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Research article

RELATIONSHIPS BETWEEN FAT AND CHOLESTEROL CONTENTS AND FATTY ACID COMPOSITION IN DIFFERENT MEAT-PRODUCING ANIMAL SPECIES

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The objective of this study was to determine the relationships between intramuscular fat, cholesterol contents and fatty acid composition in the muscles of different animal species. Intramuscular fat, cholesterol and fatty acid composition in 207 muscle samples from 129 animals of different species (pigs, beef cattle, farmed red deer, horses and geese) were determined and analysed. The obtained results indicated unequal relations between intramuscular fat and cholesterol contents and fatty acid proportions in the muscles of different animal species. The increase of intramuscular fat content resulted in higher monounsaturated and lower polyunsaturated fatty acid contents in most muscles of meat producing animals. In all the species higher fatness did not show any increase in cholesterol content and also cholesterol contents were lower as fat increased in *m. semimembranosus* of pigs and *m. pectoralis profundus* of horses. The cholesterol content positively correlated with saturated and monounsaturated fatty acids in the *longissimus* muscle with the lowest fat content found in red deer and beef cattle, whereas the correlations between these measures were negative in *m. pectoralis profundus* of horses and the breast of goose containing high fat levels. Negative correlations between polyunsaturated fatty acids and cholesterol content were found in the *longissimus* muscle of red deer and cattle, whereas these correlations in goose breast and horse meat were positive.

Key words: animal species, cholesterol, correlation, fatty acid composition, health, intramuscular fat

INTRODUCTION

Meat has a substantial role in human diet both during evolution and in the present [1-3]. Several studies reported that consumption of red meat may increase the risk of cardiovascular disease (CVD) and colon cancer thus leading to a negative perception of the role of meat in health. However, current literature data does not support the existence of an unquestionable relationship between a large intake of red meat and the

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risk of myocardial ischemia [2,4-6]. Accepting all the reported data, moderate intake of a variety of foods, including meats that are enjoyed by people, remains the best dietary advice [7]. Recently meat consumption has increased and is likely to continue to do so in the future, with poultry in particular increasing its importance globally [8]. Although pork and poultry are the two most widely consumed meats [9], modern consumers in the meat market are looking for new products and new flavours [10,11]. Meat of rare breeds and venison could address some distinctive needs of consumers as an alternative to meat sourced from conventional production systems. Since meat is described as food rich in saturated fat and cholesterol, there is a need for the evaluation of different meats and exploring ways to change the lipid composition of food. Genetic variations for intramuscular fatty acid deposition have been investigated much less compared to animal nutritional strategies [12] and this encouraged us to compare the lipids of different animal species. The objective of this analysis was to study the relationships between intramuscular fat and cholesterol contents and fatty acid composition in the muscles of different animal species.

MATERIAL AND METHODS

The animals used in the study were Lithuanian White pigs, Aubrac cattle, Vishtinės geese, farmed red deer and farm horses raised in production systems in Lithuania. In total, 207 samples from 129 animals were used. The samples were excised from different carcass sites: *m. longissimus dorsi* of pigs, red deer and cattle, *m. semimembranosus* of pigs and red deer*, m. pectoralis profundus* of horses and breast and thigh of geese after 24 h chilling. The muscle samples for fatty acid and cholesterol detection were stored at -65±2.5 °C until analysis.

Intramuscular fat was determined by the Soxhlet extraction method No 960.39 [13]. The content of intramuscular fat was expressed as weight percentage of wet muscle tissue.

Fatty acid profiles: The extraction of lipids for fatty acid analysis was performed with a mixture of chloroform/methanol as described by Folch *et al.* [14]. Methylation of the samples was performed using sodium methoxide. The FAMEs were analysed using a gas chromatograph Shimadzu GC 2010 (Shimadzu Corp., Kyoto, Japan) fitted with a flame ionization detector. The separation of methyl esters of fatty acids was affected on the capillary column Rt-2560 (100 m x 0.25 mm x 0.2μm; Restek, Bellefonte, PA, USA) by temperature programming from 140 °C to 240 °C. The column was operated at 140 °C for 5 min, then the temperature was increased to 240 °C at 4 °C/min and held for 20 min. The temperatures of the injector and detector were held, respectively, at 240° and 260 °C. The rate of flow of carrier gas (nitrogen) through the column was 1.06 ml/min. The peaks were identified by comparison with the retention times of the standard fatty acids methyl esters "37 Component FAME Mix" and trans FAME MIX k 110 (Supelco, USA). The relative proportion of each fatty acid was expressed as the

relative percentage of the sum of the total fatty acids using „GC solution" software for Shimadzu gas chromatograph workstations.

