

Effects of Energy Restriction on Growth Performance, Serum Indices, and Immune Function in Broilers: Postprint

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Abstract

This experiment aimed to investigate the effects of different levels of energy restriction on growth performance, serum indices, peripheral blood T lymphocyte subsets, and immune function in broiler chickens. Seventy-two 22-day-old Cobb broiler chickens were selected and randomly divided into three groups: a control group, experimental group 1 (15% energy restriction group), and experimental group 2 (30% energy restriction group), with 24 replicates per group and one bird per replicate. The experimental period lasted 20 days. Sampling was performed by slaughter at 28 and 42 days of age. The results showed: 1) The final body weight, average daily feed intake, and average daily gain of broilers in the 15% and 30% energy restriction groups were significantly lower than those in the control group ($P < 0.05$), and the 30% energy restriction group was significantly lower than the 15% energy restriction group ($P < 0.05$); the feed-to-gain ratio of the 30% energy restriction group during 22–42 days of age was significantly higher than that of the 15% energy restriction group and the control group ($P < 0.05$). 2) Compared with the control group, 15% and 30% energy restriction significantly increased serum immunoglobulin M (IgM) content at 28 days of age and serum immunoglobulin G (IgG) and immunoglobulin A (IgA) contents at 42 days of age ($P < 0.05$), and significantly decreased serum high-density lipoprotein cholesterol content at 28 days of age ($P < 0.05$); 30% energy restriction significantly increased serum IgA content at 28 days of age ($P < 0.05$), and significantly decreased serum total cholesterol content at both 28 and 42 days of age, as well as serum triglyceride and high-density lipoprotein cholesterol contents at 42 days of age ($P < 0.05$). 3) Compared with the control group, 30% energy restriction significantly increased the percentage of peripheral blood CD8⁺ T lymphocytes at 28 days of age ($P < 0.05$). 4) Compared with the control group, 30% energy restriction significantly increased the bursa of Fabricius index at 28 days of age and the spleen index at 42 days of age ($P < 0.05$). In

conclusion, energy restriction reduced growth performance, enhanced immune function, and decreased blood lipid levels in broiler chickens.

Full Text

Effects of Energy Restriction on Growth Performance, Serum Indexes, and Immune Function of Broilers

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Abstract: This experiment investigated the effects of different energy restriction levels on growth performance, serum indexes, peripheral blood T lymphocyte subsets, and immune function in broilers. Seventy-two 22-day-old Cobb broilers were randomly allocated to three groups: a control group, trial group 1 (15% energy restriction), and trial group 2 (30% energy restriction), with 24 replicates per group and one bird per replicate. The experimental period lasted 20 days. Broilers were slaughtered and sampled at 28 and 42 days of age. The results showed: (1) The final body weight, average daily feed intake (ADFI), and average daily gain (ADG) of broilers in both the 15% and 30% energy restriction groups were significantly lower than those in the control group ($P < 0.05$), with the 30% restriction group showing significantly lower values than the 15% restriction group ($P < 0.05$). The feed-to-gain ratio (F/G) in the 30% energy restriction group was significantly higher than that in both the 15% restriction group and the control group during the 22–42 day period ($P < 0.05$). (2) Compared with the control group, 15% and 30% energy restriction significantly increased serum immunoglobulin M (IgM) content at 28 days of age and serum immunoglobulin G (IgG) and immunoglobulin A (IgA) contents at 42 days of age ($P < 0.05$), while significantly decreasing serum high-density lipoprotein cholesterol (HDL-C) content at 28 days of age ($P < 0.05$). The 30% energy restriction significantly increased serum IgA content at 28 days of age ($P < 0.05$) and significantly decreased serum total cholesterol (TC) content at both 28 and 42 days of age, as well as serum triglyceride (TG) and HDL-C contents at 42 days of age ($P < 0.05$). (3) Compared with the control group, 30% energy restriction significantly increased the percentage of peripheral blood CD8⁺ T lymphocytes at 28 days of age ($P < 0.05$). (4) Compared with the control group, 30% energy restriction significantly increased the bursa of Fabricius index at 28 days of age and the spleen index at 42 days of age ($P < 0.05$). It is concluded that energy restriction reduces growth performance while improving immune function and decreasing blood lipid levels in broilers.

