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Original Research Article

Astaxanthin supplementation mitigated intestinal damage and immunity in overfed Pekin ducks by regulating gut morphology, intestinal inflammation, and antioxidant balance

Xueze Lv [a, b], Uchechukwu Edna Obianwuna [c], Weifang Yang [b], Ziyue Zhang [a], Keying An [a], Bozhi Shi [a], Yingchao Dong [c], Shugeng Wu [c, *], Zhaofei Xia [a, *]

[a] College of Veterinary Medicine, China Agricultural University, Beijing 100193, China
[b] Beijing General Animal Husbandry Station, Beijing 100107, China
[c] Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China

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ABSTRACT

This study explored the impact of astaxanthin (AST) supplementation on growth performance, serum lipid profile, gut morphology, and antioxidant and immune function in the intestinal mucosa of Pekin ducks subjected to overfeeding. A total of 150 male Pekin ducks at one day of age were randomly allotted into five treatment groups with five replicates of six ducks each. The control group and ad libitum group (ALG) received a basal diet while others received basal diets supplemented with AST at 40 mg/kg (Low-dose group [LDG]), 80 mg/kg (medium-dose group [MDG]), and 120 mg/kg (high-dose group [HDG]). After 1 to 14 d on basal diets (brooding phase), the ducks were assigned to the dietary treatment groups for 15 to 38 d (Grower phase) and 39 to 42 d (overfeeding period). Results indicated that AST supplementation improved final body weight and weight gain at both the grower and overfeeding phases ($P < 0.05$). Overfeeding increased the serum lipid level, altered intestinal morphology, and led to higher expression of pro-inflammatory factors and oxidative stress biomarkers while reducing antioxidant enzyme activity, associated gene expression, and anti-inflammatory factors in the duodenal and jejunal mucosa ($P < 0.05$). Additionally, overfeeding caused increased apoptotic cell counts in the duodenal and jejunal mucosa of the control group ($P < 0.05$), culminating in intestinal tissue damage and dysfunction. Dietary supplementation of AST mitigated these adverse effects, alleviated intestinal damage and promoted gut health. It exerted a hypolipidemic effect, improved villi morphometrics in the duodenum, jejunum, and ileum, and enhanced the levels of interleukin-4 (IL-4), soluble tumor necrosis factor-alpha receptor (sTNFαR), and transforming growth factor-beta (TGF-β) ($P < 0.05$). It also increased the activities of antioxidant enzymes and the mRNA expression of key antioxidant-related genes, including nuclear factor erythroid 2-related factor 2 (*Nrf2*), glutathione S-transferases (*GSTs*), and glutamate-cysteine ligase catalytic subunit (*GCLC*) ($P < 0.05$). Moreover, it reduced the expression of pro-inflammatory factors, oxidative stress biomarkers such as reactive oxygen species (ROS) and malondialdehyde (MDA), and the number of apoptotic cells in the duodenal and jejunal mucosa ($P < 0.05$). Immunoglobulin secretion and mucosal immunity were also significantly improved with AST supplementation ($P < 0.05$). Variations among the AST dietary groups suggest that a medium dosage of 80 mg/kg could effectively mitigate intestinal damage from overfeeding while enhancing growth performance, antioxidant defences, and

* Corresponding authors.
E-mail addresses: wushugeng@caas.cn (S. Wu), zhaofeixiacau@126.com (Z. Xia).
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immune responses. Our results would provide a theoretical reference for using AST as a nutritional strategy to enhance gut health in ducks exposed to overfeeding.

1. Introduction

China is the world's largest producer of meat ducks, accounting for over 4.1 billion ducks produced annually or 68% of the global production (Hou and Liu, 2021). Due to its high quality, duck meat has gained increasing attention among consumers, contributing to over 15% of the overall meat supply (Bai et al., 2022). Consumer preference and demand for roasted Pekin ducks have increased over time due to their tenderness and aromatic flavour (Bernacki and Adamski, 2001). Pekin ducks are renowned for their capacity to deposit fat in the breast and abdominal tissue, especially during the overfeeding periods (Davail et al., 2003), which enhances the content of unsaturated fatty acids linked to the meat's delicious quality (André et al., 2007; Xu et al., 2017).

Most duck farmers adopt nutritional regimens such as overfeeding to meet the rising demand and achieve better growth performance and meat quality. Various dietary interventions have been reported to enhance growth performance in different duck breeds, including the H strain (Bai et al., 2022) and Pekin (Ao and Kim, 2019; Wei et al., 2021; Hong et al., 2022). However, such a dietary regimen could increase nutrient metabolism and fat accumulation, heightening the risk of lipid peroxidation and gut mucosal damage due to free radical generation.

Research indicates that overfeeding in ducks is linked with oxidative stress-induced gut dysfunction, inflammation, behavioral changes, and alterations in metabolic and hormonal profiles (Hérault et al., 2019; Lo et al., 2020; Wei et al., 2021). These detrimental effects can impair nutrient digestibility and reduce growth performance. Nutrients for growth are supplied by diet; the gut digests and absorbs these nutrients to support proper growth, development, and physiological activities. Improved gut development facilitates digestion and nutrient absorption (Qin et al., 2019). Therefore, maintaining optimal gut health is essential for promoting the growth performance of animals.

The gastrointestinal tract is highly susceptible to oxidative stress: increasing the antioxidant function of the gut would protect the organisms against oxidative stress-induced injury. Therefore, it becomes expedient to supplement diets with natural antioxidants that could reduce lipid peroxidation and scavenge free oxygen radicals while modulating the immune response to preserve gut integrity (Li et al., 2018). Investigating the potential of specific nutrients, particularly during overfeeding phases, to mitigate intestinal dysfunction is essential. Plant extracts have been used as natural antioxidants in animal nutrition to increase animal health, performance, and welfare during oxidative stress, thus boosting resistance to diseases and oxidative toxins (Rossi et al., 2023). Thus, astaxanthin (AST), a natural pigment with high antioxidant properties, is a promising dietary intervention that could alleviate diet-induced oxidative stress in the gut and promote growth performance (Tolba et al., 2020).

