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Effect of dietary oregano essential oil on the growth, meat quality, selenium distribution, and serum biochemical traits of pigs

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SUMMARY

Natural substances of plant origin play an important role in the protection of animal health and improvement of the quality and health safety of food products of animal origin. The research was carried out to determine the effect of oregano essential oil used as a feed additive for pigs on growth performance, meat quality and chemical composition, the level of selenium in pig tissues, and selected serum biochemical indicators. The experiment was conducted on 40 fatteners of the 990 Polish Synthetic Line. The animals were divided into two groups (20 animals in each) with an equal share of gilts and barrows. The pigs in the control group (CG) received a basal diet, while the pigs in the experimental group (OG) received the same diet supplemented with 300 mg·kg^{-1} Ecodiar®, containing 5% natural oregano oil (*Origanum vulgare* ssp. *hirtum*). The study did not show a significant effect of the preparation containing oregano oil on the body weight of fattening pigs, their growth rate, or feed intake and utilization. Chemical analysis showed that pigs receiving the oregano oil supplement had significantly higher ($P \leq 0,05$) content of dry matter in the longissimus dorsi (LD) muscle. The LD muscle colour assessment showed that supplementation of the diet with oregano oil during the fattening period had a significant effect on the yellow colour component of meat (higher b* values; $P \leq 0,05$). Supplementation with oregano oil significantly reduced the serum triglyceride concentration ($P \leq 0,05$). The addition of oregano oil to the feed does not affect the fattening and carcass characteristics of pigs; however, it may have a beneficial effect on some meat quality characteristics of fatteners.

KEY WORDS: pigs, oregano oil, fattening performance, meat, selenium

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INTRODUCTION

Natural substances of plant origin play an important role in the protection of animal health and improvement of the quality and health safety of food products of animal origin (Shah et al. 2014, Alagawany et al. 2015). Owing to its broad-spectrum properties, oregano is of great interest to researchers (Bakkali et al. 2008, Embuscado 2015). Oregano essential oil contains about 120 compounds, the amounts of which depend on the species, but also on the environment in which the plant grows (Azizi et al. 2009, De Falco et al. 2013, Leyva-López et al. 2017), The main components of oregano essential oil are phenolic compounds, such as thymol and carvacrol, substances with confirmed antioxidant properties (Loizzo et al. 2009, Han et al. 2017). However, the antioxidant effect of oregano essential oil depends on the amount administered and its composition (Kulisić et al. 2004, Oniga et al. 2018).

Research by Botsoglou et al. (2009) demonstrates the safety of using oregano oil in in vivo tests and indicates its hepatoprotective properties. This oil can be a source not only of natural antioxidants, but also of substances with bioprotective properties (Sahin et al. 2004, Avila-Sosa et al. 2010). There have also been studies showing its antimutagenic and antitoxic properties (Mossa et al. 2013).

Due to its antibacterial properties, oregano can be used as a substitute for antibiotics. Oregano oil exhibits antibacterial activity against various strains of Gram (+) and Gram (-) bacteria (Grondona et al. 2014) and reduces biofilm formation by bacteria of the species *Staphylococcus aureus* and *Staphylococcus epidermidis* (Nostro et al. 2007). It can help to limit the emergence and spread of pathogenic organisms, while it is safe for animal health.

Biologically active substances from oregano also play an important role in improving the immunity and integrity of the intestinal barrier in pigs (Zou et al., 2016). The reduction of undesirable intestinal microorganisms promotes the absorption and utilization of nutrients from feed. Thymol, by protecting the intestinal microvilli responsible for nutrient absorption, has a positive effect on the secretion of endogenous digestive enzymes and on blood components (Bampidis et al. 2005, Hashemipour et al. 2013).

Lipid oxidation processes in meat negatively affect its taste, colour and nutritional value and are responsible for the formation of toxic compounds. The antioxidants contained in essential oils also improve the quality of meat (Rossi et al. 2013). As natural metabolites, phenolic compounds have been found to counteract oxidative stress in muscles (Mahfuz et al. 2021).

