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Effect of feeding season on pH, glycolytic potential, colour and myofibrillar proteins in rabbit meat

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Summary

The aim of this study was to determine the influence of feed composition, related to the rearing season, on meat quality and postmortem proteolysis. The experiment was carried out on 14 rabbits from a small traditional farm. The animals originated from the crossing of local crossbred females with males of the Belgian giant breed. The rabbits were grown in two seasons: in summer and autumn. Seven animals slaughtered in summer were fed with green forage (mainly red clover mixed with grass) and cereal grain (oat and triticale), whereas the other 7 rabbits, slaughtered in autumn, were fed carrot, beet, cooked potatoes and cereal grain. In samples of the longissimus dorsi muscle, the following traits were measured: pH values 1 h and 24 h after slaughter, the meat colour in the CIE L*a*b* system and glycolytic potential (GP). Muscular proteins were analysed by the SDS-PAGE method. It was found that the colour of the meat of animals reared in the autumn season was more intensive yellow and had a significantly higher GP. The meat of animals reared in the summer season contained significantly less α-actinin and significantly more troponin I, troponin C and myosin LC2. Feed composition can influence the course of proteolytic changes in rabbit meat, which affects the technological and sensory qualities of meat.

Keywords: rabbit, meat quality, meat proteins

Rabbit meat is one of the most preferred meat sources in several countries, especially in Europe (29% of world production), as it is an alternative to beef, pork and mutton (12). Rabbit meat is highly appreciated for its valuable nutritional and dietetic properties: low cholesterol (0.045%) and sodium, high potassium, phosphorus and magnesium. The meat is lean, and its lipids are highly unsaturated (0.6-14.4%). Moreover, rabbit meat is rich in proteins (18.1-23.7%), and its amino acids are of high biological value (8).

The effects of rearing systems on carcass traits and the quality of rabbit meat have been analysed by a number of researchers (6, 7, 19, 22). Nutrition can influence carcass fattening, intramuscular fat content and its fatty acid composition, the protein content of meat and its amino acid composition, as well as the colour of meat (22, 23). Carrilho et al. (5) observed that more fibrous and less energetic fattening diets had no effect on pH, water-holding capacity (WHC) and sensory meat traits when rabbits consumed a common pre-slaughter diet. Meat from animals fed with a low-fibre and high-energy diet was darker. Dalle Zotte et al. (9) noted that feed composition influenced the ultimate pH (pH_u) only. A slight decrease in muscle pH_u was observed in the case of less energetic diets.

Feeding can affect the range and intensity of glycolytic and proteolytic changes (9, 24). The formation of the protein/polypeptide profile determines many traits that influence the technological and sensory qualities of meat (2, 11, 14). There is currently no data on the influence of feeding on the protein/polypeptide profile of rabbit meat from small traditional farms in Poland. On such farms, animals are usually fed a seasonal feed. In summer, it consists mainly of red clover mixed with grass, oat, and triticale, whereas in winter it is based on carrot, beet, cooked potatoes, oat, and triticale.

The aim of this study was to find differences in postmortem proteolysis affected by feed composition, related to the feeding season, in rabbits from a small traditional farm. This pilot study will help better understand the influence of diet on the range and intensity of glycolytic and proteolytic changes in meat.

Material and methods

Raw material. The studies were carried out on 14 rabbits (males) from a small traditional farm. The animals

originated from the crossing of local crossbred females with males of the Belgian giant breed. The animals were kept in wooden cages (95 × 80 cm, 60 cm high) in an open area, two animals per cage (separately males and females). Seven animals grown in summer were fed with green forage (mainly red clover mixed with grass) and cereal grain (oat and triticale), whereas the other 7 rabbits, grown in autumn, were given carrot, beet, cooked potatoes and cereal grain. The animals were fed *ad libitum*.

The rabbits were weighed before slaughter. Each animal was slaughtered in the morning, following mechanical stunning. After stunning, the animals were exsanguinated in a vertical position. Following the slaughter, carcasses were manually dehided and eviscerated, weighed and chilled at 4°C. On average, the animals were 7 months old and weighed 3.72 ± 0.27 kg. The average carcass weight (without head and edible offal) was 1.92 ± 0.16 kg. Hence, the dressing percentage was around 51.58 ± 1.51%. The meat quality parameters were evaluated in samples taken from the longissimus dorsi muscle. The samples were taken at the height of the last rib.

