and -II (LDL/HDL), no statistical significance of the effect was achieved. The

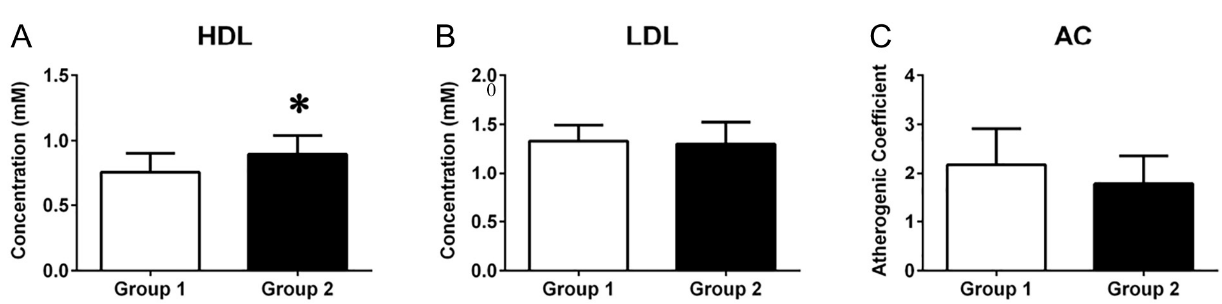

Fig. 1. Effect of black pepper dietary supplementation on pig serum lipid levels. Experimental animals were randomly assigned into group 1 (ordinary diet) and group 2 (black pepper-supplemented diet; 3 g/kg food). At the end of the experiment, i.e. as soon as the pigs from both groups reached a LBW of 100 kg, HDL and LDL blood serum levels were detected by biochemical test kits as described in the material and methods section ($N = 10$). *$P < 0.05$ versus control. AC: atherogenic coefficient.

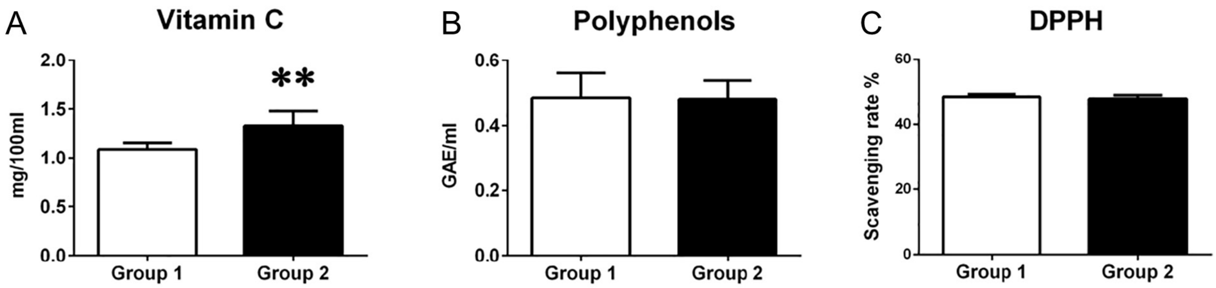

Fig. 2. Effect of black pepper-supplemented diet on anti-oxidant capacity parameters of pig serum. The study conditions were the same as described in the legend of Fig. 1. At the end of the feeding experiment, serum anti-oxidant status parameters were measured. A: vitamin C level in blood serum ($N = 8$); B: polyphenol levels in blood serum ($N = 5$); C: scavenging capacity of DPPH (N = 5). GAE: gallic acid equivalents. **P < 0.01 versus control.

Table 2
Productive performance of fattening pigs from 30 to 100 kg LBW.