Keywords: energy restriction; broilers; immunoglobulin; T lymphocyte sub-

sets; blood lipid level

It is commonly believed that under a fixed genetic background, greater feed intake leads to higher market weight in broilers. Consequently, commercial broiler production typically provides ad libitum energy at 2-3 times maintenance requirements [1], resulting in excessive fat deposition, metabolic and bone metabolism disorders, reduced immunity, increased mortality, and frequent occurrence of diseases such as sudden death syndrome and ascites [2-4]. Since fat deposition consumes more energy than lean tissue deposition, this practice reduces feed utilization efficiency [5]. Therefore, reducing fat deposition is a critical challenge in modern broiler production. Studies have demonstrated that moderate feed restriction effectively decreases the incidence of metabolic diseases like ascites and sudden death syndrome, reduces overall mortality, enhances immune function, improves meat quality, decreases feed-to-gain ratio, and increases production efficiency [6-8]. Xu et al. [9] and Li et al. [10] investigated the effects of energy restriction in broiler breeder hens on offspring performance, finding that 20% maternal energy restriction significantly increased leg muscle sarcomere length and serum total cholesterol and growth hormone levels while decreasing abdominal fat percentage in 28-day-old offspring. Research has shown that immunity and metabolism interact physiologically and pathologically: both innate and adaptive immune systems participate in non-immune diseases such as obesity, which produces metabolic and immune abnormalities that increase susceptibility to cardiovascular disease, type II diabetes, cancer, and neurodegenerative disorders; conversely, immune cells (including lymphocytes and leukocytes) are regulated by internal energy metabolism at multiple levels [11]. This interaction between immune response and metabolism reveals that proper regulation and management of energy intake to maintain a delicate balance is crucial for organismal health [12]. Energy restriction has attracted considerable attention as an effective method for extending lifespan and promoting health in humans [13]. Research on energy restriction has expanded from vertebrates to nearly all invertebrates, though results remain inconsistent and mechanisms controversial [14]. This study aimed to investigate the effects of different energy restriction levels on growth performance, serum indexes, and immune function in broilers to provide a theoretical basis for rational feed restriction application in broiler production. Additionally, as chickens serve as a model animal, studying their serum biochemical indexes and immune function may provide interspecies comparisons for exploring potential mechanisms by which energy restriction improves health and extends lifespan.

1.1 Experimental Animals and Design

One thousand 1-day-old healthy Cobb chicks were raised on wire floors and fed ad libitum with diets formulated according to NRC (1994) nutrient requirements for broiler starters under conventional management. At 22 days of age, 72 male broilers with similar body weights were selected from the 1,000 birds, individ-

ually weighed, and randomly divided into three groups: a control group, trial group 1 (15% energy restriction), and trial group 2 (30% energy restriction), with 24 replicates per group and one bird per replicate housed in individual cages.

1.2 Experimental Diets

The control group diet was formulated as pellets according to NRC (1994) nutrient requirements for broiler growers and provided daily at the recommended intake for Cobb male broilers of the corresponding age. All three groups received diets with consistent metabolizable energy levels. The nutrient composition of trial group 1 and 2 diets was adjusted (except for metabolizable energy) to control feed intake at 85% and 70% of the control group level, respectively, ensuring that energy intake was 15% and 30% lower than the control group while maintaining similar intake of other nutrients. The composition and nutrient levels of experimental diets are shown in Table 1 .

1.3 Management

The experiment was conducted at the Animal Experimental Station of Henan Agricultural University for a period of 20 days. Birds received 定量采食 (quantitative feeding) and ad libitum water access under normal management and conventional vaccination programs. Daily feed intake was recorded per replicate, and the birds' mental state, appetite, and fecal condition were monitored, with mortality recorded.

1.4 Sampling and Measurements

1.4.1 Growth Performance Feed was weighed daily to record individual feed intake. At 28 and 42 days of age, after 12 hours of fasting, birds were weighed in the morning to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) for each period based on initial weight, final weight, and daily feed intake.

1.4.2 Serum Indexes At 28 and 42 days of age, 10 birds with similar body weights were selected from each group. Blood samples were collected from the wing vein into tubes, allowed to clot at room temperature for 2 hours, and centrifuged at 3,000 r/min for 10 minutes to obtain serum. Serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) contents were determined by immunoturbidimetry. Serum glucose (GLU), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents were measured using an automatic biochemical analyzer (RA-1000, Bayer, USA).