A safe level of oregano oil for fattening pigs, according to a study by EFSA (2019), is 48 mg/kg of feed. Although much research has been done using oregano oil, not all aspects of its mechanisms of action are understood. It is worth analysing its exact effects not only on growth performance, but also on homeostasis in the body. The inclusion of oregano oil in the diet, due to its content of polyphenols, may also improve the quality of food from farm animals (Starčević et al. 2015).

Therefore, this study is aimed at verifying that oregano oil as a feed additive for pigs has a beneficial effect on growth performance, the quality and chemical composition of meat, the level of selenium in the body, and selected serum biochemical indicators.

MATERIALS AND METHODS

Animals and feeding

The experiment was conducted at the National Research Institute of Animal Production in Poland in Pawłowice, on 40 fatteners of the 990 Polish Synthetic Line. The experiment was carried out in accordance with the ethical and welfare standards laid out in EU Directive 2010/63/EU. The animals were assigned to two dietary groups matched for sex and body weight (20 animals each with an equal share of gilts and barrows). The animals were housed in individual pens (1 x 2 m). The feed was supplied ad libitum, and water was provided by nipple drinkers.

Table 1
Ingredients and nutritional value of the basal diets

Dietary components (%)	Grower (1st period)	Finisher (2nd period)
Wheat	20,00	30,00
Maize	-	10,00
Barley	25,00	-
Triticale	30,00	35,00
Soybean meal 46%	18,00	10,00
Rapeseed meal	-	6,00
Wheat bran	2,54	5,50
Limestone	1,00	1,10
Monocalcium phosphate	0,80	0,30
NaCl	0,35	0,35
L-lisyne	0,20	0,20
DL-methionine	0,04	-
L-threonine	0,05	0,02
Soybean oil	1,50	1,00
Porzyme 9300	0,025	0,025
Phyzyme XP TPT	-	0,01
Premix 0,5 %*	0,50	0,50
Nutrients ($g \cdot kg^{-1}$)		
Metabolizable energy **($MJ \cdot kg^{-1}$)	13,00	13,00
Crude protein	175,96	162,10
Lysine	10,05	9,07
Methionine+cystine	6,20	5,91
Threonine	6,60	5,95
Tryptophan	2,10	1,88
Ca	6,60	6,10
P	5,80	5,28
Na	1,57	1,56
Se (Na2SeO3)	0,30	0,30

*Vitamin-mineral premix provides (per kg basal diet): vitamin A 8000 IU; vitamin, D_3 1000 IU; vitamin E 60 mg; vitamin K_3 2 mg; vitamin B_1 2 mg; vitamin B_2 4 mg; vitamin B_6 4 mg; vitamin B_{12} 25 µg; biotin 100 µg; pantothenic acid 10 mg; niacin 20 mg; folic acid 400 µg; choline chloride 600 mg; Fe 80 mg; Mg 400 mg; Mn 40 mg; Zn 100 mg; Cu 10 mg; I 0,8 mg; Co 0,4 mg, Se 0,3 mg.
**Calculated from Pig Nutrition Standards (1993)

Pig fattening was carried out from 28±1 to 110±1 kg body weight. The basal diets (grower from 28±1 to 74±1 kg body weight and finisher from 74±1 to 110±1 kg body weight) were in accordance with Pig Nutrition Standards (1993). The ingredients and nutritional value of the feed used in the experiment are given in Table 1.

The pigs in the control group (CG) received a basal diet, while the pigs in the experimental group (OG) received the same diet supplemented with 300 mg·kg^{-1} Ecodiar® (Ecopharm Helas S.A.), containing 5% natural oregano oil (*Origanum vulgare* ssp. *hirtum*). The main constituents of oregano essential oil (OEO) according to the supplier were 81,02% carvacrol 5,42% p-cymene, 4,65% γ-terpinene, and 3,02% thymol.

During this experimental period, pigs were weighed individually three times: at the start of fattening (28 kg±1kg), at 74±1 kg body weight (BW), and before slaughter (110±1 kg). Average daily gain (g), feed intake (kg per day), and feed conversion (per kg of weight gain) were determined at the end of each fattening period for all pigs and throughout the experimental period.

