Muscle Fiber Number and Growth Performance of Pigs from Sows Treated with Ractopamine

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ABSTRACT : The goal of the trial was to evaluate the effects of ractopamine (20 ppm in the ration) given pregnant sows during three different pregnancy stages (T1: 25 to 50 d; T2: 50 to 80 d; T3: 25 to 80 d of gestation, and T4: control-no ractopamine) on fetal muscle development (through counts of the number of fibers of the *semitendinosus* muscle), on the growth and carcass characteristics of the progeny. Forty eight weaned piglets (12 per treatment) were assessed for number of muscle fibers, while performance and carcass characteristics were evaluated on a separate 48 animals (12 per treatment) grown to 100 kg. Animals produced by sows treated from 25 to 50 d of pregnancy (T1) resulted in non-significant increase of 6.85% in the number of muscle fibers in the *semitendinosus* muscle when compared to animals from the control group. Performance results were significantly different ($p<0.05$), and animals produced by sows from the T1 group gained more weight during the growth I stage (25 to 50 kg) and during the total period (6 to 100 kg) (991 vs. 903 grams, and 844 vs. 772 grams, respectively) when compared to controls. For carcass characteristics, results showed that animals produced by the T1 group of sows were heavier at slaughter ($p<0.05$) when compared to the controls (T4) (100.17 vs. 93.09 kg). There was a positive correlation between number of muscle fibers and bodyweights, carcass weights, *Longissimus dorsi* muscle depth and dressing out (0.80, 0.86, 0.67, and 0.50, respectively). Sows treated with ractopamine between 25 to 50 d of pregnancy produced piglets that performed better and had superior carcass characteristics than those produced by untreated sows. The ractopamine used for pregnancy sows (25 to 50 d) can be indicated as a device to increase the progeny performance. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 10 : 1492-1497)***

Key Words : Pigs, Muscle, Myofibers, Performance, Carcass, Beta-adrenergic Agonist

INTRODUCTION

Pigs born with fewer muscle fibers normally grow less than piglets born with more fibers, suggesting that high numbers muscle of fibers are a requirement for good growth (Dwyer et al., 1993).

Hyperplasia of fetal muscle fibers in pigs is completed around d 85 to 90 of pregnancy (Wigmore and Stickland, 1983). During the fetal development, two types of fibers are formed: primary and secondary. Primary muscle fibers grow by rapid fusion of primary myoblasts, and secondary fibers are formed on the surface of the fused primary cells. The primary fetal fibers are present at 35 d of pregnancy and the secondary fibers start their organization around 54 to 70 d of gestation (Wigmore and Stickland, 1983).

Primary fibers are more resistant to environmental influences, while secondary fibers are susceptible to various environmental factors, including nutritional and hormonal variations (Handel and Stickland, 1987). The main reason for variation in muscle fiber number is uterine malnutrition during pregnancy (Wigmore and Stickland, 1983; Handel and Stickland, 1987).

The exact mechanism through which hormones affect muscle growth is not clear yet. There may be a combined action of the different hormones affecting growth rates and the so-called nutrient partitioning substances, directing nutrients for specific muscle growth (Ricks et al., 1984). Energy partitioners like ractopamine are synthetic substances, defined as beta-adrenergic agonists belonging to the class of phenetanolamines.

The action of adrenergic compounds is partially mediated by 3',5'-adenosine monophosphate (cAMP). The catalytic unit of the adenyl-cyclase system produces cAMP, when the receptor binds to a stimulating protein, linked to the nucleotide guanine, responsible for the conversion of ATP into cAMP. Then the cAMP increasing the kinases activation which are responsible for the phosphorylation and modification of the activities of several enzymes, modulating metabolic processes such as muscular contraction, lipolysis and glycogenolysis (Moody et al., 2000).

The goal of this trial was to treat pregnant sows with ractopamine and assess possible effects on fetal muscle hyperplasia, and subsequently, on performance and carcass characteristics of the progeny.

MATERIALS AND METHODS

The trial was conducted at the pregnancy and farrowing unit of a 450-sow commercial farm located in Rolândia, Paraná State, Brazil. For growth and finishing, the pigs were transferred to the Swine Department of the Teaching Farm at the State University of Londrina, in Londrina.

