Effects of Weaning Age on Growth Performance and Serum Biochemical Indices in Wuzhishan Piglets: Postprint

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Abstract

This experiment aimed to investigate the effects of weaning age on growth performance and serum biochemical indices of Wuzhishan piglets. Ninety-six Wuzhishan piglets with similar age ($\pm 1d$)*andbodyweight*[(0.56±$0.07) kg] were selected and randomly allocated into 4 groups, with 4 replicates per group and 6 piglets per replicate. The 4 groups of piglets were weaned at 21, 28, 35, and 42 days of age, respectively. The trial commenced at 8 days of age and concluded at 56 days of age; body weight, feed intake, and serum biochemical indices were measured regularly throughout the experimental period. The results demonstrated that average daily gain (ADG) decreased to varying degrees after weaning in all groups, with earlier weaning age associated with a greater decline in ADG; the ADG of the 35-day and 42-day weaning groups during the periods of 21–28, 28–35, 35–42, and 49–56 days of age was significantly higher than that of the 21-day weaning group ($P<0.05$). Average daily feed intake (ADFI) increased significantly after weaning in all groups ($P<0.05$), and no significant differences in ADFI were observed among groups after 42 days of age ($P>0.05$). In the 21-day, 28-day, and 35-day weaning groups, serum total protein (TP), albumin (ALB), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents, as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities, all exhibited significant changes at 7 days post-weaning ($P < 0.05$), with the most pronounced fluctuations observed in the 21-day weaning group; compared with the 35-day and 42-day weaning groups, the 21-day weaning group displayed significantly reduced serum TP, ALB, IgG, and IgM contents at 28 and 35 days of age ($P < 0.05$), and significantly elevated ALT, AST, and LDH activities ($P < 0.05$); at 56 days of age, serum TP, IgG, and IgM contents remained significantly lower than those in the 35-day and 42-day weaning groups ($P<0.05$). Serum triglyceride (TG) and cholesterol (CHOL) contents in the 21-day weaning group showed significant variations between 28 and 56 days of age ($P<0.05$), whereas serum TG and

CHOL contents in the 35-day and 42-day weaning groups showed no significant changes throughout the experimental period (P>0.05) . Except for a significant difference from the 42-day weaning group at 42 days of age (P<0.05), the serum TP, ALB, IgG, IgM contents and ALT, AST, LDH activities in the 35-day weaning group showed no significant differences from the 42-day weaning group at other time points (P>0.05) . It can be concluded that Wuzhishan piglets weaned at 35 or 42 days of age are superior to those weaned at 21 days of age in terms of growth performance, serum biochemical indices, and immune function.

Full Text

Preamble

Effects of Weaning Age on Growth Performance and Serum Biochemical Indices of Wuzhishan Piglets

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Abstract: This experiment was conducted to investigate the effects of weaning age on growth performance and serum biochemical indices of Wuzhishan piglets. Ninety-six Wuzhishan piglets of similar age ($\pm1day$)$andbodyweight$[(0.56±$0.07) kg] were randomly allocated into four groups, with four replicates per group and six piglets per replicate. The four groups were weaned at 21, 28, 35, and 42 days of age, respectively. The trial ran from 8 to 56 days of age, during which body weight, feed intake, and serum biochemical indices were measured regularly. The results showed that average daily gain (ADG) decreased to varying degrees after weaning in all groups, with earlier weaning resulting in greater ADG reduction. The ADG of the 35-day and 42-day weaning groups was significantly higher than that of the 21-day weaning group during the periods of 21–28, 28–35, 35–42, and 49–56 days of age (P<0.05). Average daily feed intake (ADFI) increased significantly after weaning in all groups (P<0.05), with no significant differences observed among groups after 42 days of age (P>0.05). Serum total protein (TP), albumin (ALB), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents, as well as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities changed significantly at 7 days post-weaning in the 21-day, 28-day, and 35-day weaning groups (P<0.05), with the 21-day weaning group showing the greatest fluctuation. Compared with the 35-day and 42-day weaning groups, the 21-day weaning group exhibited significantly reduced serum TP, ALB, IgG, and IgM contents (P<0.05) and significantly elevated ALT, AST, and LDH activities (P<0.05) at 28 and 35 days of age. At 56 days of age, serum TP, IgG, and IgM contents remained significantly lower in the 21-day weaning group compared to the 35-day and 42-day weaning groups (P<0.05). Serum triglyceride (TG) and cholesterol