Sample collection

All pigs were slaughtered at 110±1 kg body weight (at the end of the fattening period). Pre-slaughter fasting did not exceed 24 h. The animals from both groups were slaughtered in a way that minimized pain, suffering and stress in accordance with applicable zootechnical procedures. Slaughter and evaluation of slaughter performance were carried out at the Pig Testing Station.

Samples were collected from all animals in each group. Blood was collected from the jugular vein (at slaughter) to obtain serum. The samples were allowed to coagulate at room temperature for 1 h. Serum was separated by centrifugation at 4°C, 2000 x g for 10 min. Samples of serum and the longissimus dorsi muscle (the last three thoracic vertebrae) were collected and stored at −20°C.

Carcass measurement

Carcass evaluation was carried out in accordance with the methodology used at Pig Testing Stations (Różycki and Tyra, 2010). The hot carcass weight was determined using a scale, about 30 min after the start of slaughter procedures. The cold carcass weight was determined after about 24 h of chilling at 2–4°C. The cold dressing percentage was calculated from the cold carcass weight. Lean meat content was evaluated according to the SEUROP grading method (Commission of the European Communities 2005), using the Sydel Capteur Gras/Maigre (CGM) apparatus (Sydel, Lorient, France). The lean meat content of the carcass was calculated automatically by the CGM apparatus using the following formula:

$$Y = 50{,}11930 - 0{,}62421X_1 + 0{,}26979X_2$$

where: Y is the estimated percentage of lean meat in the carcass; X_1 is the thickness of the fat (including the rind) between the third and fourth last ribs at 6 cm off the dorsal midline, at a trajectory perpendicular to the rind (in mm); and X_2 is the thickness of the longissimus dorsi muscle (in mm), measured at the X_1 position.

The pH_{45} (45 min post-mortem) was measured in the longissimus dorsi muscle (between the last thoracic vertebra and first lumbar vertebra) using the MATTHÄUS, pH-STAR CPU pH-meter (Matthäus, Pöttmes, Germany), with a glass electrode standardized for pH 4,6 and 7,0. Meat colour was determined in the longissimus dorsi muscle (last thoracic vertebra) 24 h after slaughter using the Minolta Chroma Meter CR-310 (Minolta Co., Ltd., Japan), in the L*a*b* system, where the L* value designates lightness, ranging from 0 for black to 100 for perfect white, and a* and b* are colour coordinates (+a* = red, –a* = green, +b* = yellow, –b* = blue). The measuring system mounted in

the probe consists of an integrated mirrored sphere connected to a flash lamp. The lamp transmits diffused light to the test sample. The light reflected from the sample is captured inside the sphere by an optically conductive wire. The reflected light beam is further transmitted to the portable unit, where it is divided into three parts and directed to carefully standardized colour filters and then to the photometric elements. At the same time, the reflection of the source light from the surface of the sphere is evaluated. The water holding capacity (WHC) of the meat was determined by the Grau and Hamm method (Hamm 1986).

Chemical analysis

The proximate chemical composition of the grower and finisher diets and the longissimus dorsi muscle were determined by standard AOAC methods (2000). Amino acids in the diet were assayed using the Beckman automatic analyser. Phosphorus (P) was assayed by the vanadium-molybdenum photo-colorimetric method, and calcium (Ca) and sodium (Na) by emission spectrometry on a Buck Scientific spectrophotometer. Total cholesterol content in the muscle was determined by an AOAC (2000a) method. Cholesterol content in the meat was determined by gas chromatography with mass spectrometry (GCMS) on a CLARUS 600 system from Perkin Elmer (USA). Samples for analysis were prepared according to AOAC method 994.10. The reference standard was cholesterol from Sigma-Aldrich (Cat. No. C8667), while 5α-cholestan-1-β-ol was used as the internal standard (Sigma-Aldrich, Cat. No. R206296).

Analysis of serum biochemical traits

Blood samples for serum were collected during slaughter from the jugular vein of all pigs. After blood centrifugation, the serum was frozen at –20°C. The following biochemical indices were determined in the thawed serum: glucose, total cholesterol, HDL fraction, LDL fraction, and triglycerides. Cholesterol, HDL cholesterol, glucose, and triglycerides were measured in the serum using BioMaxima reagent kits (Poland). The absorbance was measured using a Marcel® PRO (Bio) spectrophotometer (Poland). LDL cholesterol levels were calculated using the Friedewald formula (Friedewald et al. 1972).