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Table 1. Composition of diets[a] offered to sows

Ingredients (%)	Diets		
	Gestation	Pre-lactation	Lactation
Corn	54.700	64.180	54.980
Wheat bran	30.600	6.000	-
Soybean meal (45% CP)	11.000	23.800	31.000
Soybean oil	-	2.040	5.020
Limestone	1.350	1.080	0.920
Dicalcium phosphate	1.350	1.900	2.080
Salt	0.500	0.500	0.500
Sugar	-	-	5.000
Inert	0.001	-	-
Vitamin-trace mineral premix[b]	0.400	0.400	-
Vitamin-trace mineral premix[c]	-	-	0.400
Mineral supplement[d]	0.100	0.100	0.100
Chemical composition (%)			
CP	14.067	16.886	18.486
ME (kcal/kg)	2,865	3,170	3,375
Crude fiber	5.057	3.901	3.594
Crude fat	3.431	5.086	7.537
Calcium	0.946	0.953	0.943
Total phosphorus	0.746	0.703	0.693
Lysine	0.630	0.880	1.030

[a] As-fed basis.

[b] Supplied per kg of vitamin-mineral premix: vitamin A, 1,250,000 IU; vitamin D_3, 250,000 IU; vitamin E, 8,750 IU; vitamin K_3, 150 mg; vitamin B_1, 125 mg; vitamin B_2, 1,125 mg; vitamin B_6, 150 mg; vitamin B_{12}, 4,500 µg; folic acid, 400 mg; calcium pantothenate, 3,250 mg; niacin, 3,750 mg; biotin, 50 mg; choline, 70,000 mg; iron, 12,250 mg; copper, 5,250 mg; manganese, 8,750; zinc, 26,250 mg; iodine, 350 mg; selenium, 75 mg; antioxidants, 1,000 mg.

[c] Supplied per kg of vitamin-mineral: vitamin A, 1,000,000 IU; vitamin D_3, 250,000 IU; vitamin E, 8,750 IU; vitamin K_3, 163 mg; vitamin B_1, 125 mg; vitamin B_2, 1,125 mg; vitamin B_6, 150 mg; vitamin B_{12}, 4,500 µg; folic acid, 400 mg; calcium pantothenate, 3,000 mg; niacin, 3,500 mg; biotin, 45 mg; choline, 70,000 mg; iron, 10,500 mg; copper, 4,500 mg; manganese, 7,500; zinc, 22,500 mg; iodine, 300 mg; selenium, 75 mg; antioxidants, 1,000 mg.

[d] Supplied per kg of mineral supplement: Fe, 60,000 mg; Cu, 120,000 mg; Zn, 60,000 mg.

Paraná State.

Forty Large White×Landrace second to fourth-parity sows were used in the trial.

During pregnancy, the sows were housed in individual pens. After farrowing, the piglets were identified to allow follow-up according to the treatment of their mothers. Piglets were maintained with the sows up to weaning (at 21 d of age).

The trial was started at AI of the sows. The semen used for insemination was obtained from hybrid boars with the same genetic background.

At day 20 of pregnancy 10 sows were randomly assigned to each of four treatment groups. The periods of the treatments were based on stages of the fetal secondary muscle cells development, according Dwyer et al. (1994), been defined by pre-hyperplasia stage (25 to 50 d of pregnancy), hyperplasia stage (50 to 80 d of pregnancy) and pre-hyperplasia+hyperplasia stages (25 to 80 d of pregnancy), as follows:

Treatment 1: pregnancy feed containing 20 ppm of ractopamine from 25 to 50 d of pregnancy.

Treatment 2: pregnancy feed containing 20 ppm of ractopamine from 50 to 80 d of pregnancy.

Treatment 3: pregnancy feed containing 20 ppm of ractopamine from 25 to 80 d of pregnancy.

Treatment 4: control group, fed pregnancy feed without ractopamine.

Pregnancy and pre-lactation feeds for all four treatment groups were formulated according to requirements specified by NRC (1998) (Table 1).

Sows were fed 1.8 kg/d up to 80 d of pregnancy. From 80 d on, the sows were fed on 2.8 kg/d until farrowing.

From farrowing to weaning, sows were fed lactation feed *ad libitum*, formulated according to the NRC guidelines (1998) (Table 1).

