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Effects of Dietary Supplementation with Fermented Asparagus By-products on Serum Antioxidant Capacity, Immune Function, and Inflammatory Cytokine Levels in Sows and Suckling Piglets (Postprint)

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Abstract

This experiment aimed to investigate the effects of supplementing fermented asparagus by-products to sows during late gestation and lactation on serum antioxidant capacity, immune function, and inflammatory cytokine levels in sows and suckling piglets. Fifteen pregnant sows with similar body condition, parity, and expected farrowing dates were randomly allocated into three groups, with five replicates per group and one sow per replicate. Sows in groups A (control), B, and C were supplemented with 0, 0.25, and 0.50 kg of fermented asparagus by-products per sow per day, respectively. The trial commenced on day 85 of gestation and concluded on day 21 postpartum. Ear vein blood was collected from sows at farrowing and weaning, while anterior vena cava blood was collected from piglets at 10 and 21 days of age for determination of serum parameters. The results indicated: 1) Serum total superoxide dismutase (T-SOD) activity in group C sows was extremely significantly higher than that in the control group at weaning ($P<0.01$), while serum malondialdehyde (MDA) content was extremely significantly lower than the control group at both farrowing and weaning ($P<0.01$). Serum T-SOD activity in group B suckling piglets was significantly higher than the control group at 10 days of age ($P<0.05$), with serum MDA content being significantly lower ($P<0.05$). 2) Serum growth hormone (GH) content in group C sows was significantly higher than the control group at farrowing ($P<0.05$). 3) Serum immunoglobulin A (IgA) and immunoglobulin G (IgG) contents in group C sows were significantly higher than the control group at farrowing ($P<0.05$). 4) Serum interleukin-6 (IL-6) content in groups B and C sows was extremely significantly lower than the control group at both farrowing and weaning ($P<0.01$). Serum tumor necrosis factor-α (TNF-α) content in group C sows was significantly lower than the control group at weaning

(P<0.05). Serum TNF-α content in group C suckling piglets was significantly lower than the control group at 10 days of age (P<0.05). In conclusion, supplementation of 0.50 kg fermented asparagus by-products per day to sows can enhance their antioxidant capacity and immune function, increase GH content, reduce inflammatory responses, while simultaneously improving antioxidant capacity and reducing inflammatory responses in piglets.

Full Text

Preamble

Effects of Supplementary Feeding of Sows with Fermented Asparagus By-Product on Antioxidant Capacity, Immune Function and Contents of Inflammatory Factors in Serum of Sows and Suckling Piglets

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Abstract: This experiment was conducted to investigate the effects of supplementary feeding of fermented asparagus by-product to sows during late gestation and lactation on serum antioxidant capacity, immune function, and inflammatory factor contents in sows and suckling piglets. Fifteen pregnant sows with similar body condition, parity, and expected farrowing dates were randomly divided into three groups with five replicates per group and one sow per replicate. Sows in groups A (control), B, and C were supplemented with 0, 0.25, and 0.50 kg of fermented asparagus by-product per head per day, respectively. The trial lasted from day 85 of gestation to day 21 postpartum. Blood samples were collected from sows via ear vein at farrowing and weaning, and from piglets via anterior vena cava at 10 and 21 days of age for serum index determination. The results showed: 1) At weaning, serum total superoxide dismutase (T-SOD) activity in group C sows was extremely significantly higher than that in the control group (P<0.01), while serum malondialdehyde (MDA) content was extremely significantly lower than the control group at both farrowing and weaning (P<0.01). In group B, serum T-SOD activity in 10-day-old piglets was significantly higher than the control group (P<0.05), and serum MDA content was significantly lower (P<0.05). 2) Serum growth hormone (GH) content in group C sows at farrowing was significantly higher than the control group (P<0.05). 3) Serum immunoglobulin A (IgA) and immunoglobulin G (IgG) contents in group C sows at farrowing were significantly higher than the control group (P<0.05). 4) Serum interleukin-6 (IL-6) content in groups B and C sows was extremely significantly lower than the control group at both farrowing and weaning (P<0.01). Serum tumor necrosis factor-α (TNF-α) content in group C sows at weaning was significantly lower than the control group (P<0.05). Serum

TNF-α content in 10-day-old piglets in group C was significantly lower than the control group ($P<0.05$). In conclusion, supplementing sows with 0.50 kg of fermented asparagus by-product per day enhanced their antioxidant capacity and immune function, increased GH content, reduced inflammatory response, and simultaneously improved piglets' antioxidant capacity while reducing inflammatory response.