Selenium content

Se concentrations in samples of kidney, liver, and longissimus muscle (1 g) and serum (1 ml) were determined by mineralizing the samples in concentrated HNO_3 and $HClO_4$, and subsequently using 9% HCl. Se content was determined by spectrofluorimetry using 2,3-diaminonaphthalene (Shimadzu RF-5001 PC spectrofluorophotometer). Selenium was determined using the method described by Pilarczyk et al. (2010). The accuracy of the analytical method was verified using serum reference material (Seronorm trace elements serum L-1) and certified reference materials NCS ZC 71001 (beef liver).

Statistical analysis

The data obtained are expressed throughout as arithmetic means and standard error of the mean (SEM). The influence of the nutritional factor on the examined traits was assessed using the following linear model:

$$Y = m + ax + e,$$

where:

Y – value of trait

m – population mean

a – effect of nutritional factor

x – value of nutritional factor

e – sampling error

The normal distribution of the data was evaluated by the Shapiro–Wilk test. Statistical analysis of the data was performed by one-way analysis of variance (ANOVA) using STATISTICA 13.0 PL computer software. The significance of differences between groups was evaluated with the Duncan test.

RESULTS

Fattening performance

The study did not show a significant effect of the preparation containing oregano oil in the diet of fattening pigs on their body weight, growth rate, feed intake, or feed utilization (Table 2). However, the animals in group OG had slightly lower fattening performance parameters compared to group CG.

Table 2

Effect of the addition of oregano oil to the diet of pigs on fattening performance (n = 20)

Parameter	CG (Mean)	OG (Mean)	SEM	P-value
Initial BW (kg)	27,2	28,6	0,612	0,279
Average BW (kg)	74,7	75,1	1,078	0,867
Final BW (kg	110,2	109,6	1,253	0,821
Average daily gains (g):				
1st fattening period	754	739	13,58	0,576
2nd fattening period	721	694	21,03	0,536
Whole fattening period	740	720	11,75	0,339
Feed intake ($g \cdot day^{-1}$):				
1st fattening period	1921	1909	22,43	0,784
2nd fattening period	2518	2521	42,99	0,965
Whole fattening period	2178	2174	19,39	0,924
Feed conversion (kg) per 1 kg of weight gain:				
1st fattening period	2,57	2,63	0,630	0,645
2nd fattening period	3,54	3,80	0,126	0,313
Whole fattening period	2,96	3,05	0,045	0,288

BW – body weight

Carcass characteristics and meat quality

The carcasses of fattening pigs receiving oregano oil also had slightly lower meat content compared to the control group. However, there were no differences in the dressing percentage or hot half-carcass weight (Table 3).

Table 3
Carcass traits and physicochemical composistion of the longissimus dorsi muscle of pigs (n = 16)

Item	CG (Mean)	OG (Mean)	SEM	P-value
Lean meat content (%)	60,00	59,00	0,326	0,127
Hot carcass weight (kg)	90,00	90,00	0,991	0,867
Cold dressing percentage (%)	80,00	80,00	0,209	0,688
Dry matter (%)	25,50[a]	26,50[b]	0,231	0,028
Protein (%)	22,40	22,90	0,194	0,079
Intramuscular fat (%)	2,18	2,21	0,132	0,864
Cholesterol ($mg \cdot 100g^{-1}$)	89,50	84,70	1,418	0,088
pH 45 min	5,96	5,87	0,074	0,559
Minolta L*	52,06	54,36	0,820	0,163
Minolta a*	20,08	21,86	0,791	0,105
Minolta b*	8,70[a]	10,27[b]	0,464	0,017
WHC (% of bound water)	70,10	71,10	0,651	0,842

a, b – significant difference $P \leq 0,05$

Chemical analysis showed that pigs receiving oregano oil had significantly higher ($P \leq 0,05$) content of dry matter in the longissimus dorsi (LD) muscle. The intramuscular fat content, pH_{45} after slaughter, and water-holding capacity (WHC) in the LD muscles of the groups were similar. The LD muscle colour assessment showed that supplementation with oregano oil during the fattening period had a significant effect on the yellow colour component of meat, i.e. higher b* values ($P \leq 0,05$) compared to group CG. Higher values for lightness and redness were also obtained for the meat of pigs from group OG, but the difference was not statistically significant. Oregano oil containing thymol and carvacrol reduced the cholesterol content in the meat by about 5%.

