

## Effects of Compound-Specific Egg Yolk Antibodies on Growth, Diarrhea, and Immune Function in 18- to 28-Day-Old Calves (Postprint)

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### Abstract

This study aimed to investigate the effects of dietary supplementation with compound-specific egg yolk antibody (IgY) on growth performance, diarrhea, and immunity in 18- to 28-day-old calves. Twenty newborn Chinese Holstein bull calves were selected and fed fresh milk before 10 days of age, then randomly divided into two groups: the control (CON) group received a basal diet, while the experimental (IGY) group received the basal diet supplemented with 45 mg/d of compound-specific IgY. The preliminary period was from 11 to 17 days of age, during which a transition to milk replacer was conducted, and the formal trial period was from 18 to 28 days of age. Body weight was measured before morning feeding at 18 and 28 days of age, daily feed intake was recorded, and fecal scores were assessed. On the final day of the experiment (28 days of age), blood samples were collected from the jugular vein before morning feeding to determine serum total antioxidant capacity (T-AOC), alkaline phosphatase (AKP) activity, and concentrations of immunoglobulins, nitric oxide (NO), and lysozyme (LZM). The results showed that although dietary supplementation with compound-specific IgY had no significant effect on average daily gain or feed-to-gain ratio ( $P>0.05$ ), it significantly reduced fecal scores and diarrhea rate ( $P<0.05$ ). Compared with the CON group, the IGY group significantly increased serum concentrations of immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) ( $P<0.01$ ) by 7.19%, 5.71%, and 8.50%, respectively. The T-AOC in the IGY group was significantly higher than that in the CON group ( $P<0.01$ ), whereas the NO concentration was significantly lower ( $P<0.05$ ). Serum LZM concentration in the IGY group was significantly lower than that in the CON group ( $P<0.01$ ), but compound-specific IgY had no significant effect on serum AKP activity ( $P>0.05$ ). These results suggest that dietary supplementation with compound-specific IgY enhances immune capacity and resistance to external stress, reduces diarrhea rate, and promotes calf health

by increasing immunoglobulin concentrations and antioxidant capacity, as well as exerting its own antigen-antibody reactions.

## Full Text

### Effects of Composite Specific Immunoglobulin of Yolk on Growth, Diarrhea and Immunity of Calves Aged 18 to 28 Days

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#### Abstract

This study investigated the effects of dietary supplementation with composite specific immunoglobulin of yolk (IgY) on growth performance, diarrhea incidence, and immune function in calves aged 18 to 28 days. Twenty newborn Chinese Holstein bull calves were selected and fed fresh milk until 10 days of age, then randomly assigned to two groups. The control group (CON) received a basal diet, while the treatment group (IGY) received the basal diet supplemented with 45 mg/d of composite specific IgY. A pre-experimental period from 11 to 17 days of age was used for milk replacer transition, followed by the formal experimental period from 18 to 28 days of age. Body weight was recorded at 18 and 28 days of age, feed intake and fecal scores were recorded daily. On the final day of the experiment (day 28), blood samples were collected before morning feeding via jugular venipuncture to determine serum total antioxidant capacity (T-AOC), alkaline phosphatase (AKP) activity, and concentrations of immunoglobulins, nitric oxide (NO), and lysozyme (LZM). The results showed that dietary supplementation with composite specific IgY had no significant effect on average daily gain or feed-to-gain ratio ( $P > 0.05$ ), but significantly reduced fecal scores and diarrhea incidence ( $P < 0.05$ ). Compared with the CON group, the IGY group significantly increased serum concentrations of immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) by 7.19%, 5.71%, and 8.50%, respectively ( $P < 0.01$ ). Serum T-AOC in the IGY group was significantly higher than that in the CON group ( $P < 0.01$ ), while NO concentration was significantly lower ( $P < 0.05$ ). Serum LZM concentration in the IGY group was significantly lower compared to the CON group ( $P < 0.01$ ), though composite specific IgY had no significant effect on serum AKP activity ( $P > 0.05$ ). These results indicate that dietary supplementation with composite specific IgY enhances immune capacity and resistance to external stress by increasing serum immunoglobulin concentrations and antioxidant capacity, while exerting its inherent antigen-antibody reactions, thereby reducing diarrhea incidence and promoting calf health.

