

Effect of Dietary *Acanthopanax Senticosus* Polysaccharide on Performance, Immunological, and Somatotropic Response in Piglets Challenged with Lipopolysaccharide

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Abstract. Major objective of the current study was to evaluate the effect of *Acanthopanax senticosus* polysaccharide (ASPS) on growth and immunological, somatotropic response of piglets challenged with lipopolysaccharide (LPS). A 2 × 2 factorial design including dietary treatment (0 or 800 mg/kg ASPS) and immunological challenge (LPS or saline injection) was conducted with 64 piglets assigned to two dietary treatments. Half of the piglets of per dietary treatment were injected with LPS or saline on day 14. Blood were obtained on day 2 after LPS challenge for lymphocyte proliferation and 3h after injection for analysis of IL-1, IL-6, TNF- α , PGE₂, cortisol, GH, IGF-I and IgG. ASPS did not affect growth performance prior to LPS injection ($P>0.05$). From day 15 to 21, suppressed ADG and ADFI were observed by LPS ($P<0.01$). In contrast, ASPS improved these parameters ($P<0.05$), LPS and ASPS interaction was found on ADG and ADFI ($P<0.05$). Lymphocyte proliferation was promoted by LPS ($P<0.01$) or ASPS ($P<0.05$). but IgG was not affected ($P>0.05$) by ASPS or LPS throughout 21-day trial. ASPS depressed IL-1 ($P<0.05$), IL-6 ($P<0.05$), TNF- α ($P<0.05$) among LPS-treated piglets, ASPS and LPS interaction were observed on these indices ($P<0.05$). ASPS decreased PGE₂ ($P<0.05$) and cortisol ($P<0.05$) concentrations as well as increased concentrations of IGF- I ($P<0.05$) and GH ($P<0.05$) of LPS-treated piglets, and interaction between ASPS and LPS were observed for PGE₂ ($P<0.05$), cortisol ($P<0.05$) and IGF- I ($P<0.05$). This study suggest that ASPS mediated the release of pro-inflammatory cytokines and invoked pituitary hormone, PGE₂ during immunological challenge period, which might enable pigs to achieve better performance.

Keywords: Herbal extract, Stress, Growth, Cytokines, Metabolism, Weaned pigs

1. Introduction

The failure of commercially reared food animals to obtain their genetic potential for growth and efficiency of gain is undoubtedly attributable partly to continual pathogenic and nonpathogenic immunological stress, which directs nutrients away from tissue growth in support of immune-related processes [1]. The consensus among many researchers that decreased performance and changes in metabolism characterizing immunological stress are coordinated by cytokines released by excessive activated immune system [2]. Particularly, interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF- α) referred to as pro-inflammatory cytokines have profound behavioral, neuroendocrine, and metabolic effects [1] and [2]. Hence, mediated cytokines released by immunomodulator for potential performance is of considerable economic importance.

Acanthopanax senticosus polysaccharide (ASPS), a tan powder, is an active ingredient isolated from a traditional herbal medicine *Acanthopanax senticosus* (AS). Many previous studies have shown in humans and rodents, ASPS played a powerful role in immunomodulatory activity *in vivo* and *in vitro* [3] and [4]. However, to date, few investigations referred to effect on immunomodulatory activity of ASPS has been carried out in livestock. In the present study, the experiment was conducted to determine the effect of dietary

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ASPS on performance, immunological, and somatotropic response of piglets experimentally immunologically challenged with lipopolysaccharide.

2. Materials and Methods

2.1. Preparation and Analysis of ASPS

ASPS was prepared using ethanol precipitation methods with some modification [5]. Polysaccharide content was 92.74% measured by the phenol sulfuric acid method [6].

2.2. Animals, Diets, Experimental Design

The experimental was a 2×2 factorial design lasting for 21-day approved by pig association of Liaoning Province. The main factors included diet supplementation (0 or 800 mg/kg ASPS) and immunological challenge (LPS or saline injection). 64 crossbred barrows (Duroc, Large White, Landrace), weaned at 28±3 days of age, with average body weight of 7.25±0.21 kg were assigned to two dietary groups with 8 replicate pens (2 barrows and 2 gilts per pen). On d 14, half of the pigs of per dietary treatment were intraperitoneally injected with 100 ug/kg BW of LPS (*Escherichia coli* serotype 055:B5, Sigma) or equivalent amount of sterile saline. The basal diet containing no antibiotic was formulated according to NRC (1998), presented in Table I.

