Effects of Dietary Linoleic Acid Level in Wheat-Soybean Meal Diets on Growth Performance, Lipid Metabolism, Antioxidant Function, and Immune Function in Goslings (Postprint)

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Abstract

This experiment investigated the effects of linoleic acid levels in wheat-soybean meal diets on growth performance, lipid metabolism, antioxidant function, and immune function in goslings, aiming to further explore the appropriate linoleic acid level in wheat-soybean meal diets. Three hundred and sixty 1-day-old Wulong geese were randomly divided into 6 groups with 6 replicates per group and 10 geese per replicate (half male and half female). Group I (control group) was fed a basal diet with 0.52% linoleic acid, while the experimental groups (Groups II–VI) were fed experimental diets with linoleic acid levels of 0.72%, 0.92%, 1.12%, 1.32%, and 1.52%, respectively. The experimental period lasted for 4 weeks. The results showed that: 1) Body weight (BW), average daily gain (ADG), and feed-to-gain ratio (F/G) of all experimental groups were superior to those of the control group, with the highest BW and lowest F/G observed when dietary linoleic acid level was 1.12%. 2) Serum total cholesterol (TCH) content in Groups IV and V was extremely significantly lower than that in the control group ($P<0.01$), serum triglyceride (TG) content in Groups IV and V was significantly lower than that in the control group ($P<0.05$), serum high-density lipoprotein cholesterol (HDL-C) content in Groups IV and V was extremely significantly higher than that in the control group ($P<0.01$), and serum low-density lipoprotein cholesterol (LDL-C) content in Group IV was significantly lower than that in the control group ($P<0.05$). 3) Total antioxidant capacity (T-AOC) and activities of total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) in serum and liver of Group VI were extremely significantly higher than those in the control group ($P<0.01$). 4) Bursa of Fabricius index in Group IV was extremely significantly higher than that in the control group ($P<0.01$), serum immunoglobulin G (IgG) content in Group

III was significantly higher than that in the control group (P<0.05); 14 days after immunization, avian influenza antibody titer in Group III was extremely significantly higher than that in the control group (P<0.01). It can be concluded that appropriate linoleic acid level in wheat-soybean meal diets has significant effects on growth performance, lipid metabolism, antioxidant function, and immune function in goslings; it is recommended that the appropriate linoleic acid level in wheat-soybean meal diets for goslings be 1.12%.

Full Text

Effects of Linoleic Acid Level in Wheat-Soybean Meal Diets on Growth Performance, Lipid Metabolism, Antioxidant Capacity, and Immune Function of Goslings

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Abstract

This experiment investigated the effects of dietary linoleic acid level in wheat-soybean meal diets on growth performance, lipid metabolism, antioxidant capacity, and immune function of goslings to determine the optimal linoleic acid level. A total of 360 one-day-old Wulong geese were randomly allocated into 6 groups with 6 replicates per group and 10 goslings per replicate (half male and half female). Group (control) received a basal diet containing 0.52% linoleic acid, while experimental groups through received diets with linoleic acid levels of 0.72%, 0.92%, 1.12%, 1.32%, and 1.52%, respectively. The experiment lasted for 4 weeks.

The results showed: (1) Body weight (BW), average daily gain (ADG), and feed-to-gain ratio (F/G) in all experimental groups were superior to those in the control group, with maximal BW and minimal F/G observed at 1.12% dietary linoleic acid. (2) Serum total cholesterol (TCH) in groups and was significantly lower than in the control group (P<0.01), serum triglycerides (TG) in groups and were significantly lower (P<0.05), serum high-density lipoprotein cholesterol (HDL-C) in groups and was significantly higher (P<0.01), and serum low-density lipoprotein cholesterol (LDL-C) in group was significantly lower (P<0.05). (3) Total antioxidant capacity (T-AOC) and activities of total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) in serum and liver were significantly higher in group compared to the control group (P<0.01). (4) The bursa of Fabricius index in group was significantly higher than in the control group (P<0.01), serum immunoglobulin G (IgG) content in group was significantly higher (P<0.05), and avian influenza antibody titer in group was significantly higher than in the control group 14 days post-immunization (P<0.01).

