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**PORCINE SOMATOTROPIN AND DIETARY LYSINE
INFLUENCE BONE MINERALIZATION AND MECHANICAL
PROPERTIES IN FINISHING SWINE¹**

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Summary

The femur, a rib, and third phalanx of the second digit from the front foot were collected from 108 barrows (initial wt = 125 lb) and analyzed to determine the effects of porcine somatotropin (pST) and dietary lysine on bone mineralization and mechanical properties. In Exp. 1, pigs were injected daily with 4 mg pST and fed diets containing .6, .8, 1.0, 1.2, or 1.4% lysine; 1.0% Ca; and .8% P. Control pigs (placebo injection) received the .6% lysine diet. Bone wall thickness (BWT) of the femur increased but ash content decreased as dietary lysine level increased. Stress values and ash content of the rib also decreased with increasing dietary lysine level. In Exp. 2, pigs were injected with either 4 or 8 mg/d pST and fed diets containing .8, 1.0, 1.2, or 1.4% lysine; 1.1% Ca; and 1.0% P. Control pigs received the .8% lysine diet. Increasing pST dosage increased BWT of the femur, but decreased ash content. In the rib, increasing pST dosage reduced stress values, modulus of elasticity, and ash content. Increasing lysine level resulted in increased BWT of the femur and decreased ash content of rib, femur and phalanx. These data indicate that pST administration in conjunction with increasing lysine levels decreases bone mineralization but increases BWT of finishing pigs.

(Key Words: Porcine Somatotropin, Lysine, Finishing Pigs, Bone Mechanical Properties.)

Introduction

Porcine somatotropin (pST) exerts its effects on growth performance and carcass traits by altering carbohydrate, protein, lipid, and mineral metabolism. These changes result in improvements in growth rate, efficiency, and carcass quality of pigs treated with pST. Although the changes in protein and adipose tissue growth are well documented, little is known about the effects of pST on bone development. Recent research has reported mobility problems in gilts following long-term (up to 65 d) daily administration of 70 μ g/kg body weight pST. Because pST increases the lysine requirement of finishing pigs, Ca and P allowances also may be affected. Therefore, this study was conducted to examine the effects of pST administration and dietary lysine level on bone mineralization and mechanical properties in finishing pigs.

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²Pitman-Moore, Inc., Terre Haute, IN 47808.

Experimental Procedures

A total of 108 barrows (initial wt = 125 lb) was utilized in two experiments. Pigs were allotted by weight and ancestry to experimental treatments and were housed in 15 × 4 ft pens in an open-front building with solid concrete floors. Drip coolers were controlled thermostatically, and feed and water were provided ad libitum.

Table 1. Chemical Analyses of Control Diets, % (Exp. 1 and 2)^a

Component	Exp. 1	Exp. 2
DM	89.82	89.07
CP (N × 6.25)	17.78	17.80
Ether extract	13.52	9.93
Crude fiber	6.60	5.60
Ash	4.30	5.60
Ca	1.00	1.10
P	.90	1.00
<u>Essential and semiessential amino acids:</u>		
Arginine	1.82	1.32
Cystine	.33	.31
Histidine	.61	.45
Isoleucine	.72	.90
Leucine	1.52	1.64
Lysine	.60	.80
Methionine	.35	.45
Phenylalanine	.87	.95
Tyrosine	.33	.74
Threonine	.80	.90
Tryptophan	.22	.22
Valine	.89	.90

^aAnalyzed values are expressed on an as-fed basis.

Experimental treatments consisted of daily injections of 4 mg recombinant pST in combination with a corn-sesame meal diet (.6% lysine, 17.7% CP; Table 1) or diets containing .8, 1.0, 1.2, or 1.4% lysine provided by L-lysine HCl additions. There were two pigs per pen and three pens per treatment. Calcium and P levels were calculated to be 1.0 and .8%, respectively, and were identical in all diets. The .6% lysine diet was formulated to contain twice the recommended level for amino acids (other than lysine), vitamins, and minerals for finishing pigs (110 to 240 lb; NRC, 1988). Pigs were injected daily in the extensor muscle of the neck until the pen mean weight reached 230 ± 5 lb, at which time injections were terminated and

a 7-d withdrawal period was initiated. Pigs were slaughtered in three groups at 7-d intervals. The femur, third rib, and second phalanx of the third digit were removed from the right side of each carcass, labeled and frozen for later analyses.

In Experiment 2, 72 pigs were allotted to experimental treatments arranged in a 2 x 4 factorial with control treatment. Main effects included pST dosage (4 or 8 mg/d) and dietary lysine level (.8, 1.0, 1.2 and 1.4%). There were two pigs per pen and four pens per treatment. Control pigs (placebo injection) received the .8% lysine diet. All nutrients other than lysine were formulated to exceed NRC (1988) recommendations for finishing pigs by 200%. Levels of Ca and P were 1.1 and 1.0%, respectively. All other procedures were as described for Exp. 1, with the exception that the 1st rib was collected instead of the 3rd.