Table I: Composition of the basal diet (as-fed basis)

| Items | Content | Items | Content |
|----------------------------|---------|--|---------|
| Ingredients(g/kg) | | Ingredients(g/kg) | |
| Corn | 524 | Choline chloride | 1 |
| Soybean meal (440 g CP/kg) | 240 | Complex acidifying agent | 3 |
| Whey power | 80 | Vitamin and mineral pre-mix ¹ | 10 |
| Fish meal | 40 | Nutrient content ² | |
| Soybean oil | 23 | ME (Mcal/kg) | 3.44 |
| Sucrose | 50 | Crude protein | 191 |
| Limestone | 8 | Calcium | 8.6 |
| Dibasic calcium phosphate | 12 | Available phosphorus | 6.5 |
| Salt | 3 | Lysine | 13.6 |
| L- Lysine | 3.5 | Methionine | 3.8 |
| DL- Methionine | 0.5 | Methionine+cystine | 7.8 |
| L-Threonine | 1.8 | Threonine | 8.6 |
| Tryptophan | 0.2 | Tryptophan | 2.2 |

¹Provided the following mg/kg complete diet: retinol 3.6;cholecalciferol 0.075; alpha-tocopherol 60; phytylmenaquinone 3; cyanocobalamin 0.04; riboflavin 7.5; niacin 45; pantothenic acid 30; folic acid 1.2; thiamine 3; pyridoxine 4.8; biotin 0.22; Zn 120; Fe 120; Cu 15; Mn 50; I 0.6;Se 0.3

²Crude protein, calcium, phosphorus are analyzed values. The others were calculated.

2.3. Sampling Procedure

On day 14 and 21, body weight of each pig and food consumption per pen were measured to calculate average daily gain (ADG), average daily feed intake (ADFI) and feed/gain (F/G). On day 14 ,peripheral blood were sampled from one pig selected randomly per pen and centrifuged at 3500×g at 4°C for 5 min to prepare plasma at 3 h post-injection, and plasma was stored in centrifuge tubes at -80°C for determination of interleukin-1(IL-1), interleukin-6(IL-6),tumor necrosis factors-alpha(TNF-α), prostaglandin E₂ (PGE₂), cortisol, growth hormone(GH), insulin-like growth factor-I (IGF-I) and IgG. On 15, another share of peripheral blood samples was collected into vacuum tubes to analysis of lymphocyte proliferation.

2.4. Determination of Parameters

Lymphocyte proliferation assay: Lymphocyte proliferation stimulated by Con A was measured according to the method of Mosmann (1983) with some modifications.

Plasma parameters:Plasma IL-1, IL-6, TNF-α, GH, IGF-I, PGE₂, IgG and cortisol concentrations were determined using ELISA kit (R&D System, USA) following the indication of the manufacturer.

2.5. Statistical Analysis

Two-way analysis of variance (ANVOA) was performed using the General Linear Models (GLM) procedure of SPSS software (SPSS, 11.5). Duncan's multiple range test was used to compare differences among the treatment groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Growth Performance

Table II summarized the growth performance results. ASPS did not affect growth performance prior to challenge ($P > 0.05$). From day 15 to 21, suppressed ADG ($P < 0.01$) and ADFI ($P < 0.01$) were observed by LPS. Correspondingly, these parameters were improved ($P < 0.05$) by ASPS, and interaction between LPS \times ASPS was found on ADG ($P < 0.05$) and ADFI ($P < 0.01$), which suggested that ASPS alleviate depressed growth by LPS. There was no effect on F/G ($P > 0.05$) by whether ASPS or LPS. Taking into account data between different treatments, LPS-treated pigs fed 800 mg/kg ASPS had higher ADG and ADFI compared with pigs fed control diet ($P < 0.05$).

Table II. The effects of ASPS on growth performance of weaned piglets challenged with LPS