1.4.3 Peripheral Blood T Lymphocyte Subsets At 28 and 42 days of age, 10 birds with similar body weights were selected from each group. Fresh anti-

coagulated blood (1 mL) was mixed 1:1 with phosphate-buffered saline (PBS) and carefully layered over 2 mL of cell separation medium. After centrifugation at 1,500 r/min for 15 minutes, the centrifuge tube contained four layers from top to bottom: plasma layer, ring-shaped milky lymphocyte layer, separation medium layer, and red blood cell layer. The lymphocyte layer was carefully collected into a tube containing 5 mL of cell washing solution, mixed thoroughly, and centrifuged at 2,000 r/min for 15 minutes. The pellet was washed twice to obtain the required lymphocytes. The percentages of peripheral blood CD3, CD4, and CD8 T lymphocytes were determined by flow cytometry.

1.4.4 Immune Organ Index At 28 and 42 days of age, 10 birds with similar body weights were selected from each group for slaughter. The thymus, spleen, and bursa of Fabricius were dissected and weighed to calculate the immune organ index using the formula:

Immune organ index = immune organ weight (g) / live body weight (kg)

1.5 Statistical Analysis Data were analyzed using one-way ANOVA with SPSS 20.0 software. Duncan's multiple comparison test was used for significance testing, with $P < 0.05$ as the criterion for significant difference. Results are expressed as mean \pm standard deviation (mean \pm SD).

2.1 Effects of Energy Restriction on Growth Performance of Broilers

As shown in Table 2, during the 22-28 day period, the final weight, ADFI, and ADG of broilers in trial groups 1 and 2 were significantly lower than those in the control group ($P < 0.05$), with trial group 2 significantly lower than trial group 1 ($P < 0.05$). No significant differences in F/G were observed among groups ($P > 0.05$). During the 22-42 day period, the final weight, ADFI, and ADG in trial groups 1 and 2 were significantly lower than in the control group ($P < 0.05$), with trial group 2 significantly lower than trial group 1 ($P < 0.05$). The F/G in trial group 2 was significantly higher than in trial group 1 and the control group ($P < 0.05$), while no significant difference was observed between trial group 1 and the control group ($P > 0.05$).

2.2 Effects of Energy Restriction on Serum Indexes of Broilers

As shown in Table 3, at 28 days of age, serum IgM content in trial groups 1 and 2 was significantly higher than in the control group ($P < 0.05$), with no significant difference between the two trial groups ($P > 0.05$). Serum IgG content showed an increasing trend in trial groups 1 and 2 compared with the control group ($P > 0.05$). Serum IgA content in trial group 2 was significantly higher than in the control group ($P < 0.05$). At 42 days of age, serum IgG and IgA contents in trial groups 1 and 2 were significantly higher than in the control group ($P < 0.05$).

As shown in Table 4 , at 28 days of age, serum TC content in trial group 2 was significantly lower than in the control group ($P < 0.05$), while trial group 1 showed a decreasing trend ($P > 0.05$). Serum HDL-C content in trial groups 1 and 2 was significantly lower than in the control group ($P < 0.05$), with trial group 2 significantly lower than trial group 1 ($P < 0.05$). No significant differences were observed in serum GLU, TG, or LDL-C contents among groups ($P > 0.05$). At 42 days of age, serum TC content in trial group 2 was significantly lower than in the control group ($P < 0.05$). Serum TG and HDL-C contents in trial group 2 were significantly lower than in trial group 1 and the control group ($P < 0.05$). Serum GLU content in trial groups 1 and 2 showed a decreasing trend compared with the control group ($P > 0.05$).

2.3 Effects of Energy Restriction on Peripheral Blood T Lymphocyte Subsets of Broilers

As shown in Table 5 , at 28 days of age, the percentages of peripheral blood CD3 and CD4 T lymphocytes in trial groups 1 and 2 were higher than in the control group ($P > 0.05$). The percentage of CD8 T lymphocytes in trial group 2 was significantly higher than in both the control group and trial group 1 ($P < 0.05$). At 42 days of age, the percentages of CD3 , CD4 , and CD8 T lymphocytes in trial groups 1 and 2 were higher than in the control group, but the differences were not significant ($P > 0.05$).

2.4 Effects of Energy Restriction on Immune Organ Indexes of Broilers

As shown in Table 6 , at 28 days of age, the bursa of Fabricius index in trial group 2 was significantly greater than in the control group ($P < 0.05$), while no significant difference was observed between trial group 1 and the control group ($P > 0.05$). No significant differences were found in thymus or spleen indexes among groups ($P > 0.05$). At 42 days of age, the spleen index in trial group 2 was significantly greater than in trial group 1 and the control group ($P < 0.05$). No significant differences were observed in bursa of Fabricius or thymus indexes among groups ($P > 0.05$).