	Groups Indexes	Period 1 (30 to 60 kg)						Period 2 (60 to 100 kg)						Period 1 + 2 (30 to 100 kg)					
		Group 1			Group 2			Group 1			Group 2			Group 1			Group 2		
			C	x		C	x		C	X		C	x		C	x		C	x
Feed intake per capita daily	Feed, kg	2.059	5.46	1.32	2.063	5.91	1.43	3.195	6.18	1.50	3.200	6.11	1.48	2.573	6.08	1.48	2.583	5.93	1.44
	Metabolizable Energy, MJ	26.477	5.46	1.32	26.534	5.91	1.43	39.366	6.18	1.50	39.427	6.12	1.48	32.285	6.08	1.48	32.415	5.93	1.44
	Protein, g	327.157	5.46	1.32	327.858	5.91	1.43	481.211	6.18	1.50	481.964	6.11	1.48	405.180	6.08	1.48	398.280	5.93	1.44
	Weight at the beginning of the period, kg	27.000	13.73	3.33	27.059	13.16	3.19	62.471	13.42	3.25	61.765	10.75	2.61	27.000	13.73	3.33	27.059	13.16	3.19
	Weight at the end of the period, kg	62.471	13.42	3.25	61.765	10.75	2.61	101.882	2.16	0.52	101.824	2.65	0.64	101.882	2.16	0.52	101.824	2.65	0.64
	Average daily growth, g	0.669	17.93	4.35	0.655	12.74	3.09	0.904	9.36	2.27	0.905	13.86	3.36	0.777	10.43	2.53	0.766	9.05	2.19
Consumption per kg gain	Feed, kg	3.168	18.94	4.59	3.194	13.36	3.24	3.576	13.81	3.35	3.610	15.53	3.77	3.354	14.88	3.61	3.403	13.04	3.16
	Metabolizable Energy, MJ	40.737	18.94	4.59	41.079	13.35	3.24	44.050	13.81	3.35	44.472	15.53	3.77	42.099	14.88	3.61	42.714	13.04	3.16
	Protein, g	503.358	18.94	4.59	507.583	13.85	3.24	538.475	13.81	3.35	543.631	15.53	3.77	517.259	14.88	3.61	524.815	13.04	3.16

C: coefficients of variation; x: the average, x-bar.

gained results nevertheless suggest that black pepper may have the potential to positively affect the incidence of CVD. Previous research found that the formation and metabolism of HDL involves ABCA1, apoA1, lecithin-cholesterol acyltransferase (CETP), ATP-binding cassette transporter G1 (ABCG1), and scavenger receptor class B type I (SR-BI), among others (Zannis et al., 2015). By promoting the secretion of cellular free cholesterol to the extracellular acceptor apoA1, ABCA1 play an essential role in the nascent HDL formation. In a previous work, we reported that piperine, the major bioactive constituent of black pepper fruits, up-regulates ABCA1 levels significantly but has no effect on ABCG1 and SR-BI (Wang et al., 2017). Piperine-induced ABCA1 upregulation might be the underlying mechanism explaining the observed black pepper-mediated enhancement of blood serum HDL levels, since the cholesterol efflux-mediating activity of ABCA1 results in the production of HDL from lipid-poor apolipoprotein A-I, which serves as acceptor of the effluxed cholesterol. However, unambiguous corroboration of such underlying mechanism would need follow-up experiments using the pure compound piperine as a dietary supplement. Aline the same line, as a key regulator of HDL metabolism, CETP plays an important role by transferring cholesterol from HDL to apolipoprotein B-containing lipoproteins, but a previous study reported that black pepper extract displayed no inhibition of CETP activity (Jin and Cho, 2011).

