Comparative Investigations on Omega-3 Fatty Acids Treatments on Physicochemical Properties of Meat from Rabbits with Experimentally Induced Visceral Obesity

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Abstract. *The effect of omega-3 polyunsaturated fatty acids (PUFA) on meat physicochemical properties were investigated in rabbits with experimentally induced obesity by castration. forty-two male New Zealand White rabbits were divided into seven groups (n = 6): Group A – castrated, fed a full-diet and treated with krill oil; Group B – castrated, fed a full-diet and treated with fish oil; Group C – castrated, fed a full-diet and untreated; Group D – non-castrated, non-treated, fed a full-diet; Group E – castrated, fed a restricted diet (50%) and treated with krill oil; Group F – castrated, fed a restricted diet (50%) and treated with fish oil; and Group G – castrated, fed a restricted diet (50%) and untreated. experimental rabbits were 3-month-old and received feed for fattening rabbits. Krill and fish oil were applied per os as gelatin capsules containing 600 mg omega-3 PUFA over 60 days. During the processing of carcasses, individual samples of Longissimus Lumborum muscle (LL) and Semimembranosus muscle (SM) were collected. The results from the present experiment showed that castration and full-diet feeding of rabbits had beneficial effects on the quality of meat lipids and proteins. The castration of rabbits receiving a full diet had a most pronounced effect on fat accumulation in LL meat. The opposite tendency was demonstrated in rabbits with diet restriction in both studied muscles: statistically, significant differences were observed between treated and untreated rabbits. The higher fat content of LL and SM was associated with the omega-3 PUFA supplementation on the one hand and with diet restriction on the other. At the same time, castrated rabbits fed with a full diet exhibited statistically significantly higher protein content of LL and SM.*

Introduction

Rabbit farming is a branch of animal husbandry producing various valuable nutritional products. The most crucial role in this aspect is rabbit meat due to its low-fat content. Its low cholesterol, but high macro -, trace elements (calcium, potassium, phosphorus, sodium, cobalt, iron, copper and zinc) and B group vitamins content have determined the dietetic properties of the rabbit meat. In addition, it is a low-energy product of animal origin, as 100 g of rabbit meat provide only 160–170 kcal vs 195–380 kcal for beef and 260–330 kcal for pork meat (Bivolarski, 2012). Therefore, the world scientific community pays particular attention and spends a considerable amount of financial and human resources in the detailed study of effects of genetic, physiological, nutritional and other factors on growth, development, metabolism and meat quality in rabbits (Bivolarski et al., 2011; Hou et al., 2020; Ivanova, 2015; Maetrens et al., 2008; Palazzo et al., 2020; Ouyed et al., 2008; Vachkova, 2008).

Rabbits increase their weight until 100–120 days of age. Therefore, this period of intensive growth is most appropriate from an economic point of view for feed conversion per unit weight gain. As the age of rabbits advances, the feed expenditure increases substantially and is the highest after four months of age (Marinov et al., 2009).

The effect of feed restriction on physicochemical properties of rabbit meat depends on the level of restriction, feed quality, the duration of feeding, the age of animals. In rabbit farming practice, feed restriction is applied from 10 to 35 days, with 40%–90% of the total diet (Bivolarski, 2012; Bovera et al., 2008; Di Meo et al., 2007; Ivanova, 2015). Restricted feeding of rabbits results in stunted growth, and after return to regular feeding, the animals do not attain the necessary body weight. Consequently, compensatory growth and its effect on live body weight depend on the intensity and duration of feed restriction (Chodova et al., 2013). The authors concluded that the rabbits could not regain their body weight more prolonged and more considerable feed restriction. In contrast, moderate feed restriction did not affect them.

