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The influence of feed additive "Activo" on the content of total lipids and their classes profile in liver and skeletal muscles of pigs

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None to declare.

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The research methodology had been approved by the Bioethics Committee of the Institute of Animal Biology of the National Academy of Agricultural Sciences of Ukraine (Protocol no. 93-01 from 03.06.2021).

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With an increase in meat consumption the requirements for its quality also rise. As pork is the second most popular meat in the world, producers of this product are trying to improve its quality. The purpose of our research was to determine the effect of the feed additive "Activo", containing essential oils of cinnamon, rosemary and oregano, and chili pepper extract, on the total lipids content and their individual class profile in liver and skeletal muscles of pigs. The study was conducted on pigs of the large white breed, weighing 70 kg, divided on two groups, according to the piglets-analogues principle, which then were divided into control and experimental groups. Pigs of both groups received standard feed (SF) intended for this age group. Pigs of the experimental group received the feed additive "Activo" in an amount of 0.1 kg/t of feed in addition to SF. The duration period of experiment was 40 days. Samples of liver, *longissimus dorsi*, and *latissimus dorsi* muscle tissues were used as material for the study. The total lipid content was determined using gravimetric method, and individual lipid profile using thin-layer chromatography on silica gel plates. The study has shown that the total lipid content in the liver, long and broad back muscles of pigs of the experimental group was 1.66 and 1.95 ($P \leq 0.001$) and 1.26 ($P \leq 0.05$) times higher, respectively, than in animals of the control group. The relative cholesterol esters content in the liver lipids of the experimental group pigs was 1.9 ($P \leq 0.001$) times higher than in the control group. Instead, in the *latissimus dorsi* muscle, a lower content of triacylglycerols and a higher content of unesterified fatty acids ($P \leq 0.05$), esterified cholesterol ($P \leq 0.05$), and especially phospholipids ($P \leq 0.001$) were admitted. It was determined significant decrease of unesterified cholesterol content in the *latissimus dorsi* muscle ($P \leq 0.001$) and increase of phospholipids content ($P \leq 0.001$) under the effect of the researched feed additive.

Key words: lipids, phospholipids, triacylglycerols, essential oils, feed additive, lipid metabolism

Introduction

Growth and development intensity in pigs depends on a large number of factors, including the genetic potential, housing conditions, and feeding. Nowadays feed production technologies have made significant progress in pig production development. First of all, it is the use of new feed additives, in particular substances of natural origin [3]. Over the past decade, the number of additional supplements included in the pig diet has increased significantly. Since 2006, this trend has received a new impetus, as the use of hormones and growth promoting antibiotics has been banned [2]. Antibiotics are often used where the housing conditions are not ideal, which lead

to a number of bacterial diseases. Another reason of the feed antibiotics use was animal growth promoting. Furthermore, no one paid attention to the fact that the use of growth promoting antibiotics negatively affects the production of quality offspring, increases the cost of animal keeping and the feed cost, and deteriorates the livestock and poultry products quality. Along with the use of enzymes, probiotics, medium-chain fatty acids, plant products in the form of essential oils have also been used [4]. A significant progress has been made in the research of various essential oil-based supplements and their effects on various metabolic pathways in animals, poultry, and fish [1, 7], especially on pig productivity and meat quality [2] in recent times. One of these additives is "Activo", which is a combination of biologically active substances extracted from aromatic herbs and spices. This feed additive contains: cinnamon oil, rosemary oil, chili pepper extract and oregano oil. According to many researchers, these components have antioxidant and anti-inflammatory [6] and hepatoprotective properties [8], stimulate the activity of digestive enzymes and gastric juice secretion, improve feed conversion [9], and exhibit bactericidal and antioxidant properties, inhibiting the growth and development of pathogenic microflora [5].

The purpose of the study was to determine the effect of the biologically active feed additive "Activo" on the total lipids content and their individual classes profile in the liver and skeletal muscles of piglets.

