Effects of Montmorillonite and Yeast Culture Complex on Growth Performance, Serum Biochemical Indices, Immune Function, and Antioxidant Capacity in Weaned Piglets: Postprint

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Abstract

This study aimed to investigate the effects of dietary supplementation with montmorillonite and yeast culture complex as replacements for antibiotics and zinc oxide (ZnO) on growth performance, serum biochemical indices, immune function, and antioxidant capacity of weaned piglets. A total of 180 Duroc × Landrace × Yorkshire weaned piglets at 31 days of age with an average body weight of (6.78±0.19) kg were randomly allocated into 5 groups with 6 replicates per group and 6 piglets per replicate (half male and half female). The control group was fed the basal diet supplemented with antibiotics (500 g/t of 15% chlortetracycline and 200 g/t of 50% olaquindox) and ZnO (3 kg/t), group I was fed the basal diet supplemented with antibiotics, ZnO, and montmorillonite and yeast culture complex (1.5 kg/t), group II was fed the basal diet supplemented with ZnO and montmorillonite and yeast culture complex, group III was fed the basal diet supplemented with antibiotics and montmorillonite and yeast culture complex, and group IV was fed the basal diet supplemented with montmorillonite and yeast culture complex. The experimental period lasted 35 days. The results showed: 1) During days 15-35 of the experiment, the average daily feed intake of weaned piglets in groups III and IV was significantly higher than that in the control group and group I ($P<0.05$); during days 15-35 and 1-35 of the experiment, the average daily gain of groups II, III, and IV was significantly higher than that of the control group ($P<0.05$). 2) Compared with the control group, at days 14 and 35 of the experiment, serum alkaline phosphatase (ALP) activity of weaned piglets in groups III and IV was significantly decreased ($P<0.05$), and serum alanine aminotransferase (ALT) activity of groups II, III, and IV was significantly decreased ($P<0.05$); there were no significant differences in serum total protein (TP), albumin (ALB), urea nitrogen

(UN) contents, and aspartate aminotransferase (AST) activity among all groups (P>0.05). 3) Compared with the control group, at day 14 of the experiment, serum immunoglobulin A (IgA), tumor necrosis factor- (TNF-), and interferon- (INF-) contents in all treatment groups were significantly increased (P<0.05), serum immunoglobulin M (IgM) and interleukin-4 (IL-4) contents in groups II and III were significantly increased (P<0.05), and serum interleukin-1 (IL-1), interleukin-2 (IL-2), and interleukin-6 (IL-6) contents in group I were significantly increased (P<0.05); at day 35 of the experiment, serum immunoglobulin G (IgG) and TNF- contents in all treatment groups were significantly increased (P<0.05), serum IgM content in groups I and IV was significantly increased (P<0.05), and serum INF- and IL-6 contents in groups I, II, and III were significantly increased (P<0.05). 4) Compared with the control group, at day 14 of the experiment, serum total antioxidant capacity (T-AOC) in groups II and III was significantly increased (P<0.05), and serum glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities in all treatment groups were significantly increased (P<0.05); at day 35 of the experiment, serum T-AOC in groups III and IV was significantly increased (P<0.05), serum SOD activity in group III was significantly increased (P<0.05), and serum malondialdehyde (MDA) content in all treatment groups was significantly decreased (P<0.05). It can be concluded that dietary supplementation with montmorillonite and yeast culture complex can improve growth performance, and enhance immune function and antioxidant capacity of weaned piglets.

Full Text

Effects of Montmorillonite and Yeast Culture Complex on Growth Performance, Serum Biochemical Indexes, Immune Function and Antioxidant Capacity of Weaned Piglets

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Abstract

This study investigated the effects of dietary montmorillonite and yeast culture complex as replacements for antibiotics (AB) and zinc oxide (ZnO) on growth performance, serum biochemical indexes, immune function, and antioxidant capacity of weaned piglets. One hundred and eighty 31-day-old "Duroc × Landrace × Large White" weaned piglets with an average body weight of (6.78±0.19) kg were randomly assigned to five groups with six replicates per group and six piglets per replicate (half male and half female). Piglets in the

