



Effect of dietary supplementation with amino acids on boar sperm quality and fertility



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ABSTRACT

The aim of this study was to evaluate the effects of dietary supplementation with amino acids on sperm quality and fertility rates after insemination with boar semen. Twelve Yorkshire boars were paired by age and allocated to one of two dietary treatments composed of total lysine levels of 0.64% (T1) and 0.96% (T2), with the lysine: methionine: threonine: tryptophan: valine ratio in the diets set to 100:27:73:19:69 through the addition of synthetic amino acids. Semen was collected twice weekly (phase 1, 1–12 wk); every other day (phase 2, 13–16 wk); twice weekly (phase 3, 17–26 wk); and daily (phase 4, 27–28 wk). Semen was collected from boars during phase 3 and used to inseminate 64 multiparous sows. Our results showed that sperm concentration and total sperm cells were greater in boars in T2 than in boars in T1 in phases 2 and 4 ($P < 0.05$). Sperm motility parameters, morphologically normal sperm, and acrosome integrity in T2 boars were greater than those in T1 boars ($P < 0.05$) during the experiment. Free amino acid concentrations in seminal plasma increased in T2 boars ($P < 0.05$). Furthermore, sows inseminated with semen collected from T2 boars gave birth to more live piglets than those inseminated with semen collected from T1 boars ($P = 0.04$). In conclusion, supplementation of boar diet with amino acids improves sperm quality, and subsequently increases fertilization capacity and the number of live piglets.

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1. Introduction

The use of artificial insemination (AI) by pork producers has greatly increased over the past decade. For AI of swine, increasing the quality of sperm produced by boars is of primary importance (Waberski et al., 2008), as the production of high-quality sperm are crucial due to the limited number of insemination doses that can be obtained from one ejaculate (Ciereszko et al., 2000). Semen quality is not only

a proxy measure of boar fertility but also has sire effects on pig production in terms of the reproductive performance of sows (Smital, 2009; Huang et al., 2010). The production and quality of semen depends on genetic or intrinsic factors, including breed (Wolf, 2009), age (Huang et al., 2010), testicular size (Clark et al., 2003), environmental extrinsic factors (e.g., temperature) (Ciereszko et al., 2000; Yeste et al., 2010) and photoperiod (Yeste et al., 2010). Moreover, semen collection rhythm (Pruneda et al., 2005) and nutrition (Yeste et al., 2010) greatly influence semen quality.

Nutrition affects boar libido, sperm output, semen quality, and fertility status (sow pregnancy rate and offspring litter sizes). Low protein diets, especially in combination

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with low energy intake, have been shown to significantly reduce boar interest in mounting a dummy sow and ejaculation events (Stevermer et al., 1961). However, boar diets that contain excessive protein content may lead to corpulence, and increases in the concentration of blood urea, the number of abnormal sperm (Louis et al., 1994a), and serum skatole concentrations (Lin et al., 1992). Proteins are composed of amino acids; therefore, protein quality is largely dependent on the amino acid content and the bioavailability of amino acid (Kim et al., 2009). Different ratios of amino acids have substantial effects on the reproductive performance of boars (Ren et al., 2015). Increasing dietary lysine (Lys) from 0.86 to 1.03%, for instance, improved boar semen quality (Rupanova, 2006); other research has shown that diets supplemented with threonine (Thr) improved semen quality in both rams (Wilson et al., 2004) and boars (Close and Roberts, 1993), whereas inadequate tryptophan (Trp) led to the reduction of sperm number (Castrogiovanni et al., 2014). Moreover, diets supplemented with Trp can significantly improve motility of ram spermatozoa (Pichardo et al., 2011).

Sperm quality is closely correlated with boar fertility. Semen from boars fed a fortified selenium diet had a higher fertilization rate in mature gilts (Marin-Guzman et al., 1997). Lys, methionine (Met), Thr, Trp and valine (Val) are essential amino acids for pigs fed corn-soy based diets, but it is not clear whether supplementation of feed with these amino acids can improve boar sperm quality and fertility. It is reasonable to speculate that any dietary treatment that changes the composition of semen or seminal fluid may also influence the sperm quality. Speight et al. (2012) suggests that supplementation with organic selenium slowed the decrease in sperm motility post extension. In boars, viability of sperm stored at 15 °C was improved by increasing DHA levels in semen, a response to dietary fatty acid supplementation (Penny et al., 2000). It is not known whether or not supplementing diets with compound amino acids can increase boar fertility. Therefore, the objectives of this study were to determine (a) the effects of dietary amino acid level with the same amino acid patterns on semen quality and (b) the fertility of boar sperm when used for AI.