Materials and Methods

The experiment was conducted at a pig farm located in the Odesa region, Ukraine. Pigs of large white breed, of the same age, weighing 70 kg, were divided into two groups: control and experimental, 43 animals each by the principle of pairwise analogues. Pigs were housed in the same box under the same conditions and consumed standard feed (SF) 380673220006-C5. The pigs of the experimental group were fed (SF) and supplemented feed additive "Activo" in the amount of 0.1 kg/t of finished feed.

Samples of liver tissue, long and broad back muscles taken from the animals at the end of the experiment were used for biochemical studies.

Duration of the experiment was 40 days. At the end of the study animals of both groups were control-slaughtered and samples of liver, *longissimus dorsi* and *latissimus dorsi* muscles were taken for laboratory studies.

The study is fully complied with the ethical requirements for the use of animals in experimental research (Strasbourg, 1986; Kyiv, 2002), and the research methodology was approved by the Bioethics Committee of the Institute of Animal Biology of the National Academy of Agricultural Sciences of Ukraine (Protocol no. 93-01 from 03.06.2021).

The total lipids content in the studied samples was determined by the weight method after extraction with a chloroform-methanol (2:1) mixture according to the Folch method. Separation of lipids into separate fractions was performed by thin-layer chromatography on glass plates. Lipid samples were applied to the plate with a microdispenser and placed in chromatographic chambers. After drying, the chromatograms were developed in a chamber saturated with iodine vapor. For the lipids quantification, the layers were scraped off the plates in test tubes, hydrochloric acid was added, and the mixture was heated up to 105°C. The optimal amount of each fraction was measured on a spectrophotometer. Separate classes of lipids were identified by comparison with a chromatogram with purified standards [7].

The Student's *t*-test and *Microsoft Excel* software was used for the statistical calculation of digital data.

Results of the Study

The results of the studies showed that the feed additive "Activo" supplementation to the feed of the experimental group pigs had a significant effect on total lipids content and their individual classes profile in the liver and skeletal muscles (tables 1–3).

Thus, the total lipid content in the liver, *longissimus dorsi*, and *latissimus dorsi* muscles of the experimental group pigs was 1.66 and 1.95 ($P \leq 0.001$) and 1.26 ($P \leq 0.05$) higher comparing to control group. These data indicate the effect of the studied feed additive on the processes of lipid synthesis in the pigs' organism, which led to the accumulation of fat in the researched organs and tissues.

On the one hand, such an increase in the lipids amount in the researched tissues can be explained by fats presence in the feed additive [9], on the other hand, we did not find a significant increase in the relative content of triacylglycerols in the lipids composition of all samples. At the same time, a significant decrease in the content of this class lipids was noted among the lipids of the *longissimus dorsi* samples. In particular, the relative content of triacylglycerols decreased by 1.65 times ($P \leq 0.001$) compared to the control values.

The total lipid content increase in pig liver samples was due to an increase of cholesterol esters amount (table 1). Thus, the relative content of cholesterol esters in the liver lipids of pigs fed with the feed additive was 1.9 times higher ($P \leq 0.001$) than in the liver lipids of pigs of the control group.

The results of these studies indicate that the feed additive promotes, on the one hand, the intensification of fatty acid synthesis in the liver, and, on the other hand, their transport from the liver to other organs with blood. As for the relative content of other lipids classes in the liver — phospholipids, unesterified fatty acids and triacylglycerols — no significant changes were recorded under the influence of the feed additive, only a tendency to their decrease was found, but these data are not statistically significant.

