Muscle Structure, Protein Metabolism, and Nucleic Acid Content in Response to pST in Pigs

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SUMMARY: The effects of a longterm treatment with porcine somatotropin (pST) in Landrace pigs on the development of muscle structure characteristics, protein metabolism and nucleic acid concentration were examined in finishing pigs from about 120 to 200 days of age. Biopsy samples were taken from the longissimus muscle of each 60 barrows, gilts and boars at the start of treatment and after 5 and 10 weeks. The injection of 2 mg and 4 mg pST/d stimulated the hypertrophy of muscle fibres resulting in up to 11% thicker fibres at the end of treatment. In general, fibre type frequencies were not affected by pST. The cell-free translation was stimulated by 10% to 30%. Proteolysis studied in barrows was reduced by 7% to 12%. Higher RNA concentrations (by 16% and 26%) and DNA/RNA ratios were found after 10 weeks treatment. DNA concentration, DNA/protein ratio and the muscle fibres' nucleus-plasma-ratio not significantly changed although some small decreases were seen. The results suggest that the pST induced muscle fibre hypertrophy is caused by an increase of protein synthesis, both on the level of translation and transcription. In part proteolysis in muscle is inhibited by pST.

INTRODUCTION: The administration of purified exogenous porcine somatotropin (pST) to pigs causes a considerable repartitioning of feed energy from fat to meat. For example, after daily pST-injections (4 mg) for 10 weeks to finishing pigs the fat content of the carcass decreased relatively down to 61%, whilst the meat percentage increased up to 117% (ENDER et al.,1990). Structural changes on the cellular level and some of the basic mechanisms of growth as protein synthesis, proteolysis, and nuclei proliferation must have been occured. Consequently, the aim of this study was to investigate basic cellular processes related to the increased protein accretion in skeletal muscle after longterm pST administration. Simultanously, the timecourse of changes in relevant traits has been followed up during the longterm application using a simple biopsy technique.

MATERIALS and METHODS: Finishing Landrace gilts, barrows , and boars of about 120 to 200 days of age were used in this study. They were penned individually and fed a commercial diet (POPPE et al., 1990). 60 animals of each sex were divided randomly into three experimental groups of each 20 pigs. Treatments were 0, 2 and 4 mg pST (donated by Pitman-Moore, Inc.) injected daily i.m. dissolved in arginine buffer (pH 6,4). Gilts, barrows and boars were injected from 113/115/114 days of age over a period of 74/75/68 days respectively. The animals were slaughtered after one week withdrawal at about 200 days of age. Longissimus samples were taken by shot biopsy technique (WEGNER et al., 1988) one day after the first injection, after five weeks and after ten weeks. Samples were taken near the 13/14th thoracic vertebrae and were quickly frozen in liquid nitrogen. Histological and histochemical techniques as well as microscopical evaluation were carried out as described by REHFELDT et al.(1987). Serial transverse sections were stained for DPNH-tetrazolium reductase and acid-preincubated ATPase in a combined technique and for chromalum-carmin-eosin. In barrows and gilts translational activity was determined by cell-free polysomal translation assay (SCHRÖDER, 1989) and protein was analyzed according to PETERSON (1977). Ca^{2+}-dependent protease activity (WEIKARD and SCHRÖDER, 1985) was measured in barrows only. Nucleic acid concentrations were analyzed in gilts using modified procedures of MUNRO and FLECK (1966) and RICHARDS (1974). Difference between the treatment means was regarded as significant for $P < 0,025$ with student's t-test.

RESULTS and DISCUSSION: As result of the 10 weeks of pST-injections to gilts, barrows, and boars the muscle area of longissimus enlarged up to 6-21% and 16-21% after daily injections of 2 mg and 4 mg respectively (table 1). This was mainly caused by stimulated hypertrophy of muscle fibres indicated as higher mean fibre diameters(figure 1). This response to pST, which was greatest in barrows, showed no dose dependency at the end of treatment (about 200 days). Following the fibre growth by threefold biopsy showed that in barrows muscle fibres responded later to 2 mg pST/d compared with 4 mg pST/d. All fibre types took part in the stimulated hypertrophy to a similar extent. Moreover, pST did not affect the frequencies of slow twitch oxidative (STO), fast twitch oxidative (FTO), and fast twitch glycolytic fibres (FTG) in summary, so that pST induced shifting in energy producing metabolism or contractile properties of the muscle cannot be concluded in general(table 1). Fibre type frequencies are known to be related to meat quality; the PSE-condition (pale, soft, exudative) is connected with extremely high percentages of FTG fibres. Corresponding to the unchanged or even diminished FTG proportions, no significant deterioration in meat quality traits of longissimus muscle as pH, remission value, drip loss etc. was found in response to pST (ENDER et al.,submitted).

