Effects of Pea Protein Powder on Growth Performance, Nutrient Apparent Digestibility, Digestive Organ Development, and Serum Biochemical Indices in 1- to 4-Week-Old Gaoyou Ducks (Postprint)

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Abstract

This experiment aimed to investigate the effects of pea protein powder on growth performance, nutrient apparent digestibility, digestive organ development, and serum biochemical indices of 1- to 4-week-old Gaoyou ducks. A total of 160 one-day-old Gaoyou ducks were selected and randomly divided into 4 groups with 4 replicates per group and 10 ducks per replicate. The control group (Group C0) was fed a corn-soybean meal basal diet, while the experimental groups (Groups C1, C2, and C3) were fed experimental diets in which 3%, 6%, and 9% of the soybean meal in the basal diet was replaced with pea protein powder, respectively. The experimental period lasted 28 days. The results showed: 1) The average daily gain and average daily feed intake of Groups C1, C2, and C3 were significantly lower than those of the control group ($P<0.05$); the feed-to-gain ratio of Group C1 was significantly higher than that of the control group ($P<0.05$), while the feed-to-gain ratios of Groups C2 and C3 showed no significant difference from the control group ($P>0.05$). 2) The apparent digestibility of crude protein (CP) and total phosphorus (TP) in Groups C1, C2, and C3 showed no significant difference from the control group ($P>0.05$). The apparent digestibility of crude fiber (CF), ether extract (EE), and calcium (Ca) in Group C2 showed no significant difference from the control group ($P>0.05$), while the apparent digestibility of CF, EE, and Ca in Groups C1 and C3 were significantly lower than those in the control group ($P<0.05$). 3) Pea protein powder had no significant effect on the relative weight of the proventriculus, pancreas, and duodenum ($P>0.05$). The relative weight of the gizzard in Group C1 was significantly higher than that in the control group ($P<0.05$). The relative weights of the jejunum and ileum in Group C2 were significantly higher

than those in the control group (P<0.05). The duodenal density, jejunal density, and ileal relative length in Group C2 were significantly higher than those in the control group (P<0.05). 4) Pea protein powder had no significant effect on serum albumin (ALB), glucose (GLU), and low-density lipoprotein cholesterol (LDLC) contents (P>0.05). The serum total protein (TP) content in Group C3 was significantly higher than that in the control group (P<0.05). The serum urea nitrogen (UN) contents in Groups C2 and C3 were significantly higher than that in the control group (P<0.05). The serum triglyceride (TG) contents in Groups C1, C2, and C3 were significantly higher than that in the control group (P<0.05). The serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDLC) contents in Groups C1 and C3 were significantly lower than those in the control group (P<0.05). In summary, using pea protein powder to replace soybean meal in diets reduced the average daily gain and average daily feed intake of 1- to 4-week-old Gaoyou ducks; however, replacement with 6% pea protein powder had no significant effect on nutrient apparent digestibility, resulted in a lower feed-to-gain ratio than the control, and contributed to improved small intestinal organ development indices, influencing changes in protein and fat metabolism in vivo. It is recommended that 6% pea protein powder be used as the recommended inclusion level in Gaoyou duck diets.

Full Text

Effects of Pea Gluten Meal on Growth Performance, Nutrient Apparent Digestibility, Digestive Organ Development and Serum Biochemical Indexes of Gaoyou Ducks Aged 1-4 Weeks

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Abstract: This experiment investigated the effects of pea gluten meal on growth performance, nutrient apparent digestibility, digestive organ development, and serum biochemical indexes of Gaoyou ducks aged 1–4 weeks. A total of 160 one-day-old Gaoyou ducks were randomly allocated into 4 groups with 4 replicates per group and 10 ducks per replicate. The control group (C0) received a corn-soybean meal basal diet, while experimental groups (C1, C2, C3) received diets in which 3%, 6%, and 9% pea gluten meal replaced soybean meal in the basal diet. The trial lasted 28 days.