It is acknowledged that the early castration of male animals increases the slaughter yield, the amount of

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lipids in fat depots and increases meat fat content. In addition, the meat of castrated male rabbits had a higher water-holding capacity (WHC) and higher pH values (Ribarski et al., 2013). Most of the studies on the effects of castration on physicochemical properties of meat were performed in rams, boars and bulls, whereas data in rabbits are scarce (Ribarski et al., 2013). The meat of castrated male rabbits and females is more tender and leaner than intact males (Lebas et al., 2000).

The purpose of the present study was to investigate the effect of omega-3 polyunsaturated fatty acids on the physicochemical properties of the meat of rabbits with experimentally visceral obesity (castration) on a complete- or restricted diet.

Material and methods

The experiments were conducted with 42 male New Zealand White rabbits, divided into seven groups (n = 6): Group A – castrated, fed a full-diet and treated with krill oil; Group B – castrated, fed a full-diet and treated with fish oil; Group C – castrated, fed a full-diet and untreated; Group D – intact, fed a full-diet and untreated; Group E – castrated, fed a restricted diet (50%) and treated with krill oil; Group F – castrated, fed a restricted diet (50%) and treated with fish oil; and Group G – castrated, fed a restricted diet (50%) and untreated. experimental rabbits were 3-month-old and received feed for fattening rabbits (Table 1).

Krill and fish oil were given – *per os* as gelatin capsules containing 600 mg omega-3 polyunsaturated fatty acids (PUFA) over 60 days. At the end of the experiment, the animals were euthanised in compliance with current normative documents and the rules of the Animal Ethics Committee of the Trakia University. After carcass dissection, samples from *Longissimus Lumborum* muscle (LL) and *Semimembranosus* muscle (SM) were collected and treated by methods described by Pozahariskaja et al. (1964). The samples were first cooled at 2°C and stored for 24 h. Afterwards, meat pH was measured with a pH meter Consort C532. The pH meter was equipped with a combined penetrating electrode for meat analysis and calibrated with standard solutions (pH 4.0 and pH 7.0). The samples for pH determination were localised on the right T5 level for LL and in the middle third of the muscle for SM (Blasco et al., 1992).

The water-holding capacity (WHC) of muscles was determined as described by Grau and Hamm (1953).

Total protein, fat, ash and water (moisture) contents were analysed by classical methods of general chemical analysis of cooled meat (Vashin et al., 1999).

The statistical analysis of results was done using Statistica V.7.1 for Windows (StatSoft Inc., USA). Descriptive statistics were used to calculate means and standard errors of means. The effect of the group on studied parameters was evaluated with a one-way analysis of variance (ANOVA). The significance of the differences was determined by the post hoc LSD test and set as ($P < 0.05$).

Results

Physicochemical properties of LL meat from the four groups of rabbits fed a full diet are shown in Table 2. The moisture content was the lowest in Group C – 69.99 ± 0.69%. It was statistically significantly different from the other three groups (A, B and D). The opposite tendency was established for protein, fat, dry matter, pH and WHC of meat, which was substantially higher in Group C than in the other groups. The highest ash content was - in Group C, but it was statistically significantly different only compared with Group A. An interesting tendency was established for meat fat content. It was the highest in Group C (4.64 ± 0.40%) and Group B (3.11 ± 0.59%). Fat content was statistically significantly different between Group C and all other groups and Group B vs Groups C and D.

SM's physicochemical analysis did not reveal inter-group differences concerning fat and ash contents. Like in the case of LL, moisture content was the lowest in Group C (72.73 ± 0.45%) and statistically significantly different from the other groups. The values also differed considerably between the groups treated with krill and fish oil (A vs B) and Groups C and D (Table 3). SM protein content was the highest in Group C (23.40 ± 0.36%) and significantly different from average values recorded in Group B ($P < 0.04$)

