Effects of Yucca Extract on Growth Performance, Serum Antioxidant and Immune Indices, Fecal Nitrogen and Phosphorus Emissions, and Microbial Count in Weaned Piglets (Postprint)

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Abstract

This experiment aimed to investigate the effects of yucca extract supplementation on growth performance, serum antioxidant and immune indices, nitrogen and phosphorus emissions in feces, and microbial count in weaned piglets, in order to determine the appropriate supplementation level of yucca extract in piglet diets. A total of 120 healthy 28-day-old weaned "Duroc × Landrace × Large White" piglets with a body weight of (8.00±0.46) kg were selected and randomly allocated into 5 groups, with 4 replicates per group and 6 piglets per replicate. Group I served as the control group and was fed a basal diet; groups II–V were fed the basal diet supplemented with 90, 120, 150, and 180 mg/kg yucca extract, respectively. The experimental period lasted 28 days. The results showed that: 1) Compared with the control group, group III exhibited extremely significant increases in average daily gain (ADG) and average daily feed intake (ADFI) ($P<0.01$), and a significant decrease in feed-to-gain ratio (F/G) ($P<0.05$); groups III, IV, and V showed significant or extremely significant reductions in diarrhea rate ($P<0.05$ or $P<0.01$). 2) Compared with the control group, group III displayed significant increases in serum catalase (CAT), superoxide dismutase (SOD) activities, and total antioxidant capacity (T-AOC) ($P<0.05$). No significant differences were observed among groups in serum glutathione peroxidase (GSH-Px) activity and malondialdehyde (MDA) content ($P>0.05$). 3) Compared with the control group, group III showed a significant increase in serum nitric oxide (NO) content ($P<0.05$), and extremely significant increases in nitric oxide synthase (NOS) and inducible nitric oxide synthase (iNOS) activities ($P<0.01$); groups III, IV, and V exhibited significant increases in serum immunoglobulin G (IgG) content ($P<0.05$). 4) Compared with the control group, group III demonstrated a significant increase in thymus index

(P<0.05). No significant differences were found among groups in spleen index (P>0.05). 5) Compared with the control group, group IV showed a significant reduction in total nitrogen content in feces (P<0.05), and groups II, III, and IV displayed significant decreases in ammonia nitrogen content in feces (P<0.05). No significant differences were observed among groups in total phosphorus content in feces (P>0.05). 6) Compared with the control group, groups III and IV exhibited significant reductions in Escherichia coli count in feces (P<0.05). No significant differences were found among groups in total aerobic bacteria and Lactobacillus counts (P>0.05). In conclusion, under the conditions of this experiment, the recommended appropriate supplementation level of yucca extract in piglet diets is 120 mg/kg.

Full Text

Effects of Yucca schidigera Extract on Growth Performance, Serum Antioxidant and Immune Indexes, Fecal Nitrogen and Phosphorus Emission and Microorganism Number of Weaner Piglets

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Abstract: This experiment was conducted to investigate the effects of Yucca schidigera extract on growth performance, serum antioxidant and immune indexes, fecal nitrogen and phosphorus emission, and microorganism number in weaner piglets, and to determine the optimal supplemental level of Yucca schidigera extract in piglet diets. A total of 120 healthy 28-day-old weaner piglets (Duroc × Landrace × Large) with an initial body weight of (8.00±0.46) kg were randomly allocated into five groups with four replicates per group and six piglets per replicate. Group I served as the control and was fed a basal diet, while groups II through V were fed the basal diet supplemented with 90, 120, 150, and 180 mg/kg Yucca schidigera extract, respectively. The experimental period lasted 28 days. The results showed: 1) Compared with the control group, group III exhibited extremely significant increases in average daily gain (ADG) and average daily feed intake (ADFI) (P<0.01) and a significant reduction in feed to gain ratio (F/G) (P<0.05). The diarrhea rate in groups III, IV, and V was significantly or extremely significantly reduced (P<0.05 or P<0.01). 2) Serum catalase (CAT), superoxide dismutase (SOD) activity, and total antioxidant capacity (T-AOC) in group III were significantly higher than those in the control group (P<0.05). No significant differences were observed among groups in serum glutathione peroxidase (GSH-Px) activity or malondialdehyde (MDA) content (P>0.05). 3) Serum nitric oxide (NO) content in group III