Keywords: fermented asparagus by-product; sows; suckling piglets; serum indexes

China is a major asparagus producer, with approximately 6.96 million tons of asparagus produced in 2015, generating about 2 million tons of asparagus by-products. Asparagus by-products are processing residues rich in vitamins, polysaccharides, essential amino acids, and bioactive compounds (saponins and flavonoids) [1-2]. Previous studies have shown that feeding asparagus by-products to lactating cattle and sheep can significantly increase milk yield and improve milk quality [3], and supplementing growing-finishing pigs with asparagus by-products can enhance daily weight gain and feed conversion ratio [4]. However, fresh asparagus by-products are difficult to preserve, have high crude fiber content, and poor palatability, limiting their widespread application in animal production. Micro-storage treatment can effectively extend the preservation time of fresh asparagus by-products and reduce their crude fiber content [5]. Our research group previously found that solid-state fermentation of asparagus by-products improved their nutritional value and palatability, and supplementation to sows during late gestation and lactation enhanced intestinal peristalsis, reduced constipation, and improved milk quality [6]. However, no studies have been reported on the effects of feeding fermented asparagus by-products on serum indices of sows and their piglets. This experiment investigated the effects of supplementary feeding of fermented asparagus by-products to sows during late gestation and lactation on serum antioxidant indices, hormone levels, immunoglobulin contents, and inflammatory factor levels in sows and suckling piglets, providing technical support for the rational use of fermented asparagus by-products in healthy sow production.

1.1 Experimental Materials

Fermented asparagus by-product: Fresh asparagus by-products were washed, crushed to approximately 3 cm pieces, and naturally dried until no juice flowed between fingers when squeezed (moisture content about 65%). Straw fiber decomposition agent (mainly composed of enzyme preparations, emulsifiers, probiotics, etc.), corn meal, and soybean meal were added at 0.1%, 5.0%, and 5.0% respectively, mixed evenly, then thoroughly blended with the dried asparagus pieces. Finally, 1.0% sodium chloride solution was sprayed and mixed again. The mixture was packed into 25 kg fermentation bags, filled to 2/3 capacity, with air removed as much as possible, sealed tightly with cable ties, and fermented at room temperature for 14 days to produce fermented asparagus by-product. The main nutrient components and contents are shown

in Table 1 .

1.2 Experimental Design

Fifteen "Landrace × Yorkshire" crossbred sows with medium body condition, 2-3 parities, and similar expected farrowing dates (within one week) were randomly divided into three groups with five replicates per group and one sow per replicate. Group A (control) was fed a basal diet only, while groups B and C were fed the basal diet supplemented with 0.25 and 0.50 kg of fermented asparagus by-product per head per day, respectively. All groups received the same daily amount of basal diet, which was formulated according to NRC (1998) standards. The composition and nutrient levels of the basal diet are shown in Table 2 .

1.3 Feeding Management

The experiment was conducted at Wanjia Pig Farm, Yaoyu Town, Xinyu City, Jiangxi Province. The formal trial lasted from day 85 of gestation to day 21 postpartum. Mortality of piglets in each group was recorded during the experimental period. Healthy piglets were weighed at birth and at 21 days of age after overnight fasting. Fermented asparagus by-product was fed twice daily, and complete feed was provided 2 hours later. A 7-day pre-trial period was conducted before the formal experiment. Other feeding management and disease prevention procedures followed the normal protocols of the pig farm.