control group were fed basal diets supplemented with AB (500 g/t 15% chlortetracycline + 200 g/t 50% olaquindox) and ZnO (3 kg/t). Experimental groups I, II, III, and IV received basal diets supplemented with AB + ZnO + montmorillonite and yeast culture complex (1.5 kg/t), ZnO + montmorillonite and yeast culture complex, AB + montmorillonite and yeast culture complex, and montmorillonite and yeast culture complex alone, respectively. The experiment lasted 35 days. The results showed: (1) From days 15–35, average daily feed intake (ADFI) of piglets in groups III and IV was significantly higher than in the control and group I ($P<0.05$), while average daily gain (ADG) in groups II, III, and IV was significantly higher than the control ($P<0.05$). (2) At days 14 and 35, serum alkaline phosphatase (ALP) activity in groups III and IV was significantly decreased compared with the control ($P<0.05$), and serum alanine aminotransferase (ALT) activity in groups II, III, and IV was significantly decreased ($P<0.05$). No significant differences were observed in total protein (TP), albumin (ALB), urea nitrogen (UN), or aspartate aminotransferase (AST) among all groups ($P>0.05$). (3) At day 14, serum immunoglobulin A (IgA), tumor necrosis factor- (TNF-), and interferon- (INF-) levels in all experimental groups were significantly increased ($P<0.05$); serum immunoglobulin M (IgM) and interleukin-4 (IL-4) in groups II and III were significantly increased ($P<0.05$); and serum IL-1 , IL-2, and IL-6 in group I were significantly increased ($P<0.05$). At day 35, serum IgG and TNF- in all experimental groups were significantly increased ($P<0.05$); serum IgM in groups I and IV was significantly increased ($P<0.05$); and serum INF- and IL-6 in groups I, II, and III were significantly increased ($P<0.05$). (4) At day 14, serum total antioxidant capacity (T-AOC) in groups II and III was significantly increased ($P<0.05$), and serum glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities in all experimental groups were significantly increased ($P<0.05$). At day 35, serum T-AOC in groups III and IV was significantly increased ($P<0.05$), SOD activity in group III was significantly increased ($P<0.05$), and serum malondialdehyde (MDA) in all experimental groups was significantly decreased ($P<0.05$). These results indicate that dietary supplementation with montmorillonite and yeast culture complex can improve growth performance, immune function, and antioxidant capacity of weaned piglets.

Keywords: montmorillonite; yeast culture; weaned piglets; growth performance; immune function; antioxidant capacity

Early weaning is a key technology in intensive pig production but often causes weaning stress syndrome, leading to reduced feed intake, diarrhea, and even mortality [1]. Currently, high doses of antibiotics [2] and zinc oxide (ZnO) [3] are commonly added to piglet diets to alleviate weaning stress, but these practices cause environmental pollution and promote bacterial resistance, threatening human and animal health [4]. Therefore, developing green additives to replace antibiotics and ZnO is urgently needed. Montmorillonite, an aluminosilicate clay mineral, adsorbs bacteria and toxins due to its unique structure [5-6] and has been used in China for decades [7-9]. Yeast culture, a microecological product from anaerobic yeast fermentation, is rich in oligosaccharides

and probiotics, offering safe, green, and efficient benefits for animal production [10-11]. Studies show yeast culture improves livestock performance, immunity, stress response, and toxin adsorption [12-15]. However, limited research exists on whether combined montmorillonite and yeast culture complex can replace antibiotics and ZnO in piglet diets. This study examined the effects of this complex on growth performance, serum biochemical indexes, immune function, and antioxidant capacity in weaned piglets to evaluate its potential as an antibiotic/ZnO alternative and provide a scientific basis for its application.

1.1 Experimental Materials

The montmorillonite and yeast culture complex was provided by Amlan International (USA), containing 70% calcium montmorillonite and 30% yeast cell culture.

1.2 Experimental Design and Diets

One hundred and eighty 31-day-old "Duroc × Landrace × Large White" weaned piglets (6.78±0.19 kg body weight) were randomly allocated to five groups with six replicates per group and six piglets per replicate (half male, half female). The control group received basal diet supplemented with antibiotics (500 g/t 15% chlortetracycline + 200 g/t 50% olaquindox) and ZnO (3 kg/t). Experimental groups I, II, III, and IV received basal diets supplemented with: (I) antibiotics + ZnO + montmorillonite and yeast culture complex (1.5 kg/t); (II) ZnO + montmorillonite and yeast culture complex; (III) antibiotics + montmorillonite and yeast culture complex; and (IV) montmorillonite and yeast culture complex alone. All diets met NRC (2012) nutrient requirements for piglets. Diet composition and nutrient levels are shown in Table 1 .