was significantly increased (P<0.05), while nitric oxide synthase (NOS) and inducible nitric oxide synthase (iNOS) activities were extremely significantly elevated (P<0.01). Serum immunoglobulin G (IgG) content in groups III, IV, and V was significantly higher than that in the control group (P<0.05). 4) The thymus index in group III was significantly increased compared with the control group (P<0.05), though no significant differences were found in spleen index among all groups (P>0.05). 5) Fecal total nitrogen content in group IV was significantly reduced compared with the control group (P<0.05), while fecal ammonia nitrogen content in groups II, III, and IV was significantly decreased (P<0.05). No significant differences were observed among groups in fecal total phosphorus content (P>0.05). 6) Fecal Escherichia coli counts in groups III and IV were significantly lower than those in the control group (P<0.05), while no significant differences were found among groups in total aerobic bacteria or Lactobacillus counts (P>0.05). In conclusion, under the conditions of this experiment, the optimal supplemental level of Yucca schidigera extract in piglet diets is recommended to be 120 mg/kg.

Keywords: Yucca schidigera extract; weaner piglets; growth performance; immune index; antioxidant index; nitrogen and phosphorus

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The production of nursery piglets constitutes a critical component of swine production, with environmental conditions and feed additives being key factors influencing piglet growth performance [1]. Research has demonstrated that ammonia concentrations reaching 100 μg/kg in pig housing can exert extremely significant effects on porcine growth and development [2-3], making it imperative to identify feed additives that can simultaneously enhance piglet growth performance while reducing harmful gas emissions. Yucca schidigera extract, derived from effective components of Yucca plants, serves as a feed additive capable of reducing animal ammonia emissions, regulating intestinal microenvironment, promoting cellular nutrition, and improving animal immunity [4]. Cheeke et al. [5] reported that the primary active components of Yucca schidigera extract include saponins, polysaccharides, and yucca phenols. Ding Yongmin et al. [6] identified three potential mechanisms for saponins in Yucca schidigera extract: 1) inhibiting proliferation of harmful bacteria and reducing their populations to maintain optimal ratios between beneficial and harmful microorganisms; 2) enhancing nutrient absorption and feed utilization by altering the morphology of digestive tract epithelial cell membranes and reducing surface tension; and 3) improving animal immune function through immunomodulatory properties. Johnston et al. [7] investigated various livestock species and found that dietary supplementation with Yucca schidigera extract not only reduced concentrations of ammonia and hydrogen sulfide in animal housing but also improved feed utilization and average daily gain (ADG). Wang Li et al. [8] observed that Yucca schidigera extract supplementation decreased pig house ammonia concentrations by 33%-46%, increased lactating sow feed intake by an average of 0.5 kg/d, and significantly reduced the number of weak piglets. Min et al. [9]

reported that dietary Yucca schidigera extract improved carcass length, grade, backfat thickness, and loin eye area in pigs. Wang Baoli et al. [10] found that feeding pigs diets containing Yucca schidigera extract reduced ammonia production from excreta and decreased environmental pollution from swine operations, with an optimal addition level of 90 g/t. Colina et al. [11] investigated dietary manipulation to reduce ammonia concentrations in nursery facilities and demonstrated that feeding weaner piglets diets containing 125 mg/kg Yucca schidigera extract significantly reduced ammonia concentrations compared with the basal diet group. The present experiment was designed to investigate the effects of different supplemental levels of Yucca schidigera extract on piglet growth performance, serum antioxidant and immune indexes, fecal nitrogen and phosphorus emission, and microorganism numbers, thereby providing a scientific basis for determining the optimal supplemental level of Yucca schidigera extract in piglet diets.

