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Effect of growth rate on *post mortem* proteolysis in lamb muscles

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SUMMARY – Proteolysis in *Longissimus dorsi* (LD) and *Semi membranosus* (SM), and particularly collagen degradation was studied to examine the effect of growth rate on proteolysis of 5 low growth rate lambs (LGR) and 5 high growth rate lambs (HGR). HGR lambs had significantly higher ($P < 0.05$) live weights, carcass weights and growth rate. No significant differences were observed between muscles in non protein nitrogen (NPN) content, free hydroxyproline (OH-Proline) in fresh muscle (FM) or NPN, but LD had more total OH-Proline content than SM (758.49 mg N/g FM vs 721 mg N/g FM). NPN content at slaughtering was significantly higher ($P < 0.05$) in HGR lambs. HGR lambs had significantly higher *ante mortem* proteolysis (+6%) in the two studied muscles. NPN content increased about 15% during storage until 21 days *post mortem* (it represented 1.5% of total nitrogen). More than 80% were obtained in the first 10 days for both treatments. Lambs which had high *ante mortem* proteolysis showed high *post mortem* proteolysis. HGR lambs had also significantly higher *ante* and *post mortem* collagen degradation. At slaughtering, OH-Proline in NPN was higher ($P < 0.05$) in HGR lambs (+34%) than in LGR lambs and increased by 44% vs 29% for HGR and LGR during ageing with the most important increase during the first 3 days. Furthermore, HGR lambs had a lower ($P < 0.05$) total OH-Proline content than LGR (679.35 µg OH-Proline/g FM vs 800.14 µg OH-Proline/g FM respectively).

Key words: Proteolysis, collagen, NPN, OH-Proline, ageing, lambs, muscles.

RESUME – *"Effet de la vitesse de croissance sur la protéolyse* post-mortem *dans les muscles d'agneau". La protéolyse dans les muscles Long dorsal (LD) et le Semi membraneux (SM), et en particulier la dégradation du collagène ont été étudiées afin de déterminer l'effet de la vitesse de croissance sur la protéolyse chez 5 agneaux en croissance faible (LGR) et 5 agneaux en croissance élevée (HGR). Les HGR ont des poids vifs, des poids de carcasse et une vitesse de croissance significativement plus élevés ($P < 0,05$). Aucune différence significative n'a été observée entre muscle pour le contenu en azote non protéique (ANP), en hydroxyproline (OH-Proline) libre dans le muscle frais (MF) et dans l'ANP, mais LD possède plus d'OH-Proline totale que SM (758,49 mg N/g MF contre 721 mg N/g MF). Le contenu en ANP, à l'abattage, a été significativement plus élevé ($P < 0,05$) pour les HGR. Les HGR auraient une protéolyse avant la mort significativement plus élevée (+6%) dans les deux muscles étudiés. Le contenu en ANP a augmenté d'environ 15% durant les 21 jours de stockage après la mort (ce qui représente 1,5% de l'azote total). Plus de 80% de cette augmentation ont été obtenus dans les 10 premiers jours. Les agneaux qui ont une protéolyse ante mortem élevée ont une protéolyse* post mortem *élevée. Les HGR ont présenté également une dégradation du collagène avant et après la mort significativement plus élevée. A l'abattage, OH-Proline dans l'ANP a été plus élevée ($P < 0,05$) pour les HGR (+34%) que pour les LGR et a augmenté de 44% et 29% pour les HGR et les LGR durant la maturation avec l'augmentation la plus importante durant les 3 premiers jours. De plus, les HGR ont un contenu en OH-Proline totale plus faible ($P < 0,05$) que LGR (679,35 µg OH-Proline/g de MF contre 800,14 µg OH-Proline/g de MF respectivement).*

Mots-clés : *Protéolyse, collagène, ANP, OH-Proline, maturation, agneaux, muscles.*

Introduction

Meat ageing varies considerably between animals (Valin *et al.,* 1975) and depends on *post mortem* proteolysis. Collagen degradation was recently studied, with thermal stability modifications (Mills *et al.,* 1989), biochemical alterations (Feidt *et al.,* 1996) or ultrastructural damage (Nishimura *et al.,* 1996) reported. Few studies were conducted on the effect of livestock production on collagen evolution. Proteolysis can be estimated by NPN evolution and OH-Proline content in NPN fraction.

The aim of this experiment was to study proteolysis during ageing and the effect of *ante mortem* growth rate on this proteolysis.