1.4 Sample Collection

Sow blood samples: Blood (5 mL) was collected from the ear vein of sows within 24 h after farrowing and on the weaning day at 08:00 after overnight fasting. Samples were left at 4 °C for 30 min, then centrifuged at 3,000 r/min for 15 min. Serum was aspirated, aliquoted into 0.5 mL tubes, and stored at -20 °C until analysis.

Piglet blood samples: Blood was collected from the anterior vena cava of healthy piglets with similar body weight at 10 and 21 days of age at 08:00 after overnight fasting using the same method as for sows.

1.5 Measurement Indicators and Methods

Serum immunoglobulins [immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM)], inflammatory factors [interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α)], and hormones [growth hormone (GH), insulin (INS)] were measured by enzyme-linked immunosorbent assay. Serum total superoxide dismutase (T-SOD) activity was measured by the hydroxylamine method, and serum malondialdehyde (MDA) content was measured by the thiobarbituric acid (TBA) method. All kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.6 Data Processing and Statistical Analysis

Raw data were organized using Excel 2003 and analyzed by one-way ANOVA using SPSS 17.0 software. Differences among group means were tested for significance using Duncan' s multiple comparison method. Results are expressed as "mean ± standard error."

2.1 Effects of Supplementary Feeding of Sows with Fermented Asparagus By-Product on Growth Performance of Suckling Piglets

As shown in Table 3 , supplementary feeding of fermented asparagus by-product to sows during late gestation increased the average birth weight of piglets compared with the control group, but the difference was not significant ($P>0.05$). Compared with the control group, continued supplementation during lactation increased the average survival rate of piglets during lactation ($P>0.05$), thereby increasing the number of piglets weaned at 21 days of age ($P>0.05$). Continued supplementation during lactation had no significant effect on the average weaning weight of piglets at 21 days of age ($P>0.05$).

2.2 Effects of Supplementary Feeding of Sows with Fermented Asparagus By-Product on Serum Antioxidant Indices of Sows and Suckling Piglets

As shown in Table 4 , at farrowing, there was no significant difference in serum T-SOD activity among sow groups ($P>0.05$), while serum MDA content in group C sows was extremely significantly lower than that in groups A and B ($P<0.01$). At weaning, serum T-SOD activity in group C sows was extremely significantly higher than that in groups A and B ($P<0.01$), while serum MDA content was extremely significantly lower than that in group A ($P<0.01$). In 10-day-old suckling piglets, serum T-SOD activity in group B was significantly higher than that in group A ($P<0.05$), and serum MDA content was significantly lower ($P<0.05$). Serum T-SOD activity and MDA content in group C piglets showed no significant differences from the other two groups ($P>0.05$). At 21 days of age, there were no significant differences in serum T-SOD activity and MDA content among groups of suckling piglets ($P>0.05$).

2.3 Effects of Supplementary Feeding of Sows with Fermented Asparagus By-Product on Serum Hormone Contents of Sows and Suckling Piglets

As shown in Table 5 , at farrowing, serum GH content in group C sows was significantly higher than that in group A ($P<0.05$), while there was no significant difference between group B and group A ($P>0.05$). At weaning, there were no significant differences in serum GH content among sow groups ($P>0.05$). There were no significant differences in serum INS content among sow groups at either farrowing or weaning ($P>0.05$). No significant differences were observed in

serum GH and INS contents among suckling piglets at 10 and 21 days of age (P>0.05).

2.4 Effects of Supplementary Feeding of Sows with Fermented Asparagus By-Product on Serum Immunoglobulin Contents of Sows and Suckling Piglets

As shown in Table 6 , at farrowing, serum IgA content in group C sows was significantly higher than that in group A (P<0.05), and serum IgG content was significantly higher than that in groups A and B (P<0.05). Serum IgA, IgG, and IgM contents in group B showed no significant differences from group A (P>0.05). At weaning, there were no significant differences in serum IgA, IgG, and IgM contents among sow groups (P>0.05). No significant differences were observed in serum IgA, IgG, and IgM contents among suckling piglets at 10 and 21 days of age (P>0.05).