1.1 Experimental Materials

The Yucca schidigera extract used in this experiment was "Furongbao Natural Steroidal Saponin" produced by DPI Distribution & Processing Inc., USA, with active components comprising total soluble solids 30%, saponins 10.5%, and a B50 value 12 mg. The saponin content, which should not be less than 10.5%, is critical as many functions of Yucca schidigera extract are associated with saponins. The B50 value, determined by nitrophenol cyanide analysis, measures the ammonia-binding capacity of Yucca schidigera extract and represents the weight of extract required to bind 50% of ammonia.

1.2 Experimental Design

A total of 120 healthy 28-day-old weaner piglets (Duroc × Landrace × Large) with an initial body weight of (8.00±0.46) kg were randomly divided into five groups with four replicates per group and six piglets per replicate (half male and half female). Group I served as the control and received a basal diet, while experimental groups II through V received the basal diet supplemented with 90, 120, 150, and 180 mg/kg Yucca schidigera extract, respectively. The experimental period lasted 28 days.

1.3 Experimental Diets

The experimental diets were formulated according to the feeding standards recommended by NRC (1998). The composition and nutrient levels of the basal diet are presented in . The premix provided the following per kilogram of diet: VA 270,000 IU, VC 298 mg, VD3 1,700 IU, VE 43.75 mg, VK3 3.12 mg, VB1 1.87 mg, VB2 6.25 mg, VB6 12 mg, VB12 0.023 mg, nicotinic acid 25 mg, calcium pantothenate 15 mg, folic acid 0.65 mg, biotin 0.4 mg, choline 600 mg, Fe (as ferrous sulfate) 130 mg, Cu (as copper sulfate) 12 mg, Zn (as zinc sulfate) 120 mg, Mn (as manganese sulfate) 50 mg, Ca (as calcium sulfate) 10.35 mg,

Se (as sodium selenite) 0.16 mg, and Co (as cobalt sulfate) 0.1 mg. Digestible energy was a calculated value, while other nutrients were measured values.

1.4 Animal Management

The experiment was conducted in a fully enclosed pig house with piglets raised on nursery pens with ad libitum access to feed and water. Routine immunization and disinfection procedures were followed according to standard farm management protocols. The health status of piglets in each pen was observed daily at 08:00, 12:00, 16:00, and 20:00.

1.5 Sample Collection and Processing

Serum: On day 29 of the experiment at 07:00, six piglets from each group with body weight close to the group average were selected for blood collection (10 mL) via anterior vena cava puncture. Serum was separated by centrifugation at 3,000 r/min for 10 minutes and stored at -20°C until analysis.

Feces: For three consecutive mornings before the end of the experiment, approximately 40 g of fresh feces were collected from four healthy piglets randomly selected from each replicate, then mixed by replicate and stored in sterile self-sealing bags at -20°C.

Tissue Samples: On the morning of day 29, three piglets from each group with body weight close to the group average were euthanized via ear vein injection of chlorpromazine hydrochloride (3 mg/kg BW). The abdominal cavity was opened to remove the spleen and thymus, which were weighed after blotting blood with filter paper and removing fat.

1.6 Measurement Indicators and Methods

1.6.1 Growth Performance

All piglets were fasted and weighed at 08:00 on day 1 and day 29 to determine initial and final body weights for calculation of average daily gain. Feed intake was recorded by replicate to calculate average daily feed intake (ADFI) and feed to gain ratio (F/G). Diarrhea incidence was observed and recorded throughout the experiment to calculate diarrhea rate using the formula: Diarrhea rate (%) = 100 × cumulative number of diarrheic piglets / (total number of piglets × experimental days).

1.6.2 Serum Antioxidant Indexes

Serum superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) activities, and malondialdehyde (MDA) content were determined using assay kits purchased from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.6.3 Immune Organ Index

Immune organ index (g/kg) = immune organ weight / pre-slaughter live body weight.

1.6.4 Serum Immune Indexes

Serum nitric oxide (NO), immunoglobulin G (IgG) content, nitric oxide synthase (NOS), and inducible nitric oxide synthase (iNOS) activities were determined using assay kits purchased from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions.

