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# Effect of different amino acid patterns on semen quality of boars fed with low-protein diets

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ABSTRACT

The objective of the present study was to determine the effect of different amino acid patterns on the semen quality of boars fed with low-protein diets. Twenty-four boars were randomly divided into 4 groups (HP, LP1, LP2, and LP3). HP boars received 17% crude protein diet with a lysine:threonine:tryptophan:arginine (Lys:Thr:Trp:Arg) ratio of 100:50:20:104. Other boars received 13% CP and similar Lys levels (0.84%) with Lys:Thr:Trp:Arg ratios of 100:50:20:71, 100:76:38:71, and 100:76:38:120 for LP1, LP2, and LP3, respectively. These results showed sperm motility in the LP3 group was higher than in HP group during the 13–22 week period. The total sperm number, acrosome integrity ratio, and the effective total sperm number in LP3 and LP2 was higher than in other groups, and the abnormality ratio was lower than in other groups during the 13–18 week period. During 19–22 week period, in LP1 and LP3 groups, total sperm number and effective total sperm number were higher than in other groups, abnormality ratio was lower, and acrosome integrity ratio was higher than in the HP group. Nitric oxide synthase activity of seminal plasma and nitric oxide concentration of spermatozoa were significantly higher in the LP3 group than in other groups. Furthermore, mRNA expression of androgen receptor in testes was up-regulated in LP3. In conclusion, we suggest that the optimum ratio of Lys:Thr:Trp:Arg in a 13% CP diet for boars is 100:76:38:120, which results in similar or better reproductive performances than a 17% CP diet.



## 1. Introduction

Semen quality in boars plays an important role in pig breeding. In practice, boars are typically fed a high crude protein (CP) diet due to its importance for boars. However, excessive protein in the diet may cause boars to become overweight and increase blood urea and serum skatole concentrations, and abnormal sperm (Lin et al., 1992; Louis et al., 1994). Excess dietary protein in rats increased liver damage and free radical production (Evans and Halliwell, 2001). A few studies have reported that boars fed with low-protein (LP) diets have similar semen quality to those fed high-protein diets (Luce et al., 1976; O'Shea et al., 2010). It has also been reported that pigs fed with low-protein diets supplemented with amino acids achieved a similar performance to those fed with a high-protein diet (Kerr et al., 2003). Furthermore, the reduction of CP from 18% to 14% in grower pig diet reduced nitrogen (N) in urine and feces by 84.72% and 42.65%, respectively, while the reduction of CP from 15.5% to 11.5% in finisher pig diet reduced

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N in urine and faces from 59.80% to 27.98%, respectively (Tartrakoon et al., 2004). It is widely known that dietary protein has a relatively high cost and is a limited resource. Therefore, it is necessary to find an efficient way to improve semen quality, and deal with shortage of feed resources and environmental protection.

Protein quality is primarily dependent on the amino acid (AA) content and the bioavailability of AA (Kim et al., 2009). Higher bioavailability can be achieved by using proteins that provide a perfect pattern of essential and nonessential AA in the diet relative to animal requirements (King, 2000). Wang and Fuller (1990) observed that there was different N deposition depending on the amino acid pattern in feed used for growing pigs. An appropriate AA pattern can improve the efficiency of protein utilization in pregnant sows (Kim et al., 2009). Several studies have showed that diets supplemented with threonine (Thr) improved the semen quality of rams (Wilson et al., 2004) and boars (Close and Roberts, 1993). Lack of tryptophan (Trp) resulted in increased amounts of undescended testes and reduction of spermatozoon (Castrogiovanni et al., 2014), while Trp-supplemented diets can significantly improve ram spermatozoon motility (Jiménez-Trejo et al., 2012). An in vitro experiment also showed that boar sperm capacitation and acrosome reaction are improved by L-arginine (L-Arg) supplementation (Funahashi, 2002). Moreover, in humans, sperm motility was increased by adding L-Arg in an in vitro study (Carvalho et al., 2012). There are a few studies that have considered the effects of different AA ratios on boar reproduction, and one study suggested that a diet with a lysine (Lys):methionine (Met):Thr:Trp ratio of 100:60:65:19 improved the reproduction performance of boars (Kiefer et al., 2012).