Astaxanthin has demonstrated various biological functions, including anti-inflammatory, anticancer, anti-apoptotic, and neuroprotective effects (Haque et al., 2021; Kohandel et al., 2022; Si and Zhu, 2022). The study by Chang and Xiong (2020), highlighted the efficacy of AST in alleviating gastrointestinal diseases, attributable to its strong antioxidative and anti-inflammatory properties. Dietary supplementation of AST in laying hens (Shevchenko et al., 2020) and broiler chickens (Tolba et al., 2020) demonstrated significant anti-inflammatory effects and enhanced immunity, effectively mitigating oxidative stress-induced damage. The study of Ao and Kim (2019) demonstrated that AST could promote the growth performance of ducks under normal physiological conditions. Hence, the capacity of AST to alleviate oxidative stress, mitigate the progression of animal diseases, and enhance growth performance makes it a potential feed additive in poultry nutrition (Pertiwi et al., 2022). Therefore, nutritional interventions aimed at improving intestinal health and structure are increasingly vital.

There are limited research and exists a gap in knowledge regarding the effects of overfeeding on the intestinal health of Pekin ducks and the use of dietary AST to mitigate these adverse effects. This study aimed to investigate the impact of AST on growth performance, gut morphology, serum lipid profile, antioxidant capacity, immune function, and gene expression related to the intestinal mucosa in overfed Pekin ducks. The findings will offer both theoretical insights and practical guidance for utilizing AST as a nutritional strategy to enhance growth performance and overall physiology in Pekin ducks.

2. Materials and methods

2.1. Animal ethics statement

This study was carried out in strict accordance with the provisions of the Animal Welfare and Animal Experimental Ethics Review Committee of China Agricultural University. The animal ethics number is AW51213202-2-2.

2.2. Animal diets and experimental design

The commercial ducks used in the experiment were obtained from Beijing Nankou Duck Breeding Technology Company. The *Phaffia rhodozyma* (PR) was fermented in a jar and then freeze-dried, and the AST content was 20,000 mg/kg. The product (>99% purity) was obtained from Zhiyusheng. Co., Ltd. Kuming, China.

A total of 150 Pekin male ducks at one day of age were raised for 42 d in a feeding trial, divided into three phases: brooding phase (1–14 d), finishing phase (15–38 d), and overfeeding phase (39–42). On day 15, the ducks with similar body weights were selected and allotted to five treatment groups and each group had 30 ducks (five replicates of six birds each). The five treatment groups included a low-dose group (LDG; 0.2% PR + AST at 40 mg/kg plus basal diet), a medium-dose group (MDG; 0.4% PR + AST at 80 mg/kg plus basal diet), a high-dose group (HDG; 0.6% PR + AST at 120 mg/kg plus basal diet), a control group (CON; basal diet with no AST supplementation) and ad libitum feeding group (ALG; basal diet and normal feeding, i.e., no subjection to overfeeding). Throughout the feeding trial, the ducks had access to clean water and fresh feed, and all the routine immunization protocols were strictly adhered to. During the finishing phase, feeds were offered freely to all the groups while during the overfeeding phase, all animals except the ALG group were fed four times daily: 06:00,

12:00, 18:00, and 24:00, with feed quantitatively measured (400 g). The basal diets were formulated to meet the nutritional requirements of National Research Council (1994) and the China National Feeding Standard of Meat Duck (NY/T2122–2012, Table 1).

2.3. Chemical analysis of feed samples

Feed samples were analyzed for crude protein (method 990.03) and ether extract (method 954.02) using the AOAC (2012) procedures. Crude ash and crude fiber were analyzed following China National Standard (GB/T 6438-2007 and GB/T 6434–2007b, respectively). The quantification of calcium and phosphorus in the diets were respectively determined by EDTA disodium complexometric titration according to China National Standard (GB/T 6436–2018) and (GB/T 6437–2018), utilizing optical emission spectrophotometry with an inductively coupled plasma-optical emission spectrometry (Agilent 5800 ICP-OES, Palo Alto, USA). The amino acids methionine and lysine values in feed were determined by the conventional acid hydrolysis method according to China National Standard (GB/T 18246–2019). The metabolizable energy (ME) was calculated according to the China National Feeding Standard of the Meat Duck (NY/T 2122–2012).

2.4. Growth performance

On d 15, all the animals were weighed to obtain the initial body weight (IBW) before being allotted to various dietary groups. On a daily basis, the ducks were monitored, and feed consumption was recorded. On d 38 and 42, respectively, the animals were deprived of feed for 8 h, then the body weight was recorded. The final body weight (FBW), average daily gain (ADG), average daily feed intake (ADFI), and feed-weight gain ratio were calculated.

Table 1
Ingredients and nutritional composition of feed ingredients (% dry matter basis).

Item	0–14 d	15–38 d	39–42 d
Ingredients			
Corn	60.85	65.93	72.77
Soybean meal	30.00	24.00	15.00
Wheat bran	5.00	6.00	8.00
$CaHPO_4$	1.50	1.00	1.20
Stone powder	1.00	1.00	1.20
Salt	0.30	0.30	0.30
DL-Met	0.15	0.15	0.18
L-Lys	0.20	0.12	0.15
Premix[1]	1.00	1.50	1.20
Total	100.00	100.00	100.00
Nutritional composition[2]			
Crude protein	18.72	16.74	13.38
Ether extract	2.89	3.00	3.14
Ash	2.81	2.55	2.18
Crude fiber	3.80	3.65	3.44
Calcium	0.84	0.93	0.92
Phosphorus	0.45	0.42	0.39
Methionine	0.45	0.42	0.41
Lysine	1.20	0.99	0.82
ME, kcal/kg	2741	2782	2820

[1] The premix provides the following ingredients per kilogram of diet: Cu ($CuSO_4$-$5H_2O$) 10 mg Fe ($FeSO_4$-$7H_2O$) 60 mg; Zn (ZnO) 60 mg; Mn ($MnSO_4$-H_2O) 80 mg; Se ($NaSeO_3$) 0.2 g; I(KI) 0.2 mg; Cr (Cr_2O_3), 0.15 mg; choline 1000 mg; vitamin A 10,000IU; vitamin D 33,000 IU; vitamin E 20 IU; vitamin K 32 mg; thiamine 2 mg; riboflavin 8 mg; choline 1000 mg; vitamin K_3 2 mg; vitamin B_6 4 mg; vitamin B_{12} 0.06 mg; pantothenic acid 20 mg; nicotinic acid 50 mg; folic acid 1 mg; biotin folic acid 1 mg; biotin 0.2 mg.