Black pepper is also known as a rich source of anti-oxidants, which can inhibit and neutralize free radicals (Srinivasan, 2007). A number of different diseases have been associated with pro-oxidative processes, and improvement of anti-oxidant defenses of the body have been considered as preventive and therapeutic strategies (Hertog et al., 1993; Yeung et al., 2019). Previous studies have shown that the level of plasma vitamin C could be elevated by simultaneous piperine supplementation (Vijayakumar and Nalini, 2006a), which was confirmed in principle by our data in this study. The black pepper diet used in our study exhibited no effect on polyphenol concentration in serum and on DPPH scavenging capacity. These two anti-oxidant parameters are dose-dependent (Agbor et al., 2007), and our used dose of black pepper might have been too low to induce significant changes. Nevertheless, piperine, essential black pepper oil, and oleoresin of black pepper, also have been shown to possess the direct ability to scavenge superoxide anion radicals and hydroxyl radicals, and resist linoleic acid lipid peroxidation (Kapoor et al., 2009). Therefore, presence of these functional ingredients in black pepper suggests it as a potential candidate to ameliorate oxidative stress and to scavenge free radicals (Mittal and Gupta, 2000). Notability, another study indicated that black pepper essential oil could inhibit key enzymes relevant to type-2 diabetes and hypertension for its anti-oxidative properties, suggesting it as a potential agent for management and/or prevention of these two diseases (Oboh et al., 2013).

As a major type of animal in used in agriculture, pigs are not only the main contributor for the production of meat and meat products, but also a good animal model for human diseases. The cardiovascular system, digestive system, nutritional needs and mineral metabolism of pigs are similar to human, as well as parts of anatomy, physiology and biochemistry of body (Gonzalez et al., 2015). Black pepper supplementation-related research using the porcine model can not only evaluate its utility to modify the health status of the animals, but can also indicate possible benefits for human health, and even give hints for possible higher quality of animal products obtained upon black pepper supplementation (all of such putative benefits need to be corroborated with future research). Due to its relevance for human health, the porcine model is overall considered to be of a transformative value in biomedical research and development (Kuzmuk and Schook, 2011). Moreover, like in humans, animal health is also closely related to food, and animal health improvement might result in higher quality animal products (if this would be the case for the quality of meat in black pepper supplemented animals is an interesting question that needs to be addressed with future research). The use of food supplementation in piglets in particular is also very suitable for evaluation of putative effects on growth rate and feed utilization.

In recent years, natural spices become increasingly attractive as a food supplement in animal feeding. Generally, feed is used to ensure the general dietary energy for animals, and the addition of certain dietary supplements (e.g., spices) may influence animal feed intake, meat production, animal health, and even give hints for putative human health benefits. Black pepper has widely known anti-microbial properties, and the extracts from its fruits and leaves exhibit strong anti-bacterial activity against certain plant pathogenic bacteria and common microorganisms in food. For example, inhibitory effects on *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger* have been shown in several studies (Awuah and Kpodo, 1996; Mandeel, 2005; Morsy and Abd El-Salam, 2017; Zarringhalam et al., 2013). Also, this spice has been shown to have a positive effect in normalizing digestive system function and can help a hypofunctioning digestive system by promoting the secretion of hydrochloric acid in stomach (Myers et al., 1987). On the other hand, black pepper has also been shown to inhibit intestinal chloride secretion and gas hyperplasia to reduce constipation, diarrhea and the abdominal pain (Pongkorpsakol et al., 2015). In our study, the growth of the animals from the two groups was quantified by continuous monitoring of feed intake and LBW through the study course. Although the black pepper-supplemented diet had no significant effects on average daily growth in both the growing and the fatting period, it showed a tendency to increase daily food intake and promote animal feeding probably by stimulating appetite and enhancing digestive functions.

5. Conclusion

In this study, we found that milled black pepper fruits supplementation has positive effects throughout the growth of pigs: (1) The supplementation elevated serum HDL levels significantly, suggesting that this biologically active spice may play a potential role in lipid regulation and cardiovascular protection; (2) The level of vitamin C in blood serum is increased significantly in the supplemented group, indicating that the black pepper supplementation might boost anti-oxidant defenses. Considering the various known biological activities of this phytogenic dietary additive, its effects on immune and digestive parameters deserve to be studied in more detail in future work. In case that the beneficial effects of black pepper supplementation can be translated into other animal models and human subjects, it might prove to be of a high benefit to develop functional foods and food supplements exploiting the various health-promoting effects of black pepper and its constituents.

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Declaration of competing interest

The authors declare no conflict of interest.

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