Effects of Alfalfa Crude Polysaccharides Processed by Different Drying Methods on Production Performance, Egg Quality, and Antibody Content in Serum and Eggs of Laying Hens: Postprint

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Abstract

This experiment was conducted to investigate the effects of alfalfa crude polysaccharides processed by different drying methods on production performance, egg quality, organ indices, and antibody contents in serum and eggs of laying hens. Fifty-four Jinghong No. 1 laying hens at 38 weeks of age were selected and randomly allocated into 3 groups with 18 replicates per group and 1 bird per replicate, housed individually in cages. The control group was fed a corn-soybean meal basal diet, while the experimental groups were fed the basal diet supplemented with 1,000 mg/kg of alfalfa crude polysaccharide 1 (ACPS1) obtained by microwave vacuum drying and alfalfa crude polysaccharide 2 (ACPS2) obtained by vacuum drying, respectively. The experimental period lasted 65 days. The results showed that: 1) During days 28–36 of the experiment, the average daily feed intake of hens in the ACPS1 group was significantly increased ($P<0.05$); dietary supplementation with alfalfa crude polysaccharides processed by different drying methods had no significant effect on laying rate or average daily gain at any stage ($P>0.05$). 2) On day 63 of the experiment, the yolk color of hens in the ACPS1 group was significantly increased ($P<0.05$); on day 30, dietary supplementation with alfalfa crude polysaccharides processed by different drying methods had no significant effect on shell color, yolk color, albumen height, or Haugh unit of laying hens ($P>0.05$). 3) The liver index of hens in the ACPS1 group was significantly increased ($P<0.05$), whereas the ovary index of the ACPS2 group was significantly decreased ($P<0.05$); furthermore, both the liver index and ovary index of the ACPS1 group were significantly higher than those of the ACPS2 group ($P<0.05$). Dietary supplementation with alfalfa crude polysaccharides processed by different drying methods had no significant effect on spleen index or oviduct index of laying hens ($P>0.05$).

4) The serum immunoglobulin Y (IgY) content of hens in the ACPS1 group was significantly increased (P<0.05), and the serum Newcastle disease virus antibody (NDV-Ab) content, yolk NDV-Ab content, and albumen immunoglobulin A (IgA) and immunoglobulin M (IgM) contents in both ACPS1 and ACPS2 groups were significantly increased (P<0.05); moreover, the yolk NDV-Ab content and albumen IgA and IgM contents in the ACPS1 group were significantly higher than those in the ACPS2 group (P<0.05). These results indicate that dietary supplementation with alfalfa crude polysaccharide can deepen yolk color and increase antibody contents in serum and eggs, with ACPS1 demonstrating superior efficacy.

Full Text

Effects of Crude Polysaccharides Isolated from Alfalfa Processed by Different Drying Methods on Performance, Egg Quality, and Antibody Contents in Serum and Eggs of Laying Hens

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Abstract

This experiment was conducted to investigate the effects of crude polysaccharides isolated from alfalfa (ACPS) processed by different drying methods on production performance, egg quality, organ indices, and antibody contents in serum and eggs of laying hens. Fifty-four 38-week-old Jinghong No. 1 laying hens were randomly allocated to 3 groups with 18 replicates per group and 1 hen per replicate housed individually in cages. The control group was fed a corn-soybean meal basal diet, while the experimental groups were fed the basal diet supplemented with 1,000 mg/kg ACPS obtained by microwave vacuum drying (ACPS1) or decompression drying (ACPS2). The experiment lasted for 65 days. The results showed that: 1) Compared with the control group, the average daily feed intake of hens in the ACPS1 group was significantly increased during days 28–36 (P<0.05). Dietary supplementation with ACPS processed by different drying methods had no significant effects on egg production rate or average daily gain at any stage (P>0.05). 2) On day 63, yolk color in the ACPS1 group was significantly increased (P<0.05). On day 30, dietary ACPS had no significant effects on eggshell color, yolk color, albumen height, or Haugh unit (P>0.05). 3) The liver index in the ACPS1 group was significantly increased (P<0.05), while the ovarian index in the ACPS2 group was significantly decreased (P<0.05). Moreover, both the liver index and ovarian index in the ACPS1 group were significantly higher than those in the ACPS2 group (P<0.05). Dietary ACPS had no significant effects on spleen index or oviduct index (P>0.05). 4) The serum immunoglobulin Y (IgY) content in the ACPS1 group was significantly in-

