Effects of Candida krusei on Growth Performance, Immune Function, Blood Parameters, and Manure Deodorization in Broiler Chickens: Postprint

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Abstract

Candida krusei (LSA) is a yeast strain isolated from chicken manure by our research team that possesses ammonia nitrogen degradation capability. This experiment aimed to investigate the effects of dietary supplementation with different levels of LSA on growth performance, immune function, blood indices, and fecal/urinary deodorization in broiler chickens. A total of 240 healthy 1-day-old Arbor Acres (AA) broiler cockerels were selected and randomly divided into 4 groups, with 6 replicates per group and 10 birds per replicate. The control group was fed a basal diet without antibiotic supplementation, while the three experimental groups were fed the basal diet supplemented with 1.0×10 , 1.0×10 , and 1.0×10 CFU/kg LSA, respectively. The experimental period lasted 42 days, with ad libitum access to feed and water throughout. The results showed: 1) No significant differences were observed among groups in average daily feed intake, average daily gain, average body weight, or feed-to-gain ratio ($P > 0.05$). 2) Dietary LSA supplementation had no significant effect on thymus index or bursa of Fabricius index in broilers ($P > 0.05$), but supplementation with 1.0×10 CFU/kg LSA significantly increased the spleen index ($P < 0.05$). 3) Dietary LSA supplementation significantly reduced serum uric acid and plasma ammonia levels ($P < 0.05$), but had no significant effect on biochemical indices such as serum total protein, albumin, and globulin ($P > 0.05$). 4) Dietary LSA supplementation had no significant effect on fecal/urinary moisture content or total nitrogen content ($P > 0.05$), but supplementation with 1.0×10 CFU/kg LSA significantly reduced fecal/urinary ammonia nitrogen content ($P < 0.05$). In conclusion, LSA had no adverse effects on growth performance, immune organs, or blood indices in broilers; dietary LSA supplementation can enhance immune function, improve nitrogen metabolism, reduce ammonia nitrogen content in

feces and urine, and decrease environmental pollution.

Full Text

Effects of *Candida krusei* on Growth Performance, Immune Function, Blood Indices and Manure Deodorization of Broilers

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Abstract: *Candida krusei* (LSA) is a yeast strain with ammonia nitrogen degradation capability screened from chicken manure by our research team. This study aimed to evaluate the effects of dietary supplementation with different levels of LSA on growth performance, immune function, blood indices and manure deodorization in broilers. A total of 240 one-day-old healthy Arbor Acres (AA) male broilers were randomly allocated to 4 groups with 6 replicates per group and 10 birds per replicate. The control group was fed a basal diet without antibiotics, while three experimental groups were fed the basal diet supplemented with 1.0×10 , 1.0×10 and 1.0×10 CFU/kg LSA, respectively. The experiment lasted for 42 days with ad libitum access to feed and water throughout the period. The results showed that: 1) There were no significant differences in average daily feed intake, average daily gain, average body weight or feed-to-gain ratio among all groups (P>0.05). 2) Dietary LSA supplementation had no significant effect on thymus index or bursal index of broilers (P>0.05), but supplementation with 1.0×10 CFU/kg LSA significantly increased spleen index (P<0.05). 3) Dietary LSA supplementation significantly reduced serum uric acid and plasma ammonia contents (P<0.05), but had no significant influence on biochemical indices such as serum total protein, albumin and globulin (P>0.05). 4) Dietary LSA supplementation had no significant effect on manure moisture content or total nitrogen content (P>0.05), but supplementation with 1.0×10 CFU/kg LSA significantly decreased manure ammonia nitrogen content (P<0.05). These results indicate that LSA has no adverse effects on growth performance, immune organs or blood indices of broilers; dietary LSA supplementation can enhance immune function, improve nitrogen metabolism, reduce ammonia nitrogen content in manure, and decrease environmental pollution.