Serum biochemical analysis and tissue selenium content

There were no significant differences in the selenium concentration in the examined tissues between the groups (Table 4). However, the serum selenium concentration was elevated by 16% in group OG in relation to group OG. Supplementation with oregano oil significantly reduced the serum triglyceride concentration ($P \leq 0,05$). In group OG, the total and LDL cholesterol concentrations as well as the concentration of glucose in the serum decreased.

Table 4

Selenium content in the organs of pigs and biochemical parameters in the serum (n = 16)

Parameter	CG (Mean)	OG (Mean)	SEM	P-value
Selenium content (mmol ·l^{-1}):				
- serum	2,77	3,22	0,129	0,094
- liver	0,50	0,56	0,025	0,273
- longissimus muscle	0,09	0,10	0,004	0,461
- kidney	1,33	1,30	0,039	0,800
Biochemical parameters (mmol· l^{-1}):				
- total cholesterol	2,49	2,28	2,865	0,151
- HDL cholesterol	1,05	1,09	0,037	0,609
- LDL cholesterol	1,19	1,02	0,073	0,226
- Triglicerydes	0,68^{a}	0,52^{b}	0,036	0,023
- Glucose	5,18	5,07	0,357	0,878

a, b – significant difference $P \leq 0,05$

DISCUSSION

Fattening performance

This study found no significant effect of the preparation containing oregano oil on the growth and feed conversion of fattening pigs (Table 2). The studies of other authors (Simitzis et al. 2010; Ranucci et al. 2015, Forte et al. 2017) also showed no significant effect of oregano oil on the daily gains and final body weight of fattening pigs. There was no evidence (Simitzis et al. 2010) of an increase in the growth rate of fatteners following the use of different levels of oregano oil (0,25; 0,5 and 1 ml·kg^{-1} of feed). As in the present study, fattening pigs fed with 0,2% oregano oil added to the diet did not attain higher body weight gain or better feed utilization than pigs from the control group (Forte et al. 2017). However, a study by Zou et al. (2016) using 25 mg OEO·kg^{-1} of feed showed better daily gain (ADG) and feed conversion per kg of daily gain (F/G). Another study (Cho et al. 2012) found that carvacrol, which is the main component of OEO, reduces body weight and weight gain in mice. In a study on fish, diet supplementation with oregano oil was not shown to affect functional traits (Cararo et al. 2017). Essential oils improve digestion and nutrient absorption by protecting the intestinal villi and by enzymatic stimulation (Bampidis et al. 2005, Hasemipour et al. 2013). In the present study, this effect did not translate into growth rate or feed utilization per kg of weight gain in the fatteners.

Carcass characteristics and meat quality

In this study, the addition of oregano to the diet of pigs showed a tendency to reduce meat content. Similar results were observed in another study (Hofmann et al. 2022), in which significantly lower ham weight was obtained in pigs receiving a diet supplemented with oregano. Chemical analysis of the longissimus dorsi muscle showed that the addition of OEO significantly increased the dry matter content ($P \leq 0,05$) in the muscle. Studies using oregano oil in the diet of fattening pigs have not investigated its effect on the chemical composition of the longissimus dorsi muscle. The significantly higher dry matter content in the longissimus dorsi muscle of pigs in group OG may result from the

properties of oregano reducing undesirable intestinal microbes, thereby promoting the absorption of nutrients (Bampidis et al. 2005).

As in other studies (Simitzis et al. 2010; Ranucci et al. 2015; Forte et al. 2016), the addition of OEO to the diet was not shown to significantly affect the pH_{45} of the longissimus dorsi muscle of fattening pigs (Table 3).