3.1 Effects of Energy Restriction on Growth Performance of Broilers

The results showed that 15% and 30% energy restriction significantly reduced final body weight, ADFI, and ADG, with the 30% restriction group showing lower values than the 15% restriction group. During the 22-28 day period, no significant differences in F/G were observed among groups, but during the 22-42 day period, the 30% energy restriction group exhibited a significantly higher F/G than the 15% restriction group and control group, while the 15% restriction group had the lowest F/G. Zhao [15] reported that Hubbard broilers subjected to energy or feed quantity restriction showed significantly lower ADG and higher F/G than ad libitum-fed birds, consistent with our findings. Urdaneta-Rincon et al. [16] demonstrated that feed restriction at 5%, 10%, and 15% from 5-42 days

of age significantly reduced body weight at 42 days compared with ad libitum feeding. Saleh et al. [17] found that feeding broilers different energy gradient diets (with consistent energy-to-crude protein ratios) according to maintenance requirements during the early growth period (7-14 days) significantly reduced body weight at 14, 21, 42, and 63 days and feed intake compared with ad libitum feeding, while significantly improving feed conversion efficiency and energy utilization. Miao et al. [18] fed broilers diets with energy levels of 12.12 and 13.37 MJ/kg until 35 days of age and found that energy restriction reduced ADG and improved feed conversion efficiency during 1-21 days, with no significant effect during 22-35 days, partially consistent with our results. Deaton [19] reported that 10% feed restriction from 7-14 days had no significant effect on body weight at 41 days or growth rate throughout the experimental period, possibly due to: (1) the short restriction period of only one week, and (2) the low restriction level of 10%. Short-term, low-level restriction may allow broilers to exhibit compensatory growth later in life.

3.2 Effects of Energy Restriction on Serum Indexes of Broilers

Immunoglobulins play crucial roles in the adaptive immune system as pathogen-defense molecules. The typical molecular structure consists of two identical heavy (H) chains and two light (L) chains, each divided into variable and constant regions. In poultry, immunoglobulins are classified as IgA, IgG, and IgM based on their heavy chains. Immunoglobulins are extensively involved in immune responses, and their concentrations directly affect immune competence. Our results showed that 15% and 30% energy restriction significantly increased serum IgM content at 28 days of age and serum IgG and IgA contents at 42 days of age. Few studies have examined the effects of energy restriction on serum immunoglobulin levels in broilers. Xu et al. [20] reported that 10% feed restriction in adult male striped hamsters for 21 days significantly increased serum IgG and IgA contents. Nayak et al. [21] found that 20% and 40% energy restriction in rats significantly increased the numbers of IgG, IgM, and IgA cells in the spleen. Ebersole et al. [22] reported that energy restriction significantly increased serum IgM and IgG contents in rhesus monkeys, consistent with our results. However, Lara-Padilla et al. [23] showed that alternate-day fasting for 18 weeks significantly reduced small intestinal and serum IgA contents in mice compared with ad libitum feeding, contrary to our findings. These discrepancies may be attributed to differences in restriction levels and feeding methods, though specific mechanisms require further investigation.

Fasting serum glucose, TG, HDL-C, LDL-C, and TC contents serve as metabolic markers closely associated with fat deposition and metabolic disease occurrence. Serum TG and glucose levels are positively correlated with carcass fat content in poultry [24-25]. Our results showed that 15% energy restriction significantly reduced serum HDL-C content at 28 days of age, with decreasing trends in serum TC, TG, and LDL-C contents, but no significant effect on serum glucose. The 30% energy restriction significantly reduced serum TC and HDL-C contents

at 28 days of age, with decreasing trends in serum TG and LDL-C contents, but no significant effect on serum glucose. At 42 days of age, 30% energy restriction significantly reduced serum TC, HDL-C, and TG contents. Zhao [15] reported that energy restriction significantly reduced serum TG, cholesterol, and HDL-C contents in broilers without affecting serum glucose, consistent with our results. Wang [26] found that 30% energy restriction significantly reduced serum TG and glucose contents and markedly decreased serum cholesterol and HDL-C contents, partially consistent with our findings. Chen [27] induced insulin resistance in rats through high-calorie diets and found that energy restriction significantly reduced serum TG and cholesterol contents, generally consistent with our results. Wei et al. [28] studied 100 healthy volunteers who consumed a low-fat, low-energy, low-sugar, low-protein, high-unsaturated fatty acid diet for five consecutive days per month over three cycles, observing reductions in fasting serum glucose, TG, and LDL-C contents without significant changes in HDL-C. These findings suggest that energy restriction improves lipid metabolism and reduces body fat deposition.

