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ORIGINAL PAPER

Influence of sex on meat chemical composition and the content of vitamins A, E and cholesterol in the *longissimus dorsi* muscle of hybrid pigs*

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Abstract

Pork is one of the most consumed meats in Poland, which highlights the importance of research into its composition. Consumers often pay attention to quality. The aim of the study was to determine the effect of sex on the content of vitamins E and A and cholesterol in the *longissimus dorsi* muscle (*musculus longissimus dorsi*, LD) of DanBrad hybrid pigs. A total of 48 samples were collected: 12 each from the carcasses of gilts, entire males, surgically castrated and immunocastrated pigs. Surgical castration was performed between the 2nd and 5th day of the piglets' lives and immunocastration was carried out by intramuscular injection of Improvac 8 and 4 weeks before slaughter. During fattening, the animals were kept in group pens in a bedding-free system. The criterion for division was the sex of each individual. Feed and water were available *ad libitum* during fattening. A four-phase feeding system was used, based on complete feed mixtures. Slaughter was carried out in accordance with applicable meat industry regulations. Samples of the *longissimus dorsi* muscle from the lumbar region between the 3rd and 4th vertebrae were taken from cooled right half-carcasses. The content of vitamins A and E, and cholesterol in the tested samples was determined. The chemical composition was also analysed. The highest content of tocopherols was found in the meat of immunocastrated pigs and the lowest in gilts. Statistically significant differences were found between the retinol content in surgical castrated and entire males. In the LD muscle samples of gilts, the cholesterol content was statistically significantly higher than in the samples from males castrated either surgically or immunologically. The results of the study indicated significant and highly significant variation in the basic chemical composition between LD samples from animals in the different groups. Further studies of larger meat sample sizes are needed to confirm or refute the current findings.

Keywords: cholesterol and vitamins, sex, pigs, chemical composition, meat quality

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INTRODUCTION

Pig production aims to obtain high-quality meat while meeting the growing consumer demands for animal welfare, nutrition, and the nutritional value of the final product (Karpiesiuk et al. 2019). As pork remains the most consumed meat in Poland, research on its chemical composition and nutritional value seems important. Meat of slaughter animals is considered a high-quality product owing to its protein, phosphorus, and iron content. Additionally, meat is an important source of the B-group vitamins, as well as macro- and microelements. Some vitamins, such as A and B_{12}, are supplied exclusively by animal products, as they are not present in plant tissues (Świąder et al. 2011).

One approach to improving pig production and animal welfare is the breeding of fattening pigs divided by sex, which means keeping male and female animals in separate pens, buildings, or farms (Borell et al. 2020, Škrlep et al. 2020, Dobiesz et al. 2023). In Poland, most farms keep gilts and pigs, and less frequently males. Differences in metabolism, growth rate, and hormonal balance between males and females result in varying daily gains, fat levels, meatiness, and slaughter efficiency (Kozera et al. 2023*a*).

Meat plays an important nutritional role as a source of vitamins, minerals, protein, and fat. Of particular importance are vitamin A and vitamin E, which are among the most significant substances with antioxidant properties accumulated in animal tissues (Blicharski et al. 2013, Babicz, Kasprzyk 2019).

Vitamin E (tocopherols and tocotrienols) is a lipid-soluble vitamin, which is not produced by the bodies of animals or humans. Eight forms are distinguished, among which α-tocopherol and γ-tocopherol perform the most important physiological functions because of their strong antioxidant properties (Brigelius-Flohé 2021). Vitamin E stabilizes cell membranes, is involved in the regulation of gene expression related to lipid metabolism and cholesterol transport (Galmés et al. 2018), supports immune system function (Han, Meydani 2006), and reduces lipid peroxidation by neutralizing ROS – reactive oxygen species and RNS – reactive nitrogen species (Wang, Quinn 1999). Vitamin E supplementation also improves the color and stability of meat by inhibiting myoglobin oxidation.