Material and methods

Animals

The experimental period was 21 days. Ten 123 day-old male lambs (INRA 401) received hay *ad libitum,* with 5 low growth rate animals (LGR) receiving hay only and 5 high growth rate animals (HGR) hay + 900 g/d of concentrate. Animals were weighed every week and before slaughter. Offered and refused feed were measured every day. Animals were knocked out mecanically and bled out. An initial sample of each muscle (*Longissimus dorsi*, LD; *Semi membranosus*, SM) was taken to estimate muscle *ante mortem* proteolysis. The carcasses were stored 1.5 h after slaughtering at 4°C. Further samples were taken (50-100 g) at 0 days and 24 h *post mortem*, dipped in 0.1 g/l sodium azide and then placed in vacuum pouches, stored at 4°C and sampled at 3, 10, 17 and 21 days *post mortem*. For analysis, only the internal part of the muscle was used.

Biochemical analysis

Peptide extraction

Peptide extraction was done according to the Feidt *et al.* (1998) method. Non Protein Nitrogen (NPN) content was determined using Kjeldahl on automatic Vapodest (Gerhardt) in triplicate for each day of ageing. Total nitrogen content (TN) was measured on 1 g of homogenised muscle.

Hydroxyproline of NPN fraction

OH-Proline of NPN fraction was chromatographed by HPLC after PITC derivatization according to a modification of Bidlingmeyer *et al*. (1984) method on each muscle of 4 lambs (2 of each treatment). NPN OH-Proline concentration was expressed as µg OH-Proline/g fresh meat.

Total collagen content in fresh muscle (TC)

Total collagen content was calculated from the OH-Proline content of the muscle measured using the method of Bonnet and Kopp (1986).

Statistical analyses

Results were treated by 3 way variance analysis (muscle, growth rate and slaughtering day).

Results and discussion

Animal performance

Live weights were evaluated at slaughtering and carcass weights without the head. At slaughtering, HGR lambs had significantly ($P < 0.005$) higher live weights, carcass weights and growth rate than LGR lambs (Table 1).

Table 1. Performance

Lamb	Lamb weight (kg)		Carcass weight (kg)		Growth rate (g/d)	
	m†	S††	m	S	m	S
HGR	30.76	2.63	11.69	1.57	265	37.15
LGR	25.72	2.96	8.56	0.42	47	34.51

†m = mean.
††S = standard deviation.

Growth rate effect on muscle proteolysis

The statistical model showed that there was no significant difference between muscle in NPN content (Fig. 1). At slaughtering, NPN content was significantly higher ($P < 0.05$) in HGR lambs (3.03 g/kg versus 2.85 g/kg for HGR and LGR respectively) and could indicate a higher *ante mortem* proteolysis. HGR lambs had significantly higher proteolysis (+6%) in these two muscles. NPN content increased about 15% between 0 and 21 days *post mortem*. NPN content represented about 10% (10.05 ± 0.55 versus 9.72 ± 0.49 for HGR and LGR lambs respectively) of total nitrogen (TN) content, which was about 32.70 mg N/g FM for HGR lambs and 31.49 mg N/g FM for LGR lambs. Thus, NPN increase during ageing represented 1.5% of TN. The increase of NPN content was probably due to the production of small peptides and amino acids (Mikami *et al.,* 1991) by *post mortem* proteolysis. More than 80% of increase was obtained in the first 10 days of ageing for the two treatments. It seems that *post mortem* proteolysis was correlated with *ante mortem* proteolysis. Lambs (HGR) which had high *ante mortem* proteolysis showed high *post mortem* proteolysis. This was a similar conclusion to that found for steers by Fishell *et al.* (1985) who thought that this relationship may be mediated partly through the turnover and (or) maturation rate of intramuscular collagen. Cattle fed high-energy diets grow more rapidly and had increased rates of protein turnover, which may affect collagen solubility and (or) myofibril fragmentation (Wu *et al.,* 1981).

