Short communication

Influences of lipopolysaccharide-induced immune challenge on performance and whole-body protein turnover in weanling pigs

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Abstract

The objective of this study is to characterize the influence of immune stress induced by lipopolysaccharide (LPS) on protein utilization and turnover for early-weaned pigs. A total of 15, crossbred weanling pigs (initial body weight 10.15±0.39 kg) were assigned to one of three treatments. Pigs were injected with LPS and fed ad libitum (LPS-challenge), or injected with endotoxin-free physiological salt solution (PSS) and fed ad libitum (control), or injected with PSS and fed the same amount of feed as LPS-challenged pigs (pair-feed). All pigs received a 4-d nitrogen balance trial. On d 1 and 3 of the trial, LPS-challenged pigs were injected intramuscularly with 200 μg/kg BW of LPS dissolved in 1 ml PSS. Pigs in other treatments were injected with 1 ml PSS. 15N-Glycine (5 mg/kg BW) was gastrically infused after the second injection. Feces and urine were collected daily to determine the N output throughout the duration of the trial. Lymphocyte blastogenesis (LB) and serum immunoglobulins (IgA, IgG, IgM) were also detected to illustrate the LPS-induced immune responses. Results indicated that the injection of LPS significantly (P<0.01) elevated the LB and resulted in lower average daily gain (ADG), feed intake (ADFI) and efficiency of feed utilization than control pigs. Pair-fed pigs had higher performance than LPS-challenged pig but poorer than control pigs. Injection of LPS also resulted in significantly (P<0.01) lower nitrogen intake and efficiency of utilization than controls, and more fecal N excretion than Pair-fed pigs (3.19±0.85 vs. 2.19±0.67 g/d, P<0.05). The whole-body nitrogen flux (4.34±0.19 vs. 11.35±0.12 g N/kg BW0.75/d) and N accretion (5.57±0.59 vs. 10.17±1.12 g Pr/kg BW0.75/d) were acutely (P<0.01) reduced as the feed intake decreased, but there was no significant difference between LPS-challenged and pair-fed pigs. Injection of LPS markedly (P<0.05) increased the protein degradation (16.76±1.09 vs. 14.53±1.24 g N/kg BW0.75/d). It is concluded that LPS-induced immune challenge depresses growth performance and feed utilization efficiency by enhancing protein degradation rate and decreasing protein utilization for body protein retention.

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Keywords: Pigs; LPS; Immune challenge; Protein turnover; Nutrient availability

1. Introduction

Infectious diseases or inflammation markedly reduce voluntary feed intake and redistribute nutrients away from the growth process for support of immune system functions (Wannemacher, 1977). Similarly, acute activation of the immune system via exposure to pathogenic or nonpathogenic antigens results in lower body growth rate, and efficiency of feed utilization in chicks (Klasing et al., 1987) and pigs (Van Heugten et al., 1994).
Changes in protein metabolism include depressing skeletal muscle protein synthesis (Jepson et al., 1986), stimulating skeletal muscle protein degradation (Klasing et al., 1987), and increasing liver and heart protein synthesis (Ballmer et al., 1991). These changes in metabolism suggest altered nutritional requirements during immunologic challenge. A best understanding of the interactions between nutrient metabolism and immunity is essential for regulating immune response and improving animal welfare and production by nutritional strategies (Cook et al., 1993). However, the determination of protein requirements for immune-challenged pigs is very complicated due to the dearth of information on whole-body protein turnover under such a circumstance.

The present study was conducted to determine the effect of LPS-induced immune challenge on performance and whole-body protein turnover in weanling pigs.

2. Materials and methods

2.1. Animals and animal cares

The experimental protocols used in this study were approved by the Sichuan Agricultural University Institutional Animal Care and Use Committee. A total of 15 crossbred (Landrace × Rongchang) weanling pigs (average initial weight was 10.15 ± 0.39 kg) were used. Pigs were vaccinated with swine fever at 21 days old. Sows of the piglets were received no vaccinations during pregnancy. Neither antibiotics nor antibiotics nor vaccines were administrated to the pigs during the experiment. Pigs were allocated to one of three treatments on the basis of Ad libitum (control), or injected with PSS and fed the same diet as LPS-challenged pigs (pair-feed).