In conclusion, appropriate dietary linoleic acid level in wheat-soybean meal diets significantly affects growth performance, lipid metabolism, antioxidant capacity, and immune function in goslings. The recommended optimal linoleic acid level for goslings fed wheat-soybean meal diets is 1.12%.

Keywords: linoleic acid; gosling; growth performance; lipid metabolism; antioxidant capacity; immune function

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Introduction

Linoleic acid is an essential fatty acid and the initial member of the n-6 polyunsaturated fatty acid (PUFA) series. It plays crucial roles in immune regulation, lipid metabolism, and cardiovascular disease prevention [1]. However, China currently lacks nutrient requirement standards for meat geese, and parameters for linoleic acid requirements remain undefined, making research on linoleic acid nutrition in geese particularly important. Balnave [2] reported that linoleic acid deficiency in chicks dramatically reduces disease resistance, increases water consumption, severely impairs growth, and causes liver enlargement due to fat accumulation, with increased eicosatrienoic acid content in most tissues leading to decreased arachidonic and linoleic acid levels. Cunnane [3] found that linoleic acid deficiency in pigs resulted in slow growth, dermatitis, fragile capillaries, increased water loss, reduced immunity, and various cardiovascular diseases, with these symptoms appearing in most pregnant sows and growing pigs. McDowell [4] noted that insufficient linoleic acid supply disrupts basal metabolism by replacing membrane linoleic acid, altering mitochondrial structure, and blocking oxidative phosphorylation. Pentieva et al. [5] demonstrated that PUFAs reduce blood lipids, decrease the metabolic burden caused by hyperlipidemia, and effectively lower serum cholesterol and body fat deposition. Yang et al. [6] found that dietary fish oil and corn oil improved catalase (CAT) and superoxide dismutase (SOD) activities in broiler spleens and increased SOD activity at 21 days of age. The NRC (1994) [7] recommended a linoleic acid requirement of 1% for geese aged 1-4 weeks. Currently, few studies on linoleic acid requirements for geese exist worldwide. While linoleic acid is generally not deficient in corn-soybean meal diets for geese, wheat-based diets are deficient in linoleic acid, resulting in poorer growth performance compared to corn-based diets. Therefore, this study used Wulong goslings to investigate the effects of different linoleic acid levels on growth performance, lipid metabolism, antioxidant capacity, and immune function, aiming to determine the optimal linoleic acid level in wheat-soybean meal diets and provide a theoretical basis for establishing nutrient standards and scientific feed resource utilization in China.

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1. Materials and Methods

1.1 Experimental Animals and Design A total of 360 healthy one-day-old Wulong geese were randomly divided into 6 groups with 6 replicates per group and 10 goslings per replicate (5 males and 5 females). Group served as the control group and received a basal diet containing 0.52% linoleic acid. Experimental groups through received diets with linoleic acid levels of 0.72%, 0.92%, 1.12%, 1.32%, and 1.52%, respectively, adjusted using corn oil, palm oil, and tallow to maintain consistent fatty acid composition ratios among groups. The experimental period lasted 4 weeks.

1.2 Experimental Diets The basal diet was formulated according to NRC (1994) poultry nutrient requirements. Diet composition and nutrient levels are presented in Table 1 . The linoleic acid level in the basal diet was measured as 0.52% using gas chromatography.

Table 1 Composition and Nutrient Levels of Experimental Diets (Air-Dry Basis) %

Note: The multivitamin and trace elements provided the following per kg of diet: VA 1,500 mg, VD3 200 IU, VE 12.5 mg, VK3 1.5 mg, VB1 2.2 mg, VB2 5.0 mg, nicotinic acid 65 mg, pantothenate 15 mg, biotin 0.2 mg, VB6 2 mg, folic acid 0.5 mg, choline 1,000 mg, Fe 90 mg, Cu 6 mg, Mn 85 mg, Zn 85 mg, I 0.42 mg, Se 0.3 mg, Co 2.5 mg. Linoleic acid was a measured value, while other nutrient levels were calculated values.

1.3 Management Practices Goslings were raised indoors in floor pens. The house was thoroughly disinfected regularly to ensure healthy growth and development. Birds had free access to feed and water, with feed provided in small amounts frequently. Growth and development were observed daily. Sampling, weighing, and counting were performed accurately.