The results showed: (1) Average daily gain (ADG) and average daily feed intake (ADFI) in groups C1, C2, and C3 were significantly lower than in the control group (P < 0.05). The feed-to-gain ratio (F/G) in group C1 was significantly

higher than in the control group ($P < 0.05$), while no significant differences were observed between groups C2 and C3 and the control group ($P > 0.05$). (2) Apparent digestibility of crude protein (CP) and total phosphorus (TP) did not differ significantly among groups ($P > 0.05$). Group C2 showed no significant differences from the control in apparent digestibility of crude fiber (CF), ether extract (EE), and calcium (Ca) ($P > 0.05$), whereas groups C1 and C3 had significantly lower digestibility of CF, EE, and Ca than the control group ($P < 0.05$). (3) Pea gluten meal had no significant effect on relative weights of the proventriculus, pancreas, or duodenum ($P > 0.05$). Group C1 exhibited significantly higher relative gizzard weight ($P < 0.05$), while group C2 showed significantly higher relative weights of jejunum and ileum ($P < 0.05$) and significantly greater duodenal density, jejunal density, and relative ileal length ($P < 0.05$). (4) Pea gluten meal did not significantly affect serum albumin (ALB), glucose (GLU), or low-density lipoprotein cholesterol (LDLC) levels ($P > 0.05$). However, group C3 had significantly higher serum total protein (TP) content ($P < 0.05$), groups C2 and C3 had significantly higher serum urea nitrogen (UN) content ($P < 0.05$), and groups C1, C2, and C3 had significantly higher serum triglyceride (TG) content ($P < 0.05$). Serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDLC) levels in groups C1 and C3 were significantly lower than in the control group ($P < 0.05$).

In conclusion, dietary inclusion of pea gluten meal to replace soybean meal reduced ADG and ADFI in 1–4 week-old Gaoyou ducks. However, 6% replacement with pea gluten meal did not significantly affect nutrient apparent digestibility, resulted in a lower F/G than the control, enhanced intestinal organ development indices, and altered protein and fat metabolism. A 6% replacement level is recommended for Gaoyou duck diets.

Keywords: pea gluten meal; Gaoyou ducks; growth performance; nutrient apparent digestibility; digestive organs; serum biochemical indexes

Introduction

The shortage of protein feed resources has become a key constraint on the sustainable and healthy development of modern animal husbandry in China. Soybean meal is the most widely used plant protein feed in livestock and poultry diets, but its frequent price fluctuations, large supply-demand gap, and concerns regarding anti-nutritional factors and genetically modified safety affect the stable supply of protein feed. Therefore, accelerating the utilization of unconventional protein feed resources such as pea gluten meal and continuously strengthening nutritional value assessment and safety evaluation of soybean meal alternatives have important practical value. Protein resources such as miscellaneous meals, processing by-products, and scraps are gradually being developed as soybean meal substitutes.

As one of China's important edible legumes, peas yield starch, fiber, and protein

that can serve as raw materials for the food industry as well as in textile, pharmaceutical, and chemical applications. Pea gluten meal, a by-product of pea vermicelli processing, offers advantages including relatively high nutritional value, low price, and abundant resources, making it suitable as an unconventional protein feed to partially replace fish meal, soybean meal, and other ingredients. Its development and utilization have gradually gained attention. However, most recent research has focused on whole Canadian yellow peas and pea starch, with limited studies on domestic pea gluten meal.

To expand the application scope of pea gluten meal, research on its feeding applications in livestock and poultry diets is essential. Rational utilization of pea gluten meal requires extensive and in-depth systematic investigation. Currently, only a few studies have examined its use in pig and chicken diets, with no reports on duck diets. Therefore, this experiment replaced partial soybean meal with pea gluten meal in diets for 1–4 week-old Gaoyou ducks to investigate its effects on growth performance, nutrient apparent utilization, digestive organ development, and serum biochemical indicators, providing a technical foundation for the effective application of pea gluten meal in duck and poultry production.