The addition of oregano oil did not significantly affect the lightness or redness of the meat colour, but significantly increased its yellowness ($p \leq 0,05$). These results were not confirmed in research on growing pigs conducted by Simitzsis et al. (2010), in which oregano oil was administered for 35 days at various concentrations (0,25; 0,5 and 1 $ml \cdot kg^{-1}$). Janz et al. (2007) also showed that OEO supplementation did not affect the lightness (L*) of the meat colour of pork.

Colour is one of the most important quality characteristics of meat. The natural colour of meat results from its content of myoglobin and haemoglobin. Meat components that affect its colour are highly susceptible to oxidation. The variability of the b* parameter in pork depends almost exclusively on the relative content of chemical forms of myoglobin, with oxymyoglobin having the highest yellowness (b*), metmyoglobin lower yellowness, and deoxymyoglobin the lowest (Lindahl et al., 2001, Karamucki et al. 2013). Diet supplementation with oregano essential oil may indirectly affect the colour of meat by reducing haemoglobin oxidation and activating mechanisms that modify the distribution of pigment in animal tissues (Botsoglou et al. 2002, Simitzis et al. 2008, Garcia-Galicia et al. 2020). According to some authors (Brewer et al. 2001, Karamucki et. al. 2013), the yellowness of the colour depends mainly on the pH of meat, which affects the intensity of redox processes. A slight decrease in the pH of the meat of animals receiving oregano oil could increase the amount of the oxidized form of oxymyoglobin and thus significantly increase the yellowness of meat, with a slight increase in lightness and redness.

Serum biochemical analysis and tissue selenium content

This study found no significant effect of the addition of oregano oil on the concentration of selenium in pig tissues. There are also no studies confirming these dependencies, as the effect of oregano oil on the selenium content in pigs has not been investigated. However, it has been shown that active substances can affect the absorption of minerals by improving the functioning of the gastrointestinal tract (Zou et al. 2016). This may result in a slightly increased (by about 16%) concentration of selenium in the serum. A slight increase in the serum selenium concentration may also be associated with an increase in selenium-containing antioxidant enzymes (Tan et al. 2015, Samarghandian et al. 2016). A study by Reshadi (2020) has shown that the use of oregano oil increases serum antioxidant status. This is due to the widely documented antioxidant properties of carvacrol, the main component of oregano essential oil (Hashemipour et al. 2013, Leyva-López et al. 2017).

The present study showed a significant decrease ($P \leq 0,05$) in triglyceride concentration in group OG. Similar results were obtained in studies on poultry (Caceda-Gallardo et al. 2020, Mendoza-Ordoñez et al. 2020). Increasing the concentration of oregano oil to 200 $mg \cdot kg^{-1}$ causes a further reduction in the concentration of triglycerides ($P \leq 0,05$) in the serum of turkeys (Mendoza-Ordoñez et al. 2020). Carvacrol, the main component of oregano oil, reduces plasma triglycerides (Lee et al. 2003). A slight, statistically insignificant decrease was also obtained for the level of total cholesterol and its fractions following the use of OEO (Table 4). The use of OEO in studies on poultry (Migliorini et al. 2019, Caceda-Gallardo et al. 2020, Mendoza-Ordoñez et al. 2020) also had no

hypocholesterolaemic effect. Essential oils can improve digestion and nutrient absorption through enzymatic stimulation (Bampidis et al. 2005). Thymol has been shown to lower cholesterol by inhibiting the production of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which regulates cholesterol synthesis (Elson 1995). A reduction in cholesterol synthesis also decreases the concentration of LDL cholesterol. The lack of hypocholesterolaemic effect of the OEO used may be due to the relatively small amount of this compound compared to carvacrol, whose content is much higher. Consequently, the effect of lowering serum triglycerides is more pronounced.

CONCLUSIONS

The results of this study suggest that supplementation of pigs' diet with oregano oil does not affect growth performance or carcass quality. It increases the yellowness of meat and the content of dry matter, while decreasing the concentration of triglycerides in the serum. While oregano oil supplementation had a limited effect on the performance characteristics of pigs, it influenced meat quality parameters. At the same time, it did not negatively affect the selenium content in the bodies of fattening pigs.

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