1.4 Measurement Indicators

1.4.1 Growth Performance At 08:00 each weekend, birds were fasted for 6 hours (water provided) and weighed by replicate. Feed consumption was recorded by replicate using the "empty trough method" each weekend. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated. Mortality and culling were recorded daily to calculate the death and culling rate.

1.4.2 Serum Lipid Metabolism Indicators At the end of week 4, 10 mL of blood was collected from the wing vein and centrifuged at 3,000 r/min to prepare serum samples. Serum triglycerides (TG), total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents were measured using a UV-1100 spectrophotometer.

1.4.3 Antioxidant Indicators At the end of week 4, two goslings per replicate were randomly selected. Blood was collected from the wing vein and centrifuged at 3,000 r/min to prepare serum samples, which were aliquoted and stored at -40°C. After blood collection, birds were slaughtered and liver samples were collected to prepare 10% liver tissue homogenates. Serum and liver total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD) activity, malondialdehyde (MDA) content, and glutathione peroxidase (GSH-Px) activity were measured using a UV-1100 spectrophotometer.

1.4.4 Immune Indicators **1.4.4.1 Immune Organ Indices:** After blood collection, birds were euthanized by jugular exsanguination. The thymus, spleen, and bursa of Fabricius were excised to determine immune organ indices using the formula: Immune organ index (g/kg) = fresh immune organ weight (g) / live body weight (kg).

1.4.4.2 Serum Immunoglobulin Content: Serum immunoglobulin A (IgA), immunoglobulin M (IgM), and immunoglobulin G (IgG) contents were determined by immunoturbidimetry using assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.4.3 Avian Influenza Antibody Titer: At 36 days of age, blood was collected from the wing vein at 7, 14, 21, and 28 days post-vaccination and centrifuged to obtain serum for antibody titer determination. Avian influenza antibody titers were measured using the hemagglutination and hemagglutination inhibition test (HA-HI).

1.5 Statistical Analysis Data were analyzed using one-way ANOVA with LSD multiple comparisons in SPSS software. $P<0.05$ and $P<0.01$ were considered significant and highly significant, respectively.

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2. Results

2.1 Effects of Dietary Linoleic Acid Level on Growth Performance As shown in Table 2 , dietary linoleic acid supplementation highly significantly affected F/G and ADG ($P<0.01$) and significantly affected BW ($P<0.05$), with all showing quadratic relationships ($P<0.05$). Specifically, BW in group was highly significantly higher than in group ($P<0.01$) and significantly higher than in groups and ($P<0.05$). ADG in groups , , and was significantly higher than in groups , , and ($P<0.05$). F/G in groups , , and was highly significantly lower than in groups , , and ($P<0.05$). No significant differences in ADG or F/G were observed among groups , , and ($P>0.05$), and no significant differences in ADFI were found among all groups ($P>0.05$).

These results indicate that BW, ADG, and F/G in experimental groups were all superior to the control group, with maximal BW and minimal F/G achieved at 1.12% dietary linoleic acid. Therefore, a quadratic curve was fitted between BW

(Y) and dietary linoleic acid level (X) using data from groups to , establishing the regression equation:

$$Y = 1.206 + 0.264X - 0.110X^2 \ (R^2 = 0.621, P = 0.000)$$

From this regression equation, the maximum BW was achieved at 1.2% dietary linoleic acid in wheat-soybean meal diets.

Table 2 Effects of Dietary Linoleic Acid Level on Growth Performance of Goslings

Note: In the same row, values with the same small letter or no letter superscripts indicate no significant difference (P>0.05), adjacent small letters indicate significant difference (P<0.05), and alternate small letters indicate highly significant difference (P<0.01). The same applies below.