At weaning, 12 piglets from each treatment (six barrows and six gilts) were selected and euthanized by an intraperitoneal injection of pentobarbitone followed by exsanguinations. Muscle *semitendinosus* was dissected out from each animal and a complete transverse slice 1 cm thick was taken from the muscle and stored for 24-h in Bouin solution, and then preserved in 70% alcohol. The total cross-section of the mid-portion of the muscle was stained with H&E (hematoxylin-eosin). The total area of the muscle was measured using the Image-Pro Plus software, version 4.5.1.22 and an HP 4c Scanner. A digital Pro-series 3-Chipcolor camera and an Olympus BX 50 microscope were used to capture eight fields, randomly. Then, were counted a total mean of 2.346 fibers per muscle, corresponding to 0.6% of the surface area of the muscle. Total number of muscle fibers was estimated from the relation between total surface area of the muscle and surface area of the counted fields.

For performance evaluation, 12 animals from each treatment group (six barrows and six gilts) were randomly selected at weaning. Average treatment weight was used as the parameter for the selection of pigs. A total of 48 piglets at an average starting weight of 6.270±0.868 kg were housed in pairs in pens designed for two animals. The whole group was fed *ad libitum* with the same feeds up to slaughter, formulated to meet their requirements (NRC, 1998) (Table 2). The feeds were formulated according to the growing stage of the pigs: initial I (6 to 10 kg of bodyweight), initial II (10 to 20 kg of bodyweight), growing I (20 to 50 kg of bodyweight), growing II (50 to 80 kg of bodyweight), and finishing (80 to 100 kg of bodyweight).

For performance evaluation, the following parameters were measured: average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR).

Table 2. Composition of diets[a] of the experimental of performance evaluation

Ingredients (%)	Phases				
	Initial I	Initial II	Growing I	Growing II	Finishing
Corn	45.600	54.990	69.646	77.504	84.924
Soybean meal	23.400	35.800	26.440	19.471	12.850
Limestone	-	0.740	0.920	0.758	0.772
Dicalcium phosphate	-	1.620	0.880	0.711	0.550
Salt	-	0.500	0.250	0.250	0.250
Sugar	5.000	3.000	-	-	-
Soybean oil	1.000	2.800	1.280	0.794	0.133
L-lysine -HCl 78%	-	-	0.110	0.062	0.071
DL-methionine	-	-	0.024	-	-
Vitamin-trace mineral premix[b]	-	-	0.400	0.400	0.400
Mineral supplement[c]	-	-	0.050	0.050	0.050
Vitamin-mineral premix[d]	25.000	-	-	-	-
Vitamin-trace mineral premix[e]	-	0.400	-	-	-
Mineral supplement[f]	-	0.150	-	-	-
Chemical composition (%)					
CP	19.640	20.686	18.000	15.500	13.200
ME (kcal/kg)	3,270	3,230	3,300	3,300	3,300
Crude fiber	3.153	3.900	2.980	2.783	2.573
Crude fat	4.735	5.425	4.402	4.035	3.540
Calcium	0.803	0.787	0.600	0.500	0.450
Total phosphorus	0.672	0.637	0.500	0.450	0.400
Lysine	1.340	1.190	0.950	0.750	0.600
Methionine	-	-	0.250	0.258	0.228

[a] As-fed basis.

[b] Supplied per kg of vitamin-mineral premix: vitamin A, 1,000,000 IU; vitamin D_3, 250,000 IU; vitamin E, 2,750 IU; vitamin K_3, 625 mg; vitamin B_1, 300 mg; vitamin B_2, 1,050 mg; vitamin B_6, 275 mg; vitamin B_{12}, 3,750 μg; folic acid, 150 mg; pantothenic acid, 3,500 mg; niacin, 5,750 mg; choline, 25,000 mg; selenium, 75 mg; growth promoter, 7.5 g; antioxidants, 2.5 g.

[c] Supplied per kg of mineral supplement: Fe, 90,000 mg; Cu, 16,000 mg; Mg, 30,000 mg; Zn, 140,000 mg; Co, 200 mg; I, 850 mg; Se, 120 mg.