2. Materials and methods

All procedures with animals were approved by the Biosafety and Animal Care and Use Committees of Sichuan Agricultural University. The study was conducted at the Institute of Animal Nutrition, Sichuan Agricultural University in Sichuan, China.

2.1. Animals and treatments

Twelve Yorkshire boars were selected at 251 ± 4.3 (mean \pm SEM) days of age with an initial average body weight (BW) of 161.3 ± 5.6 kg. Boars were assigned to 2 treatments (6 boars per treatment) and housed individually in $2.3 \text{ m} \times 2.3 \text{ m}$ pens on partial-slatted floors. Diets were formulated to be isonitrogenous and isoenergetic with the same ratio of amino acids (Lys:Met:Thr:Trp:Val at the ratio of 100:27:73:19:69). Diet composition is shown

Table 1
Composition of diets (as-fed basis).

| Items | Treatment ^a | |
|--------------------------------------|------------------------|-------|
| | T1 | T2 |
| Ingredients (%) | | |
| Corn | 74.52 | 75.46 |
| Wheat bran | 8.00 | 8.00 |
| Soybean meal (44%) | 12.00 | 10.00 |
| Soy oil | 1.50 | 1.50 |
| Valine | – | 0.16 |
| Lysine | 0.13 | 0.61 |
| Methionine | – | 0.08 |
| Tryptophan | – | 0.06 |
| Threonine | 0.02 | 0.30 |
| Limestone | 0.75 | 0.75 |
| Calcium hydrophosphate | 2.20 | 2.20 |
| Choline chloride | 0.15 | 0.15 |
| Sodium chloride | 0.50 | 0.50 |
| Vitamins additive ^b | 0.10 | 0.10 |
| Trace minerals additive ^c | 0.13 | 0.13 |
| Nutrient levels | | |
| DE (Mcal/kg) ^d | 3.26 | 3.27 |
| CP (%) ^d | 12.89 | 12.88 |
| Ca (%) ^d | 0.86 | 0.86 |
| AP (%) ^d | 0.53 | 0.53 |
| Lys (%) ^e | 0.61 | 0.94 |
| Met (%) ^e | 0.18 | 0.24 |
| Trp (%) ^e | 0.14 | 0.20 |
| Thr (%) ^e | 0.51 | 0.72 |
| Val (%) ^e | 0.55 | 0.65 |

^a T1, total lysine levels of 0.64%; T2, total lysine levels of 0.96%.

^b Additive of trace minerals mixture provided per kg of diets: Cu, 16 mg; Zn, 165 mg; Fe, 95 mg; Mn, 30 mg; Se, 0.3 mg; I, 0.25 mg.

^c Additive of vitamins mixture provided per kg of diets: VA, 8000 IU; VD, 200 IU; VK, 5 mg; VE, 200 IU; VB1, 2 mg; VB2, 16 mg; VB6, 6 mg; VB12, 0.03 mg; Folic acid, 1 mg; Biotin, 0.3 mg; Pantothenic acid, 25 mg; Nicotinic acid, 35 mg.

^d Calculated.

^e Analyzed.

in Table 1, with total Lys levels of 0.64% (T1, in accordance with Meisinger (2010)), and 0.96% (T2). Synthetic amino acids were kept at 4 °C until fed to the boars. The daily food allowance for the entire experimental period followed the Meisinger (2010) guidelines for feeding levels at different bodyweights for maximizing sperm production.

2.2. Semen collection and evaluation

Boars were trained to mount an artificial sow, and semen was collected by gloved-hand technique (Almond et al., 1998) twice weekly (phase 1, 1–12 wk); every other day (phase 2, 13–16 wk); twice weekly (phase 3, 17–26 wk); and daily (phase 4, 27–28 wk). Boar libido as well as sperm production and quality were estimated for each ejaculate throughout the experiment.

Boar libido was assessed per the method described in Louis et al. (1994a). The time between entrance into the collection area and onset of ejaculation, and the duration of ejaculation were recorded. Semen volume was measured after straining through two layers of disposable filter membranes to remove gelatinous fraction by weighing each ejaculate and converted to volume as described by Lovercamp et al. (2013). The gelatinous fraction was weighed. Semen samples were diluted in a

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