1.1 Experimental Time and Location

The feeding trial was conducted from June to July 2015 at the Gaoyou Duck Breeding Center of Jiangsu Fengda Waterfowl Multiplication Center. Laboratory analyses were performed from September 2015 to February 2016 at the Feed Analysis and Testing Center of Jiangsu Agri-Animal Husbandry Vocational College.

1.2 Experimental Materials

Pea gluten meal used in the experiment was collected from Yantai, Shandong Province, with crude protein (CP), ether extract (EE), crude fiber (CF), calcium (Ca), and total phosphorus (TP) contents of 56.08%, 3.15%, 4.98%, 1.01%, and 0.63%, respectively.

1.3 Experimental Design and Management

A total of 160 healthy one-day-old Gaoyou ducks with similar body weight were randomly divided into 4 groups with 4 replicates per group and 10 ducks per replicate (half male and half female). The control group (C0) was fed a corn-soybean meal basal diet formulated according to NRC (1994) standards. Experimental groups (C1, C2, C3) received diets in which 3%, 6%, and 9% pea gluten meal replaced soybean meal in the basal diet. All experimental diets were in powder form; composition and nutrient levels are shown in Table 1 . The experimental period lasted 28 days.

Ducks had ad libitum access to feed and water and were vaccinated according to standard procedures. Lighting regimen: 24 h of 100 W intensity for days 1–7, gradually reduced by 1 h daily thereafter, with natural lighting after day 21.

Brooding temperature: 29–31 °C for days 1–3, decreasing by 0.4 °C daily from days 4–20 until reaching ambient temperature at day 21. Feeding frequency: 7 times daily for the first 3 days, reduced to 5 times daily after day 3, and further reduced to 3 times daily after day 7.

1.4 Sample Collection and Processing

Diet samples: Using the quartering method, 250 g diet samples were collected from each group and stored at 4 °C for later analysis.

Excreta samples: During days 26–28, fresh, well-formed excreta (100 g) were collected twice daily (09:00 and 14:00) from designated spots in each pen. After adding 10 mL of 10% sulfuric acid for nitrogen fixation, samples were stored at 4 °C. All excreta from each replicate were thawed, mixed, dried to constant weight at 65 °C, equilibrated at room temperature for 24 h, weighed accurately, ground, and passed through a 40-mesh sieve to prepare air-dried excreta samples for analysis.

Digestive organ samples: At 28 days, 2 ducks per replicate were randomly selected, fasted, and weighed before slaughter. After bleeding, the abdominal cavity was opened immediately to remove viscera. The gizzard, proventriculus, pancreas, duodenum (from pylorus to bile duct outlet), jejunum (to yolk sac remnant), and ileum (to ileocecal junction) were separated, rinsed with distilled water, and blotted with filter paper.

Serum samples: At 28 days, 2 ducks per replicate were randomly selected for blood collection (5 mL) from the wing vein. After standing for 30 min, blood was centrifuged at 3,000 r/min for 10 min at 4 °C, and serum was transferred to cryovials and stored at –20 °C.

1.5.1 Growth Performance

Ducks in each replicate were group-weighed after fasting on days 1 and 28 to calculate average daily gain (ADG). Feed consumption was recorded daily, with residual feed measured weekly to calculate average daily feed intake (ADFI). Feed-to-gain ratio (F/G) was calculated from ADG and ADFI. Mortality body weight was recorded to correct feed consumption and other indicators.