creased (P<0.05). Serum Newcastle disease antibody (NDV-Ab), yolk NDV-Ab, and albumen immunoglobulin A (IgA) and immunoglobulin M (IgM) contents were all significantly increased in both ACPS1 and ACPS2 groups (P<0.05). Furthermore, yolk NDV-Ab and albumen IgA and IgM contents in the ACPS1 group were significantly higher than those in the ACPS2 group (P<0.05). These results indicate that dietary ACPS can deepen yolk color and increase antibody contents in serum and eggs, with ACPS1 showing superior effects.

Keywords: crude polysaccharides isolated from alfalfa; laying hens; performance; egg quality; antibody content

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Since the 1960s, the immunomodulatory effects of polysaccharides have attracted considerable attention. Polysaccharides can enhance both cellular and humoral immune functions in animals, thereby promoting growth and improving production performance. Crude polysaccharides isolated from alfalfa (ACPS) are water-soluble heteropolysaccharides extracted from alfalfa stems and leaves, belonging to non-starch polysaccharides. They appear as light yellow powder, free from tannins and starch, and are acidic polysaccharides [1]. Capillary electrophoresis analysis has shown that ACPS primarily contains xylose, arabinose, glucose, rhamnose, galactose, glucuronic acid, and galacturonic acid [2], representing one of the main bioactive components of alfalfa. Previous studies have demonstrated that dietary supplementation with appropriate doses of ACPS can promote animal growth [3], improve meat quality [4], enhance immunity [5-6], reduce lipids [7], provide antioxidant effects [8], modulate intestinal microflora, and promote the proliferation of beneficial bacteria [9]. As a green and environmentally friendly feed additive, ACPS has gained increasing attention. Liu et al. [10] reported that dietary ACPS significantly improved humoral and cellular immune functions in broiler chickens. Jiang et al. [11] found that early supplementation with ACPS significantly increased serum Newcastle disease antibody titers in broilers. Zhang et al. [12] administered alfalfa polysaccharide concurrently with swine fever attenuated vaccine to piglets and observed significantly increased peripheral blood immunoglobulin G (IgG) content and enhanced resistance compared with the vaccine control group. However, the effects of ACPS on antibody contents in eggs have not been reported.

Research indicates that the biological activity of plant polysaccharides is closely related to their physicochemical composition, water solubility, molecular weight, degree of branching, viscosity, and advanced helical structure. These properties are primarily influenced by three major factors: sample processing methods, extraction parameters, and drying methods. Molecular weight and viscosity are particularly associated with drying methods [13]. Various drying techniques, including hot air drying, vacuum drying, and microwave drying, can significantly affect polysaccharide conformation, causing irreversible changes that influence their structure and biological properties [14]. Fan et al. [15] and Ma et al. [16] investigated the effects of different drying methods on the antioxidant activities of *Ganoderma lucidum* polysaccharides and *Inonotus obliquus* polysaccharides,

respectively, and found that drying methods significantly affected antioxidant activity. Wu [17] analyzed the effects of freeze drying, hot air drying, and vacuum drying on the chemical composition and antioxidant activity of finger citron polysaccharides, also demonstrating that drying methods can influence polysaccharide biological functions. However, no studies have reported on the effects of drying methods on the biological activity of alfalfa polysaccharides. Therefore, this study used Jinghong No. 1 laying hens to investigate the effects of dietary supplementation with ACPS processed by microwave vacuum drying (ACPS1) and decompression drying (ACPS2) on production performance, egg quality, organ indices, and antibody contents in serum and eggs. The objective was to identify the optimal drying method and provide a scientific basis for ACPS as a novel feed additive, which is significant for developing and utilizing high-activity ACPS.