(CHOL) contents in the 21-day weaning group varied significantly between 28 and 56 days of age ($P<0.05$), whereas no significant changes were observed in the 35-day and 42-day weaning groups throughout the experimental period ($P>0.05$). Except for significant differences at 42 days of age ($P<0.05$), no significant differences were found between the 35-day and 42-day weaning groups in serum TP, ALB, IgG, and IgM contents or ALT, AST, and LDH activities at other time points ($P>0.05$). These findings indicate that Wuzhishan piglets weaned at 35 or 42 days of age demonstrate superior growth performance, serum biochemical profiles, and immune function compared to those weaned at 21 days of age.

Keywords: weaning age; Wuzhishan piglets; growth performance; serum biochemical indices; immunoglobulin

Introduction

Early weaning has become a widely adopted advanced technology in modern swine production to improve sow reproductive efficiency and reduce feeding costs. However, the abrupt changes in physiology, nutrition, and environment during the weaning period, combined with weaning stress and pathogen invasion, often lead to "post-weaning stress syndrome"in piglets, characterized by reduced feed intake, growth retardation, diarrhea, and compromised immune function [1]. Blood physiological and biochemical indices are closely related to animal metabolism, nutrition, and health status, serving as important indicators of metabolic and health conditions in animal bodies or organs. Therefore, studying the effects of weaning age on piglet growth performance and serum biochemical indices holds significant practical importance.

Numerous studies have investigated the impact of weaning age on piglet growth and development, typically evaluating appropriate weaning ages by examining blood physiology and biochemistry, digestion, fecal characteristics, and immunological markers. Colson et al. [2] found that 28-day-old weaned piglets exhibited significantly higher daily feed intake and growth rate than those weaned at 21 days. Zhou et al. [3] reported that ADG and ADFI increased slowly during the first week post-weaning but rose significantly in the second week, with 28-day-old weaned piglets showing significantly better production performance than those weaned at 23 days, indicating that 28-day-old weaned piglets experienced less weaning stress. Zhu and Du [4] compared different weaning ages (14, 21, and 28 days) in Fanjingshan special wild boar piglets and found that earlier weaning resulted in more severe post-weaning diarrhea and negative impacts on growth performance. These findings demonstrate that weaning stress substantially affects piglet body weight, weight gain, and feed intake, with greater impacts observed at earlier weaning ages. However, Kalita et al. [5] investigated the effects of different weaning ages (28, 35, and 42 days) on piglet growth performance and found no significant differences in body weight among groups at

42 days of age, a result inconsistent with previous studies that may be attributed to differences in pig breed and diet type.

Wuzhishan pigs, native to Hainan Province, represent China' s smallest and lightest rare pig breed, characterized by small body size, strong stress resistance, genetic stability, early sexual maturity, and excellent meat quality. Recognized as a national livestock breed resource by the Ministry of Agriculture in 2000, Wuzhishan pigs constitute a unique livestock genetic resource in Hainan Province. Current research on Wuzhishan pigs has primarily focused on meat quality traits and molecular genetic characteristics. However, due to backward farming practices and low technical levels, studies on early weaning of Wuzhishan piglets have been scarce. Therefore, this experiment was designed to investigate the effects of weaning age on growth performance and serum biochemical indices of Wuzhishan piglets, to objectively evaluate the stress level associated with weaning age, and to provide theoretical and practical basis for scientifically determining the appropriate weaning age for Wuzhishan piglets.