[2] The levels of crude protein, ether extract, ash, crude fiber, calcium, phosphorus, methionine and lysine were analyzed, while metabolizable energy (ME) was calculated.

2.5. Sample collection

At the end of the feeding trial, two ducks selected from each replicate were weighed and slaughtered. Before slaughter, about 5 mL of blood was collected from the jugular vein and into a collection tube. The duodenum, jejunum, and ileum tissues were cut (about 1-2 cm), rinsed with 0.9% saline, and then fixed into a 4% formalin solution for histology analysis. Also, the mucosa of the duodenum and jejunum were removed of chyme and scraped aseptically by sterile glass slides at an angle of about 45 °C. The collected samples were placed into a tube, frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis.

2.6. Determination of serum biochemical indices

The blood collection tubes were allowed to stand at room temperature for 2 h, and then the harvested serum was centrifuged at 3000 × *g* at 4 °C for 12 min. The serum was transferred into new Eppendorf tubes and kept in the refrigerator at −20 °C for further analysis. Serum lipid indices including total cholesterol (TG), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured by an automatic biochemical analyzer (DIRUI CS-1600, Changchun, China). The assay kits used for all the above indicators were provided by Shanghai Enzyme-linked Biotechnology Co., Ltd. All the above indicators were measured following the manufacturer's instructions.

2.7. Intestinal morphology

The fixed duodenal and jejunal samples were cleaned, dehydrated, clarified, and then embedded in paraffin. The segments were cut to 5-mm thickness, mounted on glass slides, deparaffinized in xylene, rehydrated, stained with hematoxylin and eosin, and viewed under a light microscope. The protocols were according to those described by Zhou et al. (2021). Five intact intestinal villi were selected for each section, and measurements were taken using Image-ProPlus 6.0 (Media Image-Media Cybernetics, Inc., Rockville, MD, USA). The software measured the villus height (VH) and crypt depth (CD) in the slices while the villi height to crypt depth ratio was a calculated value. The average values were considered as the final measurement values.

2.8. Determination of intestinal apoptosis

The terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) method was used to detect the apoptosis of intestinal epithelial cells. The procedure, according to Jiang et al. (2019), was adopted. Briefly, the paraffinized sections of jejunal and duodenal tissues were deparaffinized with xylene twice, rehydrated with an ethanol concentration gradient, washed with PBS three times, and incubated with proteinase K at room temperature for 20 min. Then the sections were incubated with Equilibration Buffer, labelled with incubation buffer, terminated with labelling, and washed at 37 °C for 60 min in the dark. The sections were stained with 4′,6-diamidino-2-phenylindole (DAPI) staining solution and sealed with 10% glycerol-NaHCO3. The stained sections were photographed and observed under a fluorescence microscope. The Image-pro plus 6.0 software was used to select the green fluorescent nuclei with the same labelling as the uniform standard for determining the positive cells in all the photos. The blue nuclei with the same labelling of DAPI were selected as the total cells so that the number of positive cells and the total number of cells were obtained from the analysis

of each photo. The percentage of positive cells (positive cells/total cells × 100) was calculated as the apoptosis rate (%).

2.9. Determination of intestinal cytokines, antioxidant capacity, and immune function

The collected duodenal and jejunal mucosa samples were used to determine the levels of pro-inflammatory factors including interleukin beta and 6 (IL-1β, IL-6), interferon gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and anti-inflammatory factors including interleukin 4 (IL-4), soluble tumor necrosis factor-alpha receptor (sTNFαR) and transforming growth factor-beta (TGF-β); the antioxidant and oxidative cues including catalase (CAT), total antioxidant capacity (T-AOC), glutathione (GSH), glutathione reductase (GR), reactive oxygen species (ROS), malondialdehyde (MDA) and interferon stimulating protein (ISP), and the immunity indices including immunoglobulin M and G (IgM and IgG) and secretory immunoglobulin A (sIgA). These indices were analyzed using an ELISA kit (Shanghai Meilian Biotechnology Co., China), and all protocols were followed per the manufacturer's instructions.

2.10. Quantitative reverse transcription PCR (qRT-PCR) analysis

Total RNA was extracted from 0.1 g of the duodenal and jejunal tissue samples using TRIzol reagent (Thermo Fisher Scientific, Shanghai, China) according to the manufacturer's instructions, and then reverse-transcribed into single-stranded cDNA using the TIANScriptIIRT Kit (TIANGEN, Beijing, China). The gene expressions of nuclear factor erythroid 2-related factor 2 (*Nrf2*), glutathione S-transferases (*GSTs*), and glutamate-cysteine ligase catalytic subunit (*GCLC*) were determined using qRT-PCR with specific primers (Table 2). Quantitative PCR analyses were performed using the Step One Plus Real-time PCR system (Applied Biosystems, Foster City, CA, USA). After initial denaturation at 95 °C for 10 min, 40 cycles of amplification were carried out, followed by the generation of melt curves that could be used to verify the amplification specificity. The samples were tested in triplicate. The $2^{-\Delta\Delta Ct}$ method was used to calculate the relative gene expression levels and the glyceraldehyde-3-phosphate dehydrogenase (*GADPH*) was used as a housekeeping gene. All procedures used were according to those described by Yu et al. (2023).

2.11. Statistical analysis

Statistical analysis was done with the SPSS 26.0 version (SPSS Inc., Chicago, IL, USA). One-way ANOVA with orthogonal linear and quadratic contrasts, followed by Tukey's multiple comparison test was performed, which examined statistical differences among treatments. Statistical significance was considered at a *P*-value less than 0.05. Images were processed by GraphPad Prism 9.0. The experimental model applied was as follows:

$$Y_{ij} = \mu + T_i + R_j + \varepsilon_{ij},$$

where Y_{ij} is the observed value of the dependent variable (e.g., growth performance, serum lipid profile, antioxidant activity, cytokine expression, etc.) for the *i*-th treatment and j-th replicate; μ is the overall mean of the response variable across all groups and replicates; T_i is the effect of the *i*-th treatment group, which includes different doses of AST (low-dose, medium-dose, high-dose, control, ad libitum group); R_j is the random effect of the *j*-th replicate within each treatment to account for variability among replicates; ε_{ij} is the residual error.