1.1 Experimental Materials

The ACPS used in this experiment was provided by the Feed Additive Research Laboratory of the Institute of Animal Science, Chinese Academy of Agricultural Sciences. The extraction was performed using water extraction, alcohol precipitation, and deproteinization methods. The specific procedures were as follows: Purple alfalfa was harvested, sun-dried, and baled, then cut into 40–60 cm segments and weighed. Eight times the weight of water was added, and the mixture was extracted at 100 °C for 4 h with circulation, filtered through a 200-mesh sieve, concentrated under reduced pressure, precipitated with alcohol, deproteinized with 3% trichloroacetic acid, and precipitated with alcohol again. ACPS1 and ACPS2 were then obtained by microwave vacuum drying and decompression drying, respectively, and finally vacuum-packaged for storage.

1.2 Experimental Animals and Diets

Fifty-four 38-week-old Jinghong No. 1 laying hens were selected as experimental animals. The experimental diets were formulated with corn and soybean meal as the main ingredients, with nutrient levels determined according to the NRC (1994) recommendations for laying hens. The composition and nutrient levels of the basal diet are shown in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis), %

Notes:
1) The premix provided the following per kg of diet: Mn 63.6 mg, Zn 69 mg, Fe 30 mg, Cu 6.25 mg, I 0.4 mg, Se 0.2 mg, VA 8,000 IU, VD_3 3,000 IU, VE 15 IU, VK_3 2 mg, VB_1 2 mg, VB_2 4 mg, VB_6 4 mg, VB_{12} 0.01 mg, calcium pantothenate 12 mg, nicotinic acid 40 mg, folic acid 1 mg, biotin 0.1 mg, choline 212.5 mg.
2) Nutrient levels were all calculated values.

1.3 Experimental Design and Management

A single-factor completely randomized design was employed. Fifty-four 38-week-old healthy Jinghong No. 1 laying hens with similar body weight and egg production rate were randomly divided into 3 groups with 18 replicates per group and 1 hen per replicate. Hens were housed individually in cages labeled with group and replicate numbers. The control group received the corn-soybean meal basal diet, while the experimental groups received the basal diet supplemented with 1,000 mg/kg ACPS1 or ACPS2. The experiment included a 7-day pre-trial period followed by a 65-day trial period. On day 30, all hens were immunized via intramuscular injection with Newcastle disease live vaccine (LaSota strain).

The feeding trial was conducted in the animal facility of the Institute of Animal Science, Chinese Academy of Agricultural Sciences during winter, from November 11, 2014, to January 15, 2015. A three-tier stacked cage system was used with individual housing. Feed was provided three times daily with ad libitum access to feed and water. Lighting consisted of natural light supplemented with artificial light for a constant 16-h photoperiod (controlled by a poultry house lighting controller). House temperature and humidity were recorded daily, and hen health status was monitored with mortality recorded promptly. Manure was removed every other day.

1.4 Measurement Indicators and Methods

1.4.1 Component Contents of ACPS The contents of moisture, crude protein, and crude ash in the two ACPS samples were determined according to GB/T 6435-2014, GB/T 6432-94, and GB/T 6438-2007, respectively. Polysaccharide content was measured using the phenol-sulfuric acid method with glucose as the standard. Flavonoid content was determined by the sodium nitrite-aluminum nitrate-sodium hydroxide colorimetric method with rutin as the standard. Saponin content was measured by the 5% vanillin-glacial acetic acid colorimetric method with oleanolic acid as the standard.

1.4.2 Production Performance During the experiment, daily egg production was recorded per replicate. Feed consumption was recorded on days 27, 36, 50, and 64 to calculate average daily feed intake (ADFI). Body weight was measured on days 1, 28, and 65 after overnight fasting to calculate average daily gain (ADG).