2.5 Effects of Supplementary Feeding of Sows with Fermented Asparagus By-Product on Serum Inflammatory Factor Contents of Sows and Suckling Piglets

As shown in Table 7 , at farrowing, there were no significant differences in serum IL-1β content among sow groups (P>0.05). At weaning, group C sows had the lowest serum IL-1β content, which was significantly lower than that in group B (P<0.05) but not significantly different from group A (P>0.05). Serum IL-6 content in groups B and C sows showed no significant differences at farrowing and weaning (P>0.05), but was extremely significantly lower than that in group A at both time points (P<0.01). At farrowing, there were no significant differences in serum TNF-α content among sow groups (P>0.05). At weaning, serum TNF-α content in group C sows was significantly lower than that in group A (P<0.05), while group B showed no significant difference from group A (P>0.05). In 10-day-old suckling piglets, serum TNF-α content in group B showed no significant difference from group A (P>0.05) but was significantly higher than that in group C (P<0.05). At 21 days of age, there were no significant differences in serum TNF-α content among groups of suckling piglets (P>0.05). No significant differences were observed in serum IL-6 and IL-1β contents among suckling piglets at 10 and 21 days of age (P>0.05).

3.1 Effects of Supplementary Feeding of Sows with Fermented Asparagus By-Product on Number of Weaned Piglets

The number of piglets weaned per sow per year is one of the most commonly used and important indicators for evaluating modern pig farm production performance and is a primary measure of farm economic benefits—the more weaned piglets, the higher the economic returns. Improving the survival rate of suckling piglets is a crucial measure for increasing the number of weaned piglets, and the disease resistance of suckling piglets directly affects their survival rate. Studies have shown that increasing immunoglobulin content in sow milk plays

an important role in enhancing the disease resistance of suckling piglets, and supplementary feeding of fermented asparagus by-product to lactating sows can effectively increase immunoglobulin content in colostrum and milk while reducing pro-inflammatory factor content [6]. When suckling piglets consume milk with high immunoglobulin content, it helps improve their disease resistance and survival rate, which is one of the important reasons why supplementary feeding of fermented asparagus by-product in this experiment effectively increased the number of weaned piglets.

3.2 Effects of Supplementary Feeding of Sows with Fermented Asparagus By-Product on Serum Antioxidant Indices of Sows and Suckling Piglets

T-SOD and MDA are biochemical markers reflecting the body' s antioxidant capacity. T-SOD is an important antioxidant enzyme that can scavenge endogenous free radicals and reduce damage to tissue cells caused by free radicals. MDA attacks unsaturated fatty acids on cell biomembranes, leading to lipid peroxidation and exerting toxicity on cells. Studies have shown that asparagus polysaccharides have strong free radical scavenging effects and exhibit good antioxidant activity at the levels of rat serum, cells, and organelles [7]. Asparagus flavonoids can protect T-SOD from inactivation [8], reduce MDA content [9], and repair damaged tissues and cells [10]. Ethanol extract from asparagus bases can significantly increase serum T-SOD activity in mice, and high doses can reduce serum MDA content [11]. Compared with the control group, sows supplemented with fermented asparagus by-product showed significantly increased T-SOD activity in milk and a downward trend in MDA content, with milk antioxidant substances mainly derived from blood [6]. The results of this experiment showed that supplementing sows with 0.50 kg of fermented asparagus by-product per head per day extremely significantly increased serum T-SOD activity at weaning and extremely significantly reduced serum MDA content at both farrowing and weaning, indicating that supplementary feeding of fermented asparagus by-product can improve the antioxidant capacity of sows. Antioxidant substances in sow serum can be transferred to milk, and the antioxidant content in milk may affect the antioxidant capacity of piglets. Mao et al. [6] found that supplementary feeding of fermented asparagus by-product could significantly increase T-SOD activity in sow milk and reduce MDA content. This experiment showed that supplementing sows with 0.25 kg of fermented asparagus by-product per head per day significantly increased serum T-SOD activity and reduced serum MDA content in 10-day-old suckling piglets, demonstrating that supplementary feeding of fermented asparagus by-product to sows can simultaneously improve the antioxidant capacity of both sows and suckling piglets.