2.2 Effects of Dietary Linoleic Acid Level on Lipid Metabolism As shown in Table 3 , dietary linoleic acid supplementation highly significantly affected serum TCH and LDL-C contents ($P<0.01$), both showing quadratic relationships ($P<0.05$). Serum TCH in groups and was highly significantly lower than in group ($P<0.01$), and serum LDL-C in group was significantly lower than in group ($P<0.05$). Dietary linoleic acid significantly affected serum TG content ($P<0.05$), showing a linear relationship ($P<0.05$), with serum TG content tending to decrease as dietary linoleic acid level increased. Serum TG in groups and was significantly lower than in group ($P<0.05$). Dietary linoleic acid significantly affected serum HDL-C content ($P<0.05$), showing a quadratic relationship ($P<0.05$), with serum HDL-C in groups and being highly significantly higher than in group ($P<0.01$).

These results demonstrate that at 1.12% dietary linoleic acid, serum TCH and TG contents were lowest, serum HDL-C content was highly significantly increased, and serum LDL-C content was significantly decreased, with no significant effects on lipid metabolism indicators when linoleic acid exceeded 1.32%. This indicates that appropriate linoleic acid level in wheat-soybean meal diets plays a significant role in ensuring normal lipid metabolism in geese.

Table 3 Effects of Dietary Linoleic Acid Level on Lipid Metabolism of Goslings (mmol/L)

2.3 Effects of Dietary Linoleic Acid Level on Antioxidant Capacity As shown in Table 4 , dietary linoleic acid supplementation highly significantly affected serum T-AOC and GSH-Px activity ($P<0.01$), both showing quadratic relationships ($P<0.05$). Serum T-AOC and GSH-Px activity in group were highly significantly higher than in group ($P<0.01$). Dietary linoleic acid significantly affected serum T-SOD activity ($P<0.05$), showing a linear relationship ($P<0.05$), with serum T-SOD activity tending to increase as dietary linoleic acid level increased. Serum T-SOD activity in group was highly significantly higher than in group ($P<0.01$). No significant differences in serum MDA content were observed among groups ($P>0.05$).

As shown in Table 5 , dietary linoleic acid significantly affected liver T-AOC and T-SOD and GSH-Px activities ($P<0.05$), all showing quadratic relationships ($P<0.05$). Liver T-AOC and T-SOD and GSH-Px activities in group were highly significantly higher than in group ($P<0.01$). No significant differences in liver MDA content were observed among groups ($P>0.05$).

These results indicate that at 1.12% dietary linoleic acid, T-AOC and T-SOD and GSH-Px activities in both serum and liver were highly significantly increased, demonstrating that appropriate linoleic acid level in wheat-soybean meal diets importantly enhances antioxidant capacity in goslings.

Table 4 Effects of Dietary Linoleic Acid Level on Serum Antioxidant Capacity of Goslings

Table 5 Effects of Dietary Linoleic Acid Level on Liver Antioxidant Capacity of Goslings

2.4 Effects of Dietary Linoleic Acid Level on Immune Function

2.4.1 Immune Organ Indices As shown in Table 6 , dietary linoleic acid significantly affected the bursa of Fabricius index ($P<0.05$), showing a quadratic relationship ($P<0.05$). The bursa of Fabricius index in group was significantly higher than in group ($P<0.05$), and that in group was highly significantly higher than in group ($P<0.01$). Spleen and thymus indices in all groups showed a trend of increasing then decreasing as dietary linoleic acid level increased.

These results indicate that 1.12% dietary linoleic acid improved immune organ indices in goslings.

Table 6 Effects of Dietary Linoleic Acid Level on Immune Organ Indices of Goslings

2.4.2 Serum Immunoglobulin Content As shown in Table 7 , dietary linoleic acid highly significantly affected serum IgG content ($P<0.01$), showing a quadratic relationship ($P<0.05$). Serum IgG content in group was significantly higher than in group ($P<0.05$), and that in group was highly significantly higher than in group ($P<0.01$). Serum IgM and IgA contents in all groups showed a trend of increasing then decreasing as dietary linoleic acid level increased.

These results indicate that 1.12% dietary linoleic acid increased serum immunoglobulin content in goslings.

Table 7 Effects of Dietary Linoleic Acid Level on Serum Immunoglobulin Content of Goslings

2.4.3 Avian Influenza Antibody Titer As shown in Table 8 , dietary linoleic acid significantly affected avian influenza antibody titer at 14 days post-immunization (P<0.05), showing a quadratic relationship (P<0.05). Antibody titers in groups and were significantly higher than in group (P<0.05), and that in group was highly significantly higher than in group (P<0.01). At 7, 21, and 28 days post-immunization, antibody titers in all groups showed a trend of increasing then decreasing as dietary linoleic acid level increased.