[d] Supplied per kg of vitamin-mineral premix: vitamin A, 1,500,000 IU; vitamin D_3, 450,000 IU; vitamin E, 7,500 IU; vitamin K_3, 1,500 mg; vitamin B_1, 250 mg; vitamin B_2, 1,300 mg; vitamin B_6, 375 mg; vitamin B_{12}, 5,000 μg; folic acid, 150 mg; calcium pantothenate, 4,500 mg; niacin, 7,500 mg; biotin, 22.5 mg; choline, 68,000 mg; methionine, 25,000 mg; lysine, 70,000; iron, 12,500 mg; copper, 5,250 mg; manganese, 8,750; zinc, 26,250 mg; iodine, 350 mg; selenium, 75 mg; antioxidants, 1,000 mg.

[e] Supplied per kg of vitamin-mineral premix: vitamin A, 1,000,000 IU; vitamin D_3, 150,000 IU; vitamin E, 3,000 IU; vitamin K_3, 750 mg; vitamin B_1, 150 mg; vitamin B_2, 875 mg; vitamin B_6, 250 mg; vitamin B_{12}, 4,500 μg; folic acid, 250 mg; calcium pantothenate, 2,500 mg; niacin, 5,000 mg; biotin, 7.5 mg; choline, 35,000 mg; iron, 8,750 mg; copper, 3,750 mg; manganese, 6,250; zinc, 18,750 mg; iodine, 250 mg; selenium, 75 mg; antioxidants, 500 mg.

[f] Supplied per kg of mineral supplement: Fe, 60,000 mg; Cu, 120,000 mg; Zn, 60,000 mg.

The pigs were slaughtered at 133 d of age, and the carcasses were evaluated for the assessment of the effects of the treatments of the sows on the progeny.

At slaughter, carcasses were individually evaluated, and the following parameters were recorded: live weight at slaughter (LW), carcass length (CL), backfat (BF), *Longissimus dorsi* muscle depth (MD), loin eye area (LA), hot carcass weight (CW), dressing-out (DO), carcass lean yield (CLY). The BF, MD and LA data were obtained from P_2. The CLY was calculated according Irgang et al. (1997).

The experimental design for cell counts was fully randomized, with four treatments and 12 replications per treatment. Each of the piglets was one replication. For the performance evaluation trial, the experimental design was fully randomized, with four treatments and six replications per treatment. For carcass evaluation, the experimental design was fully randomized, with four treatments and two genders and six replications per treatment.

ANOVA and Dunnett's test were performed to evaluate data related to treatments. The average of the control group was compared to all treatment averages using the GLM procedure as outlined by SAS (1998).

RESULTS AND DISCUSSION

Table 3 shows the results of the fiber counts and muscle surface area of the *semitendinosus* muscle.

There was no difference ($p>0.05$) among treatments for the evaluated parameters. However, animals produced by ractopamine-treated sows from 25 to 50 d of pregnancy had a non-significant increase of 6.86% in the number of muscle fibers when compared to the control group.

Dwyer et al. (1994) reported increased fetal muscle mass when pregnant sows were fed at higher levels (5 kg/d) when compared to the control group (2.5 kg/d). The authors reported that sows consuming increased amounts of feed

Table 3. Effects of the use of ractopamine during different pregnancy stages and influence of gender on the number of muscle fibers (MF) and on the surface area (SA) of the *semitendinosus* muscle of piglets slaughtered at 6 kg of body weight (means and overall standard deviations)

Periods of treatments	Parameters	
	MF±SD×10^{-5}	SA±SD×10^{-5} (mm^2)
25 to 50 d	4.24±0.56	2.39±0.34
50 to 80 d	3.88±0.61	2.08±0.50
25 to 80 d	4.04±0.56	2.15±0.37
Control	3.97±0.41	2.34±0.33
Gender		
Males	4.18±0.55	2.28±0.40
Females	3.89±0.50	2.20±0.40
Coefficient of variation (%)	13.60	17.90

There were no difference between treatments (p>0.05) by Dunnett's test.
SD = standard deviation

during the 30 d preceding the hyperplasia period of the secondary fibers produced piglets with 9 to 13% more secondary muscle cells, suggesting that there might be a beneficial effect of additional feeding. Growth rates, weight gain and feed conversion rates were also improved up (p>0.05) to 80 kg of live weight in their study.