Cholesterol content: The cholesterol content in meat was determined according to the extraction method described by Polak et al. [15] and followed by HPLC separation and analysis on Shimadzu 10 A HPLC system (Shimadzu Corp., Kyoto, Japan). The data collection and evaluation was performed by using LC Solution (Shimadzu Corp., Japan) operating system. The analytical column was LiChrospher 100 RP-18e, 150 x 4.6 mm, 5 mm (Alltech Associates Inc.,USA) with a guard column (LiChrospher 100 RP-18, 7.5 x 4.6 mm). The cholesterol content was expressed as mg/100 g fresh meat.

Statistical analysis: The data of different animal species from their production systems were subjected only to the descriptive statistics in SPSS 22. Pearson correlation coefficients between fat, cholesterol and selected sums of fatty acid groups were calculated. The degree of correlation was categorized into three levels: high ($r \geq 0.60$); moderate ($0.60 > r \geq 0.30$) and low ($r < 0.30$). Correlation coefficients with P-value $P < 0.05$ were considered as significant.

RESULTS

Statistical measures of fat and cholesterol contents, and sums of total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in the muscles of different animal species were calculated and presented in Table 1. In the *longissimus* muscle the highest mean and maximal content of intramuscular fat (IMF) was determined for pigs, whereas the largest range of fat in this muscle was found from 0.13 to 2.55% in red deer. The lowest content of fat in the *longissimus* muscle and the lowest range variation was found for beef. The *semimembranosus* muscle of pig hams showed higher fat content than the same muscle of the red deer. The biggest range of fat content in the *semimembranosus* muscle of pigs was from 1.27 to 9.03%. However, the highest IMF mean was found in the thigh and breast of the goose and in *m. pectoralis profundus* of horse.

The lowest contents of cholesterol were found in the *longissimus* muscle of beef cattle and in both *longissimus* and *semimembranosus* muscles of pigs, whereas the highest cholesterol content was found in the goose breast and in the *semimembranosus* and *longissimus* muscles of red deer.

The lowest saturation was found in the lipids of goose meat in comparison with other animal species. The highest and lowest percentages of total polyunsaturated fatty acids were detected in the muscles of red deer and pigs, respectively.

Pearson correlation coefficients (Table 2) showed a negative correlation between intramuscular fat and cholesterol contents in the *semimembranosus* muscle of pigs ($r = -0.578$; $P < 0.01$) and in the *pectoralis profundus* muscle of horses ($r = -0.663$; $P < 0.001$). In the other studied muscles, except the *longissimus* muscle of red deer, negative correlation

coefficients were very low. The cholesterol content in the *longissimus* muscle of red deer tended to have a positive correlation with IMF (r=0.378; P=0.069).

Table 1. Descriptive statistics for fat, cholestrol and fatty acid composition in muscles of different animal species