Vitamin A (retinol) is a lipophilic compound that plays a key role in growth, reproduction, fetal development, and cell differentiation, particularly in epithelial and connective tissue cells (Mirowski 2016, Chen et al. 2023). In many countries, including EU member states, the doses of vitamin A used in pig feeding often exceed the recommendations of the NRC (2012) by up to six or ten times. The consequences of long-term feeding or consumption of excessive amounts of vitamin A in human and animal nutrition include skin paralysis, bone and joint pain, and enlargement of the liver and spleen

(Teister et al. 2010). Studies indicate that retinol supplementation affects its accumulation in tissues, although most experiments were conducted during a limited fattening period (Olivares et al. 2009).

Cholesterol is not present in plant tissues but is present in all cells and body fluids of humans and animals, where it has structural and metabolic functions. It is a component of cell membranes, the myelin sheath, and plasma lipoproteins. It is also a precursor of bile acids, steroid hormones, and vitamin D_3. Although most cholesterol is synthesized endogenously, about one third comes from the diet. Most cholesterol is found in nervous tissue and the liver, while pork offal contains much more cholesterol than muscle tissue (Barowicz et al. 1999). The authors mentioned above also indicate that the sex of pigs does not significantly affect the cholesterol content in meat, whereas the proportion of vegetable fats in the feed may reduce its concentration in tissue lipids.

Given the possible relationship between the sex of fattening pigs and the chemical composition of meat, studies were undertaken to determine the effect of sex on the content of vitamin A, vitamin E, and cholesterol in the *longissimus dorsi muscle* of DanBred hybrid pigs.

MATERIALS AND METHODS

The material for the study consisted of muscle samples from the *musculus longissimus dorsi* (LD) of DanBred hybrid pigs, taken from cooled right half-carcasses in the lumbar region between the 3rd and 4th vertebrae. A total of 48 samples, each approximately 10 cm thick, were collected (12 samples each from gilts, surgically castrated male pigs, non-castrated and immunocastrated fattening pigs). Surgical castration was performed between the 2nd and 5th day of life, coinciding with iron administration. Immunocastration was carried out by administering two doses of Improvac vaccine intramuscularly at a four-week interval (2 ml per animal in 1 dose, i.m.; Zoetis, Belgium SA, Zaventem, Belgium). Vaccinations were given 8 and 4 weeks before the planned slaughter. All animals were housed in buildings that met welfare and biosecurity requirements (Council Directive 2008/120/EC of 18 December 2008 laying down rules for the protection of pigs, Regulation of the Minister of Agriculture and Rural Development of 12 March 2026 on measures to combat African swine fever). They were kept in group pens in a bedding-free system. Animals in each group, i.e.:

I – gilts,
II – male pigs surgically castrated before 7 days of age,
III – entire male pigs,
IV – male pigs immunocastrated with Improvac,

were fed a four-phase complete feed mixture:

- Finisher 1 for growing pigs weighing 23-43 kg,
- Finisher 2 for growing pigs with a body weight of 43-63 kg,
- Finisher 3 for finishing pigs weighing 63-85 kg,
- Finisher 4 for finishing pigs with a body weight over 85 kg.

The recipes for the individual complete feed mixtures were determined in accordance with the recommendations of the Seges Pig Research Centre (SPRC). The main ingredients were cereals and protein components. The ingredients and chemical composition of diets are presented in Dobiesz et al. (2026). The content of mineral and vitamin additives used in the compound feed is shown in Table 1.