We saw that the macroscopically larger muscle area was achieved by an increase in fibre growth detectable at the microscopic level. In which way have the basic processes of muscle growth - nuclei proliferation and protein accretion - been modified by pST to cause this stimulation of fibre growth? Cell-free translation activity as one marker for protein synthetic activity was stimulated by 10% to 30% in response to pST. This response was already evident one day after the first pST injection (point 0, figure 2) and has been maintained during the following 5 weeks of treatment. The stimulatory effect was statistically significant in

Table 1: Effects of pST on selected structural and biochemical traits of carcass and biopsy samples of m. longissimus in Landrace pigs (+ significant to control at P < 0,025)

		Control			2 mg pST/d			4 mg pST/d		
Loin area (mm²)		$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n
gilts		39,9	2,9	(20)	45,8+	4,5	(18)	46,5+	4,5	(20)
barrows		33,6	4,1	(19)	40,6+	5,2	(20)	40,8+	3,3	(18)
boars		35,8	3,9	(20)	38,0+	4,1	(20)	42,2+	4,3	(19)
FTG fibres(%) 10 wks. treatment										
gilts		77,1	4,3	(18)	75,6	3,6	(16)	74,5+	3,1	(16)
barrows		74,0	5,5	(16)	77,1	3,9	(19)	75,6	3,6	(12)
boars		73,8	5,8	(16)	72,7	5,0	(18)	72,1	4,6	(18)
Protein (mg/g) in gilts										
	0	130,7	26,6	(14)	141,5	25,0	(13)	147,3	29,6	(15)
Weeks of	5	156,9	19,1	(11)	171,3	19,2	(15)	178,2	22,2	(14)
treatment	10	178,2	21,5	(13)	183,8	19,0	(15)	193,5	21,1	(16)
DNA(mg/g)/protein(mg/g) in gilts										
Weeks of	0	2,73	0,50	(13)	2,58	0,62	(12)	2,44	0,44	(13)
treatment	5	2,05	0,27	(11)	1,85	0,32	(13)	1,75	0,36	(10)
	10	1,35	0,21	(11)	1,22	0,22	(16)	1,11	0,19	(13)
Nucleus-plasma-ratio in boars (Nuclei per mm² fibre area)										
	0	421	115	(18)	388	129	(16)	386	108	(19)
Weeks of	5	311	100	(20)	253	167	(20)	286	99	(20)
treatment	10	256	48	(13)	243	63	(17)	218	55	(16)

Fig. 1: Relative effects of pST on MLD muscle fibre diameter of Landrace pigs

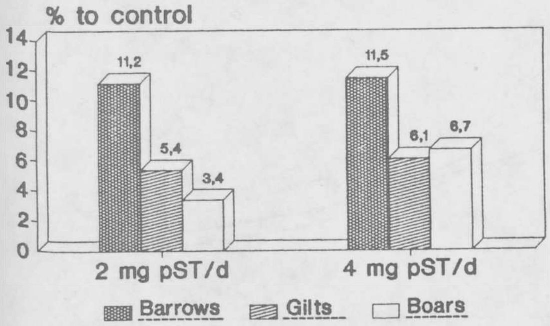
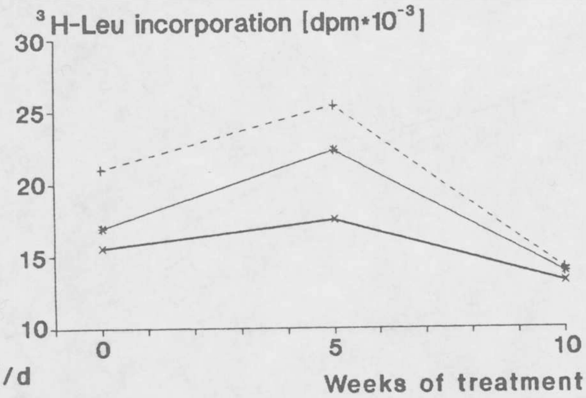