Variables	Anatomical location of muscles							
	M. longissimus dorsi				***M. semimembranosus***			
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Pork n=24								
IMF	2.11	0.53	1.11	3.19	2.57	1.65	1.27	9,03
Cholesterol	37.88	5.36	29.40	49.29	39.87	5.51	26.70	46.81
SFA	36.34	1.85	33.06	39.10	32.62	1.87	28.56	36.66
MUFA	51.80	3.61	42.97	57.45	47.46	5.59	32.38	58.38
PUFA	9.14	3.34	5.57	16.77	15.07	5.08	4.54	28.78
n-6 PUFA	8.09	2.95	4.62	15.71	13.55	4.63	3.98	26.15
n-3 PUFA	1.05	0.39	0.67	2.03	1.52	0.46	0.56	2.63
Red deer n=24								
IMF	0.96	0.57	0.13	2.55	0.92	0.68	0.13	3.40
Cholesterol	47.54	5.59	39.68	59.44	52.76	4.41	42.09	63.47
SFA	35.65	6.62	24.24	54.29	33.61	4.22	25.56	43.61
MUFA	23.47	3.49	16.99	29.67	23.86	4.12	17.78	33.14
PUFA	29.07	7.04	12.64	40.60	30.17	6.09	18.08	43.57
n-6 PUFA	20.57	5.58	8.59	30.76	21.19	4.68	12.83	30.78
n-3 PUFA	8.31	1.95	3.83	11.43	8.78	2.18	5.11	14.60
Goose meat n=30								
	Breast				Thigh			
IMF	3.56	0.90	2.10	5.88	5.22	0.94	3.76	7.73
Cholesterol	71.06	12.77	40.39	94.52	45.40	9.83	23.49	62.39
SFA	27.43	1.24	24.55	29.74	24.84	1.27	22.38	27.07
MUFA	49.50	3.33	43.45	58.72	56.17	2.26	52.05	62.78
PUFA	20.64	2.26	15.49	26.52	17.78	1.55	14.04	22.19
n-6 PUFA	18.66	1.85	14.22	22.42	16.10	1.18	12.98	18.82
n-3 PUFA	1.99	0.68	1.24	4.10	1.68	0.59	1.07	3.37
	Beef (n=25)				Horse meat (n=25)			
	M. longissimus dorsi				*M. pectoralis profundus*			
IMF	0.69	0.21	0.26	1.10	4.17	2.13	1.07	6.34
Cholesterol	36.56	5.51	28.83	46.33	45.52	9.04	26.91	63.20
SFA	33.97	4.98	22.02	42.48	32.30	1.62	28.31	35.29
MUFA	26.50	6.25	15.12	38.16	40.54	7.48	20.29	48.61
PUFA	27.85	7.13	14.23	41.42	25.02	5.24	18.87	38.74
n-6 PUFA	21.04	5.48	11.69	29.96	12.21	6.74	6.90	31.13
n-3 PUFA	6.80	2.07	2.54	11.46	12.81	2.86	6.37	17.12

IMF = intramuscular fat content(% of wet tissue); SFA, MUFA, PUFA = sum of all detected saturated, monounsaturated and polyunsaturated fatty acids (% of total FA), respectively.

Table 2. Correlation coefficients between fat content and fatty acid composition, and cholesterol content in muscles

Species	Muscle	SFA	MUFA	PUFA	n-6 PUFA	n-3 PUFA	Chole-sterol
Pork	*Longissimus*	-0.300	0.174	-0.010	-0.013	0.012	-0.050
	Semimembranosus	0.199	0.285	-0.299	-0.299	-0.295	-0.578**
Red deer	*Longissimus*	0.412*	0.458*	-0.392†	-0.390†	-0.299	0.378†
	Semimembranosus	0.257	0.109	-0.191	0.253	0.005	-0.044
Beef	*Longissimus*	0.317	0.463*	-0.465*	-0.508**	-0.256	-0.084
Horse meat	*Pectoralis profundus*	0.096	0.473*	-0.453*	-0.502*	0.351	-0.663***
Goose	Breast	-0.438*	0.565***	-0.478**	-0.466**	-0.320†	-0.093
	Thigh	-0.191	0.428*	-0.419*	-0.467**	-0.167	-0.100

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; † $0.05 \leq P < 0.10$; SFA, MUFA, PUFA = sum of all detected saturated, monounsaturated and polyunsaturated fatty acids, respectively

Intramuscular fat in the *longissimus* muscle of red deer and beef cattle was positively related with a different level of significance to the sum of saturated and monounsaturated fatty acids, while the sum of monounsaturated fatty acids in the *pectoralis profundus* of horses and in the breast of goose were negatively related to intramuscular fat. The correlation coefficients between IMF and the sum of polyunsaturated fatty acids were negative in all the studied muscles, however, a significant negative relation was estimated in the *longissimus* muscle of beef cattle (r=-0.465; P<0.05), *m. pectoralis profundus* of horse (r= -0.453; P<0.05) and in the breast (r=-0.478; P<0.01) and thigh (r= -0.419; P<0.05) of goose. The relations between the sum of n-6 PUFA and intramuscular fat reflected the relations of total PUFA to IMF. However, there was no any effect on the relations of n-3 PUFA to IMF, except for beef.