Table 1. Lipids content and their individual classes profile in pig liver (M±m, n=5)

Parameter	Groups of animals	
	Control	Experimental
Total lipids, g/kg of raw mass	1.90±0.10	3.16±0.13***
Phospholipids, %	55.77±1.33	52±1.23
Unesterified cholesterol, %	8.79±0.83	10.82±0.76
Unesterified fatty acids, %	12.31±0.97	11.73±0.84
Triacylglycerols, %	18.98±0.50	17.07±0.76
Esterified cholesterol, %	4.15±0.33	7.97±0.45***

Note. Here and further: *— P<0.05, ** — P<0.01, *** — P<0.001.

Table 2. Lipids content and their individual classes ratio in the tissue of the *longissimus dorsi* muscle of pigs (M±m, n=5)

Parameter	Group of pigs	
	Control	Experimental
Total lipids, g/kg of raw mass	1.46±0.11	2.86±0.16***
Phospholipids, %	44.05±0.82	51.48±0.39***
Unesterified cholesterol, %	9.87±0.67	8.73±0.19
Unesterified fatty acids, %	11.43±0.77	13.93±0.42*
Triacylglycerols, %	28.52±1.38	17.20±0.13***
Esterified cholesterol, %	6.13±0.45	8.66±0.65*

Table 3. Lipid content and their individual classes profile in the *latissimus dorsi* muscle tissue of pigs (M±m, n=5)

Parameter	Groups of animals	
	Control	Experimental
Total lipids, g/kg of raw mass	1.84±0.09	2.32±0.12*
Phospholipids, %	51.32±0.30	55.09±0.45***
Unesterified cholesterol, %	10.08±0.82	7.67±0.58*
Unesterified fatty acids,%	10.14±0.80	10.14±0.78
Triacylglycerols,%	21.45±1.14	22.74±0.36
Esterified cholesterol,%	7.61±0.61	4.00±0.07***

When analysing changes in the content of certain lipids classes in samples of *longissimus dorsi* muscle (table 2), in addition to the decrease in triacylglycerols noted by us, attention is drawn to the increase in the content of unesterified fatty acids (P≤0.05), esterified cholesterol (P≤0.05), and especially phospholipids (P≤0.001). These data indicate a positive effect of the feed additive containing biologically active essential oils and extracts on the processes of structural lipids synthesis, and especially phospholipids synthesis in the pigs' muscle tissue.

Similar changes were also admitted in the study of certain classes of lipids content in the *latissimus dorsi* muscle of piglets (table 3). Under the action of the researched feed additive on the background of total lipids content increase (P≤0.05) in the broad back muscle of experimental group piglets, a significant increase in phospholipids content (P≤0.001) was found. Among other data, a decrease in the unesterified cholesterol content (P≤0.001) in the pigs' *latissimus dorsi* muscle of the experimental group compared to the control group is worthy of note.

Thus, summarising the data obtained, we can conclude that the feed additive "Activo", containing essential oils, supplementation to pig diet causes a significant increase in lipid synthesis in the liver and phospholipids in skeletal muscles.