3. Results

3.1. Growth performance

The impact of AST on the growth performance of Pekin ducks during the grower and overfeeding phase are presented in Table 3.

At the end of the grower phase (38 d), there was a significant effect of AST on the FBW ($P = 0.022$) and ADG ($P = 0.029$) of the ducks, but there were no significant variations within the AST groups. However, no significant dietary effects were found for ADFI ($P = 0.082$), and F/G ($P = 0.593$). During the grower period: 15 to 38 d, ADG increased linearly and significantly ($P = 0.010$) with increases in AST supplementation. At the end of the overfeeding period (42 d), there were significant effects of AST on FBW ($P < 0.001$) and ADG ($P = 0.013$), but no significant variations due to dietary effects were recorded for F/G ($P = 0.485$). Significant effect found for ADFI ($P < 0.001$), was mainly due to feed quantity, as other groups had same quantity of feed except the ALG group. Also, during the overfeeding period: 39 to 42 d, with the increase of AST addition, there were linear ($P < 0.001$) and quadratic significant changes ($P = 0.002$) in FBW, while ADG showed significant linear changes ($P = 0.002$). The ADFI showed significant linear changes ($P = 0.004$), this linear significant change was not reflected in the AST and control groups but in the ALG group.

3.2. Serum biochemical indices

The effect of dietary AST on the serum lipid profile of Pekin ducks is shown in Table 4. There were significant effects of dietary AST on all the serum lipid profile parameters ($P < 0.05$). The ALG group recorded significantly lower values for TG, TC, HDL-C, and LDL-C ($P < 0.05$), while the control group recorded the highest values for TG, LDL-C, and lowest values for HDL-C ($P < 0.05$). Significant variations ($P < 0.05$) among the AST groups for all the lipid profile indices except for the TG were notable. For the TG levels, the MDG had the lowest value among the treatments. The TC levels were highest in LDG ($P < 0.05$), while the MDG and HDG recorded lower values. The levels of LDL-C were lower in the MDG and HDG

Table 2
Primer sequences for qRT-PCR of target genes.

Genes	Primer sequence (5′–3′)	Fragment length, bp	Temperature, °C
Nrf2	F: GCTGGAGTTAGACGAGGAGACA R: GAAGTATGCGTGCTCTGTGAAA	111	60
GSTs	F: GAATCAGGGCTGGCAGGTCA R: CAGGTGCTTTGTTGGTGAGGAT	157	60
GCLC	F: GTTAGGGTGTCCTGGATTTACGG R: GTGACGGCGTGTTCTTATCTTTA	200	60
GADPH	F: GGTTGTCTCCTGCGACTTCA R: TCCTTGGATGCCATGTGGAC	165	60

Nrf2 = nuclear factor erythroid 2-related factor 2; *GSTs* = glutathione S-transferases; *GCLC* = glutamate cysteine ligase; *GADPH* = glyceraldehyde-3-phosphate dehydrogenase.

Table 3
Effect of dietary astaxanthin on growth performance of Pekin ducks.

Item	Groups[1]					SEM	*P*-value		
	LDG	MDG	HDG	CON	ALG		Treatment	Linear	Quadratic
15–38 d									
IBW, g	776.67	779.44	776.11	778.89	773.89	1.100	0.531	0.445	0.321
FBW, g	3012.50^{a}	3029.17^{a}	3033.33^{a}	2937.50^{b}	2930.50^{b}	13.487	0.022	0.007	0.183
ADG, g/d	93.16^{a}	93.74^{a}	94.05^{a}	89.94^{b}	90.15^{b}	0.570	0.029	0.010	0.228
ADFI, g/d	211.18	212.22	216.04	210.07	209.23	0.858	0.082	0.257	0.044
F/G	2.27	2.26	2.30	2.34	2.32	0.017	0.593	0.152	0.884
39–42 d									
FBW, g	3324.60^{b}	3341.67^{a}	3331.70^{a}	3234.17^{c}	3174.17^{d}	10.040	<0.001	<0.001	0.002
ADG, g/d	106.39^{a}	104.17^{a}	96.94^{ab}	98.89^{ab}	78.89^{b}	2.904	0.013	0.002	0.210
ADFI, g	400.00^{a}	400.00^{a}	400.00^{a}	400.00^{a}	253.30^{b}	78.560	<0.001	0.004	0.186
F/G	3.86	3.83	4.19	4.14	3.67	0.104	0.485	0.954	0.127

LDG = low dose group; MDG = medium dose group; HDG = high dose group; CON = control group; ALG = ad libitum feeding group; SEM = standard error of the mean; IBW = initial body weight; FBW = final body weight; ADG = average daily gain; ADFI = average daily feed intake; F/G = feed-to-gain ratio.
[a, b] Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1] Data are expressed as the mean of 5 replicates of 6 birds each.

Table 4
Effect of dietary astaxanthin on serum lipid profile of Pekin ducks (mmol/L).

Item	Groups[1]					SEM	*P*-value		
	LDG	MDG	HDG	CON	ALG		Treatment	Linear	Quadratic
TG	3.08^{ab}	2.50^{b}	2.61^{b}	3.39^{a}	1.78^{c}	0.161	0.004	0.072	0.505
TC	4.07^{b}	3.56^{c}	3.59^{c}	4.60^{a}	3.07^{d}	0.110	<0.001	<0.001	0.311
LDL-C	2.58^{b}	1.93^{c}	1.39^{d}	3.07^{a}	1.79^{c}	0.130	<0.001	0.006	0.426
HDL-C	2.02^{b}	1.63^{c}	2.20^{a}	0.96^{d}	1.28^{c}	0.101	<0.001	0.102	0.273

LDG = low dose group; MDG = medium dose group; HDG = high dose group; CON = control group; ALG = ad libitum feeding group; SEM = standard error of the mean; TG = triglycerides; TC = total cholesterol; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol.
[a-d] Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1] Data are expressed as the mean of 5 replicates of 6 birds each.