Table 3 presents the correlations between cholesterol and the sums of fatty acids. The cholesterol content in the *longissimus* muscle of red deer and beef cattle was positively related (r=0.523; P<0.01 and r=0.603; P<0.001) to the sum of SFA, respectively. The cholesterol content was also positively related to MUFA in the *longissimus* muscle of red deer (r=0.409; P<0.05) and beef cattle (r=0.620; P<0.001). Meanwhile, the relations between these measures were negative in the *pectoralis profundus* of horses (r=-0.511; P<0.01) and in the breast of geese (r= -0.557; P<0.001). The relations with negative correlations between the sum of PUFA and cholesterol content were found in the *longissimus* muscle of red deer (r= -0.506; P<0.05) and cattle (r= -0.618; P<0.001), and conversely, *id est* positive correlations were estimated in goose breast (r= 0.564; P<0.001) and *m. pectoralis profundus* of horses (r=0.508; P<0.01). The relations between cholesterol and n-6 PUFA reflected the relations of cholesterol to total PUFA. However, such reflections of cholesterol and n-3 PUFA relations were estimated only in the *longissimus* muscle of red deer and cattle.

Table 3. Correlation coefficients between cholesterol content and fatty acid composition in muscles of different animal species

Species	Muscle	SFA	MUFA	PUFA	n-6 PUFA	n-3 PUFA
Pork	*Longissimus*	-0.089	-0.152	0.160	0.162	0.143
	Semimembranosus	-0.092	-0.303	0.272	0.269	0.292
Red deer	*Longissimus*	0.523**	0.409*	-0.506*	-0.469*	-0.477*
	Semimembranosus	0.135	0.227	-0.213	-0.327	0.131
Beef	*Longissimus*	0.603***	0.620***	-0.618***	-0.573**	-0.613***
Horse meat	*Pectoralis profundus*	-0.085	-0.511**	0.508**	0.534**	-0.328
Goose	Breast	0.161	-0.557***	0.564***	0.586***	0.276
	Thigh	-0.218	-0.086	0.202	0.287	-0.042

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; SFA, MUFA, PUFA = sum of all detected saturated, monounsaturated and polyunsaturated fatty acids, respectively

DISCUSSION

The amount of intramuscular fat and its fatty acid composition play major roles in meat quality attributes and health considerations [16,17]. Cholesterol is an important component of cell membranes, brain and steroidogenic tissues [18]. It was hypothesized that high total cholesterol content in animal food products may cause coronary heart disease [4]. Although the 2015-2020 Dietary Guidelines for Americans removed the recommendations of setting a limit to the maximum intake of 300 mg/day cholesterol, the Guidelines still advised eating as little as possible of dietary cholesterol. The emphasis is shifted away from fat quantity to fat quality and to lower dietary intake of saturated fatty acids and total cholesterol [2,4,16].

Intramuscular fat content

The present study indicated high variations of fat both between the studied animal species and the individuals of the analysed species and between different muscles. These results are in agreement with the report of Hocquette et al. [16] and Jacyno et al. [19] who showed a high variability of intramuscular fat in the *longissimus* muscle of Polish hybrid pigs and Realini et al. [20] who found large differences between pig muscles. Low intramuscular fat content of red deer found in the present study is in agreement with the review of Daszkiewicz and Mesinger [21]. Hoehne et al. [22] have reported higher intramuscular fat means and their range for Charolais and German Holstein bulls than for Aubrac bulls, however, low IMF for Aubrac is consistant with the findings of Jukna et al. [23] for the same breed. In contrast to beef and meat of red deer, the meat of Lithuanian Vishtines goose showed the highest fat content. In the present study IMF content and its difference between goose breast and thigh was higher compared with Polish native goose breeds [24]. Horse-meat is not contemplated as a popular meat but its consumption should be encouraged to

obtain a healthy product in terms of its fatty acid profile and with the aim to reduce greenhouse emissions to the atmosphere. Therefore, the consumption of horse-meat is slowly increasing in some countries based on the claim that it could be an alternative to red meat [25]. In this study the IMF content in the *m. pectoralis profundus* of the horse was quite high (4.17%). However, Belaunzaran et al. [25] have reviewed lower and higher IMF means (0.1-6.63) than in the present study.