**Keywords:** calves; immunoglobulin of yolk; diarrhea; antioxidant capacity; immunity

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## Introduction

The use of antibiotics as feed additives for treating and preventing animal diseases and promoting growth has posed significant challenges to animal production and human health in modern livestock development [1]. Identifying effective alternatives to antibiotics has become a research priority to meet the requirements for healthy, green, efficient, and environmentally sustainable livestock development. Immunoglobulin of yolk (IgY) is a specific antibody formed in egg yolk through immune responses when poultry are immunized with specific antigens, with antibodies accumulating in egg yolk via blood circulation [2]. IgY exhibits strong resistance to acid, heat, high osmotic pressure, ionic strength, and specific enzymatic degradation [3], enabling it to effectively pass through the rumen and abomasum of ruminants to exert immunological effects in the intestine, while also being absorbed through the intestinal tract into the circulatory system to function in non-intestinal tissues [2]. With advancing research, IgY is increasingly being applied as an immune enhancer in animal production. Rahimi et al. [4] and Mahdavi et al. [5] fed chicks with IgY containing anti-Salmonella and anti-Escherichia coli antibodies, respectively, and found that IgY not only inhibited pathogen adhesion to intestinal mucosa but also improved intestinal health indices and enhanced immune responses. In piglets, Luo [6] and Cheng et al. [7] supplemented diets with IgY, which effectively inhibited pathogen adhesion to jejunal epithelial cells, significantly improved growth performance and immunity, and reduced diarrhea incidence in early-weaned piglets. Vega et al. [8] and Dhama et al. [9] fed calves with specific IgY against rotavirus and found that IgY enhanced immunity and reduced diarrhea incidence and mortality by activating immune responses in intestinal mucosal antibody-secreting cells. Current research has primarily focused on the immunomodulatory effects of single-specific IgY on young livestock, while studies on composite specific IgY that protects against multiple pathogens and viruses causing complex diseases, particularly in young ruminants such as calves, remain scarce. As an antibiotic alternative, composite specific IgY holds significant importance for improving immunity in calves with underdeveloped immune systems and enhancing their adaptation to external environments. Therefore, this experiment aimed to investigate the effects of dietary composite specific IgY on diarrhea and immunity in calves, providing a theoretical basis for its application in raising young ruminants.

### 1.1 Experimental Design and Diets

This experiment employed a single-factor randomized design with two groups. The control group (CON) was fed a basal diet, while the treatment group (IGY) received the basal diet supplemented with composite specific IgY (provided by

Dalian Saimu Bioengineering Technology Co., Ltd.). The composite specific IgY was prepared by immunizing hens with two composite antigens: bacterial antigens (F4, F5, F6, F18, F41 + SLT-IIv + STa-LTb + SEM-AJ01) and viral antigens (TGEV + PEDV + RV + SEM-AJ01; SEM-AJ01 is an efficient adjuvant), with specific antibodies produced through immune responses and transferred to egg yolk. The supplementation level of composite specific IgY was 45 mg/d. The basal diet consisted of antibiotic-free and microbe-free milk replacer and starter feed, with the milk replacer produced by Beijing Precision Animal Nutrition Research Center according to the national invention patent CN02128844.5. The nutrient levels of the basal diet are presented in Table 1 .

## 1.2 Animals and Management

Twenty naturally born Chinese Holstein bull calves with birth weights of approximately 40 kg were selected and randomly divided into two groups of 10 calves each. Calves received adequate colostrum within 1 h after birth and were fed fresh milk until 10 days of age. The pre-experimental period was from 11 to 17 days of age for milk replacer transition, with complete transition to milk replacer by 18 days of age. The formal experimental period lasted from 18 to 28 days of age. The milk replacer was prepared with boiled water cooled to 50–60 °C at a dry matter concentration of 12.5%, and fed to calves when the temperature decreased to approximately 40 °C. Calves were fed twice daily (08:00 and 15:00) at 12% of body weight per day. During the experiment, composite specific IgY was added to the milk replacer emulsion according to calf body weight, mixed thoroughly before feeding. Starter feed was provided ad libitum with free access to clean water throughout the experimental period. Calves were housed individually in calf hutches with dimensions of 1.6 m × 3.6 m.

