EFFECT OF STOCKING DENSITY ON GROWTH PERFORMANCE, MEAT QUALITY AND FIBRE PROPERTIES OF *BICEPS FEMORIS* MUSCLE OF SLOW-GROWING RABBITS

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ABSTRACT

The aim of the present work was to evaluate the effect of stocking density on growth performance, meat quality and fibre properties of *Biceps femoris* (BF) muscle of Czech White rabbits. A total of 20 rabbits (40 days old) were randomly allocated to two groups (10 rabbits per treatment) and reared at a different stocking density: group SC (small cage, 40 x 50 x 43 cm) at the density of 10 rabbits/m^2 (2 animals/cage) and group LC (large cage, 60 x 80 x 43 cm) at the density of 4 rabbits/m^2 (2 animals/cage). Animals were fed *ad libitum* pelleted feed. Rabbits were weighed every 7 days and feed intake was measured every day. At the end of experiment (89 days of age), all rabbits were slaughtered and used for the evaluation of carcass and meat traits. Stocking density had no significant effect on growth performance. There were no significant differences between groups with regard to hot carcass weight or the dressing-out percentage. The proportions of both perirenal and total dissectible fat were significantly lower in rabbits reared at the lower stocking density than in rabbits reared at the higher stocking density. There were no significant differences between groups for ultimate pH values or proximate chemical composition of hind leg meat. Similarly, meat colour ($L^*a^*b^*$) assessed on the BF surface was not affected by stocking density. Hind leg meat of rabbits reared at the lower stocking density contained less lauric (P=0.008) and myristic acid (P=0.033). There were significantly higher percentages of βR fibres and αR fibres and a significantly lower percentage of αW fibres in rabbits reared at the lower stocking density. The mean cross-sectional area and diameter of βR fibres were significantly smaller in rabbits reared at the lower stocking density than in rabbits reared at the higher stocking density. It can be concluded that a lower stocking density affected fatty acid profile and fibre characteristics of BF muscle of rabbits in a beneficial way and could provide an important nutritional benefit to humans.

Key words: Rabbit, stocking density, meat, fatty acid, fibre muscle.

INTRODUCTION

It is well known that rabbit meat is highly valued for its nutritional and dietary properties. It is a lean meat with a low-fat content and less saturated fatty acids and cholesterol than other meats. Although rabbit meat offers excellent nutritional and dietetic properties in itself, it can be further fortified with bioactive compounds because rabbit diet manipulation is very effective in increasing levels of PUFA, CLA, EPA, DHA, vitamin E, selenium etc. (see review of Dalle Zotte and Szendrő, 2011).

Thus, the effect of a dietary manipulation on rabbit meat quality is well documented. Less information, however, is available on the effect of housing system, e.g. stocking density (see review of Szendrő and Dalle Zotte, 2011). In this respect, some authors have studied the effect of an alternative rearing system on some meat traits such as the ultimate pH, instrumental meat colour or fatty acid profile, mostly in extensive conditions (Preziuso *et al*., 2009; D'Agata *et al*., 2009; Lazzaroni *et al*., 2009a). More attention has been paid to the study of the stocking density on productive and carcass traits (Szendrő and Dalle Zotte, 2011).To our knowledge, no information on characteristic of muscle fibres is available.

The aim of the present work was to evaluate the effect of stocking density on growth performance, meat quality and fibre properties of *Biceps femoris* muscle of Czech White rabbits.

MATERIALS AND METHODS

Animals and experimental design

Czech White rabbits were used. This rabbit population is one of 7 Czech rabbit breeds included in the Program of Rabbit Genetic Resources. Live weight of adult Czech White rabbits is 4.0-5.0 kg (Tůmová *et al*., 2011).

A total of 20 Czech White rabbits, 40 days old at the beginning of the experiment, were randomly allocated to two groups (10 rabbits per treatment) and reared at the different stocking density: group SC (small cage, 40 x 50 x 43 cm) at the density of 10 rabbits/m^2 (2 animals/cage) and group LC (large cage, 60 x 80 x 43 cm) at the density of 4 rabbits/m^2 (2 animals/cage). Animals were kept under controlled environmental conditions and fed *ad libitum* pellet feed (dry matter 88.5%, CP 16.9%, NDF 32.8%, ether extract 3.4% and starch 13.4%). Rabbits were weighed every 7 days and feed intake was measured every day. At the end of experiment (89 days of age), all rabbits were weighed and slaughtered without previous fasting in an authorized abattoir next to the Institute of Animal Science, to avoid suffering of animals due to stress caused by long transport time, and used for the evaluation of carcass traits according to the methodology recommended by Blasco and Ouhayoun (1996). Then, the right hind leg meat was used for determination of fibre characteristic of *Biceps femoris* (BF) muscle. Samples of BF muscle were frozen in isopentane cooled by liquid nitrogen and then stored at -80^{o}C until analysis. The left hind leg meat was chilled at +4^{o}C for 24h in a ventilated room and used for the ultimate pH (pHu), meat colour, proximate chemical composition and fatty acids profile determination.