Considering the results seen by Dwyer et al. (1994), who reported better performance as a result of increased feed consumption by pregnant sows during the pre-hyperplasia stage of gestation, it is possible that the improvements seen in this trial could be the indirect result of the action of ractopamine, promoting higher levels of nutrient intakes and consequently, promoting the action of growth factors.

The number of fibers was not affected by gender. However, males had a non-significant increase of 7.46% fibers in the *semitendinosus* muscle when compared to females. Miller et al. (1975) have also reported higher number of muscle fibers in the *Longissimus dorsi* muscle in males (3.33%). This difference could explain increased slaughter weights in males when compared to females at the same age.

Table 4 shows the results for the performance evaluation.

ADG was significantly higher for the growing I phase and total periods (p<0.05) in the group of piglets from sows treated with ractopamine from 25 to 50 d of pregnancy, when compared to the controls. FCR were also improved during growing II phase (p<0.05) in the group where the sows were treated with ractopamine from 50 to 80 d of pregnancy when compared to the other groups. Final weights were significantly higher (p<0.05) for the group treated during the 25 to 50 d when compared to the control group (100.17 kg and 93.09 kg, respectively) (Table 5).

The results obtained in this trial agree with those reported by Kim et al. (1994), who studied the effects of salbutamol, a beta-adrenergic agonist and reported that sows treated during the first 38 days of pregnancy (pre-hyperplasia period) produced heavier pigs at slaughter and better weight gains when compared to the untreated controls.

In theory, the treatment between 25 and 80 d of pregnancy, was supposed to generate similar results to the treatment between 25 and 50 d, since the drug was given to the sows during the same fetal development stage (pre-hyperplasia period). However, Moody et al. (2000) reported that the sensitivity of beta-receptors to agonists is reduced

Table 4. Effects of the use of ractopamine during different pregnancy stages on progeny performance on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion rate (FCR)

Phase	Periods of treatment (day of gestation)				
	Control	25 to 50 d	50 to 80 d	25 to 80 d	Coefficient of variation (%)
Initial					
ADG (kg)	0.471^a	0.528^a	0.469^a	0.495^a	14.36
ADFI (kg)	0.775^a	0.788^a	0.739^a	0.778^a	12.10
FCR (g feed/g of gain)	1.651^a	1.505^a	1.599^a	1.580^a	9.37
Growing I					
ADG (kg)	0.903^b	0.991^a	0.911^b	0.923^b	7.20
ADFI (kg)	1.926^a	2.051^a	1.918^a	2.000^a	12.08
FCR (g feed/g of gain)	2.168^a	2.078^a	2.132^a	2.135^a	11.70
Growing II					
ADG (kg)	1.061^a	1.127^a	1.078^a	1.037^a	9.86
ADFI (kg)	2.817^a	2.980^a	2.666^a	2.651^a	8.68
FCR (g feed/g of gain)	2.672^b	2.648^b	2.473^a	2.559^b	5.90
Finishing					
ADG (kg)	0.814^a	0.857^a	0.809^a	0.808^a	13.50
ADFI (kg)	2.764^a	2.991^a	2.722^a	2.648^a	11.46
FCR (g feed/g of gain)	3.418^a	3.515^a	3.297^a	3.305^a	9.30
Total period					
ADG	0.772^b	0.844^a	0.782^b	0.779^b	7.03
ADFI	1.874^a	1.986^a	1.818^a	1.835^a	8.29
FCR (g feed/g of gain)	2.429^a	2.356^a	2.320^a	2.359^a	4.90

Means followed by different letters in the same line are significantly different (p<0.05) by Dunnett's test.

Table 5. Effects of ractopamine used during different pregnancy stages on carcass characteristics of the progeny of treated sows: slaughter weight (SW), carcass weight (CW), carcass length (CL), backfat (BF), muscle depth (MD), loin eye area (LE), dressing-out (DO) and carcass lean yield (CLY)