Key words: *Candida krusei*; broiler; growth performance; immune; blood indices; manure deodorization

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Introduction

Livestock and poultry production and manure storage generate large quantities of toxic and harmful odorous gases, posing serious threats to human and animal health as well as the sustainable development of the livestock industry [1-2]. Due to the short digestive tract of poultry, feed remains in the gastrointestinal tract for a brief period, resulting in incomplete digestion and absorption. Consequently, nearly 50% of dietary nitrogen is excreted in feces without being digested or absorbed [3], with over 50% of fecal nitrogen volatilizing as ammonia gas [4]. The direct precursor of ammonia volatilization is ammonia nitrogen in manure. Studies have shown that microbial nitrification of ammonium ions (NH) can accelerate the conversion of NH and ammonia (NH) to nitrate (NO) and nitrite (NO), thereby reducing ammonia emission from poultry manure and increasing nitrogen retention [5-6]. Our research team has isolated and screened a strain of *Candida krusei* (LSA) from chicken manure, and found that inoculating it into chicken manure can rapidly reduce ammonia nitrogen content and decrease ammonia volatilization [7].

LSA is an osmotolerant yeast strain. Microscopic observation reveals oval-shaped cells with smooth texture, fringed edges, and budding reproduction. Current research indicates that it contains abundant intracellular trehalose and exhibits high glycerol production [8-9]. LSA also belongs to the category of probiotics, demonstrating strong environmental tolerance and a wide optimal growth temperature range that aligns well with broiler rearing temperatures. Moreover, this strain is simple to culture and grows rapidly, facilitating large-scale inoculation trials [7]. However, the ammonia nitrogen degradation ability of LSA has only been verified through laboratory inoculation in poultry manure, and has not been further validated in production practice. Therefore, based on previous research, this experiment investigated the effects of feeding LSA at different supplementation levels to broiler chickens on growth performance, immune function, blood indices and manure deodorization, aiming to provide a scientific theoretical basis for the application of LSA as a novel probiotic strain in practical production.

Materials and Methods

1.1 Experimental Material LSA was provided by the Poultry Nutrition Laboratory of the Feed Research Institute, Chinese Academy of Agricultural Sciences, with a viable cell count 1.0×10 CFU/mL.

1.2 Experimental Design and Diets This experiment employed a single-factor completely randomized design. A total of 240 one-day-old healthy Arbor Acres (AA) male broilers were randomly divided into 4 groups with 6 replicates per group and 10 birds per replicate. The control group was fed a basal diet without antibiotics, while experimental groups were fed the basal diet supple-

mented with 1.0×10 , 1.0×10 and 1.0×10 CFU/kg LSA via uniform spraying. The experimental period lasted 42 days. The antibiotic-free basal diet was formulated according to the Chinese Nutrient Requirements for Broilers (NY/T 33-2004) and was provided in mash form. Its composition and nutrient levels are shown in Table 1 .

1.3 Housing Management The feeding trial was conducted at the Changping Pilot Base of the Chinese Academy of Agricultural Sciences. A three-tier cage system was used. Lighting was provided 24 h/d for days 1-7 and 23 h/d from day 8 onward. House temperature was maintained at 33°C for days 1-3, then gradually decreased by reducing heating supply until reaching 25°C from day 4 onward. Diets (mash form) were prepared weekly to ensure LSA viability, and broilers had ad libitum access to feed throughout the experimental period. Daily observations were made on bird mental status, appetite and fecal conditions, with mortality recorded. Routine vaccination procedures were followed.

1.4 Measurements 1.4.1 Growth Performance
At 08:00 on days 21 and 42 of the experiment, broilers were weighed after an 8-hour fast (with free access to water) by replicate. Feed consumption was recorded to calculate average daily feed intake (ADFI), average daily gain (ADG) and feed-to-gain ratio (F/G) for the starter, grower and overall periods.

1.4.2 Immune Organ Index
On day 42, one broiler with body weight close to the replicate average was randomly selected from each replicate, weighed and slaughtered. Immediately after dissection, the thymus, spleen and bursa of Fabricius were separated, blotted with filter paper to remove blood stains, and weighed fresh after fat removal to calculate immune organ indices. Immune organ index = immune organ weight / pre-slaughter live weight.

1.4.3 Blood Indices
On day 42, one broiler per replicate was randomly selected for blood collection. A total of 10 mL blood was collected from the jugular vein. Seven mL was placed in a disposable vacuum tube (containing separating gel and coagulant), left at 37°C for 1 hour, then centrifuged at 3,500 r/min for 10 minutes to prepare serum. Serum total protein (TP), albumin (ALB), globulin (GLB), immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), uric acid (UA), urea nitrogen (UN) contents, and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using a Hitachi 7160 automatic biochemical analyzer. The remaining 3 mL was placed in a 5 mL heparin sodium tube, shaken rapidly for full anticoagulation, and immediately centrifuged at 4°C and 3,000 r/min for 10 minutes to collect plasma. Plasma ammonia content was measured within 30 minutes using the glutamate dehydrogenase rate method.