| Item | 0 mg/kg ASPS | | 800 mg/kg ASPS | | SEM ² | P-value | | |
|-------------------------------------|--------------------|--------------------|--------------------|--------------------|------------------|---------|-------|-------------|
| | -LPS | +LPS | -LPS | +LPS | | Diet | LPS | Interaction |
| average daily gain(ADG /kg) | | | | | | | | |
| 1 to14 d | 0.227 | 0.229 | 0.235 | 0.232 | 0.011 | 0.634 | 0.953 | 0.853 |
| 15 to 21 d | 0.473 ^b | 0.330 ^a | 0.483 ^b | 0.448 ^b | 0.024 | 0.019 | 0.003 | 0.042 |
| 1 to 21 d | 0.309 ^b | 0.262 ^a | 0.318 ^b | 0.304 ^b | 0.008 | 0.007 | 0.002 | 0.054 |
| average daily feed intake(ADFI /kg) | | | | | | | | |
| 1 to14 d | 0.349 | 0.348 | 0.365 | 0.353 | 0.023 | 0.655 | 0.782 | 0.811 |
| 15 to 21 d | 0.880 ^b | 0.645 ^a | 0.871 ^b | 0.864 ^b | 0.033 | 0.013 | 0.002 | 0.008 |
| 1 to 21 d | 0.526 | 0.447 | 0.534 | 0.517 | 0.022 | 0.107 | 0.054 | 0.188 |
| feed/gain (F/G) | | | | | | | | |
| 1 to14 d | 1.536 | 1.549 | 1.559 | 1.521 | 0.110 | 0.981 | 0.913 | 0.822 |
| 15 to 21 d | 1.872 | 2.037 | 1.809 | 1.913 | 0.187 | 0.625 | 0.486 | 0.872 |
| 1 to 21 d | 1.710 | 1.703 | 1.683 | 1.709 | 0.089 | 0.905 | 0.918 | 0.857 |

¹Values in the same row with different lowercases superscripts mean statistical significant difference between treatments ($P < 0.05$). ²SEM = Standard error of the mean.

3.2. Immunological Responses and Some Hormone

Table III and Table IV show the results of immunological responses. Lymphocyte proliferation was promoted by LPS ($P < 0.01$) or ASPS ($P < 0.05$). The results also showed that plasma IgG concentration was affected ($P > 0.05$) by neither dietary ASPS, LPS challenge, nor their interaction.

Table III. The effects of ASPS on lymphocytes proliferation and IgG of weaned piglets challenged with LPS

| Item | 0mg/kg ASPS | | 800mg/kg ASPS | | SEM | P-value | | |
|---------------------------|--------------------|--------------------|--------------------|--------------------|-------|---------|-------|-------------|
| | -LPS | +LPS | -LPS | +LPS | | Diet | LPS | Interaction |
| lymphocytes proliferation | 1.630 ^a | 1.745 ^b | 1.723 ^b | 1.780 ^b | 0.018 | 0.019 | 0.003 | 0.044 |
| IgG/(g/L) | 8.740 | 8.275 | 7.915 | 8.657 | 0.258 | 0.803 | 0.692 | 0.289 |

As indicated in Table IV, LPS-challenge increased plasma pro-inflammatory cytokines IL-1 ($P < 0.01$), IL-6 ($P < 0.05$), TNF- α ($P < 0.05$). In contrast, these parameters ($P < 0.05$) concentration were decreased by ASPS. An interaction between ASPS \times LPS were observed on IL-1 ($P < 0.05$), IL-6 ($P < 0.05$) and TNF- α ($P < 0.01$). Integrated look at the data between different treatments, ASPS had significant ($P < 0.05$) effect on IL-1, IL-6, TNF- α in LPS-administration pigs, but no significant effect were found in saline-injection pigs.

Table IV. The effects of ASPS on some plasma cytokines and some hormone of weaned piglets challenged with LPS

| Item | 0 mg/kg ASPS | | 800 mg/kg ASPS | | SEM | P-value | | |
|-------------------------|----------------------|----------------------|----------------------|----------------------|--------|---------|-------|-------------|
| | -LPS | +LPS | -LPS | +LPS | | Diet | LPS | Interaction |
| IL-1 (ng/l) | 264.056 ^a | 337.86 ^b | 265.722 ^a | 270.444 ^a | 12.503 | 0.022 | 0.009 | 0.017 |
| IL-6 (ng/l) | 39.711 ^a | 56.501 ^b | 40.205 ^a | 40.015 ^a | 2.927 | 0.018 | 0.015 | 0.013 |
| TNF- α (ng/l) | 284.923 ^a | 366.279 ^b | 292.615 ^a | 279.731 ^a | 15.053 | 0.022 | 0.042 | 0.009 |
| PGE ₂ (ng/l) | 410.517 ^a | 524.506 ^b | 424.095 ^a | 431.853 ^a | 17.302 | 0.041 | 0.004 | 0.01 |
| Cortisol (ug/l) | 52.789 ^a | 62.005 ^b | 52.417 ^a | 52.624 ^a | 1.741 | 0.016 | 0.019 | 0.024 |
| GH (ug/l) | 17.122 ^a | 15.465 ^b | 17.506 ^a | 17.172 ^a | 0.455 | 0.040 | 0.049 | 0.172 |
| IGF- I (ug/l) | 122.181 ^a | 110.312 ^b | 124.020 ^a | 123.529 ^a | 2.595 | 0.013 | 0.035 | 0.049 |