1. Materials and Methods

1.1 Experimental Design

Ninety-six Wuzhishan piglets of similar age ($\pm 1 day$) and body weight [(0.56±$0.07) kg] were randomly divided into four groups with four replicates per group and six piglets per replicate (half male and half female). The four groups were weaned at 21, 28, 35, and 42 days of age, respectively. Piglets were introduced to solid feed starting at 8 days of age and followed routine immunization procedures until 56 days of age. Weaning was conducted between 20:00 and 21:00 on the designated weaning day, with sows being removed while piglets remained in their original location. During the experimental period, temperature, humidity, ventilation, and lighting in the nursery facilities met piglet requirements, and environmental sanitation conditions were maintained. Basal diets were formulated according to the "Wuzhishan Pig Farming Technical Regulations" (2007), with composition and nutrient levels shown in Table 1 .

1.2 Sample Collection and Measurement

During the experimental period, all piglets were weighed after overnight fasting at 21, 28, 35, 42, 49, and 56 days of age to calculate ADG for the periods of 21–28, 28–35, 35–42, 42–49, and 49–56 days of age. Daily observations of piglet growth were conducted, and feed intake and leftovers were recorded for each replicate to calculate ADFI for the corresponding periods.

From each replicate, two piglets (one male and one female) were randomly selected before morning feeding at 28, 35, 42, 49, and 56 days of age. Blood samples (10 mL) were collected from the anterior vena cava, allowed to clot for 30 minutes, then centrifuged at 3,000 rpm for 10 minutes. Serum was harvested and stored in 1.5 mL centrifuge tubes at -20°C for subsequent biochemical analysis.

Serum biochemical indices were determined using the following methods: serum total protein (TP) by biuret endpoint method; albumin (ALB) by bromocresol green colorimetry; alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by spectrophotometry; cholesterol (CHOL) and triglyceride (TG) by enzymatic methods; urea nitrogen (UN) by urease method; lactate dehydrogenase (LDH) by colorimetry; and immunoglobulin G (IgG) and immunoglobulin M (IgM) by immunoturbidimetry. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.3 Data Analysis

Data were analyzed using one-way ANOVA procedure in SPSS 17.0 statistical software. Duncan's multiple comparison test was used for significant differences. $P<0.05$ was considered statistically significant, and results were expressed as mean±standard deviation (mean±SD).

2. Results

2.1 Effects of Weaning Age on Growth Performance of Wuzhishan Piglets

The effects of weaning age on growth performance are presented in Table 2 . ADG decreased to varying degrees after weaning in all groups, with earlier weaning resulting in greater reduction. The ADG of the 35-day and 42-day weaning groups was significantly higher than that of the 21-day weaning group during the periods of 21–28, 28–35, 35–42, and 49–56 days of age ($P<0.05$), while no significant differences were observed among groups during 42–49 days of age ($P>0.05$). Over the entire sampling period (21–56 days of age), ADG in the 35-day and 42-day weaning groups was significantly higher than in the 21-day and 28-day weaning groups ($P<0.05$).

ADFI increased substantially after weaning in all groups. During 21–28 days of age, ADFI in the 21-day weaning group was significantly higher than in the other three groups ($P<0.05$). During 28–35 days of age, ADFI in the 21-day and 28-day weaning groups was significantly higher than in the 35-day and 42-day weaning groups ($P<0.05$). During 35–42 days of age, ADFI in the 42-day weaning group was significantly lower than in the other three groups ($P<0.05$). No significant differences in ADFI were observed among groups during 42–49 and 49–56 days of age ($P>0.05$).

2.2 Effects of Weaning Age on Serum TP, ALB, IgG, and IgM Contents

The effects of weaning age on serum ALB, TP, IgG, and IgM contents are shown in Table 3 . Serum ALB content decreased to varying degrees after weaning in all groups. From a group perspective, the 21-day weaning group showed a larger decline magnitude and longer recovery time, with significant differences

between 28 days and 49–56 days of age ($P<0.05$). In contrast, the 35-day and 42-day weaning groups remained relatively stable throughout the experimental period without significant differences ($P>0.05$). From an age perspective, serum ALB content in the 42-day weaning group was significantly higher than in the 21-day weaning group at 28, 35, 42, and 56 days of age ($P<0.05$), while the 35-day weaning group showed significantly higher ALB content than the 21-day weaning group at 28 and 35 days of age ($P<0.05$). The 42-day weaning group had significantly higher ALB content than the 35-day weaning group at 42 days of age ($P<0.05$), but no significant differences were observed among groups at 49 days of age ($P>0.05$).