Fig. 2: Effects of pST on protein synthesis in muscle biopsies of gilts



x---x Control +---+ 2 mg pST/d *---* 4 mg pST/d

Fig. 3: Effects of pST on proteolytic activity in muscle biopsies of barrows

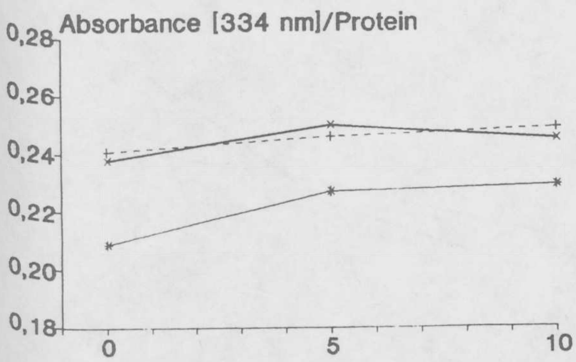
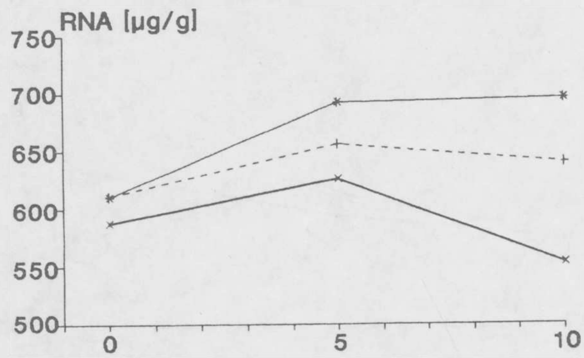