Bones were allowed to thaw overnight and cleaned manually of connective tissues. Bones were stored in plastic bags while being thawed but were allowed to dry for approximately 30 min prior to determination of mechanical properties utilizing an Instron Universal Testing Machine³. Bones were cleaned of any remaining connective tissue, extracted in petroleum ether for 48 h, and dried. From the femur, a 10 cm section was taken midshaft, and the marrow was removed prior to lipid extraction. All bones were ashed at 1,112°F for 12 h. Ash is expressed as a percentage of the dried, fat-free bone.

In order to assess the mechanical properties of bones, equations have been derived to evaluate the strength and elasticity of bones that differ in size, shape, and diameter. These equations are similar to those applied to various building materials to determine their suitability for support and durability. Bending moment refers to the actual force required to "break" or, more appropriately, bend a bone. Stress, however, not only takes into account the force required to bend the bone, but also the area and shape (cross sectional) of the bone at the point where the force is applied. Values for stress actually give a better estimate of the bone's strength than bending moment because of these considerations. Modulus of elasticity gives an estimate of the degree of flexibility of a bone. Bones with a high modulus of elasticity are very rigid, whereas bones with a lower modulus of elasticity would be more flexible. Bone wall thickness is the actual distance from the inner to the outer wall of a bone, whereas bone ash gives an indication of the degree of mineralization of a bone.

Results

Growth performance and carcass characteristics of the pigs have been described previously in detail (KSU Swine Day Rep. of Prog. 556, 1988). In Exp. 1, bending moment of the femur decreased then increased (quadratic, $P < .10$) as lysine level increased (Table 2). A similar trend ($P > .20$) was observed for stress and moment of elasticity. Ash content of the femur decreased (linear, $P < .10$), but bone wall thickness increased (linear, $P < .05$) as dietary lysine increased. Bending moment of the rib was unaffected by experimental treatment ($P > .30$); however, stress values and ash content decreased (linear, $P < .05$) with increasing lysine level. Mechanical properties and ash content of the phalanx were unaffected by experimental treatment.

³Instron Corp., Canton, MA.

Table 2. Effects of pST and Dietary Lysine on Bone Mechanical Properties and Mineralization (Exp. 1)^a

Item	Control	pST-treated, % Lysine					CV
	.6% Lysine	.6	.8	1.0	1.2	1.4	
<u>Femur</u>							
Bending moment, kg ^b	302	346	383	322	348	417	16.0
Stress, kg/cm ²	305	301	357	261	305	335	26.2
Strain	.18	.20	.19	.22	.19	.21	14.2
Modulus of elasticity, kg/cm ²	1723	1522	1843	1245	1590	1680	28.0
Ash, % ^c	69.24	69.38	69.63	67.35	68.48	67.97	2.7
Bone wall thickness, mm ^c	3.58	3.67	3.96	4.68	4.53	4.83	11.3
<u>Rib</u>							
Bending moment, kg	17	21	22	20	21	18	20.5
Stress, kg/cm ^{2d}	289	378	378	362	244	295	32.1
Strain ^b	.07	.08	.09	.10	.19	.08	24.4
Modulus of elasticity, kg/cm ²	3951	4908	4711	4476	2730	4002	55.9
Ash, % ^d	53.10	53.62	53.54	52.56	52.45	52.47	2.4
<u>Phalanx</u>							
Bending moment, kg	114	108	121	115	111	115	16.8
Stress, kg/cm ²	388	344	443	358	374	383	23.5
Strain ^d	.44	.57	.43	.47	.45	.40	21.4
Modulus of elasticity, kg/cm ²	916	653	1039	825	831	1011	30.8
Ash, %	58.19	58.01	58.74	57.30	58.03	57.59	1.5
Bone wall thickness, mm	2.04	2.25	2.04	2.29	2.33	2.17	16.0

^aData collected on bones from 36 barrows (n = 6). Linear and quadratic comparisons correspond only to pST treatments.

^bQuadratic effect of lysine (P<.10).

^cLinear effect of lysine (P<.10).

^dLinear effect of lysine (P<.05).

^eControl vs .6% lysine pST (P<.05).

In Experiment 2, no pST x lysine interactions were observed (Table 3), with the exception of bending moment of the phalanx (P<.05). Bending moment of the femur tended to increase (P>.20) with increasing pST dosage and dietary lysine level. Modulus of elasticity tended (P>.20) to decrease with increasing lysine level. Ash content of the femur was decreased (linear, P<.10) with increasing lysine level. Bone wall thickness increased as lysine level increased (linear, P<.01) and as pST dosage increased (linear, P<.10). Bending moment of the rib was unaffected by experimental treatment; however, stress and modulus of elasticity decreased as pST dosage increased (linear, P<.10). Ash content of the rib also was reduced by pST (linear, P<.01) and increasing lysine level (linear, P<.10).