1.3 Animal Management

The trial was conducted at Hunan Baiyi Zhentou Breeding Pig Farm. Pig houses were thoroughly cleaned and disinfected before the experiment. Piglets had ad libitum access to feed and water throughout the 35-day trial period, with regular pen cleaning according to standard farm management procedures.

1.4.1 Growth Performance Metrics

Daily feed intake and orts were recorded. Piglets were weighed at the start and end of the trial to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) for days 1–14, 15–35, and 1–35.

1.4.2 Serum Biochemical, Immune, and Antioxidant Indices

On days 14 and 35, six piglets per group with body weight 接近 the group mean were selected for jugular vein blood collection (10 mL) after overnight fasting. Serum was separated by centrifugation at 3,000 r/min for 10 min, aliquoted,

stored at -20°C, and sent to Beijing Huaying Biotechnology Institute for analysis of serum biochemical, immune, and antioxidant indices.

1.5 Data Processing and Analysis

Data are expressed as "mean ± standard deviation" and analyzed using one-way ANOVA in SPSS 19.0 software. Differences were considered significant at $P<0.05$.

2.1 Effects of Montmorillonite and Yeast Culture Complex on Growth Performance of Weaned Piglets

As shown in Table 2 , during days 1-14, no significant differences were observed in ADFI, ADG, or F/G among groups ($P>0.05$). During days 15-35, ADFI in groups III and IV was significantly higher than in the control and group I ($P<0.05$), while ADG in groups II, III, and IV was significantly higher than the control ($P<0.05$). The F/G in experimental groups decreased by 2.90%, 1.74%, 3.48%, and 5.23% compared with the control, respectively ($P>0.05$). Over the entire 35-day period, no significant differences were observed in ADFI among groups ($P>0.05$), but ADG in groups II, III, and IV was significantly higher than the control ($P<0.05$). The F/G in experimental groups decreased by 3.13%, 4.37%, 3.13%, and 6.25% compared with the control, respectively ($P>0.05$).

2.2 Effects of Montmorillonite and Yeast Culture Complex on Serum Biochemical Indexes of Weaned Piglets

Table 3 shows that on day 14, serum total protein (TP) and albumin (ALB) levels in experimental groups tended to increase compared with the control but without significant differences ($P>0.05$). Serum alkaline phosphatase (ALP) activity in groups III and IV was significantly lower than the control ($P<0.05$), while groups I and II showed no significant difference ($P>0.05$). Serum alanine aminotransferase (ALT) activity in all experimental groups was significantly lower than the control ($P<0.05$). No significant differences were observed in serum aspartate aminotransferase (AST) activity or urea nitrogen (UN) content among groups ($P>0.05$). On day 35, serum TP and ALB levels in experimental groups showed an increasing trend without significant differences ($P>0.05$). Serum ALP activity in groups I and II did not differ significantly from the control ($P>0.05$), while groups III and IV showed significantly lower ALP activity ($P<0.05$). Serum ALT activity in groups II, III, and IV was significantly lower than the control ($P<0.05$), but group I showed no significant difference ($P>0.05$). Serum AST activity and UN content in experimental groups tended to decrease without significant differences ($P>0.05$).

2.3 Effects of Montmorillonite and Yeast Culture Complex on Serum Immune Indexes of Weaned Piglets

Table 4 shows that on day 14, serum immunoglobulin A (IgA), tumor necrosis factor- (TNF-), and interferon- (INF-) levels in all experimental groups were significantly higher than the control (P<0.05). Serum immunoglobulin G (IgG) in groups I, III, and IV was significantly higher than the control (P<0.05), while serum immunoglobulin M (IgM) and interleukin-4 (IL-4) in groups II and III were significantly increased (P<0.05). Group I showed significantly higher serum interleukin-1 (IL-1), interleukin-2 (IL-2), and interleukin-6 (IL-6) levels (P<0.05). On day 35, serum IgG and TNF- levels in all experimental groups were significantly higher than the control (P<0.05), while serum IgM in groups I and IV was significantly increased (P<0.05). Serum INF- and IL-6 levels in groups I, II, and III were significantly higher than the control (P<0.05), but group IV showed no significant difference (P>0.05). Compared with the control, groups I and II showed no significant differences in IL-1 and IL-4 (P>0.05), while groups III and IV had significantly lower IL-1 (P<0.05) and significantly higher IL-4 (P<0.05). No significant differences were observed in IL-2 among groups (P>0.05).

