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Research article

Effect of feeding pregnant gilts fermented potato extract protein on the prenatal development and semitendinosus muscle characteristics of newborn piglets

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Abstract

The skeletal muscle, which largely forms meat, constitutes most of the body mass in pigs. Growth and meat quality mainly depend on the relationship between prenatal and postnatal development. Feed additives are used in the diet of pigs to improve production efficiency. In this study, we determined the effect of feeding pregnant pigs fermented potato extract protein on the characteristics of the skeletal muscles of newborn piglets. Ten Danish gilts (Large white x Landrace) were supplemented with or without fermented potato extract protein (5 gilts each) during the gestation period. After parturition, two neonate piglets from each sow were randomly selected, and the birth weight, organ weight, morphometrics, and the characteristics of the semitendinosus muscle, including weight, length, circumference, muscle cross-sectional area (MCSA), number of total muscle fibers, number of primary fibers, number of secondary fibers, and the ratio of secondary to primary (S:P) fibers were recorded. The piglets from sows that were fed fermented potato extract protein showed a significantly higher S:P ratio ($p = 0.02$) than those from control sows. The weights of the body and visceral organs at birth tended to be higher in the newborn offspring of the treated sows. The supplementation with fermented potato extract protein during gestation in pigs can increase the S:P ratio, which can be used to follow changes in the hyperplasia of secondary fibers in the fetus and might also affect postnatal growth.

Keywords: Fermented potato extract protein, Newborn piglets, Prenatal development, Semitendinosus muscle

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INTRODUCTION

Skeletal muscle tissue represents approximately 50% of the body mass in pigs and largely forms meat (Kalbe et al., 2017). The total number of muscle fibers is correlated with meat quality (Cerisuelo et al., 2009). Muscle growth is important in animal production. Thus, animal breeding and husbandry are essential for producing animals with more muscle fibers (Wigmore and Stickland, 1983). There are two critical phases in swine production which include the gestation (114 days before birth) and neonatal (21 days after birth) periods, for regulating growth performance (Ji et al., 2017). The nutrition of pregnant pigs might also affect the development of the fetus (Rehfeldt et al., 2011). Thus, a nutritious diet is provided to pregnant sows or gilts to improve fetal growth (Ji et al., 2017). However, prenatal development depends on the maternal nutritional supply, as well as, on the regulation of hormones and growth factors (Rehfeldt and Kuhn, 2006).

Skeletal muscle consists of a mixture of myogenic cells, including primary fibers and secondary fibers. Primary fibers are formed during embryonic development between days 35 and 60 of gestation, while secondary fibers are formed during fetal development (day 55–90 of gestation) and appear around each primary fiber (Wigmore and Stickland, 1983). According to Wigmore and Stickland (1983), the number of total muscle fibers is determined before birth, and muscle hypertrophy is the enlargement of muscle mass during postnatal development. In contrast to the aforementioned study, the number of total muscle fibers is not fixed at birth, and postnatal muscle fibers called tertiary myofibers are established three weeks after birth and increase the total content of muscle fibers (Bérard et al., 2011, Adamović et al., 2014). The growth of postnatal skeletal muscles is important for improving the quality of pig meat for consumption. An increase in the size rather than the number of muscle fibers indicates poor pork quality (Rehfeldt et al., 2000; Kalbe et al., 2017).

Farmers face various problems in pig production, such as the increasing cost of feed, several diseases, and insufficient production to meet consumer demands. A high number of piglets per litter leads to high variation in piglet birth weight and a high risk of pre-weaning mortality. Feed additives are used in the swine diet to improve the efficiency of production and performance, which can increase the profitability of pig production. Lianol® is a commercially available fermented potato extract protein that can stimulate the production of insulin-like growth factor-I (IGF-I) in pig serum (Poolperm et al., 2012, Poltep et al., 2016). IGF-I is a member of a larger family of insulin-related peptides. It has a growth-promoting effect and helps to maintain adequate metabolic activity for the optimum development of the placenta and fetus of pregnant pigs (Fowden, 2003). Additionally, a positive correlation between circulating IGF-I levels and the growth rate has been shown in various species, including pigs (Götz et al., 2001). Supplementing the diet of sows with fermented potato extract protein not only increases the circulating IGF-I level but also significantly decreases the weaning-to-estrus-interval (WSI) and farrowing-to-service interval (FSI) (Benjasiriwan et al., 2013).