Technological analysis. Meat pH was measured at 1 (pH_1) and 24 (pH_{24}) hours after slaughter with a WTW-330i pH-meter (Germany). The meat colour was measured according to the CIE L*a*b* system by a CR310 Minolta Chroma Meter with a D_{65} light source (Osaka, Japan) at 48 h postmortem (21). Loin chops (2 cm in length) were cut and bloomed for 1 hour at 4°C with no surface covering prior to colour measurement (in triplicate). Muscle glycogen, glucose and glucose-6-phosphate were determined according to Dalrymple and Hamm (10), and lactate was determined according to Bergmeyer (3). Glycolytic potential (GP) was calculated according to Monin and Sellier (20). Meat samples were frozen at –80°C until subsequent analysis.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-PAGE of muscular tissue was performed according to the method of Bollag and Edelstein (4) using the STANDARD system (Kucharczyk TE, Poland). Proteins were resolved on a 12% separation gel and 5% stacking gel. Myofibrillar proteins were extracted from 20 mg of muscle, homogenized with 800 µl of a Tris-HCl buffer (pH 6.8) containing 0.375 M 2-mercaptoethanol, 3% SDS, 8 M urea, and 2 M thiourea. The muscle protein concentration was determined as total nitrogen by the AOAC method (1). Extracted proteins were dissolved 1/1 (v/v) in a Tris-HCl sample buffer (pH 6.8) containing 0.375 M 2-mercaptoethanol, 3% SDS, 8 M urea, 2 M thiourea and 0.05% bromophenol blue. The mixture was then heated for 3 min at 95°C, and 10 µl of the sample was placed in each well. Gels were first run for approximately 1 h at 75 V followed by 5 h at 150 V. Gels were stained with Coomassie Brilliant Blue R250. Image analysis and quantification were performed with GelScan v. 1.45 software (Kucharczyk TE, Poland). Molecular weight markers (Fermentas International INC, Burlington, Canada) were used to estimate the molecular weights of the proteins.

Data analysis. All values were reported as the mean ± standard deviation (SD). A one-way analysis of variance of the feeding season as a fixed effect was performed. Pearson correlation coefficients between meat quality parameters and protein quantification obtained by electrophoresis were calculated. Statistical analysis was conducted with Statistica 9.0 software (Stat Soft, Inc. version 9.0).

Results and discussion

Meat quality. No differences in meat pH were observed. The results obtained are consistent with values reported by Carrilho et al. (5) for rabbit fattening diet with diversified contents of fibre and energy. The brightness of meat colour (L*) and the intensity of red colour (a*) did not differ between the two groups of rabbits. It was found that the colour of the meat of animals reared in the autumn season was more intensive yellow (b*), and the meat had a significantly higher GP (Tab. 1). This was probably related to feed composition, as in the autumn season the feed consisted mostly of root crops rich in carotenoids and cooked potatoes rich in digestible saccharides. A diet rich in digestible carbohydrates causes an increase in glycogen stores within muscle without affecting pH (24).

Analysis of protein composition. The protein profiles of the muscle tissues from rabbits slaughtered both in the summer and the autumn seasons are provided in Fig. 1. Tab. 1 shows the quantification of each protein band. Myosin HC, α-actinin, actin, troponin T (TnT), tropomyosin, myosin LC1, troponin I (TnI), troponin C (TnC), myosin LC2 and myosin LC3 represented 22.2, 7.7, 12.7, 5.8, 12.1, 2.6, 3.2, 1.7, 5.2 and 2.3% respectively. The contents of myosin and α-actinin

Tab. 1. Characteristics of selected technological traits of meat and quantification of selected proteins ($\bar{x}$ ± SD, n = 7)

Characteristics	Season	
	summer	autumn
pH_1	6.5 ± 0.05	6.5 ± 0.18
pH_{24}	5.6 ± 0.10	5.7 ± 0.10
GP (µmol/g)	103 ± 4.20^a	110 ± 7.83^b
Colour coordinates		
L*	55.3 ± 3.15	55.5 ± 2.90
a*	17.2 ± 2.30	19.3 ± 3.01
b*	7.2 ± 0.79^a	10.1 ± 0.63^b
Myosin HC (%)	22.5 ± 2.04	21.8 ± 0.85
α-actinin (%)	7.4 ± 0.63^a	8.0 ± 0.16^b
5M (%)	3.2 ± 0.36	3.2 ± 0.24
Actin (%)	12.0 ± 3.90	13.4 ± 0.20
Troponin T (%)	5.7 ± 0.57	5.9 ± 0.36
Tropomyosin (%)	12.2 ± 2.81	12.1 ± 1.03
15M (%)	0.71 ± 0.81	0.24 ± 0.38
Myosin LC1 (%)	2.7 ± 0.67	2.5 ± 0.48
Troponin I (%)	3.4 ± 0.25^a	2.9 ± 0.09^b
Troponin C (%)	1.8 ± 0.15^a	1.5 ± 0.13^b
Myosin LC2 (%)	5.4 ± 0.29^a	4.9 ± 0.18^b
Myosin LC3 (%)	2.3 ± 0.14^a	2.2 ± 0.11^b