1.4.3 Egg Quality On days 30 and 63, eggs laid on that day were collected for quality determination within 12 h. Eggshell color was measured using a shell colorimeter (QCR, TSS, UK). Albumen height, Haugh unit, and yolk color were measured using an egg quality analyzer (EMT-2500, Robotmation, Japan).

1.4.4 Organ Indices On day 65, all hens were euthanized by carotid artery exsanguination. The liver, spleen, ovary, and oviduct were rapidly excised, weighed, and recorded for organ index calculation.

1.4.5 Antibody Contents in Serum and Eggs On the morning of day 7 post-immunization with Newcastle disease vaccine, blood was collected from the wing vein using vacuum blood collection needles into 5-mL tubes. After room temperature incubation until serum separation, serum was collected and aliquoted into 1.5-mL tubes for storage. Serum IgY, IgA, IgM, and NDV-Ab contents were determined by double-antibody sandwich ELISA using kits purchased from Beijing Qisong Biological Technology Co., Ltd., following the manufacturer' s instructions.

On the same afternoon, all eggs were collected and numbered for determination of total and content values of IgY and NDV-Ab in yolk and IgA and IgM in albumen using the same ELISA method. Pretreatment of egg white and yolk was performed as follows:

Yolk pretreatment: Yolk was separated using an egg separator, placed in a clean petri dish, rinsed with distilled water, dried with filter paper, and the chalazae and vitelline membrane were removed. Yolk volume was recorded and yolk liquid was collected. Yolk antibodies were prepared according to the method described in reference [18]: 2 mL of yolk liquid was mixed with 4 mL of Dulbecco' s phosphate-buffered saline (D-PBS) by vortexing, then 6 mL of chloroform was added and thoroughly mixed to form an emulsion. The mixture was centrifuged at 1,000×g for 30 min at room temperature, resulting in three layers: a bottom lecithin layer, a middle semi-solid emulsion layer, and a top aqueous layer containing immunoglobulins. The supernatant was collected, aliquoted, and stored at -20 °C until analysis.

Albumen pretreatment: Eggs were broken at the small end and gently rotated to facilitate albumen flow into a graduated cylinder. Remaining albumen was transferred using a Pasteur pipette, and albumen volume was recorded. Albumen antibodies were prepared according to the method described in reference [13]: 2 mL of albumen was mixed with 4 mL of D-PBS by vortexing, then 0.21 g of powdered polyethylene glycol 8000 (PEG-8000) was added to achieve a final concentration of 3.5% (w/v) and mixed until completely dissolved. The sample was centrifuged at 14,000×g for 10 min at room temperature, and the supernatant containing immunoglobulins was collected, aliquoted, and stored at -20 °C until analysis.

1.5 Data Processing and Analysis

Experimental data were processed using Excel 2010. Component contents of ACPS processed by different drying methods were analyzed by t-test using SAS 9.2 software, while other data were analyzed by ANOVA using the ANOVA procedure. Duncan' s multiple comparison test was used for intergroup comparisons, with $P<0.05$ considered statistically significant. Egg production rate was arcsine-transformed before ANOVA.

2.1 Component Contents of ACPS Processed by Different Drying Methods

As shown in Table 2 , the contents of polysaccharides, flavonoids, and saponins in ACPS1 were significantly higher than those in ACPS2 ($P<0.05$), while the moisture content was significantly lower ($P<0.05$). No significant differences were observed in crude protein or crude ash contents between the two ACPS products ($P>0.05$).

Table 2 Component contents of crude polysaccharides isolated from alfalfa processed by different drying methods, %

Note: In the same row, values with the same or no letter superscripts indicate no significant difference ($P>0.05$), while different lowercase letter superscripts indicate significant difference ($P<0.05$). The same applies to the following tables.