References

1. Grytsyniak II, Smolianinov KB, Yanovych VG. Lipid metabolism in fish. Lviv, Triada plyus, 2010: 335 p. (in Ukrainian)
2. Huang C, Chen D, Tian G, He J, Zheng P, Yu J, Mao X, Huang Z, Yan H, Wang Q, Wang H, Yu B. Effects of dietary plant essential oil supplementation on growth performance, nutrient digestibility and meat quality in finishing pigs. *J Anim Physiol Anim Nutr*. 2022; 106 (6): 1246–1257. DOI: 10.1111/jpn.13673.
3. Hunchak A, Hunchak VM, Ratych IB. Biological effects of plants extracts in the poultry. *Sci Mess LNUVMBT Ser Vet Sci.* 2015; 17 (3): 19–31. Available at: https://nvlvet.com.ua/index.php/journal/article/view/513 (in Ukrainian)
4. Modern technologies for feeding pigs without the use of antibiotics. Electronic resource. 2019. Available at: https://vita.biz.ua/suchasni-tehnologiyi-godivli-svynej-bez-vykorystannya-antybiotykiv
5. Leyva-López N, Gutiérrez-Grijalva EP, Vazquez-Olivo G, Heredia JB. Essential oils of oregano: Biological activity beyond their antimicrobial properties. *Molecules.* 2017; 22 (6): 989. DOI: 10.3390/molecules22060989.
6. Pagliari S, Forcella M, Lonati E, Sacco G, Romaniello F, Rovellini P, Fusi P, Palestini P, Campone L, Labra M, Bulbarelli A, Bruni A. Antioxidant and anti-inflammatory effect of cinnamon (*Cinnamomum verum* J. Presl) bark extract after *in vitro* digestion simulation. *Foods*. 2023; 12 (3): 452. DOI: 10.3390/foods12030452.
7. Prudius TY, Kyryliv YI, Barylo BS. Effectiveness of the use of "Activo" essential oil extracts in the geese diet. *Modern Poultry Farming*. 2016: 12–16. (in Ukrainian)
8. Rašković A, Milanović I, Pavlović N, Ćebović T, Vukmirović S, Mikov M. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. *BMC Complement Altern Med*. 2014; 14: 225. DOI: 10.1186/1472-6882-14-225.
9. Reichling J, Schnitzler P; Suschke U; Saller R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral and cytotexic onopenties-an overview. *Fobsch Komplementmed*. 2009; 16 (2): 79–90. DOI: 10.1159/000207196.
10. Vlizlo VV (ed.), Fedoruk RS, Ratych IB. *Laboratory Research Methods in Biology, Animal Husbandry and Veterinary Medicine*. A guide. Lviv, Spolom, 2012: 764 p. (in Ukrainian)

Вплив кормової добавки «Активо» на вміст загальних ліпідів та їх окремих класів у печінці та скелетних м'язах свиней

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Зі збільшенням споживання м'яса зростають і вимоги до його якості. Оскільки м'ясо свинини є другим за популярністю у світі, то виробники цього продукту стараються підвищувати його якість. Мета наших досліджень полягала у з'ясуванні впливу

кормової добавки «Активо», яка містить олію кориці, олію розмарину, екстракт перцю чілі, олію орегано, на вміст загальних ліпідів і співвідношення їх окремих класів в печінці та скелетних м'язах свиней. Дослідження проводили на двох групах свиней великої білої породи масою 70 кг, розділених за принципом поросят-аналогів на контрольну та дослідну групи. Свині обох груп отримували стандартний комбікорм (СК), призначений для цієї вікової групи. Свині дослідної групи додатково до СК отримували кормову добавку «Активо» у кількості 0,1 кг/т комбікорму. Дослід тривав 40 діб. Матеріалом для досліджень слугували зразки тканин печінки, довгого м'яза спини, широкого м'яза спини. Загальний вміст ліпідів визначали гравіметричним методом, окремі класи ліпідів — методом тонкошарової хроматографії на пластинках із силікагелем. Дослідження показали, що загальний вміст ліпідів у печінці, довгому та широкому м'язах спини свиней дослідної групи був, відповідно, в 1,66 і 1,95 (P≤0,001) та 1,26 (P≤0,05) раза більший, ніж у тварин контрольної групи. Відносний вміст естерів холестеролу у ліпідах печінки свиней дослідної групи був у 1,9 (P≤0,001) раза вищий, ніж у контролі. Натомість у широкому м'язі спини зафіксовано менший вміст триацилгліцеролів та більший — неестерифікованих жирних кислот (P≤0,05), етерифікованого холестеролу (P≤0,05) і особливо фосфоліпідів (P≤0,001). За дії досліджуваної кормової добавки у широкому м'язі спини суттєво зменшився вміст неестерифікованого холестеролу (P≤0,001) та збільшився вміст фосфоліпідів (P≤0,001).

Ключові слова: ліпіди, фосфоліпіди, триацилгліцероли, ефірні олії, кормова добавка

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