Fig. 4: Effects of pST on RNA concentration in muscle biopsies of gilts

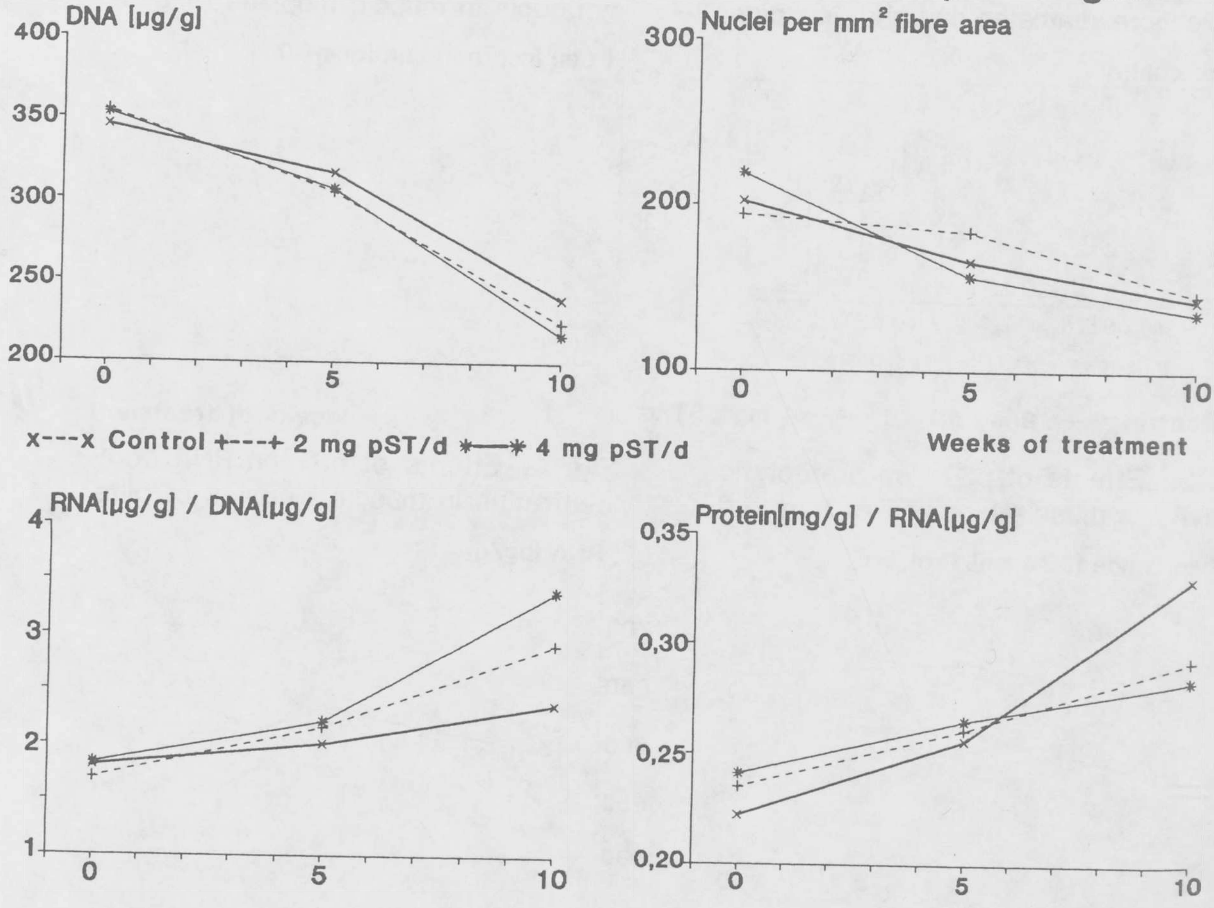


barrows only, not in gilts. At the end of treatment (10 weeks) translation activity had returned to control values in gilts, partly in barrows (2 mg pST/d), indicating that translation was stimulated by pST mainly in the first 5 weeks of the longterm application.- The neutral Ca^{2+}-dependent protease activity in barrows seemed to be unchanged by 2 mg pST/d and was decreased significantly by 4 mg pST/d(by 7-12%) suggesting a dose dependent response(fig.3).

Protein concentration in muscle seemed to be somewhat higher in the treatment groups (by 3%-14%) compared with controls, but without significance ($P > 0,025$, table 1). Treatment with pST resulted in significantly higher RNA concentrations by 16% and 26% respectively in the 2 mg- and 4 mg-group at the end. The developmental decline of RNA concentration in the control animals during weeks 5 to 10 has not occured in the treatment groups (figure 4). In contrast, DNA concentrations, nucleus-plasma-ratios and DNA/protein ratios showed no significant changes due to pST (figure 5, table 1), although those traits were lowered by pST in some instances. All compositional changes mentioned above were also reflected in a significant increase of RNA/DNA ratio at 10 weeks suggesting higher transcriptional activity due to pST in the latter 5 weeks of the experiment. On the other hand, the protein/RNA ratio was apparently decreased by pST at the end of treatment(by 11% and 14%). This is consistent with the result that translation activity measured per unit RNA declined to control values between 5 and 10 weeks of treatment.

CONCLUSIONS: The results suggest that pST promoted lean growth in pigs was achieved by stimulating the hypertrophy of skeletal muscle fibres without change in fibre type frequencies. There are two different phases in the mode of action of porcine somatotropin on protein synthesis in muscle which have to be distinguished. At the start of the experiment,

Fig. 5: Muscle biopsy parameters in response to pST in gilts



protein synthesis has been affected immediately at the level of translation. This short-term regulation effect on the efficiency of protein synthesis continued up to 5 weeks of treatment. Afterwards, a modulation of the capacity of protein synthesis seems to have occured. Normally, the capacity for protein synthesis as measured by RNA concentration declines with age (control animals), whereas in response to pST the cellular changes observed in M.longissimus indicate increased capacity. The increased RNA concentration and rather the higher RNA/DNA ratio suggest that the activation of the transcription process seems to be involved in the later steps of the pST response. Furthermore, the almost unchanged DNA concentration, DNA/protein and nucleus/plasma ratios indicate that the basic processes of muscle growth - protein accretion and nuclei proliferation - were enhanced by porcine somatotropin to nearly the same extent with little selective effect on protein accretion.

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