Although, Götz found that IGF-I and its receptors were localized in porcine skeletal muscle fibers (Götz et al., 2001), the effect of supplementation with fermented potato extract protein on muscle tissue is unclear and understudied. Based on the results of the aforementioned studies, in this study, we determined the effect of the supplementation of the diet of pregnant gilts with a fermented potato extract protein on the growth, as well as, on the morphological and histological characteristics of semitendinosus muscle in their newborn offspring.

MATERIALS AND METHODS

Animals and experimental design

The experiment was conducted on a commercial pig farm in the Ratchaburi province, Thailand. Danish gilts (Large white x Landrace; n = 10) were supplemented with or without fermented potato extract protein (Lianol®). The extract contains essential amino acids (g/16 g N) as follows: lysine 5.1, methionine 1.5, cystine 1.0, and isoleucine 3.8 (Lianol®, Huvephamar Ltd.; Antwerp, Belgium). Briefly, all pregnant gilts were fed a common diet (Table 1) and divided into two groups: control and treatment groups (n = 5 gilts per group). Following the manufacturer's instructions, the supplement was added to the feed at a dose of 10 g/gilt/day of feed at 7 a.m. during the gestation period (0–114 days).

After parturition, two neonates from each sow were randomly selected, and all piglets (n = 20) were euthanized by intravenous injection of sodium thiopental (100 mg/kg body weight). All experimental procedures conducted in this study were approved by The Animal Care and Use Committee at the Faculty of Veterinary Medicine, Kasetsart University, Thailand (ACKU 00557).

Table 1 The composition and nutrient levels of the diet.

Items	Gestation diet
Ingredients	**(%)**
Cassava (80% starch)	20.00
Wheat 10.8P	18.00
Corn	10.00
Rice bran	10.00
Palm kernel meal	10.00
Full fat baked beans	9.00
Soybean meal 46.5%	7.50
Solvent extracted rice bran	7.00
Palm oil	4.00
Mono dicalcium phosphate P 21%	2.00
Calcium carbonate	1.40
L-Lysine	0.32
DL-Methionine	0.11
L-Threonine	0.15
L-Tryptophan	0.02
Salt	0.40
Premix	0.10
Nutrient levels	
Crude protein	14.06
Fat	8.68
Fiber	5.52
Salt	0.49
Calcium	1.01
Phosphorus	0.44
Lysine	0.90
Methionine + Cystine	0.55
Threonine	0.62
Tryptophan	0.18
Metabolizable energy (kcal/kg)	3156.66

Sample Collection

Eight visceral organs, including the brain, heart, stomach, kidneys, spleen, liver, lungs, and intestine, were excised from all piglets. Then, their weights were recorded for data analysis. The semitendinosus muscles (ST) were dissected and weighed, and their length and circumference were measured.

The ST muscle tissues were collected, embedded in an optimal cutting temperature (OCT) compound (Tissue-tex®, O.C.T compound, Sakura Finetek; Torrance, CA), and stored at –80 °C for further analysis. The tissues were then cut into thin slices (10 µm thick) using the Cryostat (Leica Model, CM 1950) and stained for histological and histochemical analyses.