($P < 0.05$) compared to the LDG. Whereas for HDL-C levels, the HDG recorded the highest values ($P < 0.05$), while the LDG and MDG recorded lower values ($P < 0.05$). The linear probabilities for TC ($P < 0.001$) and LDL-C ($P = 0.006$), showed significant decreasing changes with increasing AST dosage.

3.3. Intestinal morphology

The effect of dietary AST on the intestinal morphology of Pekin ducks is presented in Table 5, while the histomorphology chart is presented in Fig. 1.

There was a significant influence of dietary AST on villi height for all the intestinal segments ($P < 0.05$), duodenum ($P < 0.001$), ileum ($P = 0.002$), and jejunum ($P < 0.001$). Among the AST groups, the effect of MDG and HDG were significantly higher ($P < 0.05$) than LDG for both duodenum and jejunum, while LDG was numerically higher in the ileum but not statistically different. Also, the effect of LDG was significantly lower ($P < 0.05$) and comparable to the control group. The influence of dietary AST on crypt depth for all the intestinal segments was markedly significant ($P < 0.05$): duodenum ($P < 0.001$) and ileum ($P = 0.036$), whereas the effect in the jejunum was not significant ($P = 0.057$). Within the AST group, there were no significant variations for the duodenal values, whereas in the ileum,

Table 5
Effect of dietary astaxanthin on the intestinal morphology of Pekin ducks.

Item	Groups[1]					SEM	*P*-value		
	LDG	MDG	HDG	CON	ALG		Treatment	Linear	Quadratic
VH, μm									
Duodenum	740.42^{c}	1027.40^{a}	1001.25^{b}	709.70^{c}	1090.17^{a}	23.011	<0.001	0.025	0.686
Ileum	804.30^{a}	724.20^{b}	655.47^{c}	546.80^{d}	594.48^{d}	20.070	0.002	<0.001	0.206
Jejunum	501.36^{c}	708.37^{b}	900.66^{a}	511.99^{c}	714.45^{b}	13.921	<0.001	0.024	<0.001
CD, μm									
Duodenum	301.89^{c}	304.27^{c}	350.31^{b}	417.29^{a}	383.70^{b}	7.970	<0.001	<0.001	0.452
Ileum	314.59^{b}	350.04^{a}	263.57^{d}	293.31^{c}	317.38^{b}	8.501	0.036	0.400	0.196
Jejunum	292.60	338.01	353.40	337.48	305.90	6.940	0.057	0.600	0.004
VH/CD									
Duodenum	2.47^{bc}	3.50^{a}	3.23^{ab}	1.70^{c}	2.80^{ab}	0.562	<0.001	0.070	0.127
Ileum	2.39	2.17	2.62	2.21	2.02	0.093	0.316	0.291	0.321
Jejunum	1.73^{c}	2.25^{ab}	2.43^{a}	1.53^{c}	2.38^{a}	0.061	<0.001	0.191	0.433

LDG = low dose group; MDG = medium dose group; HDG = high dose group; CON = control group; ALG = ad libitum feeding group; SEM = standard error of the mean; VH = villi height; CD = crypt depth; VH/CD = villi height to crypt depth ratio.
[a-d] Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1] Data are expressed as the mean of 5 replicates of 6 birds each.

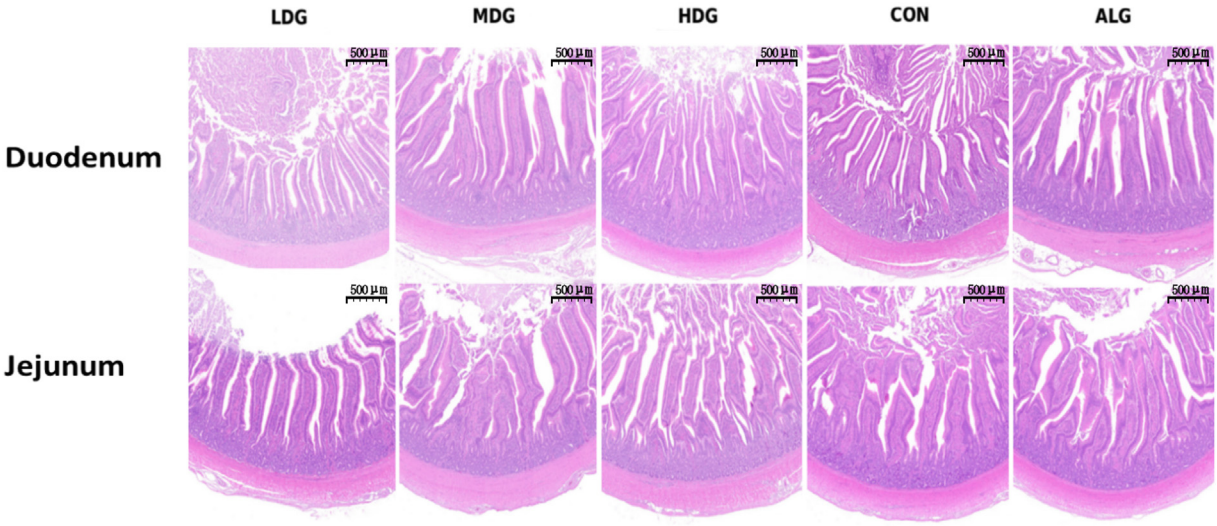

Fig. 1. Effect of dietary astaxanthin on intestinal histomorphometry (magnification, 40 ×; scale bar, 500 μm). LDG = low dose group; MDG = medium dose group; HDG = high dose group; CON = control group; ALG = ad libitum feeding group.

the HDG recorded the lowest value. The impact of dietary AST on the villi height to crypt depth ratio was significant for duodenum ($P < 0.001$) and jejunum ($P < 0.001$) but not for ileum ($P = 0.316$). Within the AST group, the MDG recorded the highest value in the duodenum, followed by HDG. In contrast, the LDG recorded the lowest value and significantly differed from the MDG ($P < 0.05$). In the jejunum, within the AST group, the HDG recorded the highest value and was significantly different ($P < 0.05$) from the MDG and LDG. The LDG recorded the lowest value and was comparable to the control group. The linear probability for the VH of the 3 segments; duodenum ($P = 0.025$), ileum ($P < 0.001$) and jejunum ($P = 0.024$), showed significant changes while the VH of jejunum also showed quadratic significant changes ($P < 0.001$). The CD of duodenum showed a linear significant decreasing change ($P < 0.001$) with increasing dose of AST. Moreover, the value of CD in the jejunum showed quadratic significant changes ($P = 0.004$).

