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Original article

Microstructure of skeletal muscles of growing calves fed silage-based *vs* hay-based diets. II. Fibre type distribution

B Kłosowski [1*], K Bidwell-Porębska [2], D Kłosowska [1*], J Piotrowski [2**]

[1] *Meat and Fat Research Institute, Warsaw, Laboratory of Meat Science, Bydgoszcz;*
[2] *Polish Academy of Sciences, Institute of Genetics and Animal Breeding, Jastrzebiec, 05-551 Mroków, Poland*

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Summary — As described in part I, samples of musculus longissimus dorsi, semimembranosus and semitendinosus were obtained post-slaughter from 2-week, 3-month and 10-month-old bull calves. The 2-week-old calves were fed milk only. All the remaining animals were fed grass silage or hay *ad lib* and a restricted amount of concentrate from 2 weeks of age onwards. Muscle fibres were differentiated according to Ziegan (1979) into fast-twitch glycolytic, fast-twitch oxidoglycolytic and slow-twitch oxidative fibres (FTG, FTO and STO, respectively). The percent distribution of individual types of fibres was estimated as related to the calves age and diet. The most numerous were always fast-twitch glycolytic fibres, the lowest values being observed in the 2-week-old calves. The effect of the diet on fibre percentage distribution in 3-month-old calves differed from that found in 10-month-old animals. This research suggests that a hay-free diet based on grass silage alters the microstructure of skeletal muscles, which thus might also affect the quality of meat.

calf / skeletal muscle fibre / silage / hay

Résumé — Microstructure des muscles squelettiques chez des veaux recevant de l'ensilage ou du foin. II. Pourcantage des fibres musculaires. *Des échantillons de muscles* longissimus dorsi, semimembranosus *et* semitendinosus *furent collectés, comme décrit dans la partie I à la suite de l'abattage de veaux de 2 semaines, et de taurillons de 3 mois et de 10 mois. Les veaux âgés de 2 semaines avaient été nourris au lait, tandis que les veaux restants reçurent à volonté de l'ensilage de graminées ou du foin, ainsi q'une dose limitée d'aliment concentré. Conformément à la méthode de Ziegan (1979), ont été distinguées les fibres musculaires glycolytiques et oxydatives glycolytiques se contractant rapidement, et les fibres oxydatives à contraction lente. Les plus nombreuses étaient toujours les fibres glycolytiques à contraction rapide – les valeurs les plus basses concernant les veaux de 2 semaines. L'influence de la ration sur la proportion de fibres dans les muscles des veaux de 3 mois différait de celle observée sur des taurillons de 10 mois. Ce travail suggère qu'une ration sans foin, à base d'ensilage de graminées, influence la microstructure des muscles squelettiques et pourrait dont agir sur la qualité de la viande.*

veau / fibre de muscle squelettique / ensilage / foin

* Present address: Technical and Agricultural University, Faculty of Animal Science, Mazowiecka 28, 85-084 Bydgoszcz, Poland
** Correspondence and reprints

INTRODUCTION

The fibre is the basic unit of skeletal muscle and comprises 75 to 90% of the total muscle mass (Hegarty, 1971). It has been proved in domestic animals that the total muscle fibre numbers remain unchanged from birth or shortly before, and throughout the animal's life (Staun, 1963; Ashmore and Addis, 1972). Further growth of the muscle is a result of changes in the proportions between fibre types and of an increase in myofibre size (Ashmore *et al*, 1972; Kłosowski and Kłosowska, 1987).

It is well established in cattle that the proportions between muscle fibre types change with age and body weight (Cornforth *et al*, 1973; Johnston *et al*, 1975; Kłosowski and Kłosowska, 1988). However, few studies have been made on young, growing animals to determine the influence of different nutrition programmes. Meanwhile, despite the extensive use of silages in cattle nutrition, the effect of all-silage forage programmes on the organism is often controversial. The negative influence of organic acid concentrations and low pH on the digestive tract is generally accepted (Ballarini, 1974; Pellegrini *et al*, 1982a). Vitamin A, mineral and iodine deficiencies, known to be connected with silage nutrition (Hemken and Vandersall, 1967; Pellegrini *et al*, 1982a, b), may also cause "dystrophic regressive phenomena in the skeletal muscle fibres" (Pellegrini *et al*, 1982c) and probably influence the market and technological value of the meat. In part I of this study we have investigated the effect of silage-based or hay-based diets on the growth of fibres of selected skeletal muscles in calves (Kłosowski *et al*, 1992). Part II examines the percentage distribution of fibre types.

