S1P06.WP

MICROSTRUCTURAL BASIS OF THE REPARTITIONING PROCESS INDUCED BY pST IN GROWING-FINISHING PIGS UP TO 150KG LIVE WEIGHT

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Please refer to Folio 3.

INTRODUCTION

The administration of porcine somatotropin (pST) to pigs increases muscle growth, improves feed efficiency and decreases fat deposition (e.g., Etherton *et al.*, 1986). The major mechanism involved is known to be the repartitioning of feed energy from fat to protein accretion. All studies so far carried out on this problem used traditional live weight end points of 100 to 115kg. Normally, prolonged fattening leads to unfavourable meat/fat ratios in the carcass because of the increasing fat deposition. It was clearly shown that it is possible to produce heavy lean pigs in spite of prolonged fattening when pST was administered (Ender *et al.*, 1991). As a rule, the results of the repartitioning process can only be examined at slaughter. Thus, the aim of this study was to examine the structural basis of the prolonged repartitioning process following the development of skeletal muscle fibres and fat cells of backfat in live pigs by sequential biopsy.

MATERIAL AND METHODS

Castrates of East German Landrace pigs were fattened up to 150kg live weight. They were fed a high energy diet containing 11.2g lysine/kg DM. 60 pigs were divided randomly into three experimental groups, which were treated with a placebo (control), 2 to 4mg pST/d (changing the dose on day 42 of treatment) or 4mg pST daily from 114 to 216 days of age. The pST (Pitman-Moore, Inc., USA) was injected intramuscularly and was dissolved in an arginine buffer (pH6.4). The pigs were slaughtered when they attained about 150kg live weight after a one week withdrawal from pST.

Longissimus dorsi and backfat samples were taken by the shot biopsy technique (Schoberlein, 1976; Wegner and Ender, 1990) one day after the beginning of treatment (115 days) and again after 5, 10 and 15 weeks (all groups), or 19 weeks (controls only) near the 13/14th thoracic vertebra alternating left and right sides. After measurement of the backfat thickness, samples from the two superficial fat layers and from muscle were cut quickly and frozen in liquid nitrogen. Serial transverse sections (10µm) from the frozen ***m.longissimus*** were stained for NADH-tetrazolium-reductase and acid-stable ATPase (pH4.2) or for haemalum-eosin. Backfat sections (10µm) were mounted on slides and remained unstained. Histological and histochemical techniques and microscopy were carried out as described by Wegner and Ender (1990) and Rehfeldt *et al.* (1993). Differences between treatment means were regarded as significant for $P<0.05$ with student's t-test.

RESULTS AND DISCUSSION

Live weight development and carcass value

As shown by the live weight data (Figure 1), the growth rate was higher in pST-treated animals in both test groups compared to the controls. At the end of treatment, near the last biopsy term, the pST-animals were between 15 and 16kg heavier (11 to 12%; $P<0.005$) than the age-related control animals. No differences were obtained using 2 increasing to 4mg pST/d compared with 4mg pST/d throughout the treatment period. The results of the

repartitioning process, not apparent from live weights, are reflected in data obtained by carcass analysis at slaughter. This shows marked differences in meat and fat content whereas meat quality, with the exception of tenderness, was not influenced (Table 1). The time course of the repartitioning process was determined by the examination of biopsy samples of backfat and muscle taken sequentially during growth.

Growth of longissimus muscle fibres

Diameter

The muscle fibres of pST-treated pigs showed a higher growth rate compared to control pigs until the 10th week of treatment as indicated by the increase in fibre diameter (Figure 2). From week 10 fibre growth in treated animals slowed up and seemed to attain the plateau earlier than the controls thereby diminishing the difference in diameter between the groups. As seen by the growth of the different fibre types (Figure 3) this was especially true for the white fibres which represent the major type in *m.longissimus*. They did not grow beyond 125µm on average with a maximum value of 155µm. In contrast, the oxidative red and intermediate fibres of pST-treated animals continued to grow slightly between weeks 10 and 15, although growth rates declined here too. These results indicate that pST accelerates muscle fibre growth indeed, but does not stimulate the fibres to grow beyond a maximum value, which is attained about five weeks earlier than in controls, in this way shortening the growth period for muscle fibres.