Serum TP content showed similar trends to ALB, decreasing to varying degrees after weaning in all groups. From a group perspective, the 28-day weaning group exhibited a significant decrease in serum TP content at 7 days post-weaning ($P<0.05$). The 35-day weaning group showed a significant reduction in TP content at 42 days of age ($P<0.05$), which returned to pre-weaning levels by 49 days of age ($P>0.05$). The 42-day weaning group remained stable throughout the experimental period without significant differences ($P>0.05$). From an age perspective, serum TP content in the 35-day and 42-day weaning groups was significantly higher than in the 21-day weaning group at 28, 35, and 56 days of age ($P<0.05$). The 42-day weaning group had significantly higher TP content than the other three groups at 42 days of age ($P<0.05$), while no significant differences were observed among groups at 49 days of age ($P>0.05$).

From a group perspective, serum IgG and IgM contents in the 21-day, 28-day, and 35-day weaning groups changed significantly after weaning ($P<0.05$), whereas the 42-day weaning group remained relatively stable throughout the experimental period without significant differences ($P>0.05$). From an age perspective, compared with the 21-day weaning group, the 28-day weaning group showed significantly higher serum IgG content at 28 and 42 days of age ($P<0.05$) and significantly higher IgM content at 28 days of age ($P<0.05$). The 35-day weaning group exhibited significantly higher serum IgG and IgM contents at 28, 35, 49, and 56 days of age ($P<0.05$). The 42-day weaning group showed significantly higher serum IgG content at 28, 35, 42, and 56 days of age ($P<0.05$) and significantly higher IgM content at 28, 35, 42, 49, and 56 days of age ($P<0.05$).

2.3 Effects of Weaning Age on Serum TG, CHOL, and UN Contents

The effects of weaning age on serum TG, CHOL, and UN contents are presented in Table 4 . From a group perspective, serum TG content in the 21-day and 28-day weaning groups varied significantly between 28 and 56 days of age ($P<0.05$), while no significant changes were observed in the 35-day and 42-day weaning groups throughout the experimental period ($P>0.05$). From an age perspective, serum TG content in the 28-day weaning group was significantly higher than in the 21-day weaning group at 28 days of age ($P<0.05$). The 35-day weaning group showed significantly higher TG content than the 21-day weaning group at 28 and 35 days of age ($P<0.05$). The 42-day weaning group exhibited significantly

higher TG content than the 21-day weaning group at 28, 35, 42, and 49 days of age ($P<0.05$). No significant differences in serum TG content were observed among groups at 56 days of age ($P>0.05$).

From a group perspective, serum CHOL content in the 21-day weaning group varied significantly between 28 and 56 days of age ($P<0.05$), while no significant changes were observed in the 28-day, 35-day, and 42-day weaning groups throughout the experimental period ($P>0.05$). From an age perspective, serum CHOL content in the 21-day weaning group was significantly higher than in the other three groups at 28 days of age ($P<0.05$). The 35-day weaning group showed significantly higher CHOL content than the 42-day weaning group at 49 days of age ($P<0.05$). No significant differences in serum CHOL content were observed among groups at other time points ($P>0.05$).

From a group perspective, serum UN content in the 21-day, 28-day, and 35-day weaning groups increased to varying degrees at 7 days post-weaning, with all groups showing significantly lower UN content at 56 days of age compared to 7 days post-weaning ($P<0.05$). In contrast, no significant differences in serum UN content were observed in the 42-day weaning group before and after weaning ($P>0.05$). From an age perspective, no significant differences in serum UN content were observed among groups throughout the experimental period ($P>0.05$).