2.2 Effects of ACPS Processed by Different Drying Methods on Production Performance of Laying Hens

As shown in Table 3 , during days 28-36, the average daily feed intake of hens in the ACPS1 group was significantly higher than that in the control group ($P<0.05$), with no significant difference from the ACPS2 group ($P>0.05$). No significant differences in average daily feed intake were observed among groups during other periods ($P>0.05$). Dietary ACPS processed by different drying methods had no significant effects on egg production rate or average daily gain at any stage ($P>0.05$). However, during days 1-65, the average daily gain in the ACPS1 and ACPS2 groups increased by 85.7% and 34.7%, respectively, compared with the control group.

Table 3 Effects of crude polysaccharides isolated from alfalfa processed by different drying methods on performance of laying hens (n=18)

2.3 Effects of ACPS Processed by Different Drying Methods on Egg Quality of Laying Hens

As shown in Table 4 , on day 63, yolk color in the ACPS1 group was significantly higher than that in the control group ($P<0.05$), with no significant difference from the ACPS2 group ($P>0.05$). Albumen height and Haugh unit in both ACPS1 and ACPS2 groups were increased compared with the control group, but the differences were not significant ($P>0.05$). On day 30, dietary ACPS processed by different drying methods had no significant effects on eggshell color, yolk color, albumen height, or Haugh unit ($P>0.05$).

Table 4 Effects of crude polysaccharides isolated from alfalfa processed by different drying methods on egg quality of laying hens (n=18)

Note: Eggshell color values represent luminous reflectance, calibrated before detection (white, 83.6%; black, 0).

2.4 Effects of ACPS Processed by Different Drying Methods on Organ Indices of Laying Hens

As shown in Table 5 , compared with the control group, the liver index in the ACPS1 group was significantly increased ($P<0.05$), while the ovarian index in the ACPS2 group was significantly decreased ($P<0.05$). Moreover, both the liver index and ovarian index in the ACPS1 group were significantly higher than those in the ACPS2 group ($P<0.05$). Dietary ACPS processed by different drying methods had no significant effects on spleen index or oviduct index ($P>0.05$).

Table 5 Effects of crude polysaccharides isolated from alfalfa processed by different drying methods on organ indices of laying hens (n=18)

2.5 Effects of ACPS Processed by Different Drying Methods on Antibody Contents in Serum, Yolk, and Albumen of Laying Hens

As shown in Table 6 , serum IgY content in the ACPS1 group was significantly higher than that in the control group ($P<0.05$), with no significant difference from the ACPS2 group ($P>0.05$). Serum NDV-Ab content in both ACPS1 and ACPS2 groups was significantly increased compared with the control group ($P<0.05$). Total and content values of NDV-Ab in yolk were significantly increased in both ACPS1 and ACPS2 groups ($P<0.05$), with yolk NDV-Ab content in the ACPS1 group significantly higher than that in the ACPS2 group ($P<0.05$). Total and content values of IgA and IgM in albumen were significantly higher in both ACPS1 and ACPS2 groups compared with the control group ($P<0.05$), with albumen IgA and IgM contents in the ACPS1 group significantly higher than those in the ACPS2 group ($P<0.05$).

Table 6 Effects of crude polysaccharides isolated from alfalfa processed by different drying methods on antibody contents in serum, egg yolk, and albumen of laying hens (n=18)

3.1 Effects of ACPS Processed by Different Drying Methods on Production Performance and Egg Quality of Laying Hens

Plant polysaccharides can affect animal growth, development, production performance, and product quality through multiple mechanisms, including enhancing immunity [19-20], providing antioxidant effects [21], exerting anti-inflammatory [22-23] and anti-infective activities [24-25], promoting proliferation of beneficial intestinal microorganisms such as *Lactobacillus* and *Bifidobacterium* [26], improving crude protein metabolism and accelerating protein synthesis [27], and reducing stress responses [28]. Liu et al. [29] reported that dietary supplementation with 0.5% alfalfa polysaccharide significantly increased average daily feed intake and average daily gain while reducing feed conversion ratio in heat-stressed New Zealand male rabbits during days 1-21. Ouyang et al. [4] found that dietary supplementation with 1.0% and 1.5% water-soluble alfalfa polysaccharide significantly increased average body weight and average daily gain while reducing feed-to-gain ratio in broiler chickens, with the 1.0% group

