Effects of Soybean Isoflavones and Astragalus Polysaccharides on Production Performance, Serum Biochemical Parameters, Immune Indices, and Milk Composition in Lactating Sows: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary supplementation of soybean isoflavones (SI) and Astragalus polysaccharides (ASP) on the performance, serum biochemical and immune indices, and milk composition of lactating sows. A single-factor experimental design was adopted. Seventy-two healthy second-parity "Landrace × Large White" crossbred sows with good body condition and similar farrowing age were selected and randomly divided into 4 groups, with 6 replicates per group and 3 sows per replicate. SI and ASP were mixed at a ratio of 1:5. The control group was fed a basal diet without SI and ASP supplementation, while the experimental groups were supplemented with the SI and ASP mixture at 100, 200, and 300 mg/kg to the basal diet, respectively. The experimental period lasted 21 d. The results showed that: 1) The average daily feed intake of lactating sows in the 100 and 200 mg/kg groups was significantly higher than that of the control group ($P<0.05$), and the total 21-day milk yield of the 200 mg/kg group was significantly higher than that of the control group ($P<0.05$). 2) The serum total protein content of the 200 mg/kg group was significantly higher than that of the control group ($P<0.05$). The serum urea nitrogen content of all experimental groups was significantly lower than that of the control group ($P<0.05$). The serum triglyceride content of the 100 and 200 mg/kg groups was significantly lower than that of the control group ($P<0.05$). The serum immunoglobulin G and interleukin-2 contents of all experimental groups were significantly higher than those of the control group ($P<0.05$). The serum immunoglobulin A content of the 200 and 300 mg/kg groups was significantly higher than that of the control group ($P<0.05$). 3) The milk fat percentage and milk protein content of all experimental groups were

significantly higher than those of the control group (P<0.05). The lactose percentage of the 300 mg/kg group was significantly higher than that of the control group and the other two experimental groups (P<0.05). The lactose percentage of the 100 and 200 mg/kg groups was also higher than that of the control group, but the difference was not significant (P>0.05). It can be concluded that dietary supplementation of SI and ASP can improve the performance, serum biochemical and immune indices, and milk composition of lactating sows. Under the conditions of this experiment, the appropriate supplementation level of SI and ASP was 200 mg/kg.

Full Text

Effects of Soybean Isoflavone and Astragalus Polysaccharide on Production Performance, Serum Biochemical and Immune Indexes, and Milk Composition of Lactating Sows

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Abstract

This trial was conducted to investigate the effects of dietary supplementation with soybean isoflavone (SI) and astragalus polysaccharide (ASP) on production performance, serum biochemical and immune indexes, and milk composition of lactating sows. Using a single-factor experimental design, 72 healthy "Landrace × Large White" crossbred sows in their second parity with good body condition and similar farrowing dates were randomly allocated into 4 groups, with 6 replicates per group and 3 sows per replicate. SI and ASP were mixed at a ratio of 1:5. The control group was fed a basal diet without SI and ASP supplementation, while the experimental groups were fed the basal diet supplemented with 100, 200, and 300 mg/kg of the SI and ASP mixture, respectively. The experimental period lasted 21 days. The results showed: 1) The average daily feed intake of lactating sows in the 100 and 200 mg/kg groups was significantly higher than that of the control group (P<0.05), and the 21-day total milk yield of the 200 mg/kg group was significantly higher than that of the control group (P<0.05). 2) The serum total protein content of the 200 mg/kg group was significantly higher than that of the control group (P<0.05), the serum urea nitrogen content of all experimental groups was significantly lower than that of the control group (P<0.05), the serum triglyceride content of the 100 and 200 mg/kg groups was significantly lower than that of the control group (P<0.05), the serum immunoglobulin G and interleukin-2 contents of all experimental groups were significantly higher than those of the control group (P<0.05), and the serum immunoglobulin A content of the 200 and 300 mg/kg groups was

significantly higher than that of the control group (P<0.05). 3) The milk fat percentage and milk protein content of all experimental groups were significantly higher than those of the control group (P<0.05); the lactose percentage of the 300 mg/kg group was significantly higher than that of the control group and the other two experimental groups (P<0.05), while the lactose percentage of the 100 and 200 mg/kg groups was also higher than that of the control group but the difference was not significant (P>0.05). In conclusion, dietary supplementation with SI and ASP can improve the production performance, serum biochemical and immune indexes, and milk composition of lactating sows. Under the conditions of this experiment, the appropriate supplementation level of SI and ASP is 200 mg/kg.