Histomorphology of the semitendinosus muscle

The frozen sections of the ST muscle tissue were stained with hematoxylin and eosin (H&E) and stained for myofibrillar adenosine triphosphatase (mATPase) after pre-incubation in an acidic or alkaline solution (pH 4.6 and 10.4), following a previously described method (D'Angelis *et al*., 2014). For mATPase staining, tissue slides were pre-incubated with an acidic (5 min) or alkaline solution (15 min) at room temperature (RT) and rinsed twice in the wash solution (0.18 M calcium chloride ($CaCl_2$) in 0.1 M sodium barbital solution). Then, they were stained with an ATPase solution (pH 9.4) at RT for 50 min and washed thrice with 1% $CaCl_2$ solution for 10 min each time. Next, 2% cobalt chloride ($CoCl_2$) was added to each jar for 5 min. All slides were stained with 2% ammonium sulfide $(NH_4)_2S$ for 20–30 seconds until the sections appeared very dark; then, they were rinsed with tap water. The samples were dehydrated in 70%, 80%, 95%, and absolute ethyl alcohol for 5 min each and cleaned with xylene I, II, and III for 5 min each. The coverslips were mounted on the tissue slides using Canada balsam (Sigma-Aldrich).

Morphometric analysis of the semitendinosus muscle

In each sample, five randomly selected areas covering a total area of 3.48 mm^2 were analyzed at 200x magnification, following a previously described method (Tristán et al., 2009). The average of the total muscle cross-sectional area (MCSA) of the semitendinosus muscle was used to calculate the circumference of the muscle. Based on mATPase staining, the number of primary and secondary muscle fibers depended on the type of pre-incubation. For pre-incubation in an acidic solution, the primary fibers corresponded to the number of dark-stained fibers, while the secondary fibers corresponded to the number of light-stained fibers. For pre-incubation in an alkaline solution, the primary fibers corresponded to the number of light-stained fibers, while the secondary fibers corresponded to the number of dark-stained fibers. The images were analyzed using the ImageJ software, and the total number of muscle fibers, including primary and secondary fibers, was calculated. The ratio of the secondary to primary muscle fibers (S:P) was calculated to determine the effect of nutrition on the susceptible component (the secondary fibers), excluding the influence of the genetic component (the primary fibers) (Dwyer et al., 1994). The total number of muscle fibers was the sum of the primary and secondary fibers per MCSA. The MCSA was calculated from the circumference using the equation D = C/¶; r = D/2; A = r^2, where D denotes the diameter, C denotes the circumference, r denotes the nuclear radius, and A denotes the area of MCSA.

Statistical analysis

The prenatal development and morphometric profiles of the semitendinosus muscle of newborn piglets from treated sows and sows in the control group were compared. The data were expressed as the mean ±standard deviation, and the differences were considered to be statistically significant at $p < 0.05$. The parameters between groups were compared by conducting t-tests in the statistical software R version 4.1.2 (R Core Team, 2021).

RESULTS

Prenatal development

The mean birth weights and the organ weights of piglets of treated and control sows are presented in Table 2. None of the parameters associated with fetal development differed significantly between the piglets of treated and control sows, although the values of body and organ weights were slightly higher in the offspring of treated sows than those of the control sows.

Table 2 The prenatal development parameters of the newborn offspring of the treated and control sows.

	Maternal treatment		p-value
Items	**Control**	**Treatment**	
Birth weight (g)	1150.60 ±406.76	1264.52 ±374.33	0.79
Brain (g)	27.05 ±2.40	28.93 ±3.15	0.38
Liver (g)	30.57 ±11.32	39.47 ±14.13	0.25
Lung (g)	15.51 ±4.92	19.81 ±6.07	0.20
Spleen (g)	1.21 ±0.60	1.76 ±0.81	0.17
Kidney (g)	8.15 ±4.44	10.62 ±2.87	0.27
Heart (g)	7.08 ±2.73	8.82 ±2.83	0.28
Stomach (g)	5.45 ±1.57	7.14 ±3.01	0.22
Intestine (g)	32.69 ±15.29	36.45 ±15.93	0.86

Muscle characteristics

The stained microscopic images of the ST muscle sections showed that the clusters of muscle fibers consisted of the primary (P) fibers, which were large and had central nuclei surrounded by numerous smaller secondary (S) fibers (Figure 1). The mATPase staining of the transverse muscle sections was performed to differentiate the primary and secondary muscle fibers based on color differences. The primary fibers had a lighter stain and were surrounded by the darker secondary fibers after pre-incubation in an alkaline solution (Figure 1A). In contrast, after pre-incubation in an acidic solution, the primary fibers were darker and were centrally located in a cluster of lighter secondary fibers (Figure 1B).