Table 1

Mineral and vitamin components of the supplementary mixture

Component	Finisher 1	Finisher 2	Finisher 3	Finisher 4
Vitamin A (IU kg^{-1})	6500	6400	6347	3250
Vitamin D_3 (IU kg^{-1})	1500	1477	1465	750.0
Vitamin E (mg kg^{-1})	18.75	18.46	18.31	9.380
Ferrous (III) sulphate monohydrate (mg kg^{-1})	80.00	78.77	78.12	40.00
Manganese oxide (mg kg^{-1})	24.00	23.63	23.44	12.00
Copper (II) sulphate pentahydrate (mg kg^{-1})	15.00	14.77	14.65	7.500
Copper (II) Alumina Chelate Hydrate (mg kg^{-1})	0.000	0.000	5.740	5.470
Zinc oxide, zinc sulphate monohydrate (mg kg^{-1})	60.00	59.08	58.59	30.00
Hydrated Zinc Glycine Chelate (mg kg^{-1})	0.000	0.000	11.47	10.93
Calcium iodate anhydrous (mg kg^{-1})	0.400	0.390	0.390	0.200
Sodium selenate (mg kg^{-1})	0.200	0.200	0.200	0.100

The growing-finishing pigs were slaughtered after 94 days of fattening. After fattening, the animals were transported to an abattoir, where they were slaughtered.

The basic chemical composition of the *longissimus dorsi* muscle (water and dry matter content, total protein, crude fat, crude ash) and energy value were analysed on a near-infrared (NIR) FoodScan™ 2 Lab analyser (Foss A/S, Denmark), in accordance with PN-A-82109 (2010). To determine the content of tocopherols (α-, β-, γ-, δ-) and retinol (vitamin A), meat samples (LD) were minced (meat grinder, Ultra-Turrax T25 homogenizer), weighed (1 g) into plastic tubes with a capacity of 50 cm^3 (Falkon), then 1.2 cm^3 of 20% aqueous ascorbic acid solution and 2 cm^3 of saponifying mixture (375 g KOH, 750 cm^3 H_2O, 450 cm^3 methanol) were added and mixed (Vortex). The samples were saponified in a water bath at 80°C for 15 min using a shaker (200 rpm). After cooling, 5 cm^3 of 35% ethanol, 5 cm^3 of 10%

NaCl, and 4 cm^3 of *n*-hexane were added; the samples were shaken for 1 min after each addition (Vortex). Afterwards, the samples were centrifuged at 2000 × g for 15 min (MPW-350R centrifuge), and an amount of 3 cm^3 of the supernatant was transferred into a 10 cm^3 tube. Extraction with 4 cm^3 *n*-hexane was repeated, and further 3-4 cm^3 of supernatant were collected after centrifugation. The extracts were combined and evaporated to dryness under a nitrogen atmosphere. The residue was dissolved in 1 cm^3 of *n*-hexane. Samples were analysed by HPLC on a SHIMADZU chromatograph using a reversed-phase system (Nucleosil C18 column), with a methanol and water mobile phase (95:5, v:v), flow rate 1 cm^3 min^{-1}, temp. 40°C; UV-vis detector at 336 nm for retinol content and RF detector, excitation wavelength 295 nm, emission 330 nm for tocopherol content. The content of retinol and tocopherol was calculated based on the peak area of external standards (±)-α-tocopherol (DL-all-rac α-tocopherol), β-tocopherol, (+)-γ-tocopherol, (+)-δ-tocopherol; retinol, synthetic vitamin A alcohol (SIGMA_ALDRICH, Missouri, USA (PN-EN-ISO 6867:2002; PN-EN-ISO 14565:2002, Hőbgerg et al. 2002, Fredriksson Eriksson, Pickova 2007, Xu 2008). The content of the analysed vitamins in muscle samples was expressed in mg kg^{-1}.