Treatments	Parameters							
	SW (kg)	CW (kg)	CL (cm)	BF (cm)	MD (cm)	LE (cm^2)	DO (%)	LCY (%)
25 to 50 d	100.17^{a}	73.30^{a}	93.71^{a}	1.53^{a}	6.12^{a}	41.82^{a}	73.14^{a}	59.73^{a}
50 to 80 d	94.17^{b}	68.48^{a}	91.77^{a}	1.51^{a}	5.94^{a}	41.76^{a}	72.65^{a}	59.72^{a}
25 to 80 d	93.03^{b}	68.34^{a}	92.73^{a}	1.56^{a}	5.88^{a}	39.85^{a}	73.25^{a}	59.68^{a}
Control	93.09^{b}	67.95^{a}	92.88^{a}	1.57^{a}	5.69^{a}	37.09^{a}	72.96^{a}	59.66^{a}
Gender								
Males	97.42^{a}	71.37^{a}	93.61^{a}	1.58^{a}	5.99^{a}	40.58^{a}	73.23^{a}	59.71^{a}
Females	92.94^{b}	67.70^{b}	91.97^{a}	1.51^{a}	5.82^{a}	39.69^{a}	72.79^{a}	59.68^{a}
CV (%)	7.07	8.30	2.98	22.27	10.72	16.02	2.30	0.35

Averages followed by different letters in the same column are significantly different ($p<0.05$) by Dunnett's test.
CV = Coefficient of variation.

Table 6. Correlations between the measured parameters

	Carcass weight	Muscle depth	Muscle surface area	Slaughter weight
Number of fibers	0.86	0.67	0.60	0.80
p	**	*	NS	**

* $p<0.05$. ** $p<0.01$. NS: Non-significant.

after exposure for long periods (longer than 28 d). In addition, this prolonged use (25 to 80 d of pregnancy) could have affected the availability of substrate for muscle cells (negative balance). It can be supposed that the sow was in a catabolic state, in an attempt to counteract this negative balance. Also, there is no information about the action of ractopamine on the hormones during the pregnancy and the relationship between these hormones and the hyperplasia of fetal muscle.

Table 5 shows the results for carcass composition.

There was a significant difference ($p<0.05$) among treatments for slaughter weight for the T1 group (drug fed from 25 to 50 d of pregnancy) when compared to the control group. No difference was seen for the other measured parameters ($p>0.05$). However, the results for muscle depth and loin eye area (*Longissimus dorsi* muscle), backfat and carcass weight of treated animals (mainly from the group fed with the drug between 25 to 50 d of pregnancy) were numerically better when compared to the control group. Kim et al. (1994) reported significant improvements ($p<0.05$) in the loin eye area in the progeny of sows treated with salbutamol, a beta-adrenergic agonist, from 0 to 38 d of pregnancy, when compared to the controls. As to gender, males had significantly higher slaughter and carcass weights ($p<0.05$), when compared to females. This could suggest that males may have higher number of muscle fibers. However, there were no differences among genders ($p>0.05$) for the other measured parameters. In general, the values were higher in males, as expected, considering the differences in nutritional requirements, growth rates, and tissue deposition rates. From the economic standpoint, it can be anticipated that the benefits of treating pregnant sows using the T1 treatment will be translated into higher revenues for the producers and improved carcass quality.

Table 6 shows the correlation values between the different parameters measured in this trial.

There was a positive correlation (r = 0.60) between number of muscle fibers and the cross-sectional surface area for the *semitendinosus* muscle, showing that the larger the surface, the higher the number of fibers. However, this cannot be used as a general rule, since different piglets may have the same weight at weaning, but varying numbers of fibers.

There were also high positive ($p<0.05$) correlations between the number of muscle fibers and the variables slaughter weight, carcass weight and *Longissimus dorsi* depth (0.80, 0.86 and 0.67, respectively), showing if the number of fibers in *semitendinosus* muscle is increased, this may result in improvements both in growth rates and carcass yields.

In conclusion, the treatment of pregnant sows with ractopamine at a 20 ppm dose level during the pre-hyperplasia stage resulted in non-significant increase of 6.85% in the number of fibers in the *semitendinosus* muscle. Pigs from ractopamine-treated sows (from 25 to 50 d of pregnancy) gained more weight and were heavier at slaughter than pigs produced by untreated sows (991 vs. 903 grams, and 100.17 vs. 93.09 kg, respectively).

IMPLICATIONS

This experiment demonstrated that pigs born from sows treated with ractopamine during 25 to 50 d of pregnancy can present faster growth rates than pigs born from untreated sows. This suggests an increased muscle deposition for pigs' carcasses and improved economic viability.

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