Keywords: soybean isoflavone; astragalus polysaccharide; lactating sows; production performance; serum biochemical and immune indexes; milk composition

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Modern breeding practices have intensified sow selection to improve reproductive performance, resulting in increased litter sizes. Consequently, the production performance and milk yield of lactating sows must also continuously improve to meet the growth demands of piglets. Since the primary nutrition and early immune protection for nursing piglets are derived from sow' s milk, regulating early piglet growth through milk composition is crucial for achieving optimal production performance. Soybean isoflavone (SI), also known as plant estrogen, is a flavonoid compound formed as a secondary metabolite during soybean growth and represents a biologically active substance. Astragalus polysaccharide (ASP), composed of hexuronic acid, glucose, fructose, rhamnose, arabinose, galacturonic acid, and glucuronic acid, exhibits antiviral, antitumor, anti-aging, anti-radiation, anti-stress, and antioxidant properties. Both SI and ASP are plant extracts with multiple biological activities that can enhance animal immunity and reproductive performance, promote mammary gland development, increase milk secretion, and are characterized by being non-toxic and residue-free. As a new generation of feed additives, they meet the requirements for safe production. Britt et al. demonstrated that dietary supplementation with appropriate levels of SI increased uterine and follicle weights in female rats while elevating follicle-stimulating hormone and gonadotropin levels. Huang et al. reported that ASP supplementation in tilapia diets increased intestinal villus length, muscular layer thickness, and crypt depth, while also increasing the number of intestinal mucous cells and intraepithelial lymphocytes. Although numerous studies have investigated SI and ASP individually, no reports have examined their combined effects. Therefore, this trial investigated the combined application of SI and ASP in lactating sows to determine whether they could further improve overall production performance, using changes in production performance, serum biochemical and immune indexes, and milk composition as evaluation criteria to provide a theoretical basis for practical application.

1.1 Experimental Materials

SI with a purity of 40.35% (CAS No. 574-12-9) and ASP with a purity of 55% (CAS No. 9005-38-3) were purchased from Shaanxi Haoyang Biological Technology Co., Ltd. Lactating sows ("Landrace × Large White" crossbred) were provided by Harbin Da Dongbei Animal Husbandry Group. Enzyme-linked immunosorbent assay kits for total protein, albumin, urea nitrogen, glucose, triglycerides, immunoglobulin G, immunoglobulin A, and interleukin-2 were purchased from Shanghai Haling Biological Technology Co., Ltd.

1.2 Experimental Design and Management

Seventy-two second-parity "Landrace × Large White" crossbred sows with similar genetic background, good health status, and close farrowing dates were randomly divided into 4 groups with 6 replicates per group and 3 sows per replicate. The control group was fed a basal diet without SI and ASP, while the experimental groups were fed the basal diet supplemented with 100, 200, and 300 mg/kg of the SI and ASP mixture (SI:ASP = 1:5, formulated based on the optimal individual supplementation levels for sows). All experimental sows were housed in the same building under identical management conditions, with ad libitum access to feed and water, and routine immunization protocols. Piglets were raised in elevated farrowing crates with heating pads. Sows began receiving the experimental diets after farrowing, and the trial lasted 21 days, with piglets weaned at 21 days of age.

1.3 Experimental Diets

Diet formulation complied with the "Feeding Standard of Swine" published by the Ministry of Agriculture in 2011. The composition and nutrient levels of the basal diet are presented in Table 1 . The premix provided the following per kg of diet: VA 20,000 IU, VD 2,000 IU, VE 60 IU, VK 2 mg, VB1 10 mg, pantothenic acid 20 mg, nicotinic acid 50 mg, VB6 5 mg, VB12 40 g, folic acid 1.5 mg, biotin 0.15 mg, VC 200 mg, choline chloride 600 mg, Mn 75 mg, Fe 140 mg, Cu 8 mg, I 0.4 mg, Se 0.3 mg. Metabolizable energy was a calculated value, while other nutrients were measured values.

1.4 Measurements

1.4.1 Production Performance Daily feed intake was recorded from the start of the experiment to calculate average daily feed intake. Litter weight at birth was recorded, and individual piglet weights were measured on days 1 and 21 to calculate average daily gain. The 21-day total milk yield was estimated based on the conversion that 1 kg of piglet weight gain requires approximately 3 kg of milk. Fecal consistency and mortality were observed daily to calculate piglet diarrhea rate and mortality using the following formulas: Diarrhea rate (%) = [(number of diarrheic piglets × days of diarrhea) / (total number of

piglets × experimental days)] × 100; Mortality (%) = (number of dead piglets / total number of piglets) × 100.