To determine the cholesterol content, LD samples were homogenised (Ultra-Turrax T25 homogeniser), 1 g was weighed (Falcon tubes), and 3 cm^3 of 10% KOH solution in ethanol (ethanol:H_2O, 1:1, v:v) with the addition of 200 mg l^{-1} L-ascorbic acid. The samples were mixed vigorously (Vortex) for 1 min, then 5 cm^3 of a mixture of *n*-hexane and ethyl acetate (9:1; v:v) were added. The samples were again vigorously mixed for 1 min, then centrifuged at 2000 × g, 4°C, for 5 min. The top layer was collected for the 100 cm^3 Soxhlet stocks. The extraction was repeated by adding a further 5 cm^3 of the n-hexane and ethyl acetate mixture (9:1; v:v) to the lower layer. The samples were mixed, centrifuged again, and the top layer was collected into a Soxhlet flask. The combined extracts were evaporated to dryness (temp. 40°C, rotary evaporator Janke & Kunkel IKA – Labortechnik, Staufen, Germany). The residue was dissolved in the carrier phase (acetonitrile : methanol : dichloromethane; 75:15:10; v:v:v). The cholesterol content was determined by HPLC (Shimadzu, Kyoto, Japan); RF, column: Nucleosil C18; carrier phase: acetonitrile : methanol : dichloromethane (75:15:10; v:v:v); flow rate 1 cm^3 min^{-1}; UV-VIS detector 222 nm. The external cholesterol standard was applied (Merck Index 13.2221, Aldrich). The content of cholesterol in LD samples was expressed in mg 100 g^{-1}.

The results collected were subject to statistical analysis. A one-way analysis of variance was used, and the significance of the differences was determined by the Duncan's test ($p \leq 0.05$ and $p \leq 0.01$). The results were statistically compiled using the Statistica PL 13.5 program.

RESULTS AND DISCUSSION

The results of studies on the content of lipophilic vitamins (tocopherols and retinol) in the longest dorsal muscle showed a statistically significant variation in the levels of β-tocopherol, γ-tocopherol, and retinol in meat samples from the experimental groups.

Muscle tissue (LD) obtained from male pigs' carcasses contained more β-tocopherol than from gilts (0.023 vs 0.010 mg kg^{-1}, $p \leq 0.05$), and slightly more than LD samples from male pigs surgically castrated before 7 days of age and male pigs immunocastrated with Improvac, although this difference was not statistically significant (Table 2). The longest dorsal muscle of surgically castrated pigs had a significantly higher content of γ-tocopherol than that of entire males (0.072 vs 0.045 mg kg^{-1}, $p \leq 0.05$), and higher than in meat samples (LD) from gilts and immunocastrated fattening pigs. In the evaluated samples of the longest dorsal muscle from gilts, entire males, surgically castrated, and immunocastrated pigs, there was no statistically significant variation in the content of α-tocopherol, δ-tocopherol and total tocopherols. However, noteworthy are higher, although statistically unconfirmed, concentrations of α-tocopherol, total tocopherols in the longest dorsal muscle of immunocastrated fattening pigs compared to samples from other evaluated animals, with the lowest levels found in gilt meat.

Table 2

Vitamin A and E content and cholesterol in the *longissimus dorsi* muscle depending on the sex of fattening pigs

Specification	Gilts	Male pigs castrated surgically before 7 days of age	Entire males	Male pigs immunocastrated with Improvac
α-tocopherol (mg kg^{-1})	1.203	1.635	1.632	1.829
β-tocopherol (mg kg^{-1})	0.010^{b}	0.016	0.023^{a}	0.020
γ-tocopherol (mg kg^{-1})	0.056	0.072^{a}	0.045^{b}	0.066
δ-tocopherol (mg kg^{-1})	0.040	0.050	0.035	0.037
Sum of tocopherols (mg kg^{-1})	1.309	1.774	1.736	1.954
Retinol (mg kg^{-1})	0.220^{b}	0.300^{a}	0.214^{b}	0.229
Cholesterol (mg kg^{-1})	57.67^{A}	44.88^{B}	50.84	45.78^{B}

a, *b* – data in the same row followed by different letters differ significantly ($p \leq 0.05$), *A*, *B* – data in the same row followed by different letters differ significantly ($p \leq 0.01$)