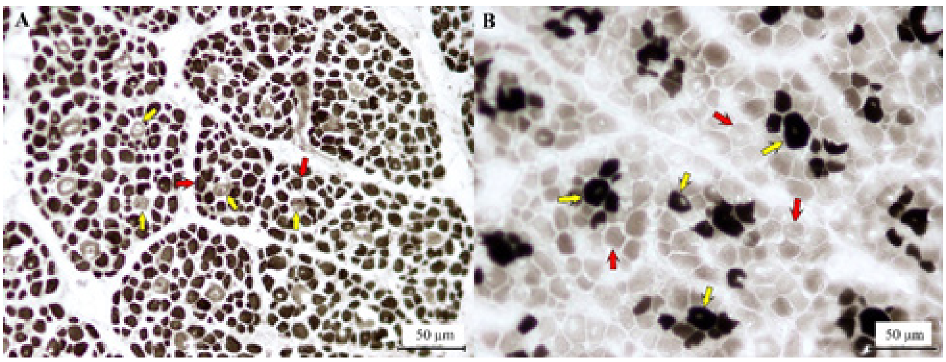

Figure 1 The photographs of the transverse sections of the semitendinosus muscle stained using mATPase from representative newborn piglets after pre-incubation in an alkaline solution at pH 10.4 (A) and pre-incubation in an acidic solution at pH 4.6 (B); magnification: 200x. The yellow and red arrows indicate the primary (P) and secondary (S) fibers, respectively.

Besides the morphological characteristics of the semitendinosus muscles, such as weight, length, circumference, and MCSA, the number of primary, secondary, and total muscle fibers, as well as, the S:P ratio from the images of transverse muscle sections were important parameters for analyzing the differences between the two groups of offspring (Table 3).

The treatment of the pregnant pigs by administering fermented potato extract protein during gestation (0–114 d) increased the muscle circumference and the MCSA in the newborn piglets, as well as, the number of total muscle fibers, although the difference between the values of the parameters recorded from the newborn piglets of treated and control sows was not significant. This might be due to the similar number of secondary fibers in both groups of piglets, although the number of primary fibers was higher in the piglets of control sows. The S:P ratio ($p = 0.02$) was significantly higher in the piglets of sows that were treated with the fermented potato extract than in the piglets of control sows.

Table 3 The morphometric profile of the semitendinosus muscle of the newborn offspring of the treated and control pigs.

	Maternal treatment		
Items	**Control**	**Treatment**	**p-value**
Muscle weight (g)	1.93 ±0.98	2.36 ±0.93	0.63
Muscle length (cm)	3.90 ±0.42	3.88 ±0.61	0.40
Muscle circumference (cm)	3.07 ±0.63	3.62 ±0.62	0.08
MCSA (cm^2)	0.76 ±0.30	1.04 ±0.35	0.09
Number of primary fibers	325.20 ±97.63	278.90 ±82.32	0.14
Number of secondary fibers	4917.30 ±1151.78	4921.80 ±1187.39	0.92
Secondary:primary ratio	15.80 ±4.30	18.04 ±2.59	0.02
Number of total muscle fibers	22635.20 ±9096.02	30430.28 ±10142.92	0.19

DISCUSSION

IGF-I regulates muscle formation and stimulates cell proliferation, differentiation, and growth in pigs. IGF-I localized in the porcine skeletal muscle fibers is correlated with plasma IGF-I (Götz et al., 2001). When IGF-I was administered to 7-day-old and 26-day-old piglets, it induced an increase in tissue protein synthesis in response to the stimulation of the skeletal muscle and other tissues in the neonate (Davis et al., 2002).