As indicated by Table IV, LPS-injection significantly increased the plasma PGE₂ ($P<0.01$), cortisol ($P<0.05$) concentration, as well as decreased the plasma GH ($P<0.05$) and IGF- I ($P<0.05$) concentrations. In contrast, dietary ASPS decreased PGE₂ ($P<0.05$) and cortisol ($P<0.05$) concentration as well as increased the plasma concentrations of IGF- I ($P<0.05$) and GH ($P<0.05$). Moreover, interaction between ASPS \times LPS were observed on PGE₂ ($P<0.05$), cortisol ($P<0.05$) and IGF- I ($P<0.05$) with exception of GH ($P>0.05$).

4. Discussion

In the present study, we took advantage of a well-documented model for inducing inflammatory response in pigs by injecting LPS, which is a component of the outer membrane of gram-negative bacteria and was successfully used in many experimental [7]. Inflammatory challenge is known to provoke a wide variety of physiological responses such as transient fever, depressed feed intake. In our study, the suppression of feed intake and growth of pigs observed following LPS challenge is consistent with the anorectic effects that accompany immune challenge of animals. To our knowledge, the current study is the first to report the effects of ASPS on stress livestock. In our study, LPS-treated pigs fed 800 mg/ kg ASPS had higher weight gain and feed intake compared with pigs fed control diet. Whereas, above effect was not found among saline-treated pigs, which indicate a positive effect of ASPS in stress condition. ADG improvement of LPS-treated pigs by ASPS in this research may be the result of increased food intake. However, the possible mechanism of food intake stimulation may be improved immune function by ASPS.

Lymphocyte proliferation is a crucial event in the activation cascade of cellular immune responses. Notably, lymphocyte proliferation was markedly increased by ASPS regardless of immunological state of pigs. In agreement with our findings, Chen et al (2011) showed that lymphocyte proliferation of mice spleen was enhanced by ASPS *in vitro*. These findings indicated critical role in enhancing immune function of ASPS. IgG was synthesized in spleen and lymphoid node, and existed mainly in serum and tissue fluid, which is an indicator of humoral immunity. Our results indicated that ASPS had no effect on humoral immune function of piglets regardless of immunological stress. These evidences indicated ASPS enhanced cellular immune response rather than humoral immune process.

In the current study, plasma IL-1, IL-6, TNF- α PGE₂ as well as cortisol were sharply elevated in response to LPS injection. In agreement with our findings, Wright et al (2000) showed that one of the first responses of immune stress animal is to release pro-inflammatory cytokines, which will induce an increase of PGE₂ in muscle and an activation of immune system. PGE₂ is a putative mediator of the febrile response to LPS [7]. Cortisol is also an important symbol of inflammatory secreted by hypothalamus-pituitary-adrenal [8]. Compared with control diet, lower concentrations of IL-1, IL-6, TNF- α , PGE₂ and cortisol due to ASPS among LPS-challenged pigs suggest alleviation of excessive activation of the immune system and diversion of more nutrients to support tissue growth. This may account for in part why LPS-treated pigs fed ASPS had higher performance compared with control diet.

Our study on pituitary hormone release of pigs, systemic levels of GH and IGF-I were depressed following LPS challenge. Hevener et al. (1997) research was in agreement with our findings. Wright et al. (2000) stated that decrease in IGF-I not only was the important symbol of inflammatory but was indicative of repartitioning of nutrients away from growth to immune function following LPS challenge. In our study, ASPS alleviated abnormal changes of somatotrophic axis of LPS-treated pigs, but did not elevate the levels of

GH and IGF-I of saline-injected pigs, which might be associated with the alleviation of excessive activation of immune system in immunological stress pigs, but did not affect the immune function of normal animals.

5. Conclusion

We draw two major conclusions from the present study. The first relates to anti-inflammatory function and improved immune function by ASPS supplementation in stress livestock. The second involves possible functional pathway of ASPS and notes that ASPS acts by mediating the release of pro-inflammatory cytokines and invoking pituitary hormone, prostaglandins et al, which profoundly enhances growth performance of immunological stress pigs.

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7. References

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