MATERIAL AND METHODS

The collecting, grouping, feeding and slaughtering of calves as well as calf performance have been described in part I of this study (Klosowski *et al*, 1992). Following the paper cited, the calves were referred to as hay diet (HD, group I and IA) or silage diet (SD, group II and IIA)-fed animals, and as "younger" (3 months old: group I and II) or "older" (10–11 months old: group IA and IIA). A group of 5 calves (2 weeks old), referred to as "newborn", was also included.

Single tissue samples were taken approximately 1 h post-slaughter from musculus longissimus dorsi (longissimus lumborum – LD), musculus semimembranosus (SM) and musculus semitendinosus (ST). In part I the sampling, storing, sectioning, preparation, and staining procedures have been described (Kłosowski *et al*, 1992). Fast twitch glycolytic (FTG), slow twitch oxidative (STO) and fast twitch oxidoglycolytic (FTO) fibres were identified according to Ziegan (1979). The percent distribution of fibre types within each muscle was estimated in 10 primary bundles of fibres of each type (approximately 400 individual fibres per sample).

The significance of the relationship between calf age or diet and fibre distribution was calculated within the groups and for each muscle separately using the χ^2 test, suitable for calculations on percent values. The information thus obtained made it possible to evaluate the differences between the general structure of individual muscles within the age and nutrition groups.

RESULTS

The data referring to the daily nutrient intake and growth rate for all the age and feeding groups have been presented in part I of this study (Kłosowski *et al*, 1992).

Figure 1 indicates that the most numerous were the FTG fibres, constituting roughly 40–54% of all fibres, the lowest values being observed for newborn calves. In the case of LD muscle, the percentage of FTG fibres remained unchanged until the animals were 90 days old and in-

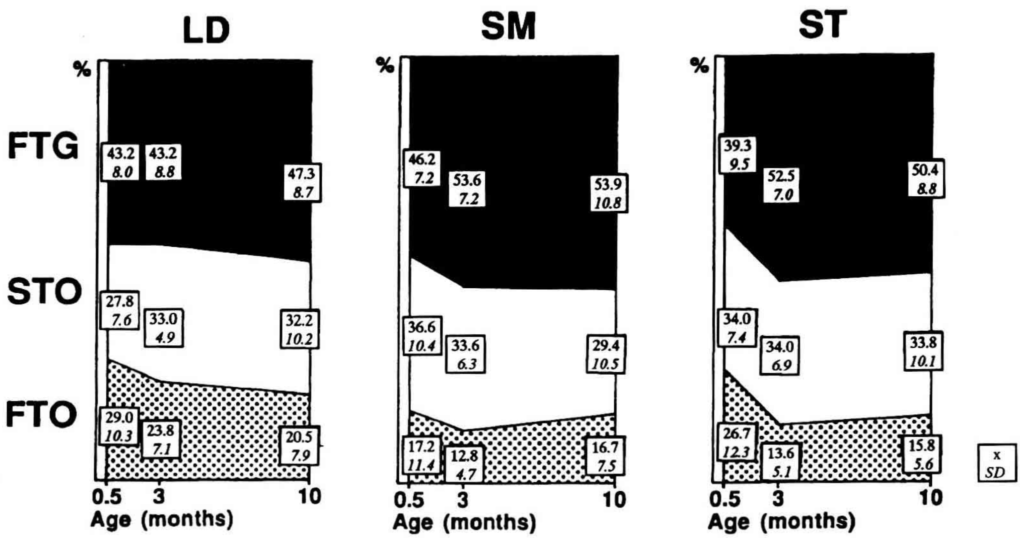

Fig 1. Percent distribution of fibre types (FTG: fast-twitch glycolytic, STO: slow-twitch oxidative, FTO: fast-twitch oxydoglycolytic) in individual muscles (LD: longissimus dorsi, SM: semimembranosus, ST: semitendinosus) as related to calf age. See table I for statistical estimations.

Table I. Significance of relationship (χ^2) between the calves age and percent distribution of fibre types presented in figure 1.