2.4 Effects of Montmorillonite and Yeast Culture Complex on Serum Antioxidant Indexes of Weaned Piglets

As shown in Table 5 , on day 14, serum total antioxidant capacity (T-AOC) in groups II and III was significantly higher than the control (P<0.05), while serum glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities in all experimental groups were significantly increased (P<0.05). No significant differences were observed in serum malondialdehyde (MDA) content among groups (P>0.05). On day 35, serum T-AOC in groups III and IV was significantly higher than the control (P<0.05), though groups I and II showed no significant difference (P>0.05). Serum GSH-Px activity in experimental groups did not differ significantly from the control (P>0.05). Serum SOD activity in group III was significantly higher than the control (P<0.05), while serum MDA content in all experimental groups was significantly lower than the control (P<0.05).

3.1 Effects of Montmorillonite and Yeast Culture Complex on Growth Performance of Weaned Piglets

This study demonstrated that dietary supplementation with montmorillonite and yeast culture complex promotes growth performance in weaned piglets. Guo et al. [16] reported that copper-loaded modified montmorillonite significantly improved growth performance and feed conversion in weaned piglets. Liu [17] found that adding 1,000–4,000 mg/kg of intercalated modified montmorillonite to weaned piglet diets produced effects comparable to 150 mg/kg chlortetracycline, with optimal results at 2,000–4,000 mg/kg. Hu et al. [18] observed similar improvements in piglet growth performance between montmorillonite and ZnO

supplementation. Tang et al. [19] reported that dietary aluminosilicate clay significantly improved growth performance and nutrient digestibility, thereby increasing ADG in piglets. Ke et al. [20] found that modified montmorillonite as an antibiotic alternative significantly improved growth performance and reduced *E. coli* and *Streptococcus* counts in jejunal contents of weaned piglets. In this study, ADFI in groups III and IV was significantly higher than the control and group I during days 15–35, while ADG in groups II, III, and IV was significantly higher than the control during days 15–35 and throughout the entire 35-day period. Therefore, dietary supplementation with 1.5 kg/t montmorillonite and yeast culture complex can replace 0.7 kg/t antibiotics or 3 kg/t ZnO in promoting growth of weaned piglets.

3.2 Effects of Montmorillonite and Yeast Culture Complex on Serum Biochemical Indexes of Weaned Piglets

Blood is a vital component of the internal environment, and changes in blood physicochemical indices reflect metabolic status and organ health [21]. Serum total protein (TP) content reflects nutritional status and protein metabolism; generally, better nutritional status increases protein synthesis and elevates serum TP [22]. Dietary modified montmorillonite in weaned piglets significantly increased red blood cell count and hemoglobin content, improving anemia and physical condition [23]. Albumin (ALB) transports raw materials for tissue synthesis and metabolic waste; serum ALB decreases when hepatic protein synthesis is impaired [24]. Studies show that 3,000 mg/kg ZnO in piglet diets increases serum TP and decreases serum UN [25-26], and similar results were reported with nano-montmorillonite supplementation [27]. In this study, serum TP and ALB in experimental groups tended to increase compared with the control without significant differences, indicating that montmorillonite and yeast culture complex can replace antibiotics or ZnO to improve protein metabolism. Urea nitrogen (UN), the end product of protein metabolism, reflects nutritional status and protein catabolism; elevated UN generally indicates reduced protein deposition [28]. At day 35, serum UN in experimental groups tended to decrease, suggesting that montmorillonite and yeast culture complex may enhance protein deposition in weaned piglets. ALT and AST are crucial transaminases; elevated blood activities indicate liver damage. Yang et al. [29] found that nano-copper-loaded montmorillonite significantly increased serum ALT activity in growing-finishing pigs without affecting AST. Other studies reported no significant effects of montmorillonite on serum ALT and AST in lactating cows [30]. In this study, serum ALT activity in groups II, III, and IV was significantly lower than the control, and serum AST activity in experimental groups tended to decrease, indicating that montmorillonite and yeast culture complex supplementation protects liver function in piglets. ALP, a key enzyme in digestion and metabolism, promotes calcium phosphate storage in bone and reflects osteoblast activity [31]. ALP activity correlates closely with zinc levels and can evaluate zinc status [32]. This study found that serum ALP activity in groups III and IV was significantly lower than the control, while groups I and II showed

no significant difference, suggesting that montmorillonite and yeast culture complex affects ALP similarly to antibiotics but less than ZnO, consistent with Wu et al. [27]; the mechanism requires further investigation.