1.6.5 Fecal Nitrogen and Phosphorus Emission and Microorganism Number

Fecal nitrogen and phosphorus emissions and microorganism numbers were determined using the method described by Tian Lixin [12]. Total nitrogen (TN) content was measured by the Kjeldahl method according to GB/T 6432−1994, and total phosphorus (TP) content was measured according to GB/T 6437.

1.6.6 Fecal Microorganism Number

Fecal microorganism counts were determined using the method described by Tian Lixin [12].

1.7 Data Processing and Statistical Analysis

Data were analyzed using SPSS 17.0 statistical software for one-way ANOVA and Duncan's multiple comparison tests. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$. Results are expressed as "mean ± standard deviation."

2.1 Effects of Yucca schidigera Extract on Growth Performance of Piglets

As shown in , the ADG and ADFI of piglets in groups II, III, IV, and V were higher than those in group I. For ADG, group III showed an extremely significant increase of 19.43% compared with group I ($P<0.01$), while groups II, IV, and V exhibited increases of 6.61%, 8.81%, and 6.88%, respectively, which were not statistically significant ($P>0.05$). Group III was significantly higher than groups II and V by 12.02% and 11.74%, respectively ($P<0.05$), but did not differ significantly from group IV ($P>0.05$). For ADFI, group III showed an extremely significant increase of 14.24% compared with group I ($P<0.01$), while group IV exhibited a significant increase of 11.37% ($P<0.05$). No significant differences were observed between group III and groups II, IV, or V ($P>0.05$). The F/G ratio was lowest in group III, which was significantly lower than all other groups ($P<0.05$), while no significant differences were found among groups I, II, IV, and V ($P>0.05$). Regarding diarrhea rate, groups IV and V showed extremely significant reductions of 57.32% and 53.48%, respectively, compared with group I ($P<0.01$), while group III exhibited a significant reduction of 42.85% ($P<0.05$). Group II showed a non-significant reduction of 28.59% ($P>0.05$).

2.2 Effects of Yucca schidigera Extract on Serum Antioxidant Indexes of Piglets

As presented in , serum CAT activity in groups II, III, IV, and V increased by 4.96%, 8.06%, 6.13%, and 4.72%, respectively, compared with group I, with all differences being significant ($P<0.05$). Serum SOD activity in group III was significantly higher than that in group I by 6.89% ($P<0.05$). No significant differences were observed among groups in serum MDA content or GSH-Px activity ($P>0.05$). Serum T-AOC in groups II, III, IV, and V increased by 6.17%, 9.52%, 6.85%, and 5.41%, respectively, compared with group I, with all differences being significant ($P<0.05$).

2.3 Effects of Yucca schidigera Extract on Serum Immune Indexes of Piglets

As shown in , serum IgG content in groups III, IV, and V increased by 6.80%, 7.77%, and 5.83%, respectively, compared with group I, with all differences being significant ($P<0.05$). Serum NO content in groups II, III, IV, and V increased by 3.03%, 17.91%, 4.34%, and 8.66%, respectively, with only group III showing a significant difference from group I ($P<0.05$). Serum NOS activity in group III was extremely significantly higher than that in group I by 26.42% ($P<0.01$), while group IV showed a significant increase of 13.40% ($P<0.05$). Serum iNOS activity in group III was extremely significantly higher than that in group I by 17.43% ($P<0.01$), while group IV exhibited a significant increase of 10.22% ($P<0.05$).

2.4 Effects of Yucca schidigera Extract on Immune Organ Index of Piglets

As indicated in , group III showed a significant increase in thymus index compared with group I ($P<0.05$), while groups II, IV, and V also exhibited increased thymus indices without significant differences ($P>0.05$). All experimental groups demonstrated increased spleen indices compared with group I, though no significant differences were observed ($P>0.05$).