3.4. Apoptosis of intestinal mucosal cells

The effect of AST on the apoptotic cell count of duodenal and jejunal epithelial cells in Pekin ducks as detected by TUNNEL staining is presented in Fig. 2. The results showed that compared with the ALG (not exposed to overfeeding), the TUNEL positivity and apoptosis of duodenum and jejunum epithelial cells were significantly increased ($P < 0.05$) in the CON group, subjected to overfeeding without supplemental AST. The supplementation of AST to Pekin ducks during the overfeeding period caused a significant reduction ($P < 0.05$) in the degree of apoptosis. Dietary AST alleviated the overfeeding-induced apoptosis on duodenal and jejunal epithelial cells.

3.5. Intestinal cytokines

The influence of dietary AST on the expression of pro-inflammatory and anti-inflammatory factors in the duodenum and jejunum is listed in Table 6. Dietary AST significantly reduced the expressions of pro-inflammatory factors (IL-1β, IL-6, TNF-α, and IFN-γ) and increased anti-inflammatory factors (sTNFαR, TGF-β, and IL-4) in both duodenum and jejunum ($P < 0.05$). The control group was notable, with the highest and lowest levels of pro-inflammatory and anti-inflammatory factors ($P < 0.05$), respectively.

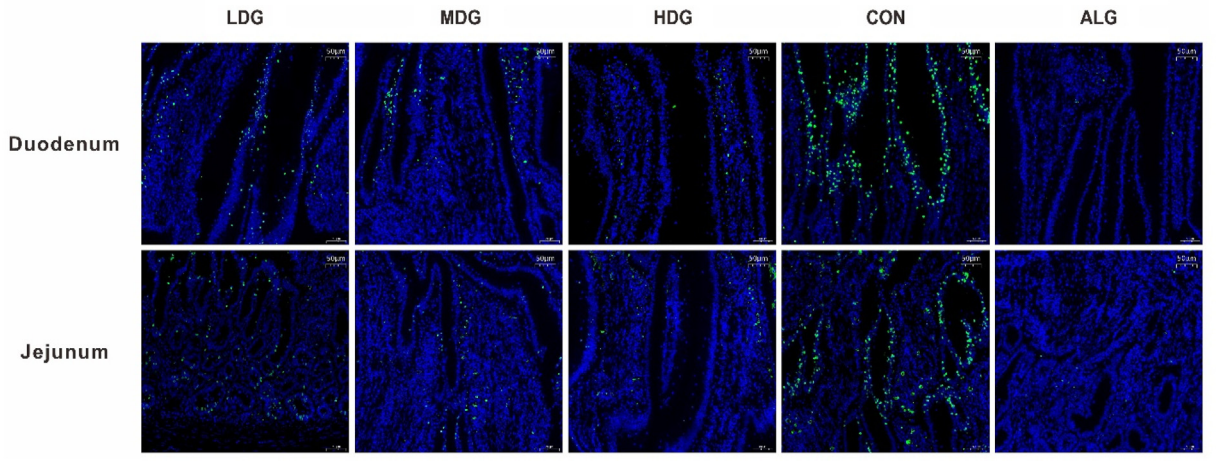

Fig. 2. Effect of dietary astaxanthin on the apoptosis level of duodenal and jejunal mucosal cells (magnification, 40 × ; scale bar, 500 μm). LDG = low dose group; MDG = medium dose group; HDG = high dose group; CON = control group; ALG = ad libitum feeding group.

Table 6
Effect of dietary astaxanthin on expression levels of pro-inflammatory and anti-inflammatory factors in the duodenum and jejunum of Pekin ducks (pg/mL).

Item	Groups[1]					SEM	*P*-value		
	LDG	MDG	HDG	CON	ALG		Treatment	Linear	Quadratic
IL-1β									
Duodenum	58.89^{d}	64.45^{b}	62.17^{b}	71.78^{a}	60.08^{c}	1.312	0.003	0.004	0.215
Jejunum	60.60^{ab}	62.39^{b}	63.67^{b}	69.50^{a}	57.82^{c}	0.920	<0.001	<0.001	0.008
IL-6									
Duodenum	23.37^{c}	24.07^{b}	24.32^{b}	28.07^{a}	24.98^{b}	0.430	<0.001	0.004	0.177
Jejunum	22.92^{b}	22.33^{b}	22.58^{b}	28.35^{a}	24.22^{b}	0.522	<0.001	0.012	0.442
TNF-α									
Duodenum	94.79^{c}	95.88^{c}	100.38^{b}	109.40^{a}	97.94^{b}	1.463	0.003	0.021	0.725
Jejunum	89.10^{b}	92.50^{b}	89.80^{b}	108.74^{a}	90.17^{b}	1.742	<0.001	<0.001	0.320
IFN-γ									
Duodenum	46.95^{c}	48.38^{b}	49.80^{ab}	50.61^{a}	47.95^{b}	0.394	0.011	0.016	0.556
Jejunum	45.10^{c}	46.32^{b}	44.20^{c}	49.48^{a}	47.64^{b}	0.125	0.011	0.014	0.545
sTNFαR									
Duodenum	2.57^{c}	3.20^{a}	3.11^{a}	2.69^{c}	2.97^{b}	0.062	<0.001	<0.001	0.108
Jejunum	2.86^{b}	2.99^{a}	2.95^{a}	2.58^{c}	2.82^{b}	0.013	<0.001	0.009	0.223
TGF-β									
Duodenum	49.91^{d}	66.54^{a}	60.07^{b}	54.68^{c}	47.27^{d}	1.571	<0.001	0.006	0.228
Jejunum	63.81^{b}	65.78^{a}	66.52^{a}	51.54^{c}	62.03^{b}	1.270	<0.001	<0.001	0.015
IL-4									
Duodenum	670.15^{a}	642.10^{b}	631.83^{c}	576.20^{d}	645.32^{b}	7.382	<0.001	0.003	0.176
Jejunum	613.13^{c}	623.26^{c}	656.57^{b}	591.78^{d}	679.05^{a}	9.231	0.005	0.191	0.521

LDG = low dose group; MDG = medium dose group; HDG = high dose group; CON = control group; ALG = ad libitum feeding group; SEM = standard error of the mean; IL = interleukins; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; sTNFαR = soluble tumor necrosis factor alpha receptor; TGF-β = transforming growth factor-beta.
[a-c] Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1] Data are expressed as the mean of 5 replicates of 6 birds each.