Explanation: a, b significant difference at $P \leq 0.05$

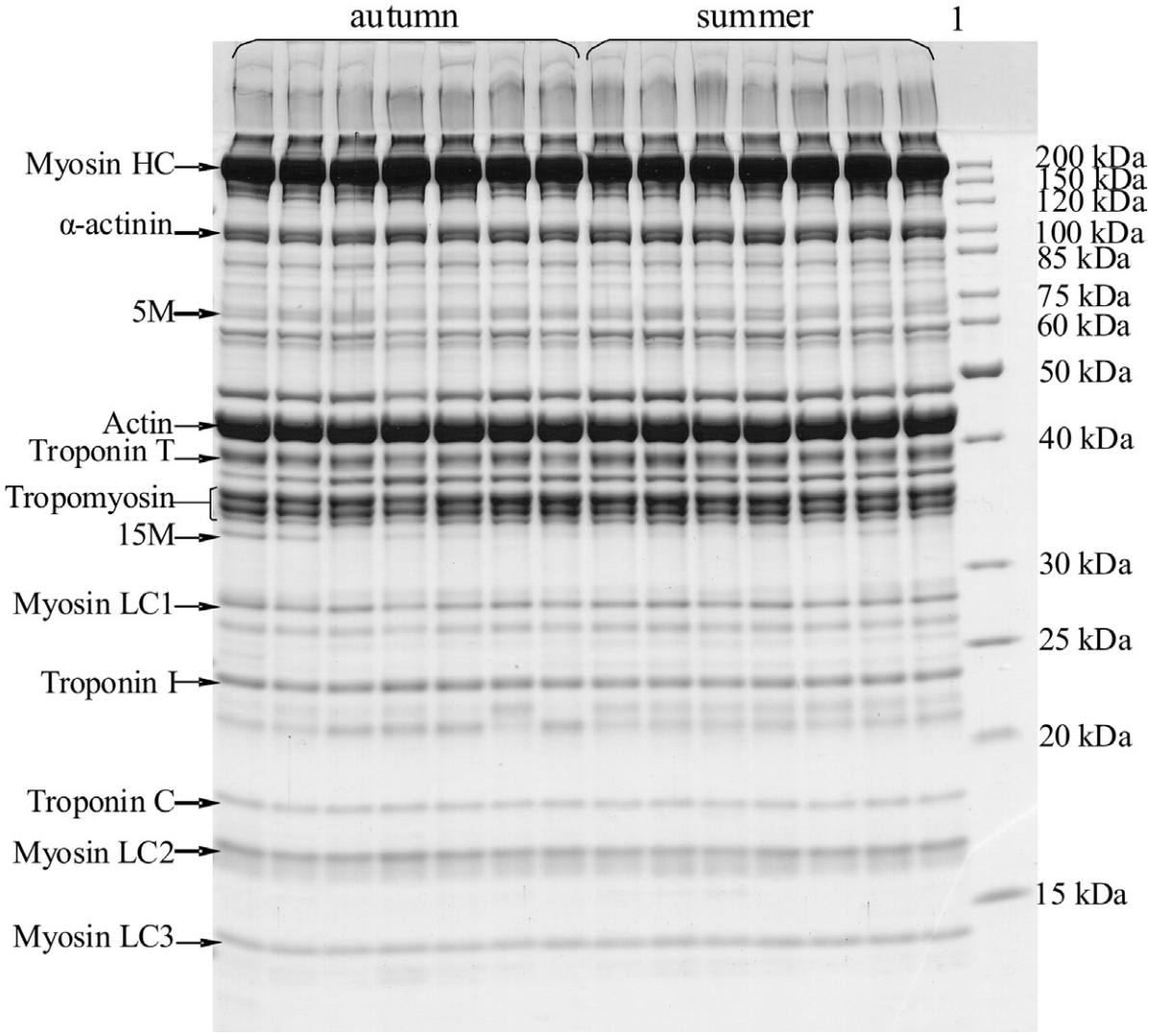

Fig. 1. SDS-PAGE electrophoresis of the degradation profile for proteins from the rabbit longissimus dorsi muscle depending on the feeding season, 1 – standard of protein size

were close to values obtained by Gil et al. (13) for the longissimus dorsi muscle from a synthetic rabbit line. Those authors determined the proportion of these proteins as 24.6 and 6.6%, respectively. The average content of actin in the meat of rabbits examined in the present study was about 20% lower than the results presented by Gil et al. (13). This may be due to the greater degradation of this protein during meat ageing. Moreover, these differences may have been caused by the different origin of muscle tissue. For instance, the composition of bovine meat proteins depends on the age of the animal, the type of muscle and the storage time of frozen meat (18).