Animals compared	*Musculus longissimus dorsi*	*Musculus semimembranosus*	*Musculus semitendinosus*
Newborn and all younger calves	xx	xx	xx
Newborn and all older calves	xx	x	xx
All younger and all older calves	x	NS	xx

x: $P < 0.05$; xx: $P < 0.01$; NS: non significant.

creased slightly in the older calves. In the SM and ST muscles the percentage of FTG fibres increased already in the younger calves and remained fairly constant in the older animals. The percentage distribution of FTO fibres presented a picture con-

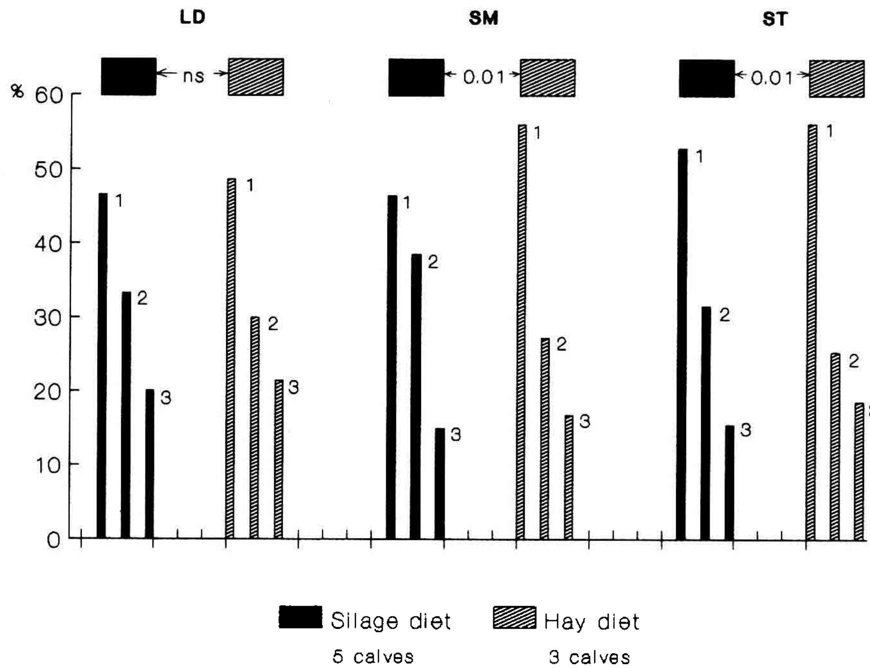

Fig 2. Percent distribution of fibre types (1: fast-twitch glycolytic, 2: slow-twitch oxidative, 3: fast-twitch oxidoglycolytic) in individual muscles (as in figure 1) of 3-month-old calves, as related to diet. 0.01 and 0.05: significance of relationship (χ^2); ns: non significant.

trary to that of the FTG: it was markedly highest in newborn, and lowest in younger calves, the differences related to age being most marked in the SM and ST muscles. The differences in percent distribution of STO fibres between age groups were the smallest and the least regular.

Using the χ^2 test for comparing newborn to all younger as well as all older calves (table I), a highly significant relationship was demonstrated between calf age and the percent distribution of fibre types in each of the three muscles. However, when comparing the younger and older calves the relationship was less convincing, as it was found significant only for LD and ST muscles.

No significant effect of diet was proved when comparing feeding systems within younger calves (fig 2). However, in older calves (fig 3) such an effect was found to be significant for the SM and ST muscles. This was caused principally by the demonstrated differences in the percentage share of FTG and STO fibres between the nutrition groups.