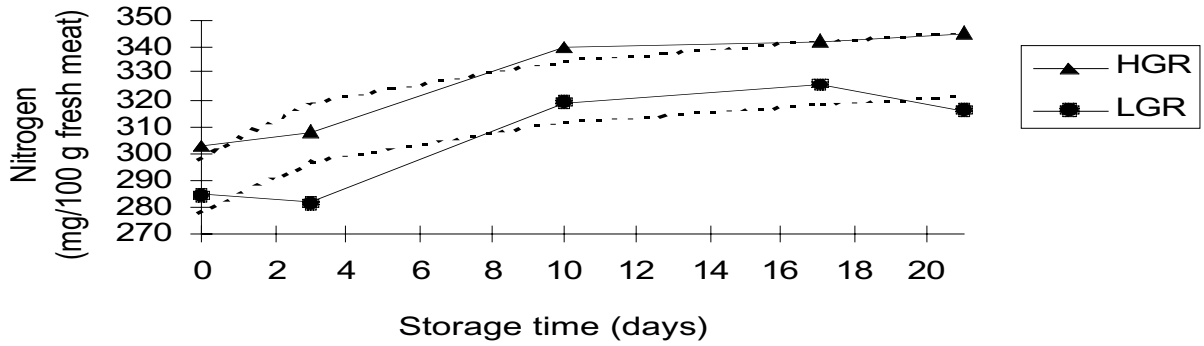

Fig. 1. NPN content during ageing. Tendency curves (dotted lines) were calculated with STAT-ITCF. HGR [y = 11.15 ln (t + 1) + 298; r_ = 0.89); LGR (y = 13.96 ln (t + 1) + 278; r_ = 0.75].

Connective tissue degradation

At slaughtering, free OH-Proline was higher ($P < 0.05$) in HGR lambs (+34%) than in LGR lambs (Fig. 2). OH-Proline is a collagen specific amino acid. Free OH-Proline is not incorporated into collagen -chains during collagen synthesis (Kivirikko, 1970), therefore, OH-Proline release is an indicator of collagen degradation. The highest level of OH-Proline indicated that HGR lambs had a significantly higher *ante* and *post mortem* collagen degradation. This content increased during storage time. NPN OH-Proline content increased by 44% versus 29% for HGR and LGR lambs respectively, between 0 and 21 days. The total increase of free OH-Proline in fresh muscle represented +1.78 µg/g FM for HGR and + 0.89 µg/g FM for LGR lambs. It seems that HGR lambs developed less connective than myofibrillar tissues. Total OH-Proline content was lower ($P < 0.05$) in HGR lambs than in LGR (679.35 µg OH-Proline/g FM ± 29.94 versus 800.14 µg OH-Proline/g MF ± 42.85 respectively).

Conclusion

HGR lambs had the highest *ante* and *post mortem* collagen degradation. *Post mortem* proteolysis was correlated with *ante mortem* proteolysis. The study of peptides appearance, their molecular weight and the localisation of OH-Proline in these peptides could allow to better understood these phenomena.

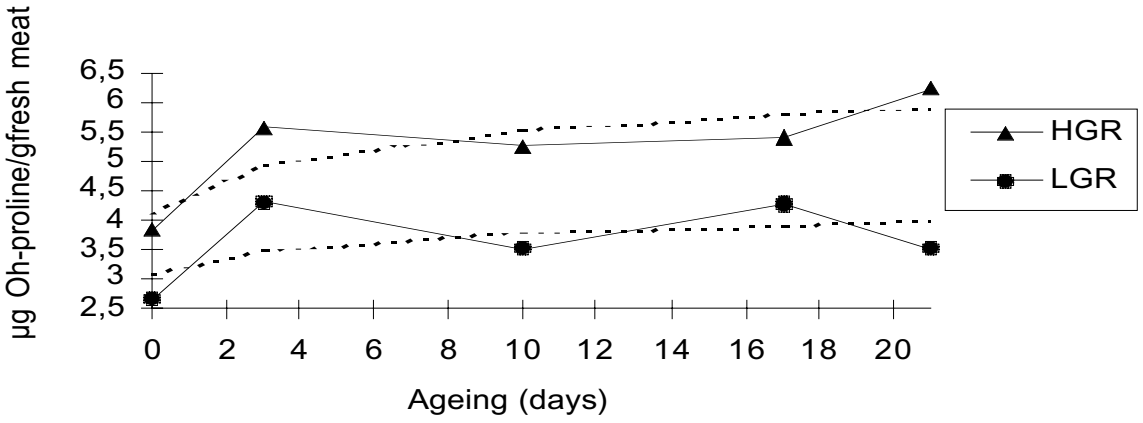

Fig. 2. OH Proline content in NPN fraction in fresh muscle. Tendency curves (dotted lines) were calculated under STAT ITCF. HGR [y = 0.585 ln (t + 1) + 4.12; r_ = 0.72]; LGR [y = 0.292 ln (t + 1) + 3.08; r_ = 0.31].

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