3.3 Effects of Supplementary Feeding of Sows with Fermented Asparagus By-Product on Serum Hormone Contents of Sows and Suckling Piglets

GH can promote the metabolism of three major nutrients and improve the body' s ability to absorb dietary nutrients [12]. Increasing GH content in pigs can significantly improve weight gain and sow milk yield [13]. Rutin has weak estrogenic effects, and estrogen can exert indirect effects by regulating the secretion of GH and other hormones [14]. Milk-derived INS can stimulate the growth of newborn piglet small intestine and affect its tissue morphology [15]. Studies have found that supplementary feeding of fermented asparagus by-product to sows had no significant effect on GH and INS contents in milk [6]. The results of this experiment showed that serum GH content in group C sows at farrowing was significantly higher than that in the control group, indicating that supplementing sows with 0.50 kg of fermented asparagus by-product per head per day promoted GH production in sows. This may be related to the weak estrogenic function of asparagus flavonoids during farrowing, which increased GH content in sows. However, there were no significant differences in serum GH and INS contents among suckling piglets at 10 and 21 days of age, possibly because supplementary feeding of fermented asparagus by-product to sows did not change GH and INS contents in sow milk.

3.4 Effects of Supplementary Feeding of Sows with Fermented Asparagus By-Product on Serum Immunoglobulin Contents of Sows and Suckling Piglets

Immunoglobulins have antibody activity and are widely present in intracellular fluid and external secretions. They can bind to pathogenic microorganisms in the body to eliminate pathogens and reduce damage to the organism. IgG is an important immunoglobulin, accounting for about 75% of total immunoglobulins [16], and plays a vital role in maintaining animal health by neutralizing bacteria, resisting viruses, and enhancing phagocytosis of pathogens by immune cells. Studies have shown that asparagus plants contain various substances such as saponins and polysaccharides that can enhance animal immune function [8,17-18]. The results of this experiment showed that serum IgA and IgG contents in group C sows at farrowing were significantly higher than those in the control group, indicating that supplementing sows with 0.50 kg of fermented asparagus by-product per head per day can increase serum immunoglobulin content in sows and strengthen their resistance to pathogenic microorganisms.

3.5 Effects of Supplementary Feeding of Sows with Fermented Asparagus By-Product on Serum Inflammatory Factor Contents of Sows and Suckling Piglets

IL-1β, IL-6, and TNF-α are three important interleukins. IL-1β is mainly produced by macrophages, but B lymphocytes and natural killer (NK) cells can also produce it, promoting antigen presentation for antibody formation. IL-6

can stimulate B cells to produce antibodies, induce T cell proliferation and differentiation, and participate in immune response, acting as a trigger for inflammatory reactions. TNF-α is an important inflammatory mediator produced by monocytes and macrophages during inflammatory processes. Cai et al. [19] found that supplementation with flavonoids could improve inflammatory responses and reduce pro-inflammatory factor content in serum, positively affecting health. Asparagus extract can improve cellular immune capacity and increase NK cell activity in mice [20]. Asparagus flavonoids can alleviate inflammatory responses [21]. The results of this experiment showed that serum IL-6 content in groups B and C sows was extremely significantly lower than that in the control group at both farrowing and weaning, and serum TNF-α content in group C sows at weaning was significantly lower than that in the control group, indicating that supplementary feeding of fermented asparagus by-product can reduce inflammatory factor content in sow serum.

Conclusion

1. Supplementing sows with 0.50 kg of fermented asparagus by-product per head per day can significantly increase serum T-SOD activity at weaning and serum GH, IgA, and IgG contents at farrowing in sows, while significantly reducing serum MDA and IL-6 contents at farrowing and weaning and serum TNF-α content at weaning.
2. Supplementing sows with 0.25 kg of fermented asparagus by-product per head per day can significantly increase serum T-SOD activity and reduce serum MDA content in 10-day-old suckling piglets. Supplementing sows with 0.50 kg of fermented asparagus by-product per head per day can significantly reduce serum TNF-α content in 10-day-old suckling piglets.

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