Table 1. Ingredients and chemical composition of the diet in rabbits

Diet ingredients	Value	Diet ingredients	Value
Moisture (%)	11.00	Calcium (%)	0.80
Crude protein (%)	16.70	Total phosphorus (%)	0.65
Crude fat (%)	4.00	Sodium (%)	0.15
Crude fibre (%)	15.00	Metabolisable energy (kcal)	2500
Crude ash (%)	5.20	Vitamin A/retinol/ (IU/kg)	13000
Lysine (%)	0.65	Vitamin D3/cholecalciferol/ (IU/kg)	1600
Methionine (%)	0.32	Vitamin E/Dl-alpha Tocopheryl acetate/ (mg/kg)	60
Methionine (%) + Cysteine (%)	0.63	Copper (mg/kg)	10

Table 2. Physicochemical characteristics of *Longissimus lumborum* muscle

Parameters	Groups				Statistical significance among groups					
	Group A Mean ± SEM	Group B Mean ± SEM	Group C Mean ± SEM	Group D Mean ± SEM	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
Moisture, %	74.44 ± 0.17	73.63 ± 0.13	69.99 ± 0.69	74.57 ± 0.20	NS	0.001	NS	0.001	NS	0.001
Protein, %	21.82 ± 0.32	22.12 ± 0.46	24.23 ± 0.41	22.25 ± 0.08	NS	0.001	NS	0.001	NS	0.001
Fat, %	2.86 ± 0.18	3.11 ± 0.59	4.64 ± 0.40	2.01 ± 0.23	NS	0.01	NS	0.01	NS	0.001
Dry matter, %	25.76 ± 0.27	26.37 ± 0.13	30.07 ± 0.71	25.40 ± 0.21	NS	0.001	NS	0.001	NS	0.001
Ash, %	1.08 ± 0.04	1.14 ± 0.04	1.20 ± 0.05	1.17 ± 0.02	NS	0.05	NS	NS	NS	NS
pH	5.55 ± 0.13	5.34 ± 0.06	6.63 ± 0.07	5.29 ± 0.04	NS	0.001	0.03	0.001	NS	0.001
WHC, %	19.52 ± 0.79	18.82 ± 0.33	27.36 ± 1.28	20.84 ± 1.17	NS	0.001	NS	0.001	NS	0.001

Table 3. Physicochemical characteristics of the *Semimembranosus* muscle

Parameters	Groups				Statistical significance among groups					
	Group A Mean ± SEM	Group B Mean ± SEM	Group C Mean ± SEM	Group D Mean ± SEM	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
Moisture, %	74.68 ± 0.25	73.61 ± 0.12	72.73 ± 0.45	74.68 ± 0.29	0.04	0.001	NS	NS	0.03	0.001
Protein, %	21.71 ± 0.29	22.37 ± 0.38	23.40 ± 0.36	21.76 ± 0.35	0.01	NS	NS	0.04	NS	0.01
Fat, %	2.79 ± 0.16	2.90 ± 0.45	2.79 ± 0.27	2.49 ± 0.36	NS	NS	NS	NS	NS	NS
Dry matter, %	25.64 ± 0.20	26.40 ± 0.12	27.30 ± 0.45	25.49 ± 0.29	NS	0.001	NS	0.05	NS	0.001
Ash, %	1.14 ± 0.06	1.13 ± 0.06	1.11 ± 0.05	1.08 ± 0.08	NS	NS	NS	NS	NS	NS
pH	5.26 ± 0.06	5.44 ± 0.09	6.29 ± 0.09	5.58 ± 0.10	NS	0.001	0.03	0.001	NS	0.001
WHC, %	22.51 ± 0.28	19.88 ± 0.84	28.33 ± 2.29	17.54 ± 0.68	NS	0.01	0.03	0.001	NS	0.001

and Group D ($P < 0.01$). A similar difference was established between Groups A and B ($P < 0.01$).

The dry matter content of SM exhibited the same trend of change as LL dry matter. The highest average values among all groups were recorded in Group C (27.30 ± 0.45%). The WHC of SM was the highest again in Group C (28.33 ± 2.29%) and significantly different vs Group A ($P < 0.01$), Group B and D ($P < 0.001$). Also, another statistically significant difference was noted between Groups A and D ($P < 0.03$). The pH values of SM were the highest in Group C. They exhibited the same dynamics as the water holding capacity, i.e., considerable difference vs the other groups fed with a full diet and statistically significant differences between Groups A and D ($P < 0.03$).