Pigs were housed individually in metabolism cages (0.7 × 1.5 m) with woven wire flooring in an environmentally controlled room (22 to 24 °C) and were given ad libitum access to water through a water nipple. Pigs were hand-fed four times per day (8:00, 12:00, 16:00, 20:00) in bowl feeders to make sure fresh feed was available, and allowed a 5-d adjustment to the experimental diets. The diet adjustment period was followed by a nitrogen balance period, which included a 4-d collection of feces and urine to determine nitrogen output. Bodyweight and feed consumption were recorded daily throughout the duration of the trial.

2.2. Immune challenge model

Lipopolysaccharide (LPS, E. coli serotype 0.55:B5, Sigma Chemical, St. Louis, MO) was used as the challenge agent. At d 1 and 3 of the N balance period, LPS-challenged pigs were injected intramuscularly with 200 μg/kg BW of LPS dissolved in 1 ml PSS (China Shenzhen Jianghe Chemical Co.). Pigs in other treatments were injected 1 ml PSS.

2.3. 15N-Glycine infusion

At d 3 of the trial, pigs were gastrically infused with 15N-Glycine (99 at.%15N, Sigma Chemical) at a dose of 5 mg/kg BW after the injection of LPS. Pigs were maintained in metabolism cages for a 2-d collection of urine under the same conditions as the N balance period. Prior to isotope infusion, a urine sample was also collected from each pig for 12 h to determine background enrichment of 15N in urinary urea.

2.4. Experimental diet

All pigs were fed the same diet formulated to meet nutrient requirements recommended by NRC (1998) for 10- to 15-kg pigs. The diet consisted of 52.1% corn, 17.8% soybean meal, 9.5% extruded soybean, 10% spray-dried whey, 5% fish meal, and fortified with minerals and vitamins. Each ingredient was analyzed for protein and amino acid contents before formulating the diet. Diet was produced by University Research Feedmill (Yann, Sichuan, China). Analyzed composition levels were DE 14.2 MJ/kg, CP 20.0%, Ca 0.9%, available P 0.55% and total lysine 1.2%.

2.5. Sample collection and analysis

To evaluate the immune response, blood samples were collected before the first injection and 2 h after the second injection of LPS. Cellular immunity was measured using a lymphocyte blastogenesis assay (Blecha et al., 1983), as modified by Van Heugten et al. (1994). Mitogen used was phytohemagglutinin (PHA, L1668, Sigma Chemical, St. Louis, MO) at 5 μg/ml. Serum immunoglobulins (IgA, IgG, IgM) were measured by ELISA technique described by Engvall and Perlmann (1972).

Feces collected during the N balance period were pooled, freeze-dried and stored at 4 °C for N determination. Urine collected was stored at −20 °C until analysis for N.

Samples of diet, urine and feces were analyzed for N content by Kjeldahl method (AOAC, 1998). The N-retention was calculated by minus N excretion (via feces and urine) from N intake. To measure the 15N enrichment of urinary urea, a 10 ml urine sample was converted into (NH4)2SO4 solution by Kjeldahl method (AOAC, 1998). Then (NH4)2SO4 solution was transferred to a titer plate, dried with a food dehydrator, and submitted for analysis of 15N enrichment by automated mass spectrometry (Isotope Mass Spectrometer, Finnigan-MAT 251).