Analyses

Cross-sections (12 μm) from each BF sample were obtained with a cryostat at -20^{o}C. Subsequently, it was performed staining for myofibrillar ATPase after successive preincubation in alkaline buffer according to the methodology recommended by Brooke and Kaiser (1970). The fibres were typed according to the nomenclature of Ashmore and Doerr (1971) as βR (red and slow twitch fibre), αR (red and fast twitch fibre) or αW (white and fast twitch fibre). For each muscle fibre type the respective percentage, mean cross-sectional area (CSA, μm^2) and diameter (μm) were determined by using software NIS Elements AR 3.1.

The pHu was determined on the BF muscle with a portable pH-meter equipped with a glass electrode suitable for meat penetration. Instrumental meat colour expressed as L* (lightness), a* (redness) and b* (yellowness) was measured with Minolta SpectraMagic^{TM}NX on a transversal section of the BF muscle surface. Values corresponded to the average of three measurements per sample.

Meat dry matter was determined by oven drying at 105^{o}C and free fat content was obtained by extraction with petroleum ether in a Soxtec 1043 apparatus (FOSS Tecator AB, Höganäs, Sweden). The determination of free fat was carried out according to ISO 1444 (1997). Protein in meat was determined using Kjeltec Auto 1030 Analyser from the same firm. Hydroxyproline content was determined by acid hydrolysis according to Diemair (1963). Fatty acid composition of hind leg meat was determined after chloroform-methanol extraction of total lipids (Folch *et al*., 1957). Nonadecanoic acid (C 19:0) was used as an internal marker to quantify the FA present in samples. Alkaline *trans*-methylation of FA was performed as described by Raes *et al*. (2003). Gas chromatography of methyl esters was performed using a HP 6890 chromatograph (Agilent Technologies, Inc.) with a programmed 60 m DB-23 capillary column (150-230^{o}C) and a flame-ionization detector (FID). Fatty acids were identified on the basis of retention times corresponding to standards. PUFA 1, PUFA 2, PUFA 3 and 37 Component FAME Mix (Supelco, Bellefonte, PA, USA) were used as standards.

Data on the growth, carcass characteristics, proximate and fatty acid composition of hind leg meat and fiber characteristic of BF muscle of the rabbits were examined by one-way analysis of variance using

the GLM procedure of the Statistical Analysis System Institute (2001). Differences between means with $P < 0.05$ were accepted as statistically significant.

RESULTS AND DISSCUSSION

Stocking density had no significant effect on weight gain and feed conversion ratio, a finding that is consistent with the other authors who concluded that applying a lower than 16 rabbits/m^2 stocking density had no effect on the growth performance of growing rabbits (e.g. Szendrő *et al*., 2009) (Table 1). In the present study, however, feed intake was higher (P=0.066) in rabbits reared at the lower density, probably associated with a higher energy requirement due to greater physical activity. There were no significant differences between groups with regard to hot carcass weight or the dressing-out percentage. The proportions of both perirenal and total dissectible fat were significantly lower in rabbits reared at the lower stocking density than in rabbits reared at the higher stocking density. Similarly, Lazzaroni *et al*. (2009b) observed decrease of fat deposit in the pen-housed rabbits (higher disposable space) compared with the rabbits reared in the individual cages. There were no significant differences between groups for ultimate pH values or proximate chemical composition of hind leg meat (Table 2). Similarly, meat colour assessed on the BF surface was not affected by stocking density. The *Biceps femoris* muscles of rabbits reared at the lower stocking density, however, exhibited lower L^* values (P=0.088) than those rabbits reared at the higher stocking density. This is consistent with the findings of Preziuso *et al*. (2009) who reported a lower lightness of BF muscle in rabbits reared in outdoor cages at the lower stocking density than in rabbits reared at the higher stocking density.

Table 1: Effect of stocking density on growth performance[1] and carcass characteristics of rabbits.

	Stocking density		RMSE[2]	*P*-value
	10 rabbits/m^2	4 rabbits/m^2		
Growth performance				
Live weight 40 d (g)	846	809	84	0.549
Live weight 89 d (g)	2702	2652	216	0.758
Weight gain (g/d)	39.5	39.9	4.9	0.907
Feed intake (g/d)	114.9	126.7	7.5	0.066
Feed conversion	3.08	3.19	0.26	0.582
Carcass characteristics				
HCW[3] (g)	1568	1608	181	0.652
Perirenal fat (g/kg HCW)	15.9[a]	9.5[b]	3.7	0.010
Total dissectible fat[4] (g/kg HCW)	25.1[a]	14.9[b]	4.3	0.001
Dressing-out[5] (%)	59.4	60.6	1.9	0.182

Means in the same row with different letters differ significantly (P<0.05). [1]40-89 days old. [2]Root Mean Square Error (n = 10 rabbits per group). [3]hot carcass weight. [4]total dissectible fat includes the scapular, inguinal and perirenal fat. [5]hot carcass weight/slaughter weight x 100.