Fatty acid profiles

A comparison of the fatty acid composition in the studied species showed that PUFA percentages of horse-meat were somewhat similar to those found in beef and this was different from the data obtained by other authors for *longissimus* and *gluteus medius* muscles [25]. However, n-3 PUFA percentages showed that horse-meat had the highest values compared to other studied species and this is consistent with the comparisons between horse-meat and beef in the literature [25]. There is an opinion that ruminant meats are high in SFA and low in unsaturated fatty acids [26]. Brugiapaglia et al. [27] indicated strong cattle breed influence on fat and fatty acid composition in beef. This fact may explain the discrepancy between lower percentages of SFA and higher percentages of PUFA in the present study and the data found in literature. The proportions of saturated and polyunsaturated lipids in red deer meat in the present study were similar to those reported by Polak et al. [15]. owever, these proportions were in contrast with the data of Daszkiewicz and Mesinger [21] who have found red deer to be higher in SFA and lower in PUFA. In the present study, differences in relative amounts of fatty acids were found between pig muscles and this is in agreement with the findings of Realini et al. [20]. The proportions of fatty acids in the pork from Lithuanian White pigs were similar to those reported for the hybrid pigs by Jacyno et al. [19], but different to those reported by Hanczakowska et al. [28]. Despite the fact that goose is a minor component of poultry production, there is also sufficient interest in goose meat for nutrition diversification. The muscles of Vishtines goose had the lowest proportion of saturated fat compared with all our studied species and also lower SFA and higher MUFA proportions compared with Polish local geese [24]. However, in the present study the differences between breast and thigh were greater than indicated by Haraf et al. [24]. Relationships between the increase in fat amount and saturated (SFA), monounsaturated (MUFA) and decrease in polyunsaturated (PUFA) proportions were reported by De Smet et al. [29], Yang et al. [30], and Wood et al. [31]. In several studies [32,33], the heritability estimates of fatty acid composition in cattle indicated that fatty acids are determined genetically. Moreover, it is known that IMF enriched in SFA and MUFA are resulting mainly from de novo synthesis and PUFA is resulting mainly from the diet [32]. This fact is likely to be applicable for other species, too. Moreover, variability for fatty acid proportions is only partly dependent on the intramuscular fat content. In the present study different relationships were estimated between IMF and fatty acid composition in the muscles of different species. A positive moderate correlation between IMF and SFA was

estimated only in red deer *longissimus* muscle, however, positive correlation coefficients between fatness of muscles and MUFA were also in agreement with the literature data [22, 29] for the *longissimus* muscle of red deer and beef cattle, for the *pectoralis profundus* of horse and for the breast and thigh of goose. According to the calculated correlation coefficients, higher fatness resulted in lower levels of PUFA and this is also consistent with the data reported in literature. However, in the present study such relationships were not estimated for both muscles of pigs and *semimembranosus* muscle of red deer and this is in contrast with the reported data by Rauw et al.[34] and Jacyno et al.[19] for pork.

Cholesterol content

Although cholesterol content in meat can be influenced by various factors, including animal species, diet and maturity, cholesterol data for meat are usually accompanied by fat content, fatty acid composition and meat type [35]. Brugiapaglia et al. [27] did not find any differences in the cholesterol content between cattle breeds, but in our study, beef from Aubrac had lower contents of cholesterol than those reported by Brugiapaglia et al. [27]. Polak et al. [15] have reported higher contents of cholesterol in red deer meat than in the present study. The muscles of Vishtines goose had lower cholesterol content compared with Polish local geese [24]. The pork from Lithuanian pigs in the present study had lower contents of cholesterol compared to the pork from hybrid pigs reported by Polish authors [28].