showing better effects than the 1.5% group. However, some studies have reported no significant effects of plant polysaccharides on animal performance. Chen et al. [30] observed that dietary supplementation with *Astragalus* polysaccharide or *Achyranthes bidentata* polysaccharide had no significant effects on daily gain, feed intake, feed-to-gain ratio, or mortality in broiler chickens. Jiang et al. [11] also reported no significant effects of early dietary ACPS supplementation on broiler performance. The present study showed that dietary ACPS processed by different drying methods had no significant effects on laying hen performance, except for significantly increased average daily feed intake in the ACPS1 group during days 28–36, which is consistent with the findings of Chen et al. [30] and Jiang et al. [11]. The inconsistent results among studies may be related to differences in ACPS sources, extraction processes, drying methods, purity, as well as animal species, age, and rearing environment. During days 29–65, the control group exhibited negative average daily gain (-4.72 g), likely due to cold stress from low house temperatures, which increased energy expenditure for thermoregulation without a corresponding increase in feed intake, resulting in negative energy balance. In contrast, ACPS-supplemented groups showed increased average daily gain, possibly due to enhanced feed intake, improved stress resistance, and increased nutrient utilization.

Albumen height and Haugh unit are important indicators of egg freshness, with higher values indicating thicker, better-quality albumen [31]. The present study showed that albumen height and Haugh unit in both ACPS1 and ACPS2 groups were higher than those in the control group on day 63, though not significantly. This may be attributed to enhanced protein metabolism in eggs [27] induced by ACPS. Yolk color depth depends on the amount and type of carotenoids consumed by poultry, as they cannot synthesize carotenoids de novo [32]. Lutein, a common dietary carotenoid, must be hydrolyzed (saponified) in the digestive tract to its free form for absorption into blood, where it is then re-esterified and stored in yolk. Both ACPS products increased yolk color on day 63, possibly due to the antioxidant capacity of polysaccharides [33] preventing lutein oxidation and enhancing pigment deposition. The significantly higher yolk color in the ACPS1 group compared with the control group may be related to its higher polysaccharide content, warranting further investigation into polysaccharide molecular weight and structure.

3.2 Effects of ACPS Processed by Different Drying Methods on Organ Indices of Laying Hens

The spleen is the largest immune organ containing numerous immunocompetent cells. Organ weight directly reflects developmental status, which can indicate immune response capacity and disease resistance. Polysaccharides can alleviate immune organ atrophy in immunosuppressed animals and promote immune organ development [10,34-35]. Chen et al. [36] reported that oral administration of purified *Schisandra chinensis* polysaccharide significantly increased thymus and spleen indices in cyclophosphamide-induced immunosuppressed mice,

with spleen index increasing dose-dependently. Wang et al. [37] found that intraperitoneal injection of *Cordyceps militaris* polysaccharide at 70 mg/kg significantly increased thymus and spleen indices in normal mice, and all doses (17.5, 35.0, and 70.0 mg/kg) significantly increased these indices in cyclophosphamide-induced immunosuppressed mice in a dose-dependent manner. The present study showed no significant effects of dietary ACPS on spleen index, possibly due to differences in animal species, age, and health status. In immunosuppressed states, plant polysaccharides can effectively alleviate immune organ atrophy and restore normal immune function, making their effects more pronounced. In contrast, normal laying hens have fully developed immune organs with a relatively balanced immune system, resulting in less significant effects.