2.6. Calculation of protein turnover

Whole-body protein turnover, and consequently protein synthesis and degradation rates, was calculated using two-pool
3. Results

3.1. Immune response measurements

*In vitro* cellular immune response was estimated using a lymphocyte blastogenesis assay. Cell proliferation rate was not significantly different among treatments before LPS injection with average value being 9.9%, and was 20.80 ± 2.31%, 15.50 ± 2.38% and 9.80 ± 3.0% for LPS-challenged, pair-fed and control group respectively. Difference between any two groups was significant ($P<0.01$). However, humoral immune response as measured by serum immunoglobulin (IgA, IgG, IgM), was not affected by LPS injection. IgG contents were 4.28 ± 0.11 g/l, 4.24 ± 0.38 g/l and 4.16 ± 0.13 g/l, IgM were 0.47 ± 0.06 g/l, 0.49 ± 0.12 g/l and 0.59 ± 0.20, and IgA were 0.43 ± 0.06 g/l, 0.41 ± 0.02 g/l and 0.38 ± 0.03 g/l for LPS-challenged, pair-fed and control group respectively.

3.2. Growth performance

Average daily feed intake (ADFI), average daily gain (ADG) and feed efficiency ($G/F$) of the LPS-challenged pigs were decreased by 28%, 43% and 21%, respectively ($P<0.01$) compared with the control pigs. Compared with pair-fed pigs, LPS-challenged pigs had 14% lower ADG (199 ± 31g vs. 171 ± 21g, $P<0.01$) and 14% lower $G/F$ (491.6 ± 20.0 vs. 424.5 ± 33.5, $P<0.01$). (Table 1).

### 3.3. Protein utilization and turnover

Nitrogen balance trial indicated that LPS-challenged pigs had a lower ($P<0.01$) N intake, retention and apparent digestibility than control pigs. But both fecal and urinary N excretions were not affected by LPS injection ($P>0.05$). The N intake affected protein utilization of weanling pigs as indicated by a lower ($P<0.01$) BV for pair-fed pigs compared with the control pigs (57.64 ± 3.02 vs. 65.45 ± 5.04). When pigs had the same amounts of N intake, LPS injection resulted in higher ($P<0.01$) fecal N excretion (3.19 ± 0.85 vs. 2.19 ± 0.67 g/d), and lower ($P<0.01$) apparent digestibility and the ratio of retention/intake (Table 2).

Immune challenge affected the whole-body protein turnover (Table 3). Compared with the control pigs, LPS injection resulted in decreased ($P<0.01$) whole-body nitrogen flux (4.34 ± 0.19 vs. 11.35 ± 0.12 g N/kg BW0.75/d) and N accretion rate (5.57 ± 0.59 vs. 10.17 ± 1.12 g Pr/kg BW0.75/d). But the efficiency of energy utilization (as reflected by heat production) during protein accretion was elevated ($P<0.01$). The whole-body nitrogen flux and rates of protein synthesis and accretion, and the efficiency of amino acids utilization ($S/Q$) were markedly ($P<0.01$) reduced in pair-fed pigs than those of control pigs. When pigs had the same amount of feed intake, the whole-body nitrogen flux, rate of protein synthesis and $S/Q$ were not affected by LPS injection ($P>0.1$), but LPS-challenged pigs had a higher ($P<0.05$) protein degradation rate (16.76 ± 1.09)

### Table 1

**Influence of LPS challenge on growth performance of weanling pigs ($n=5$)**

<table>
<thead>
<tr>
<th></th>
<th>LPS-challenged</th>
<th>Pair-fed</th>
<th>Control</th>
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<tbody>
<tr>
<td>Initial bodyweight (kg)</td>
<td>10.09 ± 0.61</td>
<td>10.20 ± 0.26</td>
<td>10.15 ± 0.30</td>
</tr>
<tr>
<td>Final bodyweight (kg)</td>
<td>10.77 ± 0.62</td>
<td>11.00 ± 0.31</td>
<td>11.36 ± 0.30</td>
</tr>
<tr>
<td>ADFI (g)</td>
<td>403.7 ± 48.8A</td>
<td>403.7 ± 48.8A</td>
<td>562.4 ± 50.0B</td>
</tr>
<tr>
<td>ADG (g)</td>
<td>171 ± 21A</td>
<td>199 ± 31A</td>
<td>302 ± 22B</td>
</tr>
<tr>
<td>Efficiency ($G/F$, g/kg)</td>
<td>424.5 ± 33.5A</td>
<td>491.6 ± 20.0B</td>
<td>536.6 ± 10.5B</td>
</tr>
</tbody>
</table>

Note: Different superscript small letter in a row means $P<0.05$; while different capital letter means $P<0.01$. 