1.4.4 Fecal Component Analysis

Fresh fecal samples were collected at 08:00 daily from each replicate during days 40-42. During sampling, the 3-day fecal samples from each replicate were thoroughly mixed, and 200 g was taken using the quartering method and placed in ziplock bags. One portion was stored at -20°C for ammonia nitrogen determination using the salicylate-hypochlorite spectrophotometric method (GB 7481-87). The other portion was treated with 25 mL of 10% sulfuric acid per 100 g feces for nitrogen fixation, then placed directly in an oven at 105°C for 15 minutes to inactivate enzymes and microorganisms, followed by drying at 65°C. After removal, samples were allowed to equilibrate in air for 24 hours before weighing to determine moisture content. Samples were then ground, passed through a 40-mesh sieve (0.425 mm), sealed in ziplock bags and stored for total nitrogen determination using the Dumas combustion method.

1.5 Statistical Analysis Experimental data were expressed as mean ± standard deviation. One-way ANOVA was performed using the one-way ANOVA procedure in SPSS 19.0 software, with Duncan' s multiple range test used for inter-group comparisons. $P<0.05$ was set as the criterion for statistical significance.

Results

2.1 Effects of LSA on Broiler Growth Performance As shown in Table 2 , dietary LSA supplementation had no significant effect on ADFI, ADG, F/G or body weight of broilers during days 1-21 ($P>0.05$). During days 22-42, ADG and ADFI showed an increasing trend while F/G showed a decreasing trend, but none reached statistical significance ($P>0.05$). Compared with the control group, LSA tended to reduce overall F/G during days 1-42, but the difference was not significant ($P>0.05$).

2.2 Effects of LSA on Broiler Immune Organ Indices As shown in Table 3 , dietary LSA supplementation showed an increasing trend for thymus index and bursal index, but neither reached statistical significance ($P>0.05$). Supplementation with 1.0×10 CFU/kg LSA significantly increased spleen index compared with the control group ($P<0.05$).

2.3 Effects of LSA on Broiler Blood Indices As shown in Table 4 , dietary LSA supplementation had no significant effect on serum TP, ALB, GLB, IgA, IgM, IgG contents or AST and ALT activities ($P>0.05$). However, serum UA content in LSA-supplemented groups was significantly lower than in the control group ($P<0.05$). Serum UN content was reduced by 21.66%, 17.83% and 33.12% compared with the control group, respectively, but the differences were not significant ($P>0.05$). Plasma ammonia content was significantly lower than in the control group ($P<0.05$).

2.4 Effects of LSA on Broiler Manure Nitrogen Content As shown in Table 5 , dietary LSA supplementation had no significant effect on manure moisture content or total nitrogen content (P>0.05). However, compared with the control group, ammonia nitrogen content in manure was reduced by 5.86%, 51.71% and 29.32% in the three experimental groups, respectively, with the 1.0×10 CFU/kg LSA group showing a significant reduction (P<0.05).

Discussion

3.1 Effects of LSA on Broiler Growth Performance As a feed additive, yeast is gradually replacing some bacterial antibiotics. In recent years, reported yeast additives both domestically and internationally have primarily employed functional yeasts such as selenium-enriched or chromium-enriched yeast, yeast culture and yeast cell wall [10-12]. In this experiment, dietary LSA supplementation had no significant effect on growth performance during days 1-21, while ADG and ADFI during days 22-42 showed an increasing trend, though not statistically significant. These results are consistent with reports by Zhou [13] and Yu [14]. However, they differ from the findings of Xiao et al. [15], who reported that high-level yeast culture supplementation could improve ADG and ADFI and reduce F/G in 1-21 day-old broilers to varying degrees. These discrepancies may be related to additive type, dosage, rearing environment and animal breed. The greater growth-promoting effect of LSA in the later stage may be attributed to LSA being a novel strain, requiring an adaptation period for broilers.