It should be noted that there were less statistically significant differences in both studied muscles of rabbits on a restricted diet than in those fed with a full diet (Tables 4 and 5).

This tendency was most evident for protein and ash contents of SM muscles and moisture content, where the established differences were insignificant. The krill and fish oil treatments in rabbits provoked an increase in LL and SM fat content. The LL's fat content significantly differed between Groups E and G ($P < 0.01$). The moisture content of LL was the highest in Group G (75.25 ± 0.19%) and substantially different than Group E ($P < 0.05$). The WHC of the LL muscle was the lowest in Group G, exhibiting statistically, significant differences only vs Group F ($P < 0.02$). Comparing the pH values of LL, significant differences were observed between Groups E and F ($P < 0.01$) and between Groups E and G ($P < 0.03$). This parameter showed a similar pattern of change in SM as well.

It is interesting to note that the highest differences in WHC of studied muscles were established in SM. In Group G, the average WHC was the lowest in Group G (17.83 ± 0.54%) and significantly different ($P < 0.001$) from values in Groups E and F.

The correlation analysis of the results showed that the meat protein content of rabbits fed a full diet correlated positively with meat dry matter and pH in LL ($r = 0.82$ and $r = 0.70$) and SM ($r = 0.73$ and $r = 0.49$). A negative correlation was found between protein and moisture of both muscles ($r = -0.82$ and $r = -0.69$, respectively), between dry matter and moisture content ($r = -1.00$ and $r = -0.94$) and between moisture and pH ($r = -0.82$ and $r = -0.42$). There was also a positive correlation between dry matter and WHC ($r = 0.70$ in LL and $r = 0.26$ in SM), and between WHC and pH ($r = 0.78$ in LL and $r = 0.59$ in SM). In addition, in LL muscle, there

Table 4. Physicochemical characteristics of the *Longissimus lumborum* muscle

Parameters	Groups			Statistical significance among groups		
	Group E Mean ± SEM	Group F Mean ± SEM	Group G Mean ± SEM	E vs F	E vs G	F vs G
Moisture, %	74.62 ± 0.24	74.80 ± 0.19	75.25 ± 0.19	NS	0.05	NS
Protein, %	22.36 ± 0.18	22.48 ± 0.20	22.51 ± 0.14	NS	NS	NS
Fat, %	1.92 ± 0.10	1.58 ± 0.24	1.05 ± 0.23	NS	0.01	NS
Dry matter, %	25.40 ± 0.24	25.11 ± 0.21	24.75 ± 0.19	NS	0.04	NS
Ash, %	1.12 ± 0.07	1.04 ± 0.04	1.19 ± 0.07	NS	NS	NS
pH	5.20 ± 0.04	5.46 ± 0.09	5.40 ± 0.04	0.01	0.03	NS
WHC, %	19.1 ± 1.06	21.38 ± 1.13	18.00 ± 0.63	NS	NS	0.02

Table 5. Physicochemical characteristics of the *Semimembranosus* muscle

Parameters	Groups			Statistical significance among groups		
	Group E MEAN ± SEM	Group F MEAN ± SEM	Group G MEAN ± SEM	E vs F	E vs G	F vs G
Moisture, %	74.64 ± 0.13	74.50 ± 0.22	74.89 ± 0.30	NS	NS	NS
Protein, %	22.09 ± 0.19	22.07 ± 0.31	22.21 ± 0.18	NS	NS	NS
Fat, %	2.19 ± 0.11	2.29 ± 0.38	1.69 ± 0.25	NS	NS	NS
Dry matter, %	25.36 ± 0.13	25.55 ± 0.24	25.16 ± 0.31	NS	NS	NS
Ash, %	1.09 ± 0.07	1.19 ± 0.03	1.26 ± 0.08	NS	NS	NS
pH	5.31 ± 0.06	5.58 ± 0.05	5.49 ± 0.06	0.01	0.05	NS
WHC, %	22.52 ± 0.86	24.17 ± 0.91	17.83 ± 0.54	NS	0.001	0.001