In the present study, the cholesterol content in pork was also lower than that reported by Rauw et al. [34] and Jacyno et al.[19], who observed positive low (0.10) and high (0.74) correlations, respectively, between IMF and the cholesterol content in pork. This is in contrast with the negative moderate correlation coefficient (-0.58) in *semimembranosus* and very low coefficient (-0.05) in *longissimus* muscles of our pigs. Literature data on the effects of lipid content and muscle type collected under different treatments and conditions provided evidence on not only positive correlation, but also on the negative and insignificant relationship between fat and cholesterol contents [35]. Rauw et al. [34] estimated very low correlation coefficients between the cholesterol content and fatty acid composition but Jacyno et al. [19] reported positive moderate phenotypic correlation between cholesterol content and SFAs and MUFAs, and negative high correlation (-0.73) between the cholesterol content and PUFAs. These results were in agreement for the longissimus muscle of red deer and beef cattle but in contrast for pork and horse and goose meats in the present study. In the present study phenotypic correlation coefficients between the cholesterol content and fatty acid composition in pig muscles were low and insignificant. Similar studies on the relationships between the cholesterol content and fatty acid composition for other species are scarcer.

CONCLUSIONS

The obtained results indicated unequal relations between the intramuscular fat and cholesterol contents and fatty acid proportions in the muscles of different animal species. The increase of intramuscular fat content resulted in higher monounsaturated and lower polyunsaturated fatty acid contents in the most of studied muscles of different animal species. The relations between the sum of n-6 PUFA and intramuscular fat reflected the relations of total polyunsaturated fatty acids to IMF but the relations of n-3 PUFA to IMF were different. In all the species higher muscle fatness did not show any increase in cholesterol content and also cholesterol contents were lower in *m. semimembranosus* of pigs and *m. pectoralis profundus* of horses. The cholesterol content was positively related to SFA and MUFA in the *longissimus* muscle with the lowest IMF of red deer and beef cattle, whereas the relations between these measures were negative in the muscles (*m. pectoralis profundus* of horses and the breast of goose) with a high IMF content. The relations with negative correlations between PUFA and cholesterol content were found in the *longissimus* muscle of red deer cattle, and positive correlations were found in the goose breast and *m. pectoralis profundus* of horses.

Authors' contributions

RV made substantial contributions to the conception and design of the data analysis and interpretation. ŠA, ŠR and JV participated in the design of the study, sample collection and examination. BS carried out chemical analysis. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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POVEZANOST IZMEĐU SADRŽAJA MASTI I HOLESTEOLA I SASTAVA MASNIH KISELINA KOD RAZLIČITIH VRSTA ŽIVOTINJA KOJE SE KORISTE ZA PROIZVODNJU MESA

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Cilj ovih ispitivanja je bio da se odredi odnos između intramuskularno locirane masti, sadržaja holesterola i sastava masnih kiselina u mišićima različitih vrsta životinja. Određivana je i analizirana intramuskularna mast, holesterol i sastav masnih kiselina kod 207 uzoraka mišića poreklom od 129 životinja različitih vrsta (svinje, goveda, jelen, konji i guske). Dobijeni rezultati ukazuju nejednake odnose između intramuskularno locirane masti, sadržaja holesterola i proporcije masnih kiselina u mišićima različitih vrsta životinja. Povećanje sadržaja intramuskularno locirane masti rezultiralo je u većoj koncentraciji mononezasićenih masnih kiselina i smanjenju sadržaja polinezasićenih masnih kiselina kod većine mišića poreklom od vrsta životinja koje se drže radi proizvodnje mesa. Kod svih životinja, veći stepen gojaznosti i prisustva masnog tkiva, nije ukazivao na bilo kakvo povećanje sadržaja holesterola. Isto tako, sadržaj holesterola je bio manji sa povćanjem masti u *m. semimembranosus* kod svinja i *m. pectoralis profundus* kod konja. Sadržaj holesterola je bio u pozitivnoj korelaciji sa zasićenim i mononezasićenim masnim kiselinama u *longissimus* mišićima sa najmanjom koncentracijom masti koje su nađene kod jelena i goveda, dok je korelacija između ovih mera bila negativna u m. pectoralis profundus konja i grudnim mišićima gusaka koji su sadržavali velike koncentracije masti. Negativna korelacija između polinezasićenih masnih kiselina i količine holesterola, nađena je kod longissimus mišića jelena i goveda, dok su ove korelacije bile pozitivne kod grudnih mišića gusaka i kod mišićnog tkiva konja.