Table 3. Main Effects of pST Dosage and Dietary Lysine on Bone Mechanical Properties and Mineralization (Exp. 2)^a

Item	pST, mg/d			Lysine level, %			
	0	4	8	.8	1.0	1.2	1.4
Femur							
Bending moment, kg/cm	370	398	385	374	406	395	392
Stress, kg/cm ²	308	286	260	273	281	263	275
Modulus of elasticity, kg/cm ²	1523	1435	1221	1433	1233	1316	1340
Ash, % ^b	67.72	67.47	66.98	67.69	67.32	66.94	66.94
Bone wall thickness, mm ^{cd}	4.54	4.99	5.00	4.66	5.01	5.14	5.12
Rib							
Bending moment, kg/cm	62	60	61	63	60	59	58
Stress, kg/cm ^{2d}	320	278	246	269	267	261	252
Modulus of elasticity, kg/cm ^{2d}	1489	1189	873	1132	1068	1014	915
Ash, % ^{be}	55.95	54.66	54.31	54.86	54.49	54.54	54.05
Phalanx							
Bending moment, kg/cm ^{bfg}	147	123	122	124	134	117	116
Stress, kg/cm ^{2h}	465	422	347	361	436	379	361
Modulus of elasticity, kg/cm ^{2h}	1085	1027	803	830	1063	932	834
Ash, % ^b	58.68	58.58	58.26	58.68	58.58	58.36	58.05
Bone wall thickness, mm ^f	2.41	2.30	2.55	2.52	2.43	2.33	2.42

^aData collected on bones from 72 pigs.

^bLinear effect of lysine (P<.10).

^cLinear effect of lysine (P<.01).

^dLinear effect of pST (P<.10).

^eLinear effect of pST (P<.01).

^fQuadratic effect of pST (P<.10).

^gLysine × pST interaction (P<.05).

^hQuadratic effect of lysine (P<.05).

Bending moment of the phalanx of pigs injected with 8 mg/d pST was similar across lysine levels (range of 111 to 126 kg). However, phalanx bending moment of pigs injected with 4 mg/d pST was more variable (105 to 143 kg), which may have accounted for the interaction (P<.05). Stress and modulus of elasticity tended to decrease (P>.25) with increasing pST dosage, but increased then decreased (quadratic, P<.10) as lysine level increased. Ash content

of the phalanx also was decreased by increasing lysine level. Bone wall thickness of the phalanx decreased then increased (quadratic, $P < .10$) with increasing pST dosage.

Discussion

Our data indicate an alteration in bone strength and mineralization in response to pST and dietary lysine. These effects are a direct response to pST administration and changes in growth rate, because Ca and P levels were identical between treatments within experiments. Because the bones had thicker walls, bending moment was unaffected. Porcine somatotropin is known to stimulate bone growth. Therefore, bones of pST-treated pigs were thicker than those of control pigs, resulting in no differences in bending moment. However, when changes in bone diameter were accounted for in calculations for stress, actual bone strength decreased. This corresponds with the decreased modulus of elasticity observed, indicating that the faster growing pigs had more pliable bones. Growing boars have been shown to have bones with greater wall thickness but less strength and mineralization than bones of barrows or gilts. This suggests that mineralization may not be able keep up with bone matrix growth in faster growing pigs, which would explain our results. Decreased mineralization also may be caused by differences in physiological maturity based on growth rate of pST-treated pigs. Since pST administration effectively inhibits initiation of the "fattening" phase in the pig's growth curve, perhaps bone development proceeds at an enhanced rate similar to the increased protein accretion. Thus, bones of pST-treated pigs may still have been growing when those of control pigs had stopped and reached maturity.

Bones of the axial skeleton are more responsive to demineralization than long bones of the extremities. Ribs from pigs in both of the present experiments had decreased stress values; however, all bones examined showed similar trends in bending moment and modulus of elasticity. Ash content also was decreased in all bones, with the exception of the phalanx in Exp. 2.

In order to achieve maximum longevity of sows, research has suggested concentrations of Ca and P for growing gilts above those required to enhance growth performance. This would maximize bone ash and strength and provide a Ca and P reservoir to buffer against negative Ca and P balance during lactation. Extrapolation of our results suggests that longevity of pST-treated gilts kept in the breeding herd is questionable, because bone ash and strength were not maximized in barrows. However, because pST stimulated the growth of the collagen matrix, there may be potential for compensatory mineralization after gilts are selected for breeding. Further research is necessary to determine the Ca and P levels necessary to maximize bone mineralization, if pST is to be administered to replacement gilts.

In conclusion, porcine somatotropin and dietary lysine alter rate, efficiency, and composition of gain in swine. Furthermore, bone growth is affected, resulting in weaker, more elastic, and less mineralized bones in pST-treated pigs. However, bone wall thickness is increased by pST administration and increased dietary lysine level.