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model (Picou and Taylor-Robert, 1969). Total protein turnover was calculated as:

$$ Q = S + E = B + I*D. $$

Where $Q$ is protein turnover (g N/d), $S$ is the rate of protein synthesis (g/d), $E$ is the rate of urea N excretion (g/d) in urine, $B$ is the rate of protein degradation (g/d), $I*D$ is the rate of N absorption from diet (g/d), and $D$ is the digestibility of dietary N. $I$ is the N intake.

Whole-body protein turnover was used to calculate whole-body protein synthesis rate (PS) and degradation rate (PB) using the relationships:

$$ PS = S*6.25, \text{ and } PB = B*6.25. $$

Thus, PS (g/d) was calculated as $PS=(Q-E)*6.25$, and PB (g/d)=$Q-I*D)*6.25$.

To estimate the efficiency of energy utilization for protein accretion, the following relationship was used:

$$ H = (n-1)*90*4.18/100 \text{ (KJ/g)}. $$

Where $n$ is the ratio of PS/PA, and PA is the rate of protein accretion (PA=PS−PB).

2.7. Statistical analysis

Data are expressed as Means±SD. Data for all parameters determined were analyzed statistically by single factorial variance analysis using the GLM procedure of SPSS10.0 software (SPSS Inc., Chicago, Illinois). Differences with $P<0.05$ were considered to be significant.
different capital letter means

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<tr>
<td>N intake (g/d)</td>
<td>12.92±1.56^A 12.92±1.56^A 17.70±1.33^B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal N excretion (g/d)</td>
<td>3.19±0.83^a 2.19±0.67^b 2.92±0.47^ab</td>
<td></td>
<td></td>
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<tr>
<td>Urinary N excretion (g/d)</td>
<td>4.39±0.39 4.53±0.29 5.11±0.91</td>
<td></td>
<td></td>
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<tr>
<td>Apparent digestibility (%)</td>
<td>75.66±3.84^A 83.31±2.97^a 97.67±1.06^B</td>
<td></td>
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</tr>
<tr>
<td>N retention (g/d)</td>
<td>5.34±0.70^A 6.20±0.77^A 9.67±1.06^B</td>
<td></td>
<td></td>
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<tr>
<td>Retention/intake (%)</td>
<td>41.32±1.55^A 48.00±2.67^B 54.60±3.38^C</td>
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<tr>
<td>BV (%)</td>
<td>54.72±3.52^A 57.64±3.02^a 65.45±5.04^B</td>
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vs. 14.53±1.24 g Pr/kg BW^{0.75}/d) and heat production (11.46±1.83 vs. 8.18±1.10 kJ/g) than pair-fed pigs.

### 4. Discussion

The immune challenge has long been considered to be the key limiting factor for animal well being and efficiency of production. Exposure of animals to pathogenic or nonpathogenic antigens results in activated immune system and subsequently cytokine release. Cytokines such as interleuking-1 (IL-1), interleuking-6 (IL-6) and tumor necrosis factor (TNF) acts as messenger regulators to alter metabolic processes in animals (Klasing and Barnes, 1988). Metabolic shifts are characterized by redistributed nutrients away from the growth process and toward immune system function (Beisel, 1977), and subsequently result in lower body growth rate and efficiency of feed utilization.