3.2 Effects of LSA on Broiler Immune Organ Indices The thymus, spleen and bursa of Fabricius are important immune organs in poultry, and their weights can be used to evaluate immune function to some extent [16]. Some studies have found that feeding microecological preparations to chicks can significantly increase bursal and spleen indices [17]. Wang et al. [18] also reported that supplementation with *Candida utilis* in gosling diets significantly increased immune organ indices. In this experiment, dietary LSA supplementation showed an increasing trend for thymus and bursal indices, while 1.0×10 CFU/kg LSA significantly increased spleen index. Additionally, no obvious pathological changes were observed in organs such as heart, kidney, liver and spleen during necropsy. These findings indicate that dietary LSA supplementation is safe and has no adverse side effects in broilers.

3.3 Effects of LSA on Broiler Blood Indices Serum TP is primarily composed of ALB and GLB, and its content can reflect nutritional status for growth and development as well as protein metabolism level [19]. Serum ALB functions in substance transport and maintenance of plasma osmotic pressure, while serum GLB is closely related to body resistance and its content can reflect immune capacity [20]. In this experiment, LSA had no significant effect on serum TP, ALB, GLB, IgA, IgM or IgG contents. Serum non-protein nitrogenous substances UA

and UN can accurately and truly reflect protein metabolism and dietary amino acid balance in animals [21]. UN is mostly synthesized by the liver from ammonia produced by protein breakdown or absorbed from the large intestine before entering the bloodstream, and its decrease indicates prolonged nitrogen retention. UA is the end product of amino acid and nucleic acid catabolism in poultry and represents the primary nitrogen excretion pathway; its increase indicates more vigorous protein catabolism [22]. Plasma ammonia, another important indicator of nitrogen metabolism, must be maintained at extremely low concentrations, as levels exceeding normal ranges can seriously harm animal health and inhibit growth [23-24]. In this experiment, dietary LSA supplementation significantly reduced serum UA and plasma ammonia contents, while serum UN content in experimental groups showed a decreasing trend compared with the control group. The underlying mechanism is that LSA can improve nitrogen metabolism and increase nitrogen utilization efficiency, thereby reducing ammonia emission and improving house environment [25]. LSA can also utilize intestinal ammonia nitrogen and reduce ammonia absorption, consequently decreasing blood uric acid and ammonia contents. These results are consistent with studies by Xiao et al. [15] and Wang et al. [26]. Yu [14] also reported that yeast culture supplementation in broiler diets significantly reduced serum non-protein nitrogenous substance content in 35-day-old broilers. However, inconsistent results have been reported regarding yeast application in broiler diets. Some studies indicated that supplementation with 2 g/kg *Saccharomyces cerevisiae* culture in laying hen diets significantly increased serum UA content [27], while others reported that dietary *Saccharomyces cerevisiae* or live yeast had no significant effect on serum UA content in broilers [28-30]. Serum AST and ALT are important indicators reflecting cardiac and hepatic function. Under normal conditions, these enzyme activities are low in serum as they are widely present in animal cells. During pathological conditions, these enzymes are released from cells into serum, resulting in increased enzyme activity [31-32]. In this experiment, serum AST and ALT activities in experimental groups showed a decreasing trend without significant differences, indicating that dietary LSA supplementation does not damage liver or heart function and has no adverse effects on animal health.

3.4 Effects of LSA on Broiler Manure Deodorization Studies have shown that nitrogen loss from livestock manure is primarily caused by ammonia volatilization [33]. Cbaerar et al. [34] also found that ammonia volatilization increases with manure moisture content. The results of this experiment showed that dietary LSA supplementation had no significant effect on manure moisture content or total nitrogen content, but significantly reduced ammonia nitrogen content in manure and decreased ammonia volatilization. These findings are similar to those of Liu [7], who reported that inoculating LSA into chicken manure significantly reduced ammonia nitrogen content. The possible reason is that viable bacterial cells surviving in animal manure can further reduce ammonia emission and improve house environment. However, the specific mechanism

requires further investigation.

Conclusions

1. Dietary LSA supplementation had no significant effect on broiler growth performance, but 1.0×10 CFU/kg LSA significantly increased spleen index.
2. Dietary LSA supplementation significantly reduced serum UA and plasma ammonia contents in broilers.
3. Dietary LSA supplementation significantly decreased ammonia nitrogen content in broiler manure.
4. In summary, LSA as a novel feed additive is safe and has no adverse side effects when fed to broiler chickens.

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