2.5 Effects of Yucca schidigera Extract on Fecal Nitrogen and Phosphorus Emission of Piglets

As demonstrated in , group IV exhibited the lowest fecal total nitrogen content, which was significantly reduced by 4.73% compared with group I ($P<0.05$). Groups II, III, and V also showed lower values than group I, but without significant differences ($P>0.05$). Fecal ammonia nitrogen content in groups II, III, and IV was significantly lower than that in group I ($P<0.05$), with reductions of 14.71%, 17.65%, and 13.24%, respectively. Group V also showed a reduction, though not statistically significant ($P>0.05$). All experimental groups exhibited reduced fecal total phosphorus content compared with group I, but no significant differences were observed ($P>0.05$).

2.6 Effects of Yucca schidigera Extract on Fecal Microorganism Number of Piglets

As illustrated in , total aerobic bacteria counts in groups II, III, IV, and V were lower than that in group I, but without significant differences (P>0.05). Fecal Escherichia coli counts in groups III and IV were significantly lower than that in group I (P<0.05), with reductions of 4.26% and 4.81%, respectively. Groups II and V also showed reductions, though not statistically significant (P>0.05). All experimental groups exhibited higher Lactobacillus counts compared with group I, but without significant differences (P>0.05).

3.1 Effects of Yucca schidigera Extract on Growth Performance of Piglets

Average daily gain, average daily feed intake, and feed to gain ratio are the three primary indicators for evaluating piglet growth performance. The present experimental data demonstrate that dietary supplementation with Yucca schidigera extract can improve ADG and ADFI while reducing F/G in piglets. Zheng Guangyao [13] reported that Yucca schidigera extract not only enhances piglet growth performance but also significantly reduces odor concentrations in pig farms. Luo Xianglin et al. [14] investigated replacement gilts and found that supplementation with 40 mg/kg Yucca schidigera extract increased ADG by 6.2% and decreased F/G by 3.6% compared with the control group, while effectively reducing the incidence of conjunctivitis in breeding pigs, which aligns with the results of the current study. Gao Jianzhong et al. [15] reported that piglets typically develop diarrhea 3-4 days post-weaning, with diarrhea frequency peaking at approximately one week post-weaning and reaching maximum litter diarrhea frequencies of up to 32.4%, before gradually declining after two weeks. The present results are consistent with these findings, showing that diarrhea rates peaked during days 4-7 of the experiment and stabilized after day 12, with all treatment groups exhibiting lower diarrhea rates than the control group.

3.2 Effects of Yucca schidigera Extract on Serum Antioxidant Indexes of Piglets

Serum GSH-Px, SOD, and CAT activities, MDA content, and T-AOC are important indicators reflecting the antioxidant capacity of the organism [15-16]. Liu Ruxiang et al. [17] and Fan Shijun et al. [18] demonstrated that serum MDA content reflects the degree of lipid peroxidation in the body and can indirectly indicate the extent of cellular damage, with higher MDA levels indicating greater cellular injury and lower levels indicating less damage. T-AOC serves as a comprehensive indicator for measuring overall antioxidant capacity [19]. Wan Shanxia et al. [20] reported that GSH-Px exists in four different types, with serum GSH-Px being the most commonly used. Xu Xianxiang et al. [21] demonstrated that saponins in Yucca schidigera extract possess antioxidant properties, while Li Guang et al. [22] reported that saponins have immunomodulatory effects. The present results indicate that dietary supplementation with Yucca

schidigera extract can increase serum T-AOC and CAT, SOD, and GSH-Px activities while reducing serum MDA content in piglets, suggesting that Yucca schidigera extract can enhance serum antioxidant capacity under the experimental conditions.