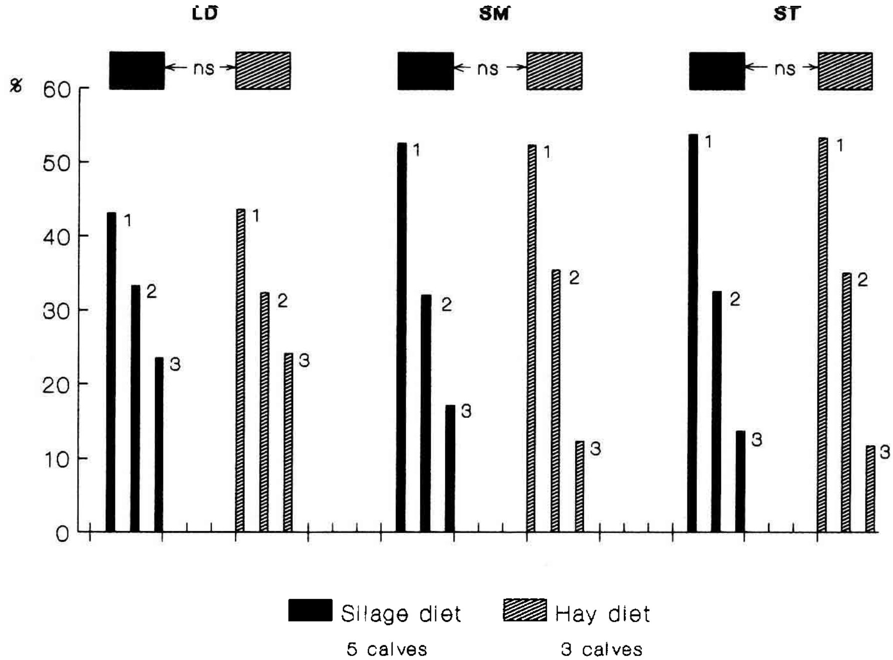

Fig 3. Percent distribution of fibre types (as in figure 2) in individual muscles (as in figure 1) of 10–11-month old calves as related to diet. Statistical estimation as in figure 2.

DISCUSSION

The percentage distribution of individual fibre types corresponds with that reported by other authors (Melton *et al,* 1971; Hunt and Hendrick, 1977; Kłosowski and Kłosowska, 1984). The influence of age is also consistent with earlier investigations. In this study changes in the percentage distribution of fibres, in particular an increase of the share of fibres characterised by a glycolytic metabolism, were also related to age and live weight, and differed for different muscles (fig 1). This can be related to their various functions and non-identical growth patterns (Ashmore *et al,* 1972; Cornforth *et al,* 1973; Richmond and Berg 1982). The differences in the percentage distribution, arising principally from an increase in the number of FTG fibres and a simultaneous decrease in the number of FTO fibres, could be attributed to a transformation between fibre types, as suggested by Ashmore *et al* (1972). The different developmental rate of LD as compared with SM and ST muscles (fig 1) was observed earlier by Kłosowski and Kłosowska (1988), who also did not observe any changes in the share of FTG fibres in the LD between 2 week- and 3 month-old calves.

The different effect of the diet on fibre distribution in younger *vs* older calves is interesting. Diet did not influence fibre type distribution in younger calves (fig 2). However, in the older animals (fig 3), those fed hay had a higher percentage of FTG and FTO, and simultaneously a lower proportion of STO fibres; only in the case of the LD muscle were no differences observed between the 2 feeding groups. Moody *et al* (1980) when comparing the percentage distribution of fibres in the LD muscle also found no significant differences between animals receiving different diets. However, in their experiment the difference in the feeding regime lay in the presence or absence of pasture. Moreover, they only examined the LD muscle, paying no attention to the different growth rates which characterise different muscles.

In the present study, it is difficult to interpret the higher percentage of FTG and FTO fibres, and the lower percentage of STO fibres in the SM and ST muscles of the older HD fed animals. The older calves from both feeding groups were slaughtered at a constant live weight of approximately 250 kg (338 *vs* 305 days of age, for HD and SD group, respectively). However, the mean DM intake (3075 *vs* 4599 g/day) and GE (59.5 *vs* 86.9 MJ/day), and thus also the mean live weight gains (617 *vs* 713 g/day) were significantly lower in the HD as compared with the SD-fed calves. Thus the differences observed in the present distribution of fibres in the ST and SM muscles could be attributed to the different daily DM and GE intake in the 2 feeding groups, and consequently to different daily LW gains. The results reported by Johnston *et al* (1981) confirm the influence of energy concentration in the diet on muscle fibre distribution, but in reverse, the higher the energy level, the higher the percentage of fibres connected with the glycolytic metabolism. Such a discrepancy may be caused by the silage diet as such. In part I of the present investigation (Kłosowski *et al,* 1992), differences have also been observed in the fibre diameters between the 2 feeding groups, which could not be adequately explained by differences in nutrient intake or growth rate. This problem needs to be examined more thoroughly, all the more so as certain investigations have shown all silage diets to cause considerable histopathological changes, not only in skeletal muscles (Pellegrini *et al,* 1982c) but also in numerous internal organs of cattle (Pellegrini *et al,* 1982a, b; Slesareva and Soloveva, 1968). The present investigation suggests that such diets can also affect the percent distribution of fibres of skeletal muscles. Whether this may influence the final quality of meat remains an interesting question, especially as Rumsey *et al* (1987) reported silage feeding to affect various beef characteristics, while Ashmore *et al* (1972) demonstrated that meat quality can be related to changes in proportions between muscle fibre types.

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