These results indicate that 0.92% dietary linoleic acid significantly increased avian influenza antibody titer.

Table 8 Effects of Dietary Linoleic Acid Level on Antibody Titre of Avian Influenza of Goslings

3. Discussion

3.1 Effects on Growth Performance Linoleic acid is a growth factor that promotes cell proliferation, providing lipids for cell membrane synthesis and water-soluble lipids for cell growth, thus promoting animal growth [8-10]. Wang et al. [9] reported that in laying ducks during early production, dietary linoleic acid levels of 0.55%, 0.75%, 0.95%, 1.15%, 1.35%, and 1.55% resulted in maximal egg production, egg weight, and daily egg mass with minimal feed-to-egg ratio at 0.95% linoleic acid. Zhang et al. [11] found that adding different levels of PUFAs to broiler diets improved feed conversion efficiency and reduced F/G, confirming that dietary PUFAs improve nutrient utilization and growth performance in broilers. The present study demonstrated that 1.12% dietary linoleic acid resulted in maximal BW and ADG with minimal F/G, indicating that appropriate linoleic acid level promotes growth and improves feed utilization in goslings.

3.2 Effects on Lipid Metabolism Research shows that PUFAs prevent cardiovascular disease, treat hyperlipidemia, and reduce cholesterol-related risks [12-13]. Stone et al. [14] confirmed that cardiovascular disease incidence correlates with saturated fatty acid intake, with higher fatty acid levels increasing serum TCH content and disease risk. Blood lipids include TCH, TG, phospholipids, and free fatty acids; excessive serum TCH and TG increase blood viscosity and cause hyperlipidemia. Lipoproteins transport lipids. HDL carries cholesterol from tissues to the liver for catabolism, converting it to bile acids or excreting it directly through bile, thereby reducing serum cholesterol. LDL transports endogenous cholesterol synthesized in the liver to peripheral tissues [15], and excess LDL leaves excessive cholesterol in arterial walls, causing atherosclerosis [16]. High LDL-C content is a major lipid risk factor for coronary heart disease, and reducing it is the primary goal of lipid-lowering therapy [17], while HDL has the opposite protective effect. Linoleic acid inhibits transcription of genes encoding fatty acid synthase and glycolysis in the liver, suppressing lipid synthesis and enhancing lipid breakdown; promotes LDL clearance; enhances hepatic fatty acid oxidation, reducing body fat deposition; and increases HDL content, which transfers intracellular cholesterol to the liver for degradation [8,18-19].

Li [8] reported that in 1-2 month-old meat rabbits, 0.9% and 1.2% linoleic acid significantly reduced serum TCH and LDL compared to 0% and 0.6% groups. Zhang et al. [20] found that n-6 PUFAs improved fat metabolism in hyperlipidemic mice, inhibiting adverse lipid deposition with better lipid-lowering effects than commercial products. Pentieva et al. [5] demonstrated that PUFAs effectively reduce blood lipids and decrease the metabolic burden of hyperlipidemia by lowering serum cholesterol and body fat deposition. However, Wang et al. [9] found that linoleic acid levels of 0.55%, 0.75%, 0.95%, 1.15%, 1.35%, and 1.55% in early-lay duck diets did not affect plasma and liver lipid parameters. The present study showed that 1.12% dietary linoleic acid significantly reduced serum TCH and TG while increasing serum HDL-C and reducing serum LDL-C, optimizing cardiovascular physiology, consistent with most previous research.