### 1.3.1 Growth Performance and Fecal Scoring

Body weight was recorded before morning feeding at 18 and 28 days of age to calculate average daily gain (ADG). Daily feed intake was recorded to calculate feed-to-gain ratio. Representative samples of milk replacer and starter feed were collected during the experiment, and nutrient composition was determined according to AOAC (2000) [10]. Gross energy (GE) was measured using a PARR-6400 automatic oxygen bomb calorimeter; crude protein (CP) content was determined using a KDY-9830 automatic Kjeldahl nitrogen analyzer; crude fat (EE) content was analyzed using an ANKOM-XT15i automatic fat analyzer; and conventional methods were used to determine dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca), and phosphorus (P) contents.

Daily health status was observed and fecal scores were assessed according to Table 2 [11]. A day with fecal score  $\leq 2$  was recorded as one diarrhea day; when fecal score  $> 3$ , electrolyte solution was administered and antibiotic treatment was applied if necessary. Diarrhea incidence and fecal index were calculated as follows:

Diarrhea incidence (%) = (number of calves with diarrhea / total number of calves) × 100

Fecal index = sum of fecal scores / total number of calves

### 1.3.2 Serum Indices

On the final day of the experiment (day 28), 5 mL of blood was collected from each calf via jugular venipuncture before morning feeding, centrifuged at 3,000 r/min for 20 min, and serum was collected and stored in 1.5 mL centrifuge tubes at -20 °C. Serum concentrations of immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA) were determined by immunoturbidimetry using a Songshang A8 automatic biochemical analyzer. Serum total antioxidant capacity (T-AOC), nitric oxide (NO), lysozyme (LZM) concentrations, and alkaline phosphatase (AKP) activity were measured by biochemical methods using a Hitachi 7600 biochemical analyzer. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute.

### 1.4 Statistical Analysis

Experimental data were analyzed using SAS 9.2 statistical software with unpaired t-tests. Differences were considered significant at  $P < 0.05$  and highly significant at  $P < 0.01$ .

## 2.1 Effects of Composite Specific IgY on Calf Growth

As shown in Table 3, there were no significant differences in initial or final body weight between the two groups during the experimental period ( $P > 0.05$ ). However, dietary supplementation with composite specific IgY tended to increase ADG ( $P = 0.082$ ). Composite specific IgY had no significant effects on average daily feed intake or feed-to-gain ratio ( $P > 0.05$ ).

## 2.2 Effects of Composite Specific IgY on Fecal Scores

As shown in Figure 1 [Figure 1: see original paper], dietary supplementation with composite specific IgY helped reduce fecal scores, with scores in the IGY group being significantly lower than those in the CON group at 20, 22, 23, and 25 days of age ( $P < 0.05$ ).

## 2.3 Effects of Composite Specific IgY on Diarrhea Incidence and Fecal Index

As shown in Table 4, although composite specific IgY did not significantly affect fecal index ( $P > 0.05$ ), diarrhea incidence in the IGY group was significantly lower than that in the CON group ( $P < 0.05$ ).

## 2.4 Effects of Composite Specific IgY on Serum Immunoglobulin Concentrations

As shown in Table 5 , dietary supplementation with composite specific IgY significantly increased serum concentrations of IgG, IgM, and IgA compared with the CON group ( $P < 0.01$ ), with increases of 7.19%, 5.71%, and 8.50%, respectively.

## 2.5 Effects of Composite Specific IgY on Serum Antioxidant Capacity

As shown in Table 6 , composite specific IgY had significant or highly significant effects on antioxidant capacity ( $P < 0.05$  or  $P < 0.01$ ). Serum T-AOC in the IGY group was significantly higher than that in the CON group ( $P < 0.01$ ), while NO concentration was significantly lower ( $P = 0.043$ ).

