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ScienceDirect
Small Ruminant Research 71 (2007) 144–149
Small Ruminant Research
www.elsevier.com/locate/smallrumres

Changes in the pyridinoline concentration of the *gastrocnemius* and *soleus* muscle in goats from 2 weeks prenatal to 24 weeks of age

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Received 27 February 2006; received in revised form 18 May 2006; accepted 29 May 2006
Available online 11 July 2006

Abstract

The objective of this study was to monitor changes in the pyridinoline concentration of collagen of the fast-twitch *gastrocnemius* and slow-twitch *soleus* muscles of Japanese Saanen male goat kids during the period from 2 weeks before birth to 24 weeks of age. The moisture concentrations of both muscles decreased and the crude protein concentration increased steadily throughout the experimental period. The percentage of total collagen in the muscular protein showed a marked decrease (80.6–41.8% in the *gastrocnemius* and 77.9–40.5% in the *soleus* muscle) during the 2 week prenatal period. Similarly, there was a decrease in soluble collagen concentration (27.8–11.6% in the *gastrocnemius* and 32.6–18.1% in the *soleus* muscle) during the prenatal period, but the decrease in total and soluble collagen concentration was slight thereafter. There was no clear tendency for change in collagen heat solubility of both muscles, and no strong relationship was identified between collagen heat solubility and pyridinoline concentration. Pyridinoline concentration in total collagen increased during the prepubertal period in both muscles, but the *soleus* muscle increased faster and had a higher concentration of pyridinoline (0.22 mol/mol collagen at 20 weeks of age) than the *gastrocnemius* muscle (0.11 mol/mol collagen at 20 weeks of age). It was found that the increase in pyridinoline concentration began during the prenatal period, and the development of a cross-linking with age was faster in the *soleus* than in the *gastrocnemius* muscle, without collagen concentration between the muscles. It is suggests that meat toughness would be improved when the proportion of fast-twitch muscular fibrils could be increased.

Keywords: Collagen; Goat; Growth; Muscle; Pyridinoline

1. Introduction

Intramuscular protein can conveniently be divided into three fractions: sarcoplasmic (water soluble), myofibrillar (salt soluble) and connective tissue (acid soluble) proteins (Koohmaraie et al., 2002). Intramuscular connective tissue is typically organized into the perimysium (90%) and endomysium (10%). The perimysium being comprised of types I and III collagen (McCormick, 1994). Tough meat has well-developed collagen fibers occupying a large portion of the thick perimysium, with the development of the collagen fibers therefore being correlated with meat tenderness (Nakamura et al., 2003).

Cross-links formed between lysine residues of neighboring collagen molecules play an important

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doi:10.1016/j.smallrumres.2006.05.013

physiological role in maintaining the mechanical stability of collagen fibers in live animals (McCormick, 1994). Cross-links increase with age (Bosselmann et al., 1995), leading to lower collagen solubility and an increase in tensile strength (Bailey, 1984). These increases in the strength of intramuscular collagen lead to a decrease in meat tenderness and result in the reduction of meat quality. Pyridinoline is a characteristic cross-link that is increasingly related to collagen content in higher percentages (Steinhart et al., 1994), while collagen solubility decreases during growth (Young and Braggins, 1993). Changes in collagen solubility with age has been demonstrated by Young and Braggins (1993), who reported the percentage of heat-soluble collagen in total collagen to decrease with an increase in chronological age of muscles in sheep. The pyridinoline concentration of collagen was also shown to significantly increase until 3 mouths of age in rats (Palokangas et al., 1992). These findings indicate collagen development with increased pyridinoline concentration in ruminants to occur during the young period, but this aspect has not been extensively researched.

Muscles consist of a slow-twitch part (type I myofibers) and fast-twitch part (type II). These two types of muscle have functional and metabolic differences (Beatty and Bocek, 1970), and the intramuscular collagen concentration differs (Zimmerman et al., 1993). Beyond this, nothing is known of the pattern of pyridinoline formation in different muscle types. This may have practical importance, as its formation may be a predictor of meat quality in mature animals.

This study compared the development of pyridinoline linkages in two functionally different muscles, the postural slow-twitch *soleus* and the locomotory fast-twitch *gastrocnemius* muscle. These muscles were used as models for fast- and slow-twitch muscles, respectively, each with a different fiber-type profile. This study thus undertook to clarify the development of intramuscular collagen in prepubertal animals with consideration of muscle types.

2. Materials and methods

2.1. Animals

Japanese Saanen male kids ($n = 25$) were assigned to six groups (four or five kids per group) on the basis of birth date. Kids were weaned at 4 weeks of age and each group was reared in a pen with free access to a concentrate diet (total digestible nutrients 70%, crude protein 15%) and ryegrass hay. The kids were slaughtered at birth, 3, 9, 16, 20 and 24 weeks of age in their respective groups. Two kids slaughtered at 16 and 24 weeks of age were excluded from this experiment because their body weights decreased due to acute diarrhea. Eight prenatal kids were obtained from five pregnant does slaughtered at 134 days of gestation (approximately 2 weeks before parturition). All kids and goats were euthanized by an overdose of sodium pentobarbital, administered intravenously. The fast-twitch *gastrocnemius* and slow-twitch *soleus* muscles were obtained from two *triceps surae* muscles on both sides of the carcasses, immediately frozen, followed by storage at −20 °C (Beatty and Bocek, 1970). Before chemical analysis, the frozen muscles (thawed at 4 °C) were completely trimmed of the epimysium, adipose tissue and tendons. The muscle belly without the tendinous portion was minced and prepared to determine the moisture and crude protein concentration and the determination of total and soluble collagen concentration and pyridinoline concentration. This study was carried out in accordance with the guide for the care and use of laboratory animals of National Agricultural Research Center for Kyushu Okinawa Region.

2.2. Collagen concentration

Soluble collagen was extracted from 4 g duplicate tissue samples by heating for 70 min at 77 °C in tubes containing 15 ml of 25% strength Ringer's solution, in a water bath (Hill, 1966). The tubes were then centrifuged at 6000 × *g* for 10 min to separate the supernatant and residue. The residue was resuspended in the Ringer's solution and centrifuged, and all the supernatant pooled. The supernatant samples and duplicate minced meat samples (7 g each) to determine the total amount of collagen in each muscle, were individually hydrolyzed in 6N HCl for 12 h at 121 °C. After neutralization, the hydroxyproline concentration of each hydrolyzed supernatant and minced meat sample was determined using a colorimetric method (Bergman and Loxley, 1963). The collagen concentration (mg/g crude protein) was calculated using the conversion factors of 7.25 (total collagen) and 7.52 (soluble collagen), respectively (Cross et al., 1973). Collagen solubility was expressed as the percentage soluble collagen of the total concentration.

2.3. Pyridinoline concentration

Pyridinoline was purified in duplicate, according to the method of Skinner (1982), and determined by the liquid chromatographic procedure of Arakawa et al. (1992). An ion exchange cellulose (Whatman CF_1, Kent, UK) slurry was prepared by mixing 10 g cellulose with 200 ml