were strong positive correlations between following the parameters: fat vs dry matter contents (r = 0.84), fat vs WHC (r = 0.56), fat vs pH (r = 0.65); protein vs WHC (r = 0.59); ash vs WHC (r = 0.44), dry matter vs pH (r = 0.82). The moisture content was strongly and negatively related to fat and WHC (r = –0.83; r = –0.72, respectively).

Fat contents of both muscles, in restricted rabbits, correlated significantly positively with dry matter (r = 0.75 and r = 0.66) and strongly negatively with moisture (r = –0.70 and r = 0.59); a negative correlation was also observed between dry matter and moisture (r= –0.98 and r= –0.99). Moreover, there was a significant negative correlation between LL moisture and WHC (r= –0.52) and LL ash vs WHC (r= –0.53).

Discussion

Rabbit meat quality depends on its physicochemical composition, is accordingly, it is significantly affected by any diet restriction. The meat's moisture, being a sum of free and bound water, is the primary muscle constituent and is influenced by the limited amount of feed (Ouhayoun and Zotte, 1996). Our results confirm that restricted feeding increased meat moisture content in both muscles, with a predominant manifestation of this tendency in castrated rabbits that did not receive krill or fish oil. In our view, this was because krill or fish oil treatments were necessary for rabbits whose diet was restricted, as evidenced by the negative correlation between fat and moisture contents of both muscles. The opposite tendency in moisture content was observed in rabbits fed with a full diet, where this parameter was the lowest in castrated untreated individuals. Fat content correlated negatively with moisture, but the most critical factor determining the low moisture content was the availability of needed energy

It should be noted that the water content of rabbit meat increased proportionally to the level of diet restriction. The moisture content of rabbits fed with a full diet was 62.3%, and in those which received 80% of the diet – 66.2% (Xiccato, 1999). Larzul et al. (2004) and Metzger et al. (2009) reported similar results, whereas Bernardini et al., 1994 affirmed that moisture correlated negatively to meat fat content. Therefore, rabbits fed a restricted diet stored less lipids in meat because of increased water content, as also shown by Xiccato (1999) found. The latter has established that more intensive diet restriction reduced meat fat within a broader range.

We found that in rabbits fed with a full diet, the lipid content in LL was higher than – in SM, which was most probably due to the specific biochemical processes in muscles with a different topographic location. The muscle fat content is essential for rabbit meat quality

(Hernandez, 2008; Blas and Wisewan, 2020), as it is primarily associated with – organoleptic properties of the product – its flavour and juiciness. Compared to meats of other species, rabbit meat is low in calories and contains less fat. Our results of lipid content in LL and SM, in full diet, fed rabbits, varied from 2.01% to 4.64% and were within the reference range reported by Hernandez and Gondret (2006) – from 0.6% to 14.4%. The slaughter age influences rabbit meat fat. Gondret et al. (1998) reported that intramuscular fat increased from 1.3% in rabbits, slaughter at 11 weeks of age. In our studies, the meat fat content of rabbits on a restricted diet was higher in SM than in LL. This reverse relationship compared to full diet-fed rabbits could be attributed to the higher LL moisture content. Despite this, the fat meat percentage in full diet-fed rabbits was substantially higher than in animals on a restricted diet. Our results are in line with those of Gondret et al. (2000), who demonstrated that rabbits with diet restriction had a considerably lower fat content in Biceps Femoris muscle, LL and SM than full dietfed rabbits. Similar results are also reported by Larzul et al. (2004). Opposite data have been established by Metzger et al. (2009), affirming that the restriction of rabbit diet did not influence the total muscle fat content of hindlimbs compared with full-fed animals. The changes in meat's fat content in restricted-fed rabbits were most probably related to the reduced enzyme activity of malate dehydrogenase and glucose-6-phosphate dehydrogenase, involved in the biosynthesis of fatty acids (Gondret et al., 1997; Zhong et al., 2021).