## 2.6 Effects of Composite Specific IgY on Serum Immune-related Enzymes

As shown in Table 7 , serum LZM concentration in the IGY group was significantly lower than that in the CON group ( $P < 0.01$ ). However, composite specific IgY had no significant effect on serum AKP activity ( $P > 0.05$ ).

## 3.1 Effects of Composite Specific IgY on Calf Growth and Diarrhea

Zhu et al. [12] reported that dietary IgY supplementation significantly increased ADG in meat ducks by elevating serum triiodothyronine and insulin-like growth factor-1 concentrations. In the present study, composite specific IgY only tended to increase ADG without significantly affecting body weight, possibly due to the short experimental period where the cumulative effects of IgY on hormone regulation had not yet reached significant levels, or due to differences in animal species. Diarrhea is a major factor affecting calf growth and development. Currently, researchers use fecal morphology characteristics, including fecal scoring, as predictive indicators of diarrhea severity to evaluate calf health status [13]. In this experiment, continuous fecal scoring revealed that dietary supplementation with composite specific IgY significantly reduced fecal scores and diarrhea incidence compared with the control group, consistent with the findings of Vega et al. [14] on specific IgY for calf diarrhea. Studies suggest that IgY can directly adhere to the fimbriae or flagella of pathogens such as *E. coli* or *Salmonella*, preventing their adhesion to intestinal mucosal epithelial cells and facilitating their excretion, thereby reducing intestinal pathogen proliferation and decreasing diarrhea incidence [2]. Additionally, oral administration of specific IgY to calves can modulate mucosal and systemic immune responses, enhancing intestinal immune defense against specific viruses, particularly in the duodenal mucosa, and reducing the incidence of viral diarrhea [14]. Furthermore, observation of overall

fecal scores and clinical symptoms revealed that dietary composite specific IgY helped delay the onset peak of diarrhea and shorten its duration when calves experienced external stress, consistent with the findings of Parreño et al. [15] on specific IgY supplementation in colostrum for calf diarrhea.

### **3.2 Effects of Composite Specific IgY on Serum Immunoglobulin Concentrations**

While diarrhea incidence and fecal index provide direct observation of calf health status, serum immunoglobulins serve as a latent indicator for evaluating animal immunity from within, and their combination facilitates comprehensive analysis of experimental results [16]. Newborn calves acquire immunoglobulins primarily through colostrum before gut closure, and adequate serum immunoglobulins are crucial for enhancing calf immunity. IgM is the major antibody in the initial phase of immune response; IgG, as the predominant antibody in serum, plays an important role in humoral immune response; IgA mediates mucosal immune responses and primarily functions through immune exclusion in coordination with the non-specific immune system [17]. Studies have shown that serum immunoglobulin concentrations in diarrheic calves are significantly lower than in healthy calves [18]. The present study demonstrated that dietary supplementation with composite specific IgY significantly increased serum IgG, IgA, and IgM concentrations. Antibody-secreting cells (ASC) are mainly distributed in the intestinal mucosal lamina propria (duodenum, jejunum, and ileum), jejunal and ileal Peyer's patches, and mesenteric lymph nodes. Parreño et al. [15] orally administered specific IgY to rotavirus-challenged calves and found significantly reduced ASC for IgM and IgA in intestinal mucosa compared with the control group, with no IgG ASC detected, and IgA ASC response was also significantly suppressed. This indicates that dietary specific IgY can directly neutralize pathogen antigens in the intestine, enhancing passive immunity [8] and maintaining serum immunoglobulins at higher levels. However, the dose-dependent inhibitory effects of passive immune antibodies on the development of antibody-mediated mucosal and systemic immune response systems warrant further attention and investigation.