3.3 Effects of Energy Restriction on Peripheral Blood T Lymphocyte Subsets of Broilers

Immune cells require nutrients such as glucose, amino acids, and fatty acids to meet their energy demands [29]. However, excessive energy intake leads to obesity, causing changes in circulating hormones and nutrients that expose immune cells to an energy-rich environment under altered metabolic hormone concentrations. This energy-rich environment affects immune cell function, resulting in an immunodeficient state and promoting inflammation [30]. T cells not only mediate cellular immunity directly but also play central roles in immune responses. The CD3 molecule is expressed on all mature T cells and serves as a common surface marker, transmitting activation signals generated by antigen binding to the T cell receptor and activating cells [31]. The CD4 molecule exists on helper T cell surfaces as a marker; CD4 T cells induce and enhance immune responses by secreting cytokines such as interleukins and interferons. CD8 T cells are markers on cytotoxic T cell surfaces, including suppressor and cytotoxic T lymphocytes that clear virus-infected cells [32]. Our results showed that 15% and 30% energy restriction increased the percentages of peripheral blood CD3 and CD4 T lymphocytes at 28 days of age, with the 30% restriction group showing significantly higher CD8 T lymphocyte percentages than the control and 15% restriction groups. This suggests that energy restriction enhances immunity, though excessive restriction may indicate an overactive immune state. At 42 days of age, 15% and 30% energy restriction increased CD3, CD4, and CD8 T lymphocyte percentages, but differences were not significant, possibly because prolonged energy restriction led to a relatively balanced state of T lymphocyte populations. Khajavi et al. [33] found that early feed restriction in heat-stressed broilers significantly increased peripheral blood CD4 helper T cell proportions and decreased CD8 cytotoxic T cell proportions at 21 days, while both cell types decreased at 42 days, partially inconsistent with our results.

Complex and coordinated signals are required to activate and maintain T lymphocyte proliferation and differentiation, determining their fate and function in a metabolism-dependent manner. Studies have shown that nutritional factors can affect lymphocyte proliferation, survival, and functional execution [34–35]. The mechanisms by which energy restriction influences lymphocytes and their secreted cytokines, and how to maintain balance among these complex cytokines, remain unclear and require further investigation.

3.4 Effects of Energy Restriction on Immune Organ Indexes of Broilers

The immune process in chickens is primarily mediated through the spleen, thymus, and bursa of Fabricius, so the developmental status of these organs directly affects immune responses. Promoting immune organ development is therefore crucial for enhancing immunity. Immune competence in chickens can be partially reflected by the relative weight of immune organs—the immune organ index—which correlates positively with immune system execution capacity. Changes in immune organ index indicate altered immune function [36]. The period before 28 days of age is critical for immune organ development and represents a key window for enhancing immune function and disease resistance [37].

Our results showed that 30% energy restriction significantly increased the bursa of Fabricius index at 28 days of age without affecting thymus or spleen indexes. At 42 days of age, 30% energy restriction significantly increased the spleen index without affecting bursa or thymus indexes. These findings align with Chen et al. [38], who reported that 30% energy restriction increased relative spleen and bursa weights in broilers. Liu [39] demonstrated that 15% energy restriction significantly increased the spleen index in Sanhuang chickens. Zhang [40] found that 30% energy restriction markedly increased the spleen index compared with ad libitum feeding. Our results are consistent with these previous studies, indicating that energy restriction can promote immune organ development to some extent.

4 Conclusion

1. Energy restriction reduces broiler growth performance in a dose-dependent manner, with greater restriction levels producing more pronounced effects.
2. Energy restriction increases serum IgM, IgA, and IgG contents while decreasing serum TG, TC, and HDL-C contents.
3. Energy restriction increases the percentages of peripheral blood CD3, CD4, and CD8 T lymphocytes.
4. Thirty percent energy restriction increases the bursa of Fabricius index at 28 days of age and the spleen index at 42 days of age.

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

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