The levels of IL-1β in the duodenum differed significantly between the AST groups and ALG ($P < 0.05$), with LDG recording significantly lowest values ($P < 0.05$) among the AST group. In the jejunum, ALG recorded the lowest values ($P < 0.05$) and no variation among the AST groups. The levels of IL-6 in the duodenum and jejunum were comparable among the AST groups and ALG ($P > 0.05$). The levels of TNF-α in the duodenum were comparable between ALG and HDG ($P > 0.05$) but not LDG and MDG ($P < 0.05$), whereas in the jejunum, there were no significant differences between the AST and ALG ($P > 0.05$). The levels of IFN-γ in the duodenum were significantly reduced in the LDG and recorded the lowest value compared to other groups ($P < 0.05$), while in the jejunum there were significant differences between ALG and LDG and HDG ($P < 0.05$). Furthermore, the levels of sTNFαR in the duodenum were significantly different between the AST groups and ALG ($P < 0.05$), higher values were recorded in the MDG and HDG compared to LDG which had the lowest value ($P < 0.05$). In the jejunum, the levels were significantly different between ALG and MDG and HDG ($P < 0.05$) but not LDG ($P > 0.05$). The levels of TGF-β in the duodenum were found to vary across the AST groups ($P < 0.05$), the MDG recorded the highest value ($P < 0.05$), followed by the HDG, while the LDG recorded the lowest value and was comparable to ALG ($P > 0.05$). Also, a similar trend was observed in the jejunum. The levels of IL-4 in the duodenum varied significantly across the AST groups with LDG and HDG recording the highest and lowest values respectively ($P < 0.05$), while comparable effects were observed between MDG and ALG ($P > 0.05$). In the jejunum, there were significant variations across the AST groups and differed significantly from the ALG ($P < 0.05$). There were significant linear changes in the duodenum and jejunum for IL-1β ($P = 0.004$, $P < 0.001$), IL-6 ($P = 0.004$, $P = 0.012$), TNF-α ($P = 0.021$, $P < 0.001$), IFN-γ ($P = 0.016$, $P = 0.014$), sTNFαR ($P < 0.001$, $P = 0.009$), and TGF-β ($P = 0.006$, $P < 0.001$). Linear significant changes were observed for levels of IL-4 in the duodenum ($P = 0.003$). In addition, IL-1β of jejunum showed quadratically significant changes ($P = 0.008$).

3.6. Antioxidant capacity of the duodenum and jejunum

The influence of AST on the antioxidant capacity of the duodenum and jejunum of Pekin ducks is presented in Table 7. Dietary AST significantly upregulated both enzymatic and non-enzymatic components: CAT, superoxide dismutase (SOD), GSH, GR, and T-AOC ($P < 0.05$). For all antioxidant cues, there were significant variations within the AST groups, both in the duodenum and jejunum ($P < 0.05$), and the highest antioxidant activities for both duodenum and jejunum were recorded with the HDG ($P < 0.05$). The control group recorded the lowest values in both segments ($P < 0.05$).

The activities of CAT in the duodenum and jejunum were lowest with the LDG and significantly different ($P < 0.05$) from MDG and HDG but were comparable to ALG in the jejunum ($P > 0.05$). The activities of SOD in both duodenum and jejunum were higher for MDG and HDG ($P < 0.05$), but LDG was comparable to ALG ($P > 0.05$). The levels of T-AOC remained comparable among the AST groups ($P > 0.05$) but differed with the ALG ($P < 0.05$). In the jejunum, there were significant variations across the AST groups ($P > 0.05$) and between AST groups and ALG ($P < 0.05$). The levels of GSH in the duodenum varied significantly across the AST groups and significant variations were found between AST groups and ALG ($P < 0.05$). Whereas, in the jejunum, the levels in the AST group were comparable to ALG ($P > 0.05$). Significant variations were notable for levels of GR in the duodenum and jejunum across the AST groups ($P < 0.05$) with HDG and LDG recording significantly higher and lower values, respectively ($P < 0.05$). There were significant linear and quadratic changes for activities of CAT ($P = 0.002$, $P = 0.001$), and GR ($P = 0.001$, $P = 0.001$) in the jejunum. The activities of SOD showed linear and quadratic changes in the duodenum ($P = 0.035$, $P = 0.010$) while only quadratic changes were found in the jejunum ($P = 0.003$). There were significant linear and quadratic changes for T-AOC levels in the duodenum ($P = 0.001$, $P = 0.018$) and in the jejunum ($P = 0.001$, $P = 0.003$). The

Table 7
Effect of dietary astaxanthin on the antioxidant capacity of the duodenum and jejunum of Pekin ducks.