1.4.2 Serum Biochemical and Immune Indexes On day 2 after the experiment ended, 10 mL of blood was collected from each sow's ear marginal vein into procoagulant vacuum tubes at 07:00, allowed to clot for 15 minutes, then centrifuged at 3,000 r/min for 30 minutes. The serum supernatant was collected and stored at -20°C for analysis. Serum total protein, albumin, urea nitrogen, glucose, triglycerides, immunoglobulin G, immunoglobulin A, and interleukin-2 were measured by enzyme-linked immunosorbent assay according to kit instructions.

1.4.3 Milk Composition Analysis On day 11 of lactation, 30 mL of milk samples were collected from the anterior, middle, and posterior mammary glands of each sow, mixed thoroughly, and stored at -20°C for analysis. Milk lactose percentage, fat percentage, and protein content on day 11 were measured using an infrared milk analyzer (Milko-Scan 134 A/B, Foss, Denmark).

1.5 Statistical Analysis

Experimental data were analyzed by one-way ANOVA using SPSS 20.0 statistical software, with Duncan' s multiple comparison test applied when significant differences were detected. Results are expressed as "mean ± standard deviation." Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$.

2.1 Effects of SI and ASP on Production Performance of Lactating Sows

As shown in Table 2 , the average daily feed intake of lactating sows in the 100 and 200 mg/kg groups was significantly higher than that of the control group ($P<0.05$). No significant differences in average daily gain of piglets were observed among groups ($P>0.05$). The 21-day total milk yield of the 200 mg/kg group was significantly higher than that of the control group ($P<0.05$), while the 100 and 300 mg/kg groups showed higher values than the control group without significant differences ($P>0.05$). Piglet diarrhea rate and mortality did not differ significantly among groups ($P>0.05$).

2.2 Effects of SI and ASP on Serum Biochemical and Immune Indexes of Lactating Sows

As shown in Table 3 , the serum total protein content of the 200 mg/kg group was significantly higher than that of the control group ($P<0.05$), while the 100 and 300 mg/kg groups showed higher values without significant differences ($P>0.05$). Serum albumin content did not differ significantly among groups

(P>0.05). Serum urea nitrogen content in all experimental groups was significantly lower than that of the control group (P<0.05), showing a decreasing trend with increasing SI and ASP supplementation levels. Serum glucose content did not differ significantly among groups (P>0.05). The serum triglyceride content of the 100 and 200 mg/kg groups was equal and significantly lower than that of the control group (P<0.05), while the 300 mg/kg group also showed lower values without significant difference (P>0.05). Serum immunoglobulin G content in all experimental groups was significantly higher than that of the control group (P<0.05), with the highest value observed in the 200 mg/kg group. Serum immunoglobulin A content of the 200 and 300 mg/kg groups was significantly higher than that of the control group (P<0.05), while the 100 mg/kg group showed higher values without significant difference (P>0.05). Serum interleukin-2 content in all experimental groups was significantly higher than that of the control group (P<0.05), with the highest value observed in the 200 mg/kg group.

2.3 Effects of SI and ASP on Milk Composition of Lactating Sows

As shown in Table 4 , the milk fat percentage of all experimental groups was significantly higher than that of the control group (P<0.05), with the 200 and 300 mg/kg groups significantly higher than the 100 mg/kg group (P<0.05), showing an upward trend with increasing supplementation levels. The lactose percentage of the 300 mg/kg group was significantly higher than that of the control group and the other two experimental groups (P<0.05), while the 100 and 200 mg/kg groups showed higher values than the control group without significant differences (P>0.05). Milk protein content of all experimental groups was significantly higher than that of the control group (P<0.05), with the 200 and 300 mg/kg groups significantly higher than the 100 mg/kg group (P<0.05), also showing an upward trend with increasing supplementation levels.

3.1 Effects of SI and ASP on Production Performance of Lactating Sows

The results indicate that dietary supplementation with SI and ASP significantly improved feed intake and milk yield of lactating sows. SI can influence the gonadal axis in lactating sows, promoting testosterone and prolactin production and release, thereby increasing milk yield and reducing piglet mortality. ASP demonstrates synergistic effects when combined with other agents for improving performance; Ling et al. reported that feeding ASP and probiotics to piglets increased survival rate by 20.0%, average daily gain by 8.57%, and reduced diarrhea rate by 20.1%. In this trial, the combination of ASP and SI improved production performance of lactating sows and average daily gain of piglets, while showing a trend toward reduced diarrhea and mortality rates.