According to Świąder et al. (2011), lipid oxidation processes occur more slowly in meat containing large amounts of vitamin E. As a result, muscle tissue is of better quality and shows reduced losses caused by oxidation, especially during extended storage. Pinelli-Saavedra (2003) found a strong correlation between the concentration of α-tocopherol in blood plasma and

the content of vitamin E in the liver, as well as between the amount of α-tocopherol consumed by the animal in feed and the concentration of vitamin E in plasma. The data show that increasing the amount of α-tocopherol in the feed ration of sows raises the concentration of vitamin E in their colostrum and milk plasma, as well as in piglet plasma and tissues, which is particularly important for young animals exposed to stress factors (Kawecka et al. 2013). Pig muscle (LD) of male pigs castrated before 7 days of age had a higher retinol content than samples from other experimental groups ($p \leq 0.05$). The longest muscle of the back of gilts had a statistically confirmed, significantly higher cholesterol content than samples from other experimental groups (57.67 mg kg^{-1}, $p \leq 0.01$) – Table 2 and higher than the 52.29 mg kg^{-1} cholesterol level determined in the studies of Blicharski et al. (2013). It should be emphasized that the cholesterol content in LD samples from males evaluated in our studies was lower than that reported by Kasprzyk (2015) in Pulawska breed, but higher than in wild boar tissues. The results of the study by Bahelka et al. (2016) indicated a significantly lower cholesterol content in the LD muscle of boars compared to muscle samples from surgically castrated males.

No significant differences in protein or fat content (%) were observed in the muscle tissues of the experimental groups. However, it is noteworthy that the highest protein content was found in the *longissimus dorsi* (LD) samples from entire males (20.96% fresh weight), whereas the lowest was recorded in samples from male pigs surgically castrated before 7 days of age (20.70% fresh weight). In a study by Zalewska et al. (2025) on the assessment of meat and fat quality in immunocastrated and uncastrated boars, the protein content was higher than in our own study, averaging 23%. A significant difference in protein content was found between heavy boars, light boars, and immunocastrated boars. LD muscle samples from animals from 4-breed crosses had a higher protein content than those reported in the studies by Kozera et al. (2016, 2023*b*), Karpiesiuk et al. (2016), and Bahelka et al. (2016). Casapu et al. (2023) reported that male carcasses had a higher protein content than female carcasses, although the differences were not statistically significant. LD muscle tissue from immunocastrated fattening pigs had the highest fat content among the samples evaluated, with LD muscle tissue from gilts containing more fat than that from male pigs surgically castrated before 7 days of age (Table 3). The study by Kozera et al. (2023*a*) also found a higher crude fat content in muscle tissue from immunocastrates than from females, which was confirmed in our study (Table 3). According to Dunshea et al. (2013), physiological conditions indicate that immunocastrated pigs show male characteristics until the second dose of the vaccine and subsequently develop female characteristics. The results presented by Batorek et al. (2012) indicated a lower crude fat content in boar meat than in surgically castrated pigs. In contrast, Bahelka et al. (2016) reported that LD samples from boars had a significantly higher crude fat content compared to the muscle tissue of surgically castrated males. According to Aluwé et al.

Table 3

Chemical composition of FOSS and physicochemical evaluation of meat from experimental fattened pigs

Specification	Gilts	Male pigs castrated surgically before 7 days of age	Entire males	Male pigs immuno-castrated with Improvac
Dry matter (%)	30,59	29,61	30,30	31,34
Total protein (%)	20.89	20.70	20.96	20.75
Crude fat (%)	8.576	7.705	7.998	9.367
Crude ash (%)	$1{,}314^{b}$	1.325^{A}	1.397^{Aa}	1.169^{B}
Water (%)	69.41	70.39	69.70	68.66
Energy value (kcal)	161.5	153.0	156.6	168.3

a, *b* – data on the same line, followed by different letters, differ significantly ($p \leq 0.05$), *A*, *B* – data on the same line, followed by different letters, differ significantly ($p \leq 0.01$)

(2015), males subjected to surgical castration have greater fat thickness than gilts and then entire male pigs, suggesting that immunocastration males also affect lipid metabolism and carcass fat content. Increasing intramuscular fat content is beneficial because it improves the sensory quality and processing value of animal products (Jankowiak et al. 2019).