2.4 Effects of Weaning Age on Serum ALT, AST, and LDH Activities

The effects of weaning age on serum ALT, AST, and LDH activities are shown in Table 5 . From a group perspective, serum ALT activity varied significantly throughout the experimental period in the 21-day, 28-day, and 35-day weaning groups ($P<0.05$), while no significant changes were observed in the 42-day weaning group ($P>0.05$). From an age perspective, serum ALT activity in the 28-day weaning group was significantly lower than in the 21-day weaning group at 28 days of age ($P<0.05$). The 35-day and 42-day weaning groups showed significantly lower ALT activity than the 21-day weaning group at 28, 35, and 49 days of age ($P<0.05$). The 42-day weaning group had significantly lower ALT activity than the other three groups at 42 days of age ($P<0.05$). No significant differences in serum ALT activity were observed among groups at 56 days of age ($P>0.05$).

From a group perspective, serum AST and LDH activities varied significantly in the 21-day, 28-day, and 35-day weaning groups ($P<0.05$), while no significant changes were observed in the 42-day weaning group throughout the experimental period ($P>0.05$). From an age perspective, serum AST activity in the 35-day and 42-day weaning groups was significantly lower than in the 21-day weaning group at 28 and 35 days of age ($P<0.05$). The 42-day weaning group showed significantly lower AST activity than the other three groups at 42 days of age ($P<0.05$). No significant differences in serum LDH and AST activities were observed among groups after 49 days of age ($P>0.05$).

Discussion

Previous studies have demonstrated that weaning age affects post-weaning production performance in piglets [6]. Gu et al. [7] investigated the effects of weaning age (17, 21, 28, and 35 days) on piglet growth performance and found that weaning age significantly affected body weight, ADG, and ADFI, with earlier weaning (21 days) causing greater reductions in these indices. Zhou et al. [3] reported that ADG and ADFI at 7 and 14 days post-weaning were significantly higher in 28-day-old weaned piglets than in 23-day-old weaned piglets, indicating that 28-day-old weaned piglets experienced less weaning stress. Zheng et al. [8] compared the effects of different weaning ages (21, 28, 35, and 42 days) on Lingao piglet performance and found that growth performance improved with increasing weaning age, with 35- and 42-day-old weaned piglets showing superior body weight, ADG, and feed conversion ratio at 60 days of age compared to those weaned at 21 and 28 days. The present study also found that ADG decreased to varying degrees after weaning in all groups, with earlier weaning resulting in greater reduction and longer recovery time. The ADG of the 21-day weaning group remained significantly lower than that of the 35-day and 42-day weaning groups during the 49–56 day period. Weaning promoted feed intake, with ADFI increasing significantly after weaning in all groups. After 42 days of age, no significant differences in ADFI were observed among groups, indicating that weaning age is an important factor affecting post-weaning feed intake and daily gain. The 21-day-old weaned piglets required longer recovery time, possibly because early weaning (21 days) caused intestinal barrier dysfunction due to weaning stress, leading to significantly reduced growth performance; however, the specific mechanisms require further investigation. Over the entire experimental period, ADG in the 35-day and 42-day weaning groups was significantly higher than in the 21-day and 28-day weaning groups, suggesting that piglets weaned at 35 and 42 days experienced less weaning stress.

Serum TP consists of ALB and globulin (GLB), and blood TP content can reflect protein metabolism status and immunity. ALB is synthesized by the liver and serves as one of the main protein sources in the body, with functions including nutrient transport and maintenance of plasma osmotic pressure stability [9]. In this study, serum TP and ALB contents decreased significantly at 7 days post-weaning in the 21-day, 28-day, and 35-day weaning groups, with the 21-day weaning group showing the greatest fluctuation and longest recovery time. Compared with the 35-day and 42-day weaning groups, serum TP and ALB contents in the 21-day weaning group were significantly reduced at 28 and 35 days of age, and serum TP content remained lower than in the 35-day and 42-day weaning groups even at 56 days of age. These results indicate that weaning after 35 days of age can promote digestion and absorption, enhance protein metabolism, and strengthen immunity. Serum immunoglobulin content or titer is an important indicator of humoral immunity. IgM is the primary antibody involved in the initial immune response, while IgG is the main antibody mediating humoral immunity in serum and can resist various bacteria and toxins

[10]. Previous studies have shown that early weaning stress can reduce humoral immunity and suppress cellular immune capacity [11]. In this study, serum IgG and IgM contents showed similar trends to TP and ALB, consistent with previous findings [12-13], indicating that piglets weaned after 35 days of age have established a relatively complete immune system with stronger capacity to produce immunoglobulins, enabling them to effectively resist weaning stress.