The benefits of supplementation with the fermented potato extract protein were demonstrated as shown in other studies, where feeding sows with the fermented potato extract protein was found to increase serum IGF-1 levels in piglets (Poolperm et al., 2012) and gilts (Benjasiriwan et al., 2013). Moreover, the piglets of sows that were fed the fermented potato extract protein (10 g per pig per day) daily showed higher birth weight and body weight at weaning compared to the piglets of control sows (Tantawet et al., 2015). Similarly, we found that the birth and organ weights of newborn piglets of supplemented sows were higher. The birth weight of piglets affects postnatal development, especially during early life (Zotti et al., 2017). Pigs with low birth weights showed lower growth performance and the least lean percentage of pig carcass at slaughter compared to pigs with high birth weights (Rehfeldt and Kuhn, 2006).

Not only dietary supplementation during gestation period can be used for the beneficial effects on fetal development, but continuously throughout lactation period can regulate growth and development of piglets through maternal milk (Zhang et al., 2019). Adding nucleotides in sow diet from late gestation through lactation periods improved growth of piglets because supplemented sow showed an increasing of lactating feed intake with lower oxidative stress levels in both sow and piglet sera when compared to non-supplemented sow and their offspring (Tan et al., 2021).

In this study, the histomorphological analysis of the semitendinosus muscle of newborn piglets showed that the central fiber of each islet originated from a primary muscle fiber surrounded by some secondary fibers at the periphery of the islets (Figure 1), which matched the findings of prenatal muscle development. The primary fibers were bigger than the secondary fibers because the number of primary fibers influences the framework of the secondary fibers (Wigmore and Stickland, 1983). The primary fibers matured to slow-twitch (type-I) fibers, and some surrounding secondary fibers matured to type-I fibers. The remaining secondary fibers matured to type-IIA and type-IIB fibers at the periphery of the islets (Lefaucheur et al., 1995). Generally, the S:P ratio can be used to follow changes in the hyperplasia of secondary fibers after 55 d (Lefaucheur et al., 2003). We found that the S:P ratio in newborn piglets of treated sows was higher. This could be due to a greater number of secondary fibers surrounding the larger primary fibers, although the number of primary fibers was lesser in the offspring of treated sows than in those of the control sows (Table 3).

Several studies on the effect of diet supplementation during pregnancy on the muscle development of the offspring have reported contrasting results. The number of primary muscle fibers of the fetus did not change when the diet of the sows was supplemented during early to mid-gestation, but the number of secondary fibers increased (Dwyer et al., 1994). Cerisuelo et al. found that increasing the quantity of food provided to pregnant sows during mid-gestation led to the development of fewer total muscle fibers, as well as, primary and secondary fibers in their offspring compared to the number of fibers in the offspring of the control sows (Cerisuelo et al., 2009). The gilts that were fed high or adequate protein diets throughout gestation produced offspring that did not show any changes in the number of total muscle fibers, whereas the offspring of gilts provided a low-protein diet had a significantly lower number of total muscle fibers (Kalbe et al., 2017).

According to the fermented potato extract protein supplementation for gestating sows could be beneficial for S:P ratio and subsequently follow changes in the hyperplasia of secondary muscle fibers of their offspring. However, adding this supplement into the sow diet will increase in total cost by approximately 203 baht per sow. If it is possible that the farmers can extract potato protein by using proper microbial fermentation method of potato residue, it can reduce feed cost. Thus, the fermented extract protein from plants has been further studied to improve the quality of feed and to be beneficial for the animal health.

CONCLUSIONS

In this study, we found that supplementing the diet of pregnant pigs with fermented potato extract protein can increase the S:P ratio of the offspring, which can be used to follow the changes in the hyperplasia of the secondary fibers in the fetus and might also affect postnatal growth.

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AUTHOR CONTRIBUTIONS

Prapassorn Boonsoongnern; Conceptualization and design the experiment, investigation, supervision, editing and finalization
Vanhnaly DALA; Investigation, methodology, formal analysis, manuscript preparation
Urai Pongchairerk; Investigation, interpretation of the study
Alongkot Boonsoongnern; Conceptualization and design the experiment, investigation
Pichai Jirawattanapong; Investigation, statistical analysis
Nattavut Ratanavanichrojn; Diet formulator, investigation

CONFLICT OF INTEREST

We have no conflict of interest.

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