3.3 Effects on Antioxidant Capacity Huang et al. [21] reported that PUFAs enhance antioxidant enzyme activity to counteract adverse effects of PUFA peroxidation. Gamma-linolenic acid converted from linoleic acid exhibits significant anti-lipid peroxidation effects by scavenging free radicals [22]. MDA is a lipid peroxide that indirectly reflects oxidation levels [23]. Antioxidant enzymes constitute the primary cellular antioxidant system, with SOD and GSH-Px being major antioxidants. T-AOC is a comprehensive indicator of antioxidant system function, reflecting the system' s compensatory capacity to external stimuli and overall free radical metabolism status [24]. Aviram [25] showed that fat increases metabolic stress and whole blood viscosity, with excessive lipids depositing in vascular endothelium, reducing oxidative enzymes and free radical elimination capacity, generating large amounts of lipid peroxidation products MDA and free radicals. Xia [26] found that adding different amounts of corn oil, linseed oil, and fish oil to laying hen diets significantly affected liver MDA content, which increased with unsaturated oil supplementation, with fish oil groups significantly higher than corn oil groups. The present results showed that 1.12% dietary linoleic acid maximized T-AOC and GSH-Px and T-SOD activities, which increased with dietary linoleic acid level, indicating that linoleic acid significantly affects lipid antioxidant capacity in geese, while serum and liver MDA content was less affected.

3.4 Effects on Immune Function Linoleic acid affects immune function through two mechanisms: First, fatty acids are important components of phospholipids that form the structural framework of cell membranes, so membrane fluidity depends on fatty acid composition [27]. Dietary fatty acid composition alters immune cell membrane composition and modulates membrane fluidity, thereby affecting poultry immunity. Second, linoleic acid is the precursor of arachidonic acid (AA). AA stimulates respiratory bursts in phagocytes and granulocytes, either by producing physiologically active eicosanoids that regulate immune responses [28] or by acting as a second messenger through itself or its contained phosphatidic acid and diacylglycerol to directly modulate immunity [29].

Immune organ development status reflects immune function capacity. Relative immune organ weight is one method to evaluate immune status; low relative weight indicates immune suppression, while high relative weight indicates enhanced immunity [30]. The thymus is a central immune organ and primary site for T lymphocyte development [31]. The spleen is a peripheral immune organ that houses various immune cells and serves as an important base for generating immune responses to antigens [32]. The bursa of Fabricius is an avian-specific central immune organ closely related to humoral immunity [33]. Numerous studies show that dietary fatty acid composition affects PUFA composition in the bursa, thymus, and bone marrow [34-36]. Wang et al. [36] found that increasing dietary PUFA content significantly accelerated growth of the pro-thymus, spleen, and bursa in chicks. The present study showed that 1.12% dietary linoleic acid significantly increased the bursa of Fabricius index, with spleen and thymus indices also showing increasing then decreasing trends, consistent with previous research.

Immunoglobulins, also called antibodies, with IgG, IgM, and IgA being the main effector molecules of humoral immunity, play important roles in the defense system, and their levels are important indicators of immune status [37]. Avian influenza antibody titer reflects the immune status against avian influenza, with higher values indicating stronger humoral immunity and disease resistance [38]. Xia [26] found that adding 1%, 3%, and 5% fish oil, 2%, 4%, and 6% linseed oil, and 2%, 4%, and 6% corn oil to laying hen diets increased serum BSA antibody titers compared to the control group without added fat. The present study showed that 1.12% dietary linoleic acid significantly increased serum IgG content, with serum IgM and IgA contents also showing increasing then decreasing trends, consistent with previous findings.

In recent years, China has imported large quantities of wheat as a strategic reserve grain for feed use. Since wheat has much lower energy and linoleic acid content than corn, its use as a primary feed ingredient yields less satisfactory production results, particularly in waterfowl feed formulation where wheat replaces corn. Therefore, determining the optimal linoleic acid level in wheat-soybean meal diets is important for guiding waterfowl production and has significant market application prospects.

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4. Conclusions

1. Experimental groups showed superior BW, ADG, and F/G compared to the control group, with maximal BW and minimal F/G achieved at 1.12% dietary linoleic acid.

2. Appropriate dietary linoleic acid level significantly ensures normal lipid metabolism in goslings.
3. At 1.12% dietary linoleic acid, serum and liver T-AOC and T-SOD and GSH-Px activities were significantly increased, along with immune organ indices and immunoglobulin content.
4. At 0.92% dietary linoleic acid, avian influenza antibody titer was increased.
5. The recommended optimal linoleic acid level for goslings fed wheat-soybean meal diets is 1.12%.

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