### **3.3 Effects of Composite Specific IgY on Serum Antioxidant Capacity**

Oxidative stress occurs when the body experiences harmful stimuli, resulting in excessive production of highly reactive molecules such as reactive oxygen species or reactive nitrogen species, where the degree of oxidation exceeds the body's capacity to clear oxidants, leading to imbalance between oxidative and antioxidant systems and consequent cellular or tissue damage [19]. Total antioxidant capacity (T-AOC) is the primary system antagonizing oxidative free radicals, restricting and eliminating excess free radicals to protect normal cellular function and maintain normal metabolism [20]. The present results showed that composite specific IgY significantly increased serum T-AOC concentration in

calves. Higher T-AOC levels facilitate timely clearance of free radicals generated during oxidation, maintaining dynamic equilibrium between oxidation and antioxidation and ensuring animal health. However, serum NO concentration in the non-supplemented group was significantly higher than in the supplemented group, possibly due to compensatory effects from plant proteins in the milk replacer causing stress responses in calves, resulting in high serum NO concentrations. Studies have also found that higher plant protein content in milk replacer intensifies this compensatory effect [20]. This suggests that dietary composite specific IgY effectively reduced compensatory responses to exogenous stress, thereby improving calf adaptation to plant proteins. These results are consistent with the fecal scoring and diarrhea findings.

### 3.4 Effects of Composite Specific IgY on Serum Immune-related Enzymes

Lysozyme (LZM), as a non-specific immune factor, participates in various immune responses and plays an important role in normal defense and non-specific immunity [21]. LZM is primarily released by mononuclear macrophages and widely distributed in animal tissues, body fluids, and secretions, exerting antibacterial and anti-inflammatory effects and enhancing immunity mainly by dissolving bacterial or fungal cell walls [22]. In this study, supplementation with composite specific IgY significantly reduced serum LZM concentration, indicating that composite specific IgY helps reduce non-specific immune responses and adverse effects of external stimuli by exerting its own antigen-antibody immune reactions, thereby improving calf adaptability. Alkaline phosphatase (AKP) enhances foreignness by altering bacterial surface structures in animal tissues, body fluids, and secretions, making them more susceptible to phagocytosis and degradation by phagocytes [16]. AKP works synergistically with LZM to enhance resistance to external pathogen infection. However, the present results showed that composite specific IgY did not affect serum AKP activity, possibly related to the physiological state of the calves at the time.

Specific IgY demonstrated positive effects in reducing calf diarrhea and improving immunoglobulin concentrations, antioxidant capacity, and immune-related enzyme activities. However, the mechanisms of immune neutralization reactions between specific IgY and pathogenic antigens in the gastrointestinal tract of young animals, and the regulatory effects of specific IgY on immune responses of intestinal mucosal immune cells require further investigation. Additionally, the positive effects of large amounts of non-specific antibodies accumulated in egg yolk during hen immunization on young animals should receive attention and be fully utilized. Systematic and in-depth research will enable better application of composite specific IgY in production.

## Conclusion

Under the conditions of this experiment, dietary supplementation with composite specific IgY enhanced calf immune capacity and resistance to external stress by increasing serum immunoglobulin concentrations and antioxidant capacity while exerting its inherent antigen-antibody reactions, thereby reducing diarrhea incidence and promoting calf health.