It is widely known that seminal plasma can serve as a biomarker of semen quality and fertility. The seminal plasma components such as alpha-glucosidase, fructose and citrate are highly related to semen quality (Said et al., 2009). Nitric oxide synthases (NOS) is a key enzyme controlling nitric oxide (NO) generation and sperm motility is improved when NO concentration increases (Lewis et al., 1996). Meanwhile, NO is an intracellular signaling molecule used to adjust sperm vitality through regulation of cyclic AMP (cAMP), and thus NOS activity decreases the intracellular cAMP level and reduces sperm motility (Belén Herrero et al., 2000). Previous research showed that sperm motility was positively correlated with sperm cAMP concentration (Das et al., 2010). However, relatively few studies have reported the effect of different amino acids model in low-proteins diets on semen quality and the possible mechanism remains unclear.

The establishment of optimum AA patterns is very important for boar reproduction and the use of low-protein AA supplemented diets that allow optimal reproductive performance of boars offers an effective means of reducing nitrogen excretion and lowers the feed cost for pig production. Therefore, the present experiments were conducted to determine the effect of different AA patterns in low-protein diets by assessing semen characteristics in order to identify the best AA feeding pattern for adult boars.

## 2. Materials and methods

### 2.1. Animals and diets

All procedures with animals were approved by the Biosafety and Animal Care and Use Committees of the Sichuan Agricultural University. Twenty-four boars [Landrace × Yorkshire, average weight (150 ± 6.70 kg)] of 9 months of age were randomly assigned to 4 treatments (HP, LP1, LP2, and LP3). Each treatment had 6 replicates with 1 boar per replicate. Boars were penned individually in pens with half-slatted floors. Before the semen quality assessment, boars were fed the corresponding experimental diet until the end of the experiment. The semen quality was assessed from May to October for a period of 22 weeks and semen in each group was collected once a week (0–12 wk), twice a week (13–18 wk), or three times a week (19–22 wk). Boars were rested for 3–4 days or 1–2 days when twice or three times weekly semen collections were carried out, respectively. Boars were exposed to ambient temperature and a natural photoperiod. The average temperature during the experiment was 23.3 °C, and supplemental ventilation for cooling during the summer months was provided by fans and a drip cooling system.

The compositions of the four diets are shown in Table 1. Boars fed with a high-protein (HP) diet received 17% of crude protein with 0.84% Lys and the Lys:Thr:Trp:Arg ratio was 100:50:19:104. Other boars received 13% CP and similar a Lys level (0.84%) with ratios of Lys:Thr:Trp:Arg of 100:50:20:71, 100:76:38:71, and 100:76:38:120 for LP1, LP2, and LP3, respectively. Vitamin, mineral, and fat intakes were similar for all treatments. All the boars were fed 2.6 kg per day (Close and Roberts, 1993). Boars were hand-fed twice a day and were provided with ad libitum access to water.

Boars were weighed once a month, and then the length and width of the testes of the boars were measured using a vernier caliper. The testicular volume was calculated by using the following formula: volume = $0.75\pi \times (\text{length}) \times (0.5 \times \text{width})^2$. The testis index was expressed as the percentage of the testicular average weight of boars divided by the total body weight (kg).

### 2.2. Semen collection and evaluation

Boars were trained to mount a dummy sow and have their semen collected by the gloved-hand method (Louis et al., 1994). Semen collections were carried out by the same person during the whole length of the experiment. The volume of semen was measured after filtering through two layers of disposable filter membranes to remove the gelatinous fraction. Semen volume was measured using a graduated cylinder, and motility and viability were subjectively assessed microscopically under ×200 magnification (Olympus, Japan). Concentration of spermatozoa was determined in 1 mL using the cytometric method in a Bürker chamber. The total number of spermatozoa per ejaculate was calculated by multiplying the volume by the concentration and the effective total number of spermatozoa per ejaculate was calculated by multiplying the total number of spermatozoa per ejaculate by the motility (Louis

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