1.5.2 Nutrient Apparent Utilization

Diet and excreta CP content was determined by the Kjeldahl method (GB/T 6432-1994), EE by Soxhlet extraction (GB/T 6433-2006), CF by filtration (GB/T 6434-2006), Ca by potassium permanganate titration (GB/T 6436-2002), TP by spectrophotometry (GB/T 6437-2002), and acid-insoluble ash (AIA) by ignition (GB/T 23742-2009). Apparent digestibility of nutrients was determined using AIA as an internal marker:

Apparent digestibility of a nutrient (%) = 100 –100 × (a × d) / (b × c)

Where: a = AIA content in diet (%); b = nutrient content in diet (%); c = AIA content in excreta (%); d = nutrient content in excreta (%).

1.5.3 Digestive Organ Development Index

Digestive organs were weighed immediately after collection, and intestinal lengths were measured. Relative organ weight, relative intestinal length, and intestinal density were calculated as:

Relative weight of digestive organ (%) = [fresh organ weight (g) / live body weight (g)] × 100
Relative intestinal length (%) = [intestinal length (cm) / live body weight (g)] × 100
Intestinal density (g/cm) = intestinal weight (g) / intestinal length (cm)

1.5.4 Serum Biochemical Indicators

Serum total protein (TP), albumin (ALB), urea nitrogen (UN), glucose (GLU), triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), and low-density lipoprotein cholesterol (LDLC) were measured using an automatic biochemical analyzer (Olympus AU2700).

1.6 Data Processing and Statistics

Data were analyzed using one-way ANOVA in SPSS 16.0. Duncan' s multiple comparison test was used for significance testing, with $P < 0.05$ indicating significant difference. Results are expressed as mean values with standard error of the mean (SEM).

2.1 Effects of Pea Gluten Meal on Growth Performance of Gaoyou Ducks

As shown in Table 2 , ADG in groups C1, C2, and C3 was significantly lower than in the control group ($P < 0.05$), with group C3 showing a 23.22% reduction. ADFI in groups C1, C2, and C3 decreased with increasing pea gluten meal substitution, with no significant differences among the three groups ($P > 0.05$) but all significantly lower than the control ($P < 0.05$). Group C3 showed the greatest reduction, with ADFI decreasing by 24.57% compared to the control. The F/G ratio in groups C2 and C3 was lower than the control but not significantly different ($P > 0.05$), while group C1 had a significantly higher F/G ratio ($P < 0.05$), increasing by 6.90%.

These results indicate that pea gluten meal significantly reduced ADG and ADFI in Gaoyou ducks, but had no significant effect on F/G except at the 3% substitution level.

2.2 Effects of Pea Gluten Meal on Nutrient Apparent Digestibility of Gaoyou Ducks

Table 3 shows that pea gluten meal had no significant effect on apparent digestibility of CP and TP ($P > 0.05$) but significantly affected digestibility of CF, EE, and Ca ($P < 0.05$). Group C2 showed no significant differences from the control in CF, EE, and Ca digestibility ($P > 0.05$), whereas groups C1 and C3 had significantly lower digestibility of these nutrients than the control ($P < 0.05$).

In summary, compared with the control group, 6% pea gluten meal substitution had no significant effect on nutrient apparent digestibility, while 3% and 9% substitution significantly reduced CF, EE, and Ca digestibility.

2.3 Effects of Pea Gluten Meal on Digestive Organ Development of Gaoyou Ducks

Table 4 reveals that pea gluten meal had no significant effect on relative weights of the proventriculus, pancreas, or duodenum ($P > 0.05$) but significantly affected relative weights of the gizzard, jejunum, and ileum ($P < 0.05$). Group C1 showed significantly higher relative gizzard weight (37.18% increase) compared to the control ($P < 0.05$). Group C2 exhibited significantly higher relative weights of jejunum and ileum (34.52% and 55.79% increases, respectively) compared to the control ($P < 0.05$).

These findings demonstrate that pea gluten meal can increase relative digestive organ weights to some extent, particularly at the 6% substitution level, which significantly enhanced jejunum and ileum relative weights.