## References

- [1] KOVACS-NOLAN J, MINE Y. Egg yolk antibodies for passive immunity[J]. *Annual Review of Food Science and Technology*, 2012, 3: 163-182.
- [2] JU Xiaojun, YANG Haiming, WANG Zhiyue, et al. Research and application of immunoglobulin of yolk in livestock and poultry production[J]. *Chinese Journal of Animal Nutrition*, 2015, 27(3): 691-697.
- [3] FU C Y, HUANG H, WANG X M, et al. Preparation and evaluation of anti-SARS coronavirus IgY from yolks of immunized SPF chickens[J]. *Journal of Virological Methods*, 2006, 133(1): 112-115.
- [4] RAHIMI S, SHIRAZ Z M, SALEHI T Z, et al. Prevention of salmonella infection in poultry by specific egg-derived antibody[J]. *International Journal of Poultry Science*, 2007, 6(4): 230-235.
- [5] MAHDAVI A H, RAHMANI H R, NILI N, et al. Effects of dietary egg yolk antibody powder on growth performance, intestinal *Escherichia coli* colonization, and immunocompetence of challenged broiler chicks[J]. *Poultry Science*, 2010, 89(3): 484-494.
- [6] LUO Danbin. Effects of chicken egg yolk immunoglobulin on growth and immune performance of early-weaned piglets[D]. Master' s thesis. Changsha: Hunan Agricultural University, 2006.
- [7] CHENG Xuehui, WANG Jie, JIANG Siwen, et al. Study on processing, storage and in vitro anti-adhesion of purified ETEC K88 fimbriae-specific egg yolk antibodies[J]. *Feed Research*, 2005(4): 3-5.
- [8] VEGA C, BOK M, CHACANA P, et al. Egg yolk IgY: protection against rotavirus induced diarrhea and modulatory effect on the systemic and mucosal antibody responses in newborn calves[J]. *Veterinary Immunology and Immunopathology*, 2011, 142(3/4): 156-169.
- [9] DHAMA K, CHAUHAN R S, MAHENDRAN M, et al. Rotavirus diarrhea in bovines and other domestic animals[J]. *Veterinary Research Communications*, 2009, 33(1): 1-23.
- [10] AOAC. Official methods of analysis[S]. 17th ed. Arlington: Association of Official Analytical Chemists, 2000.
- [11] HEINRICHS A J, JONES C M, HEINRICHS B S. Effects of mannan oligosaccharide or antibiotics in neonatal diets on health and growth of dairy calves[J]. *Journal of Dairy Science*, 2003, 86(12): 4064-4069.
- [12] ZHU Jinlan, LI Huanyou, FENG Dingyuan, et al. Effects of egg yolk antibody additives on weight gain and related metabolic hormone levels in meat ducks[J]. *Heilongjiang Animal Science and Veterinary Medicine*, 2004(12): 27-

28.

- [13] RADA V, VLKOVÁ E, NEVORAL J, et al. Comparison of bacterial flora and enzymatic activity in faeces of infants and calves[J]. FEMS Microbiology Letters, 2006, 258(1): 25-28.
- [14] VEGA C, BOK M, SAIF L, et al. Egg yolk IgY antibodies: a therapeutic intervention against group a rotavirus in calves[J]. Research in Veterinary Science, 2015, 103: 1-10.
- [15] PARREÑO V, BÉJAR C, VAGNOZZI A, et al. Modulation by colostrum-acquired maternal antibodies of systemic and mucosal antibody responses to rotavirus in calves experimentally challenged[J]. Veterinary Immunology and Immunopathology, 2004, 100(1/2): 7-24.
- [16] ZHANG Naifeng, DIAO Qiyu, LI Hui. Effects of plant protein on diarrhea and blood indices in 6-11 day-old calves[J]. Scientia Agricultura Sinica, 2010, 43(19): 4094-4100.
- [17] SUN P, WANG J Q, ZHANG H T. Effects of Bacillus subtilis natto on performance and immune function of preweaning calves[J]. Journal of Dairy Science, 2010, 93(12): 5851-5855.
- [18] STOTT G H, FELLAH A. Colostral immunoglobulin absorption linearly related to concentration for calves[J]. Journal of Dairy Science, 1983, 66(6): 1319-1328.
- [19] LI Huaying, QIU Xiaoping, LIU Xiaoying, et al. Observation of serum oxidation and antioxidant capacity indicators in patients with Graves' disease[J]. Chinese Journal of Endocrinology and Metabolism, 2011, 27(6): 495-497.
- [20] FEI Shuiying, LI Yu, LI Juntian, et al. Effects of different proportions of plant protein milk replacer on immune indices of early-weaned calves[J]. Acta Agriculturae Boreali-occidentalis Sinica, 2009, 18(6): 77-81.
- [21] HANCOCK R E W, SCOTT M G. The role of antimicrobial peptides in animal defenses[J]. Proceedings of the National Academy of Sciences of the United States of America, 2000, 97(16): 8856-8861.
- [22] DING Yahong. Preliminary development of composite whole egg powder for preventing and treating E. coli diarrhea in piglets[D]. Master's thesis. Yangzhou: Yangzhou University, 2006.

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