The results also indicate that excessive supplementation levels of SI and ASP are not beneficial, with 200 mg/kg being optimal in this trial, while 300 mg/kg tended to reduce production performance. Takashima-Sasaki et al. found that

feeding high levels of SI to pregnant rats resulted in binding to estrogen receptor β, reducing production performance and even causing offspring mortality. SI exhibits both anti-estrogenic and weak estrogenic effects, showing estrogen-like activity when endogenous estrogen levels are low by binding to estrogen receptors, but demonstrating anti-estrogenic effects when endogenous estrogen levels are high by competitively binding to receptors and reducing sex hormone bioactivity. Additionally, Hou et al. reported that adding 300 g/t ASP powder to late-gestation sow diets effectively improved production performance. The trend toward reduced performance in the 300 mg/kg group in this trial may be attributed to an excessive proportion of SI in the SI-ASP mixture. Therefore, appropriate supplementation levels of SI and ASP should be used to improve lactating sow production performance.

3.2 Effects of SI and ASP on Serum Biochemical and Immune Indexes of Lactating Sows

Serum biochemical indexes are primary indicators of animal metabolism. The results demonstrate that SI and ASP significantly increased serum total protein content in lactating sows. Total protein provides a favorable internal environment for further protein synthesis, thereby promoting protein synthesis and animal growth. The results also show that serum albumin content tended to increase with SI and ASP supplementation levels from 0 to 200 mg/kg but showed a decreasing trend at excessive supplementation levels.

Serum urea nitrogen content accurately reflects protein metabolism and amino acid balance in animals, with the lowest values indicating optimal amino acid balance and metabolic satisfaction. In this trial, serum urea nitrogen content in all experimental groups was significantly lower than that of the control group, showing a decreasing trend with increasing SI and ASP supplementation levels. Previous studies reported that ASP and SI have hypoglycemic effects and can bidirectionally regulate blood glucose, reducing blood glucose levels in diabetic rats, enhancing insulin levels, and alleviating endothelial cell damage and dysfunction. Liao and Wei demonstrated that SI and ASP reduced serum glucose content and improved vascular function in diabetic rats. This trial showed similar results, with SI and ASP significantly reducing serum glucose content in lactating sows. Triglycerides are the main component of body fat, and elevated levels can lead to lipemia. This trial demonstrated that SI and ASP supplementation significantly reduced serum triglyceride content in sows, with the most significant effect observed at 200 mg/kg.

Lamm reported that immunoglobulin A captures pathogens entering the mucosal layer by neutralizing pathogens within the mucosal epithelium and forming immune complexes in mucosal connective tissue that are excreted by epithelial cells. Immunoglobulin G is the primary immunoglobulin in serum, and its antibodies can prevent corresponding antigens from penetrating mucosal tissues. This trial showed that dietary supplementation with SI and ASP significantly increased serum immunoglobulin G and immunoglobulin A contents, thereby

enhancing immunity. Interleukin-2 promotes proliferation and differentiation of T cells, B cells, and natural killer cells, stimulates secretion of cytokines such as interferon and tumor necrosis factor, and improves cellular and humoral immune function. In this trial, serum interleukin-2 content in all experimental groups was significantly higher than that of the control group, indicating that SI and ASP significantly enhanced immune response capacity and maintained healthy status.

3.3 Effects of SI and ASP on Milk Composition of Lactating Sows

The results demonstrate that milk fat percentage and milk protein content in all experimental groups were significantly higher than those of the control group, showing an upward trend with increasing SI and ASP supplementation levels. Additionally, lactose percentage in experimental groups increased with supplementation levels, with only the 300 mg/kg group showing significant difference from the control group. Sow milk yield is the most critical factor affecting piglet growth, and sow milk is the direct source of energy and protein for nursing piglets, with milk composition changes directly affecting piglet growth and development. Li et al. found that increasing dietary SI supplementation levels increased milk fat percentage and lactose percentage, with milk protein content higher than the control group, results similar to this trial. Reports indicate that SI and ASP affect milk yield by regulating related hormone levels; ASP influences mammary cell growth through the growth hormone and insulin-like growth factor-1 axis, while estradiol promotes whey protein synthesis in mouse and dairy cow mammary tissue cultures and increases blood levels of sex hormone-binding globulin and transferrin, effects also induced by SI.

Dietary supplementation with SI and ASP can improve production performance, serum biochemical and immune performance, and milk composition of lactating sows. Under the conditions of this experiment, the appropriate supplementation level of SI and ASP is 200 mg/kg.

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Note: Figure translations are in progress. See original paper for figures.