The crude ash content in the muscle tissue (LD) of animals administered Improvac was the lowest ($p \leq 0.01$) compared to the mineral concentration (crude ash) in LD samples from entire males, male pigs castrated surgically before 7 days of age and gilts. The longissimus dorsimuscle of gilts contained significantly less ash than that of entire males (Table 3). The meat of surgically castrated pigs and entire males had a statistically significantly higher crude ash than the sample LD muscle of immunocastrated females. A different amount of this component from the last group may be related to changes in the hormonal balance after injection with Improvac. The findings of Kozera et al. (2016), Karpiesiuk et al. (2016), and studies on pigs of breeds PBZ Polish Landrace (Kasprzyk 2015), indicated a lower raw ash content than determined in our own studies (Table 3). The ash content reported by Casapu et al. (2023) was also lower than in our studies, and these authors found a higher content of this component in the carcasses of males than gilts, regardless of pig breed.

The main component of meat is water (Kropiwiec-Domańska et al. 2024). Analysis of water content showed that LD muscle samples from surgically castrated fattening pigs contained the most water, while immunocastrated males contained the least. Bahelka et al. (2016) obtained different results, with the highest water content in raw materials from males and the lowest from pigs. Opposite results to ours were reported by Nold et al. (1999). Muscle tissue (LD) samples from carcasses of animals that had been pharmacologically castrated showed the highest energy value content among pigs. According to data from Prasow et al. (2018) and Kasprzyk (2015), meat

obtained from DanBrad hybrid pigs, regardless of sex, was characterized by a higher energy value than meat from domestic breeds such as PBZ Polish Landrace, WBP Polish Large White, and Pulawska breed. A higher energy value of the slaughter material was also recorded compared to meat from Landrace fattening pigs (Casapu et al. 2023).

CONCLUSIONS

The results of the study indicated a statistically significant variation in the content of vitamins E and A in the LD muscle of pigs, determined by sex. The β-tocopherol content was higher ($p \leq 0.05$) in the LD muscle of entire males than gilts, while the γ-tocopherol content was higher ($p \leq 0.05$) in the LD muscle of pigs castrated surgically before 7 days of age than in samples from entire males. A higher content of vitamin A ($p \leq 0.05$) was also found in the LD muscle of surgically castrated fattening pigs compared to the concentration of retinol (vitamin A) in the LD muscle of gilts and male pigs. Pork loin samples from female carcasses had a higher cholesterol content ($p \leq 0.01$) than LD muscle samples from chirurgical castrated pigs and immunocastrated fattening pigs.

The results of our research indicated statistically significant, sex-dependent variation ($p \leq 0.05$ and $p \leq 0.01$) in the content of crude ash. Gender had no effect on the energy value of pork. Further studies of larger sample sizes are needed to confirm or refute the current findings. Statistical analyses indicate the need to increase the group to at least 20-25 samples of all analysed groups.

Author contributions

K.D., K.K. – conceptualization, K.D., K.K., W.K., Z.A., J.K., C.P., J.F.P. – methodology, K.K. and W.K. – validation, K.K. – formal analysis, K.K., W.K., M.B-S. – investigation, K.D., K.K., M.B-S. – resources, K.D., K.K. – data curation, K.D., K.K., W.K. – writing – original draft preparation, K.D., K.K., M.B-S. W.K., Z.A., J.K., J.F.P. – writing – review and editing, K.K, W.K. – visualization, K.K. – supervision, K.K. – project administration, K.K., W.K. – funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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