Table 5 shows that pea gluten meal had no significant effect on relative duodenal length ($P > 0.05$), but group C2 had significantly higher duodenal density than the control ($P < 0.05$). No significant effect was observed on relative jejunal length ($P > 0.05$), but group C2 showed significantly higher jejunal density ($P < 0.05$). Pea gluten meal did not significantly affect ileal density ($P > 0.05$), while group C2 had significantly higher relative ileal length (41.21% increase) compared to the control ($P < 0.05$).

Overall, pea gluten meal had minimal impact on small intestinal relative length and density, but the 6% substitution level significantly increased jejunal density and relative ileal length.

2.4 Effects of Pea Gluten Meal on Serum Biochemical Indexes of Gaoyou Ducks

Table 6 indicates that pea gluten meal significantly affected serum TP, UN, TG, TC, and HDLC contents ($P < 0.05$) but had no significant effect on ALB, GLU, or LDLC ($P > 0.05$). Specifically, group C3 showed significantly higher serum TP content (17.10% increase) than the control ($P < 0.05$). Groups C2 and C3

had significantly higher serum UN content ($P < 0.05$). All experimental groups (C1, C2, C3) showed significantly higher serum TG content ($P < 0.05$). Serum TC and HDLC contents in groups C1 and C3 were significantly lower than in the control ($P < 0.05$), while group C2 did not differ significantly from the control in these parameters.

These results demonstrate that pea gluten meal increased serum TP, UN, and TG contents, while the 3% and 9% substitution levels decreased serum TC and HDLC contents.

3.1 Effects of Pea Gluten Meal on Growth Performance and Nutrient Apparent Digestibility

Pea gluten meal is an unconventional plant protein feed with nutritional value similar to soybean meal. However, compared with other legume feeds, pea gluten meal contains anti-nutritional factors such as lectins and antigenic proteins, as well as polyphenols that impart a bitter taste, affecting palatability. This study showed that pea gluten meal substitution reduced ADG and ADFI in ducks, likely due to its palatability issues and anti-nutritional factors, with the most pronounced reduction at the 9% substitution level. Li Jinxi reported that pea gluten meal reduced weight gain and feed intake in broilers without significantly affecting F/G, consistent with our findings. Similar results were reported by Chen Yishu et al. regarding rapeseed meal substitution in duckling diets. However, Zhang Shiyuan et al. and Wu Dong et al. found no significant effects of rapeseed meal on growth performance in meat ducks and broilers, respectively. These discrepancies may be attributed to lower inclusion levels, use of double-low rapeseed varieties, or detoxification techniques such as fermentation.

Our results showed that except for the 3% substitution group, which had a significantly higher F/G ratio, other experimental groups did not differ significantly from the control. Considering ADG and ADFI together, the 6% substitution level demonstrated the highest nutrient utilization efficiency among the three pea gluten meal levels. Although pea gluten meal significantly reduced ADG and ADFI—key indicators of animal growth performance—its lower cost, non-allergenic nature, and non-GMO status, combined with the finding that the 6% substitution level produced a lower F/G ratio than the control, suggest that pea gluten meal can still serve as a soybean meal substitute to reduce production costs and ensure livestock product safety.

Nutrient digestibility results revealed no significant differences in CP and P digestibility among groups, while CF, EE, and Ca digestibility showed a trend of initial increase followed by decrease with increasing substitution levels. The 6% substitution group showed no significant differences from the control in these three nutrients, whereas the 9% substitution group had significantly lower digestibility. This indicates that among the three substitution levels, 6% pea gluten meal resulted in the highest nutrient apparent digestibility in ducks, con-

sistent with its lowest F/G ratio. This may explain why the 6% substitution group had significantly lower ADG and ADFI than the control but a lower F/G ratio. Our previous research on single-ingredient digestibility showed that ducks had significantly higher CP digestibility for soybean meal (85.77%) than for pea gluten meal (77.24%), with higher EE and Ca digestibility for soybean meal as well. However, when pea gluten meal partially replaced soybean meal in diet formulation, complementary effects among different feeds improved overall diet digestibility. In summary, 6% pea gluten meal substitution did not significantly affect the apparent digestibility of major dietary nutrients in ducks.