Blood TG and CHOL contents are important indicators for assessing lipid metabolism status [14-15]. In this study, serum TG and CHOL contents in the 21-day weaning group varied significantly between 28 and 56 days of age, whereas no significant changes were observed in the 35-day and 42-day weaning groups throughout the experimental period, suggesting that early weaning has a greater impact on lipid metabolism. UN is the main end product of nitrogenous substance (protein and amino acid) metabolism, and blood UN content can accurately reflect protein metabolism and amino acid balance in the animal body; UN content decreases when protein metabolism is favorable [16]. Early weaning can increase blood UN content in piglets [17]. The present study found that serum UN content increased significantly after weaning in the 21-day, 28-day, and 35-day weaning groups, with earlier weaning requiring longer recovery time. Significant variations in serum UN content were observed in the 21-day, 28-day, and 35-day weaning groups during 28–56, 35–56, and 42–56 days of age, respectively. These results further indicate that earlier weaning has a greater impact on normal protein metabolism, leading to enhanced protein catabolism and reduced protein deposition.

AST and ALT are important indicators for evaluating liver function and can reflect liver damage. Under normal conditions, the activities of these two enzymes in blood are low, but when the liver is damaged or under stress, these enzymes enter the bloodstream, causing increased blood activity. Therefore, their activity changes can reflect liver and heart damage and stress conditions [18-19]. Zhang et al. [20] found that serum ALT and AST activities increased to varying degrees in piglets weaned at 14 days at 7 days post-weaning, while these enzyme activities decreased to varying degrees in piglets weaned at 21, 28, and 35 days. Yang [21] compared the effects of different weaning ages (14, 21, 28, and 35 days) on blood ALT and AST activities and found no significant differences among weaning ages at 42 days of age. In this study, serum ALT and AST activities increased significantly at 7 days post-weaning in the 21-day, 28-day, and 35-day weaning groups, with the 21-day weaning group showing the greatest magnitude of change. Serum ALT and AST activities reached non-significant differences among groups at 56 and 49 days of age, respectively. Combined with the findings of Zhang et al. [20] and Yang [21], these results indicate that earlier weaning has a greater impact on serum ALT and AST activities for a certain period post-weaning, with longer-lasting effects, suggesting greater stress and liver damage in piglets.

LDH is an important enzyme in the glycolytic pathway, and serum LDH activity increases when the body experiences damage, lesions, or adverse reactions [22].

Changes in LDH activity are often used as an indicator of stress effects on piglets [12]. In this study, serum LDH activity increased significantly at 7 days post-weaning in the 21-day, 28-day, and 35-day weaning groups, with the 21-day weaning group showing the greatest fluctuation. Serum LDH activity in the 21-day weaning group was significantly higher than in the 35-day and 42-day weaning groups at 28 and 35 days of age, and all groups reached non-significant differences in LDH activity after 49 days of age. This pattern was essentially consistent with the changes in serum ALT and AST activities, indicating that earlier weaning has greater negative effects on the body, thereby affecting normal piglet growth, which aligns with previous research findings [12,21].

Conclusion

1. Compared with weaning at 21 days of age, weaning at 35 or 42 days of age can improve growth performance, increase serum TP, ALB, IgG, and IgM contents, and reduce serum AST, ALT, and LDH activities in Wuzhishan piglets.
2. Based on comprehensive analysis of all indicators, weaning Wuzhishan piglets at 35 or 42 days of age yields better results than weaning at 21 days of age.

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