The rabbit meat from the summer season contained significantly less α-actinin compared to the autumn meat from rabbits fed a diet rich in digestible carbohydrates (Tab. 1). This was probably related to the lower activity of calpains. These enzymes are responsible for degradation in the muscle Z-line that is composed of α-actinin. Rosenvold et al. (24) observed that the activity of μ-calpain was lower in meat from animals fed with diets low in digestible carbohydrates. Moreover, the rabbit meat from the autumn season with a more intense yellow colour and a higher GP contained significantly less TnI, TnC and myosin LC2 (Tab. 1). This was probably related to a higher activity of proteolytic enzymes because meat from pigs with a higher level of GP is characterised by greater fragmentation of myofibrils (16).

Correlation between muscle protein and quality traits. The residual products of proteolytic changes during meat ageing were divided into two groups: 60--85 kDa and approximately 30 kDa. Some of the bands from the first group may be unautolysed μ-calpain (80 kDa) and its autolysis products 5M (about 70 kDa) (2, 14, 15). Pearson correlation between muscle protein and meat quality traits is shown in Tab. 2. 5M polypeptide (autolysis product of μ-calpain) was negatively correlated with pH_1 and L* values. Bee et al. (2) suggested that the autolysis of μ-calpain occurs earlier in meat with faster rates of pH decline and eventually results in an earlier loss of proteolytic activity. The μ-calpain plays a key role in meat tenderisation, which affects the degradation of myofibrillar proteins. As it is well known, firm and dry meat is characterised by darker colour, thus resulting in a lower L* value (17),

which could explain the negative correlation between M5 polypeptide and L*.

Moreover, about 30 kDa polypeptide (15M) was negatively correlated with pH_{24}. These polypeptides are the product of the postmortem proteolysis of TnT (14). The activity of proteolytic enzymes increases at a sufficiently low pH. As shown by Daroit and Brandelli (11), a higher content of polypeptide could be related to a higher activity of creatine kinase.

The content of TnI in muscle was negatively correlated with GP and b*, which was consistent with results presented in Tab. 1. The value of b* was significantly negatively related with TnC and myosin LC2. As already mentioned, the rabbits in the autumn season were fed with a diet rich in carotenoids and digestible saccharides, which may contribute to an increase in GP (24). Meat with a higher level of GP is characterised by a greater fragmentation of myofibrils (16).

Significant differences in technological traits and quantification of certain proteins were observed in meat from rabbits slaughtered in different seasons. The meat of animals reared in the autumn season was characterised by a more intensive yellow colour (b*) and a significantly higher GP. This was probably related to the composition of feed, which in the autumn season was rich in carotenoids and digestible saccharides, which could cause a more intense yellow colouration and increased glycogen stores within muscles. Moreover, the rabbit meat from the summer season contained significantly less α-actinin and more TnI, TnC and myosin LC2 than the meat from the autumn season animals. These results indicate that rabbit meat can be influenced by a diversified course of proteolytic changes depending on feed composition, which can affect the technological and sensory qualities of meat.

Tab. 2. Pearson correlation between technological traits and quantification of proteins in meat

Characteristics	pH_1	pH_{24}	GP	L*	a*	b*
Myosin HC	0.42	–0.30	–0.21	0.37	–0.48	–0.20
α-actinin	–0.06	0.18	0.32	–0.07	0.14	0.41
5 M	–0.64▪	0.28	–0.19	–0.68▪	0.40	–0.21
Actin	0.00	0.18	0.27	–0.16	0.32	0.36
Troponin T	–0.26	–0.13	–0.08	0.02	–0.14	0.03
Tropomyosin	–0.16	0.31	–0.07	–0.56	–0.10	–0.48
15 M	0.05	–0.66▪	–0.12	0.49	–0.26	–0.06
Myosin LC1	–0.14	–0.51	–0.09	0.36	–0.09	–0.10
Troponin I	–0.19	–0.07	–0.65▪	–0.11	–0.27	–0.84▪
Troponin C	–0.39	–0.18	–0.40	0.01	–0.02	–0.62▪
Myosin LC2	–0.35	–0.17	–0.60	–0.13	–0.11	–0.75▪
Myosin LC3	–0.15	–0.10	–0.34	0.03	–0.37	–0.39

Explanation: ▪ $P \leq 0.05$

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