3.3 Effects of Yucca schidigera Extract on Serum Immune Indexes of Piglets

IgG exhibits antimicrobial and antiviral immunological activities [23]. Guo Junqing [24] reported that serum IgG content is positively correlated with humoral immune function. Liu Liru et al. [25] demonstrated that IgG is the primary antibody against infection and can activate complement through the classical pathway, producing antibody-dependent cell-mediated cytotoxicity (ADCC) effects and opsonization against cellular antigens. In the present study, serum IgG content in all treatment groups was higher than that in the control group, possibly because active components in Yucca schidigera extract stimulate plasma cells in the spleen and lymph nodes to produce more IgG for immune response. NO is a crucial physiological signaling molecule within and between cells that plays important roles in immune, neural, and circulatory systems. The mechanism by which polysaccharides promote NO synthesis may involve activation of lymphocytes, promotion of iNOS gene expression, and increased iNOS synthesis, thereby enhancing NO synthesis and secretion [26]. In this experiment, serum NO content and NOS and iNOS activities in all treatment groups were higher than those in the control group, consistent with the findings of Xiao Huali et al. [27] and Wang Jianwen et al. [28]. This may be attributed to components in Yucca schidigera extract that can stimulate lymphocytes, promote iNOS expression, and consequently increase serum NOS activity and NO content, thereby enhancing the animal' s immune capacity.

3.4 Effects of Yucca schidigera Extract on Immune Organ Index of Piglets

The thymus serves as the central organ for cellular immunity in piglets and is classified as a primary immune organ, while the spleen functions as a peripheral immune organ and is considered a secondary organ. The spleen contains approximately 40% T cells and 60% B cells and plays an important role in humoral immunity [29]. Rivas et al. [30] suggested that immune organ index can directly reflect the strength of an animal' s immune function. In this experiment, the thymus index in all treatment groups was higher than that in the control group, with group III showing the best results. The spleen index in all treatment groups was also higher than that in the control group, indicating that Yucca schidigera extract can promote the growth of both thymus and spleen in piglets, thereby enhancing their immune capacity.

3.5 Effects of Yucca schidigera Extract on Fecal Nitrogen and Phosphorus Emission of Piglets

Chen Huajie [31] added different levels of Yucca schidigera extract to chicken excreta to compare their effects on ammonia nitrogen content, finding that all supplementation levels significantly reduced ammonia nitrogen content in chicken excreta, with 0.024 g/L being the most effective level. These results are similar to the present findings, which showed that all treatment groups reduced fecal ammonia nitrogen content in piglets, with groups II, III, and IV achieving significant reductions. This may be because active components in Yucca schidigera extract can slow the rate of urea decomposition to ammonia, functioning similarly to urease inhibitors. Under the experimental conditions, all treatment groups reduced fecal total nitrogen and total phosphorus content, indicating that Yucca schidigera extract can improve nitrogen and phosphorus utilization in piglet diets, resulting in lower fecal nitrogen and phosphorus excretion compared with the control group.

3.6 Effects of Yucca schidigera Extract on Fecal Microorganism Number of Piglets

The piglet digestive tract harbors numerous beneficial microorganisms that play important roles in nutrient absorption, with Lactobacillus and Escherichia coli serving as primary indicators of intestinal health [32]. Murali et al. [33] and Castillo et al. [34] demonstrated that the ratio of Lactobacillus to Escherichia coli in the animal digestive tract and intestine serves as an indicator of intestinal flora balance, with the relative abundance of these organisms reflecting intestinal health status. Higher Lactobacillus counts relative to Escherichia coli can promote inhibition of pathogenic bacteria by beneficial organisms and benefit animal growth, whereas higher Escherichia coli counts can promote growth of harmful microorganisms and negatively affect animal growth. Under the experimental conditions, dietary Yucca schidigera extract reduced total aerobic bacteria and Escherichia coli counts while increasing Lactobacillus counts in feces, reflecting changes in intestinal microorganism composition and abundance. The increase in beneficial Lactobacillus and reduction in harmful Escherichia coli can regulate intestinal microflora balance in piglets.

Conclusion:

1. Dietary supplementation with Yucca schidigera extract in piglets can improve growth performance, serum antioxidant and immune indexes, reduce nitrogen and phosphorus emissions, and improve intestinal microbial balance.
2. Under the experimental conditions of this study and based on comprehensive evaluation of all indicators, the optimal supplemental level of Yucca schidigera extract in piglet diets is recommended to be 120 mg/kg.

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