Table 2: Effect of stocking density on pHu, $L^*a^*b^*$ colour values and proximate chemical composition of hind leg meat of rabbits

	Stocking density		RMSE[1]	*P*-value
	10 rabbits/m^2	4 rabbits/m^2		
pHu	5.61	5.58	0.04	0.179
L^* (lightness)	63.40	59.71	4.18	0.088
a^* (redness)	-2.19	-2.11	0.79	0.823
b^*(yellowness)	10.47	10.86	1.02	0.444
Proximate composition (g/kg)				
Dry matter	255	257	8	0.636
Protein	214	212	5	0.265
Free fat	25.9	26.1	4.8	0.401
Hydroxyproline	1.3	1.4	0.1	0.431

[1]root mean square error (n = 10 rabbits per group).

Table 3: Effect of stocking density on fatty acid profile (mg/100 g of muscle) of hind leg meat of rabbits.

	Stocking density		RMSE[1]	*P*-value
	10 rabbits/m^2	4 rabbits/m^2		
Saturated fatty acids (SFA)				
Lauric (C 12:0)	6.7^a	4.6^b	1.3	0.008
Myristic (C 14:0)	64.4^a	52.2^b	10.3	0.033
Palmitic (C 16:0)	679.6	620.3	83.3	0.179
Stearic (C 18: 0)	242.4	220.6	37.5	0.264
Total SFA	1019.1	952.9	121.3	0.157
Monounsaturated fatty acids (MUFA)				
Oleic (C 18:1n-9)	953.1	849.8	97.8	0.053
Total MUFA	1077.3	975.7	128.7	0.137
Polyunsaturated fatty acids (PUFA)				
Linoleic (C 18:2n-6)	706.6	660.3	75.3	0.141
α-linolenic (C 18:3n-3)	121.7	117.3	15.7	0.583
EPA (C 20:5n-3)	1.5	1.5	0.3	0.763
DHA (C 22:6n-3)	0.5^a	0.3^b	0.1	0.024
Total PUFA	926.8	877.9	77.5	0.228
n-6/n-3 ratio	5.75	5.94	0.52	0.467

Means in the same row with different letters differ significantly (P<0.05).[1]root mean square error (n = 10 rabbits per group).

Table 4: Effect of stocking density on fibre type distribution and fibre histomorphological characteristics of *Biceps femoris* (BF) muscle of rabbits

	Stocking density		RMSE[1]	*P*-value
	10 rabbits/m^2	4 rabbits/m^2		
Fibre type distribution (%)				
βR[2]	6.5^a	16.3^b	5.4	0.001
αR[3]	14.2^a	24.5^b	5.2	0.001
αW[4]	79.3^a	59.2^b	5.7	0.001
Fibre cross-sectional area (μm^2)				
βR	2744^a	1882^b	456	0.001
αR	1773	1739	296	0.810
αW	2882	2752	403	0.506
Diameter(μm)				
βR	58.5^a	47.9^b	5.2	0.001
αR	46.9	45.6	4.0	0.511
αW	56.0	57.6	5.8	0.569

Means in the same row with different letters differ significantly (P<0.05). [1]root mean square error (n = 10 rabbits per group). [2]*β*R (red and slow twitch fibre). [3]*α*R (red and fast twitch fibre). [4]*α*W (white and fast twitch fibre).

The fatty acid profile is presented in Table 3. Hind leg meat of rabbits reared at the lower stocking density contained less lauric acid (P=0.008), myristic acid (P=0.033), oleic acid (P=0.053) and DHA (P=0.024). Lower content of lauric and myristic acid can be explained by different rate of oxidation of individual fatty acids, a situation well documented in humans. In fact, DeLany *et al*. (2000) observed that lauric acid, medium-chain fatty acid (MCFA), was the most rapidly oxidized fatty acid, followed by the unsaturated fatty acids; the long-chain saturated fatty acids were the least oxidized. In the present study, oxidation of MCFA apparently covered a higher energy requirement in more physically active rabbits reared in cages with the higher disposable space when compared with those rabbits reared at the higher stocking density. These results indicate that rabbits reared at the lower stocking density yielded hind leg meat with the lower content of MCFA, which might provide a nutritional benefit to humans. In fact, lauric, myristic, and palmitic fatty acids are responsible for increasing total plasma and LDL cholesterol concentrations (review of Ulbricht and Southgate, 1991).

Effect of stocking density on fibre type distribution and fibre histomorphological characteristics of BF muscle of rabbits is presented in Table 4. There was a significantly higher percentage of βR fibres (red and slow twitch fibres) and αR fibres (red and fast twitch fibres) and a significantly lower percentage of αW fibres (white and fast twitch fibres) in rabbits reared at the lower stocking density. The mean cross-sectional area and diameter of βR fibres were significantly smaller in rabbits reared at the lower stocking density than in those of rabbits reared at the higher stocking density.

CONCLUSION

It can be concluded that a lower stocking density affected fatty acid profile, fibre type distribution and fibre histomorphological characteristics of BF muscle of rabbits in a beneficial way. No significant effect of stocking density on growth performance was observed.

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