The lipids in rabbit meat are composed of 36,9% saturated fatty acids (SFA) (Zotte, 2000) and 34.6% of polyunsaturated fatty acids (PUFA) from the entire fatty acid content in hind limbs (Hernandez and Gondret, 2006). Fat amount, and quality adjustment in the diet could easily change this fatty acid ratio in meat. Still, high PUFA content could harm the oxidative stability of meat (Hernandez, 2008), which is a reasonable explanation for our results concerning LL fat content in full diet-fed rabbits. The values were the highest in Group C and statistically significant vs fat contents in Groups A, B and D. There were no significant differences in SM. Therefore, castration in full diet-fed rabbits had a most remarkable influence on fat deposition in LL muscle. The opposite tendency was noted in rabbits with diet restriction for both muscles: statistically significant differences were observed between treated and untreated rabbits. The highest fat content of LL and SM was associated with omega-3 PUFA supplementation on the one hand and restricted feeding on the other. Which could be because rabbits can include dietary fatty acids in adipose and muscle tissue lipids (Hernandez and Gondret, 2006). So far, no studies have investigated the level of diet restriction on the fatty acid composition and lipid content of rabbit meat. Here we showed that the lipid quality of the rabbit meat was affected by both castration and the amount of the diet. At the same time, the castration of male rabbits resulted in the deposition of more fat, which influences the visual evaluation of carcass fattiness (Ivanova, 2015).

Rabbit meat proteins are highly nutritional as they contain all essential amino acids (Bivolarski, 2012). The meat protein content is mainly influenced by dietary protein percentage. A significant reduction of muscle-growing was reported when growing rabbits were fed low-protein rations. When they returned to a full diet, meat protein was increased (Lebas and Ouhayoun, 1987). Our data indicated that LL and SM meat protein contents were significantly higher in castrated full diet-fed rabbits. (Ribarski et al. 2013) reported similar results. Concerning restricted feeding alone, the tendency towards the highest protein content of both muscles was preserved in the castrated group compared with the groups treated with fish or krill oil.

It should be noted that the differences in groups with diet restrictions were not statistically significant. Slightly higher meat protein content was reported in rabbits fed a restricted diet versus full-fed animals (Xiccato, 1999). If the ration contained excess proteins for its energy content, nitrogen retention could be slightly improved by dietary energy supplementation until the energy/protein ratio attains a given value. If this value is exceeded because of excess energy, it will reduce the body nitrogen (Fraga et al., 1983).

WHC is an important parameter indicating the capability of meat to retain tissue water. The full-fed rabbits have the highest WHC values in muscles. The opposite tendency was observed in rabbits fed a restricted diet, i.e., the lowest values were observed in Group G. The patterns of meat WHC changes demonstrated a better potential for tissue water retention than restrictively fed rabbits. The higher loss of water from the meat of rabbits with diet restriction was attributed to limited absorption of nutrients.

The rabbit farming industry has implemented alternative production systems, which substantially increased production costs and enhanced rabbits' welfare (Szendro, 2012). The utilisation of plastic floors, elevated platforms, dual-purpose cages, gnawing sticks, etc., provides a broader and more comfortable activity area, with less boredom and fewer behavioural problems. The surgery time (8–12 min per animal) and medication costs for castration of rabbits reared for meat consumption will result in serious expenditures which could be compensated only by selling high-quality meat. Therefore, the increasing demands for rabbit meat could be met by traditional rearing technologies and alternative systems producing high-quality meat for more exigent consumers, paying a higher price.

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