Liver index is an indicator of liver health status. Chang et al. [38] reported that corn silk polysaccharide significantly reduced liver index in carbon tetrachloride (CCl_4)-induced liver injury mice, exerting hepatoprotective effects. In the present study, ACPS1 significantly increased liver index compared with the control group. This discrepancy may be attributed to the experimental conditions: the trial was conducted in winter with house temperatures of (15.30$±$1.21) °C, below the optimal range of 18–23 °C for laying hens. Cold stress increases maintenance requirements [39], and ACPS may have enhanced liver metabolic capacity by promoting protein synthesis and inhibiting protein degradation, thereby increasing hepatocyte activity and cold resistance. This is consistent with the increased feed intake without significant changes in performance observed in ACPS-supplemented groups.

The ovary is a crucial reproductive and endocrine organ responsible for oocyte production and steroid hormone synthesis. The number and morphology of follicles at all levels reflect ovarian function and reproductive endocrine status. Ovarian index is an indicator of ovarian function in poultry. Guan et al. [40] reported that *Astragalus* polysaccharide improved ovarian function and promoted follicular development and ovulation by regulating reproductive hormone levels. The present study showed that ACPS2 significantly decreased ovarian index compared with the control group, which contradicts previous findings and requires further investigation.

3.3 Effects of ACPS Processed by Different Drying Methods on Antibody Contents in Serum, Yolk, and Albumen of Laying Hens

Serum immunoglobulin content is an important indicator of humoral immunity. Polysaccharides can enhance serum immunoglobulin levels and improve immunity [41-44]. Yang et al. [26] found that dietary supplementation with 800 mg/kg *Acanthopanax senticosus* polysaccharide significantly increased serum IgG, IgA, and IgM contents in weaned piglets during days 1–21, enhancing their resistance. Zhang et al. [12] reported that intramuscular injection of 0.2 mL alfalfa polysaccharide solution concurrently with swine fever attenuated vaccine significantly promoted peripheral blood B lymphocyte numbers and IgG content in piglets. Li et al. [45] demonstrated that rice bran polysaccharide significantly promoted

serum IgG and IgA contents in Jingbai 939 chicks on days 7, 14, and 21, and IgM content on days 7 and 14, though day 21 IgM content was not significantly affected. Yu et al. [46] found that aloe polysaccharide significantly increased plasma IgG, IgA, and IgM contents in mice with oral ulcers. The present study showed that serum IgY content in the ACPS1 group and serum NDV-Ab content in both ACPS1 and ACPS2 groups were significantly increased compared with the control group, consistent with previous findings and indicating that dietary ACPS can enhance immunity by increasing serum antibody content.

Chicken egg yolk can concentrate large amounts of antibodies transferred from serum [47]. Non-specific antibodies in eggs are primarily IgY, IgA, and IgM, with serum IgY entering yolk through oocyte receptors via endocytic transport [48], while IgA and IgM are secreted by oviduct mucosa and mainly exist in albumen [49]. Studies have confirmed that yolk IgY content positively correlates with serum IgY content [50]. The present study showed that yolk IgY content was increased in both ACPS groups but not significantly different from the control group, while yolk NDV-Ab total and content values and albumen IgA and IgM total and content values were significantly higher than those in the control group. This may be related to the increased serum antibody content induced by dietary ACPS. Additionally, albumen IgA and IgM contents and yolk NDV-Ab content in the ACPS1 group were significantly higher than those in the ACPS2 group, possibly due to the characteristics of microwave vacuum drying, including low temperature, rapid and uniform drying, minimal impact on material quality, and sterilization effects. Further structural and component analysis of the polysaccharides is needed to explore these mechanisms.

Conclusions

1. Dietary supplementation with ACPS1 and ACPS2 had no significant effects on production performance of laying hens.

2. Dietary supplementation with ACPS1 significantly increased yolk color on day 63.

3. Dietary supplementation with ACPS processed by different drying methods significantly increased antibody contents in serum, yolk, and albumen of laying hens, with ACPS1 demonstrating superior effects.

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