3.2 Effects of Pea Gluten Meal on Digestive Organ Development

The effects of pea gluten meal on growth rate and nutrient digestibility may be related to differences in digestive organ development among groups. Our results showed that the 6% substitution group had significantly higher relative weights of the posterior small intestine, jejunal density, and relative ileal length compared to the control, while the 9% substitution group showed no significant differences. The effects of pea gluten meal on digestive organs generally increased then decreased with substitution level, consistent with its effects on growth performance and nutrient digestibility. Therefore, 6% pea gluten meal supplementation promoted digestive organ development, while higher substitution levels were not beneficial.

Liang Hengzhi et al. reported that Beijing ducks with high F/G ratios had significantly lower relative ileal weights than medium and low F/G groups ($P < 0.05$), suggesting a correlation between feed conversion efficiency and digestive organ development. This aligns with our finding that the 6% substitution group with the lowest F/G ratio had higher digestive organ development indices. Limited research exists on pea gluten meal effects on duck digestive organs, but similar results have been reported for other unconventional meals in poultry diets. Qiu Liangwei et al. found that fermented cottonseed meal increased small intestinal villus height and reduced crypt depth in broilers, effectively optimizing intestinal structure. Wang Baowei et al. reported that fermented grape seed meal optimized intestinal structure in 5–12 week-old Wulong geese, with the fastest intestinal tissue development at 6–8% inclusion levels.

3.3 Effects of Pea Gluten Meal on Serum Biochemical Indexes

Changes in serum biochemical indicators reflect protein, lipid, and other metabolic status in ducks. Serum TP and ALB contents reflect animal health status and protein metabolism to some extent. Our results showed an increasing trend in serum TP content with pea gluten meal substitution, with the 9% substitution group significantly higher than the control, indicating enhanced protein metabolism. Hu Yongna similarly reported that fermented rapeseed

meal substitution increased serum TP and ALB contents in broilers, suggesting enhanced protein metabolism. Although serum UN is not the primary product of nitrogen catabolism in ducks, its content reflects protein metabolic status. Our results showed that all pea gluten meal substitution levels significantly increased serum UN content. Guan Wutai et al. suggested that serum UN content reflects protein metabolic balance and negatively correlates with ADG, which validates our findings of reduced ADG and increased serum UN with pea gluten meal supplementation.

Serum GLU content showed no significant changes, indicating minimal effect of pea gluten meal on glucose metabolism, consistent with findings from Wang Huiying et al. regarding rapeseed meal substitution in Zhedong white geese. Serum TG and TC contents are common parameters for assessing lipid metabolism. Our results showed that pea gluten meal increased serum TG content while decreasing serum TC content, particularly HDLC. This may be because ducks fed pea gluten meal had vigorous metabolism, with lipid metabolites appearing in serum primarily as TG rather than TC. Further analysis suggests that pea gluten meal resulted in lipoproteins existing mainly as chylomicrons, with unchanged LDLC content but reduced HDLC.

Conclusion

Based on the experimental results, the following conclusions can be drawn: (1) Dietary pea gluten meal substitution for soybean meal reduced ADG and ADFI in 1–4 week-old Gaoyou ducks, but 6% substitution did not significantly affect nutrient apparent digestibility and resulted in a lower F/G ratio. (2) Dietary inclusion of 6% pea gluten meal promoted digestive organ development in Gaoyou ducks, with significantly higher relative weights of the posterior small intestine, jejunal density, and relative ileal length compared to the control. (3) Dietary inclusion of 6% and 9% pea gluten meal caused significant changes in serum protein and lipid metabolism indicators in 1–4 week-old Gaoyou ducks. (4) The recommended appropriate substitution level of pea gluten meal in Gaoyou duck diets is 6%.

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