3.3 Effects of Montmorillonite and Yeast Culture Complex on Serum Immune Indexes of Weaned Piglets

Immunoglobulins are primary immune molecules mediating humoral immunity and important indicators of immune function; increased serum IgA, IgM, and IgG levels indicate enhanced immunity. Studies show that high-dose yeast cell wall in weaned piglet diets increases serum IgA and IgG [33-34]. This study demonstrated that serum IgA, IgG, and IgM levels in all experimental groups were higher than the control, indicating that montmorillonite and yeast culture complex outperformed antibiotics and ZnO in enhancing immunoglobulin content. Yeast-derived -glucan is a non-specific immune stimulant that activates cytotoxic T lymphocytes (CTL), B cells, macrophages, and natural killer (NK) cells [35]. Macrophages are important immune cells that play key roles in inducing and regulating specific immune responses, secreting bioactive substances such as IL-1 and TNF- when activated. Based on cytokine expression, CD4 lymphocytes differentiate into Th1 and Th2 cells [36]. Th1 cells secrete IL-2 and IFN- to assist T cell proliferation and initiate cell-mediated immunity [37]. Yeast polysaccharides promote T and B cell proliferation and IL-1 and IL-2 production, enhancing bacterial killing in mice [38]. This study showed that serum TNF- , IFN- , IL-1 , IL-2, IL-4, and IL-6 levels in experimental groups increased compared with the control, with the most significant effects in groups I and IV. This indicates that yeast culture promotes IL-2, IL-4, and TNF- production by T lymphocytes and IFN- , IL-1 , and IL-6 production by monocytes/macrophages, consistent with Chen et al. [39] in nursery piglets and Liao [40] in broilers. Therefore, dietary montmorillonite and yeast culture complex can replace ZnO or antibiotics to increase serum TNF- , IFN- , IL-1 , IL-2, IL-4, and IL-6 levels, promote T lymphocyte and monocyte/macrophage proliferation, and effectively enhance piglet immune function.

3.4 Effects of Montmorillonite and Yeast Culture Complex on Serum Antioxidant Indexes of Weaned Piglets

Serum total antioxidant capacity (T-AOC), GSH-Px and SOD activities, and MDA content reflect the body' s antioxidant capacity [41]. T-AOC is an important indicator of overall antioxidant ability [42]. SOD is the most important antioxidant enzyme in the anti-oxidative defense system. MDA, the final metabolite of lipid peroxidation, reflects lipid peroxidation levels and indirectly indicates cellular damage [43]. Studies in fattening pigs [44] and broilers [45] demonstrated that yeast culture significantly enhances animal antioxidant capacity. This study showed that at day 14, serum T-AOC, GSH-Px, and SOD activities in groups II and III were significantly higher than the control; at day 35, serum T-AOC and SOD activity in group III were significantly higher than

the control, and serum MDA content in all experimental groups was significantly lower than the control. The antioxidant effects of yeast culture may relate to its content of vitamins (C, E), trace elements [selenium (Se), Zn, copper (Cu)], enzymes, and unknown factors [46]. Vitamin C is a water-soluble antioxidant that scavenges free radicals [47], while vitamin E donates hydrogen to radicals, inhibiting lipid oxidation chain reactions and reducing MDA production [48]. SOD is a metalloproteinase that combines with Cu, Zn, and manganese (Mn) in yeast culture to form Cu-ZnSOD and MnSOD, catalyzing superoxide anion radical disproportionation to hydrogen peroxide and preventing superoxide damage [49]. Thus, montmorillonite and yeast culture complex can replace antibiotics or ZnO and enhance antioxidant capacity.

4 Conclusion

Dietary supplementation with montmorillonite and yeast culture complex improves ADFI and ADG, enhances growth performance, and increases antioxidant capacity and immune function in weaned piglets. Based on these results, adding 1.5 kg/t montmorillonite and yeast culture complex can effectively replace antibiotics or ZnO in weaned piglet diets.

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