This study confirmed in young pigs that LPS-induced immune stress depressed significantly feed intake (by 28%), body growth rate (by 43%) and efficiency of feed utilization (by 21%). Two reasons for growth depress are low feed intake and poor feed utilization. Compared with pair-fed pigs, LPS-challenged pigs had 14% lower ADG and 14% lower G/F, suggesting that ADG decrease be completely due to the decrease of feed intake. By comparing pair-fed group and control group, we notice that 28%-decrease feed intake results in 34% lower ADG and 8% lower G/F under the condition of no immune stress. Therefore, it is estimated that, under immune stress, decreased feed intake contributes to about 70% (34%/43%) of the reduced growth rate and low feed utilization to about 30% (14%/43%). This contribution rate is consistent with the estimate by Klasing et al. (1987) in chick study which showed that LPS-challenged chicks reduced body gains and gain: feed ratios by 17.1% and 17.0%, respectively, and 70% of the reduced growth rate was attributed to decreased feed intake and 30% to inefficiencies in nutrient absorption and metabolism.

Results of protein turnover metabolism indicates that the decreases of both digestibility and utilization rate are the reason for the reduced feed utilization efficiency under immune challenge. LPS-challenged pigs had 46% higher fecal excretion and 10% lower digestibility. At the level of body metabolism, immune stress had no significant influence on protein synthesis but 15% higher protein degradation and 14% lower retention than pair-fed pigs, indicating protein degradation is the main contributor to lower retention. Since amino acids from protein degraded are re-utilized for acute phase protein synthesis in the liver and heart, urinary N excretion was not increased for LPS-induced pigs compared with pair-fed pigs. This result further confirms that animals undergoing an immune challenge have alterations in the metabolism of nutrients. The metabolic shifts of body protein include increased skeletal muscle protein degradation, decreased skeletal muscle protein synthesis, and increased liver and heart protein synthesis (Ballmer et al., 1991).

The mechanism of immune stress on feed intake and protein utilization and turnover is likely due to higher

### Table 2

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<td>H (kJ/g)</td>
<td>11.46±1.85^A 8.18±1.10^A 20.72±2.55^B</td>
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<tr>
<td>PA/PS</td>
<td>0.25±0.027^A 0.31±0.006^A 0.926±0.015^B</td>
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Q means total nitrogen flux; S and B means the quantity of whole-body protein synthesis and degradation respectively; S/Q means efficiency of amino acids utilization; PS, PB and PA means the rate of protein synthesis and degradation respectively; PA/PS means the efficiency of protein accretion. Pr is abbreviation of protein (N×6.25).
cytokine release and associated endocrine changes resulting from the immune system activation. LPS has been shown to be a potent stimulant of macrophages that produce IL-1 and TNF upon activation (Feldmann and Male, 1989). It has been reported that the administration of pro-inflammatory cytokine resulted in lower voluntary feed intake in rats (Mrosovsky et al., 1989) and in pigs (Fink et al., 1995). The reduction in voluntary feed intake is associated with an IL-1-induced release of CRH (Navarra et al., 1991) and IL-8, which serve as a potent stimulant of the lateral hypothalamus (Plata-Salaman and Borkoski, 1993). The increased protein degradation in LPS-challenged pigs is likely to meet the amino acid requirements for the synthesis of acute phase protein (Klasing, 1998). Cytokines such as IL-6, IL-1, and TNF mediate the protein synthesis and degradation by decreasing the release of anabolic hormones such as somatotropin (Honegger et al., 1991) and IGF-1 (Fan et al., 1994), increasing catabolic (glucocorticoid) hormone release (Navarra et al., 1991).

The determination of protein turnover in weanling pigs would be useful in defining the effects of metabolic modifiers on protein accretion and refining protein requirements for growing animals. It has been already reported that immune stress alters nutrient requirements (Williams et al., 1997). This study imply that regulating feed intake and providing sufficient amino acids for acute protein synthesis might be the nutritional strategies to alleviate the potential impacts of stress on animal health and production. But more researches are needed to quantify nutrient requirements for animals under stress.

Acknowledgments

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References


Further reading