Fibre type frequencies

The number of white fibres increased at the expense of the intermediate ones during the first five weeks while the red fibre percentage remained unchanged (Figure 4). In controls the percentages of all types subsequently remained constant. In pST-treated animals, however, there was a decrease in oxidative fibres (red and intermediate) and a corresponding increase in white fibres until week 15 resulting in values significantly different to controls at the end of treatment ($P<0.005$). The shift to more white fibres (+3.7%-units) occurred within the fast-twitch category only as deduced from the results of STO, FTO and FTG fibre frequencies (not shown). In a previous experiment on Landrace barrows, no differences in fibre type frequencies were observed after 75 days treatment with 2 or 4mg pST/d (Rehfeldt and Ender, 1993), and the aerobic or anaerobic capacity of pig muscle was not changed by 45d pST-treatment as measured by the activities of marker enzymes (Oksbjerg, 1992). Obviously, the glycolytic white fibre proportion is increased by pST after prolonged treatment and fattening only, whereas the contractile properties also remain unchanged under these conditions. There is an apparent conflict of results in that the percentage of glycolytic fibres increased but meat quality was not impaired with respect to the incidence of PSE (pale, soft, exudative; Table 1). From various studies is known that elevated white fibre percentages correlate to the PSE-meat condition (e.g., Fiedler and Ender, 1984) or to stress-susceptibility in pigs (e.g., Fiedler *et al.*, 1988). Obviously, changes in fibre type frequencies (metabolic types) were not large enough here to be reflected in meat quality.

Nucleus-cytoplasm-ratio

As expected the nucleus-cytoplasm-ratio of the muscle fibres, expressed by muscle nuclei number per mm^2 fibre area, considerably decreased with age during the first 10 weeks of treatment (Figure 5). This indicates that the accumulation of protein exceeds the accumulation of nuclei during growth as it is commonly recognized (Burleigh, 1980). In response to pST the nucleus-cytoplasm-ratio declined faster until week 10. At later times differences were less marked and there were no differences between treatment groups at the end of the trial. A significant difference between treatment and control groups was only observed at week 10 when fibre diameters were most different. Taken as a whole, the results show only a trend towards decreased nucleus-cytoplasm-ratio in response to pST, as previously found in studies of pST action in barrows, gilts and boars (Rehfeldt *et al.*, 1991). When the nucleus-cytoplasm-ratio is calculated not only from muscle fibre nuclei (about 65% of all nuclei) but from all nuclei in muscle cross section, no significant differences due to pST were found. Changes of the nucleus-cytoplasm or DNA/protein ratio are of interest, because it is assumed that diminished ratios, which deviate from the functional deoxyribonucleic acid unit (Cheek *et al.*, 1971), impair muscle function. Accordingly, Fiedler *et al.* (1993) found significantly reduced nucleus-cytoplasm-ratios in *m.longissimus* fibres of stress-susceptible pigs with positive halothane reaction.

Growth of backfat

Backfat thickness in control animals increased to 223% during treatment period, while only increasing to 159% or 133% in response to pST (Figure 6). The reduction by pST at the end of treatment amounted to 69% or 61% ($P<0.001$). Comparisons on a bodyweight basis to controls at 19 weeks showed a reduction to 55% or 49%. No clear dependency on the injection pattern was observed during growth, although the 2/4mg group showed a somewhat smaller reduction than the 4mg group at the end of treatment.

The deposition of lipids in backfat was drastically reduced by pST as shown by the increase in fat cell size and number (Figures 7 and 8). Periods of slowed increase and stagnation alternated in response to hormone administration as previously seen for a shorter treatment period of 75 days (Rehfeldt *et al.*, 1994). In contrast to white muscle fibre development the pST effect is evident also between weeks 10 and 15; no limits in fat accretion are attained at this point. Regarding fat cell number it must be considered that it is very difficult to distinguish between true hyperplasia and mere expansion of fat cells by lipid filling (Leat and Cox, 1980). Gurr *et al.* (1977, cited Leat and Cox, 1980) measured fat cells by microscopy of frozen sections as was done in this study and concluded that fat cell number in pigs apparently reached a plateau at 45-55 weeks of age (315-385 days), whereas incorporation of (^{3}H)thymidine into new fat cells occurred only during the first few weeks of life. Taken together with our results thus suggests that pST acts on backfat by inhibiting the growth of visible fat cells as well as the lipid filling of empty `fat cells'.

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Table 1. Selected carcass characteristics of pST-treated Landrace barrows compared to untreated controls.

	Control	2/4mg pST/d	4mg pST/d	SEM
# Animals	19	18	16	
Carcass weight, kg	121.8	118.6	119.0	9.3
Meat (%)	50.0	58.4*	59.1*	2.9
Fat (%)	34.2	22.6*	21.7*	3.2
m.Longissimus area (cm^2)	40.5	47.9*	48.3*	4.3
Backfat thickness (cm)	3.9	2.8*	2.7*	0.4
pH_{45}				
Remission value	6.2	6.2	6.4	0.4
Drip loss	25.8	25.9	26.6	3.8
Fondue loss	3.2	3.7	2.6	1.0
Shear force value	42.3	40.1	40.4	2.3
	11.1	13.1+	13.8*	2.2

Method according to Ender and Hartung (1987).
* $P<0.001$; + $P<0.005$.