Item	Groups[1]					SEM	*P*-value		
	LDG	MDG	HDG	CON	ALG		Treatment	Linear	Quadratic
CAT, μmol/g per minute									
Duodenum	29.85^{c}	38.30^{a}	35.66^{b}	21.90^{d}	35.54^{b}	1.379	<0.001	0.594	0.522
Jejunum	34.24^{c}	38.66^{b}	42.84^{a}	29.08^{d}	34.83^{c}	0.690	<0.001	0.002	0.001
SOD, U/g									
Duodenum	90.49^{b}	117.33^{a}	127.52^{a}	67.15^{c}	88.03^{b}	7.532	<0.001	0.035	0.010
Jejunum	102.76^{b}	107.83^{b}	152.39^{a}	80.11^{c}	96.39^{b}	7.244	<0.001	0.098	0.003
T-AOC, μmol/g									
Duodenum	2.11^{b}	2.20^{ab}	2.27^{a}	1.70^{d}	1.89^{c}	0.045	<0.001	0.001	0.018
Jejunum	1.80^{c}	1.98^{b}	2.12^{a}	1.40^{d}	1.73^{c}	0.041	<0.001	0.001	0.003
GSH, μmol/g									
Duodenum	1.38^{c}	1.60^{b}	1.83^{a}	1.21^{c}	1.94^{a}	0.067	<0.001	0.004	0.490
Jejunum	1.71^{b}	1.63^{b}	2.00^{a}	1.38^{c}	1.77^{ab}	0.077	<0.001	0.636	0.815
GR, nmol/g per minute									
Duodenum	1080.93^{c}	1288.19^{b}	1531.17^{a}	918.35^{c}	1352.51^{b}	58.811	<0.001	0.366	0.088
Jejunum	1013.04^{d}	1168.48^{c}	1472.21^{a}	912.99^{e}	1343.57^{b}	19.201	<0.001	0.001	0.001

LDG = low dose group; MDG = medium dose group; HDG = high dose group; CON = control group; ALG = ad libitum feeding group; SEM = standard error of the mean; CAT = catalase; SOD = superoxide dismutase; T-AOC = total antioxidant capacity; GSH = glutathione; GR = glutathione reductase.
[a-e] Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1] Data are expressed as the mean of 5 replicates of 6 birds each.

activities of GSH showed linear significant changes in the duodenum ($P = 0.004$).

In addition, the effect of dietary AST on oxidative biomarkers in the duodenum and jejunum of Pekin ducks is listed in Table 8. The efficacy of AST as a natural antioxidant was evident in significant reductions in levels of oxidative biomarkers such as ROS, MDA, and ISP in both duodenum and jejunum ($P < 0.05$). The control group recorded the highest values for all the oxidative cues in the duodenum and jejunum ($P < 0.05$). There were significant variations among the AST groups for ROS and MDA in the duodenum ($P < 0.05$), while that of ROS in the jejunum and ISP in both segments was not significant among the AST groups ($P > 0.05$). The HDG recorded the lowest values for ROS in the duodenum, whereas LDG and HDG had the lowest values for MDA in both segments, respectively. The levels of ROS in the duodenum showed linear significant changes ($P = 0.002$), while both linear and quadratic significant changes ($P = 0.017$, $P = 0.021$) were found in the jejunum. The levels of MDA in the duodenum showed both linear and quadratic changes ($P = 0.016$, $P = 0.001$). There were linear and quadratic significant changes for levels of ISP in the duodenum ($P = 0.001$, $P = 0.007$) and jejunum ($P = 0.001$, $P = 0.029$).

3.7. Immunity indices of duodenal and jejunal mucosa

The effect of AST on the immunity indices of duodenal and jejunal mucosa is presented in Table 9. The AST enhanced the secretion of mucosal immunoglobulin M and G (mIgM and mIgG), and sIgA and boosted the immunity status ($P < 0.05$). The highest and lowest values for all the mucosal immunity indices in both segments were recorded by the HDG and the control groups, respectively ($P < 0.05$). There were significant variations among the AST groups for duodenal and jejunal mIgM and jejunal mIgG ($P < 0.05$). However, there were no variations among treatments for duodenal mIgG and sIgA for both segments ($P > 0.05$). The LDG recorded lower values for mIgM in both segments and jejunal mIgG. The animals showed significant linear and quadratic changes for mIgM in the duodenum ($P = 0.028$, $P = 0.002$) while only quadratic changes ($P = 0.010$) were seen in the jejunum. The levels of mIgG showed linear and quadratic changes in the jejunum ($P = 0.001$, $P = 0.002$) while only significant linear changes were found in the duodenum ($P = 0.001$). There were linear increases in sIgA in response to AST addition for both duodenum ($P < 0.001$) and jejunum ($P < 0.001$), as well as a quadratic change in the jejunum ($P = 0.065$).

3.8. mRNA expression of antioxidant genes in the duodenum and jejunum

The effect of dietary AST on mRNA expression of antioxidant genes in the duodenum and jejunum is shown in Fig. 3. Dietary AST significantly upregulated the expression of antioxidant genes *Nrf2*, *GSTs*, and *GCLC* ($P < 0.05$). The ALG recorded the lowest expression

Table 8
Effect of dietary astaxanthin on oxidative biomarkers in duodenum and jejunum of Pekin ducks.

Item	Groups[1]					SEM	*P*-value		
	LDG	MDG	HDG	CON	ALG		Treatment	Linear	Quadratic
ROS, IU/mL									
Duodenum	217.08^{bc}	220.19^{b}	202.74^{c}	275.72^{a}	227.84^{b}	6.691	<0.001	0.002	0.650
Jejunum	251.07^{b}	242.28^{b}	246.54^{b}	285.38^{a}	243.48^{b}	3.385	<0.001	0.017	0.021
MDA, nmol/g									
Duodenum	82.56^{c}	110.60^{b}	111.11^{b}	143.45^{a}	93.40^{bc}	6.328	<0.001	0.016	0.001
Jejunum	104.92^{b}	87.55^{bc}	75.34^{c}	127.11^{a}	79.98^{c}	6.178	<0.001	0.605	0.849
ISP, pg/mL									
Duodenum	27.34^{b}	27.48^{b}	27.06^{b}	32.01^{a}	27.46^{b}	0.358	<0.001	0.001	0.007
Jejunum	27.39^{c}	27.21^{c}	27.39^{c}	33.33^{a}	29.55^{b}	0.191	<0.001	0.001	0.029

LDG = low dose group; MDG = medium dose group; HDG = high dose group; CON = control group; ALG = ad libitum feeding group; SEM = standard error of the mean; ROS = reactive oxygen species; MDA = malondialdehyde; ISP = interferon stimulating protein.
[a-c] Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1] Data are expressed as the mean of 5 replicates of 6 birds each.

Table 9
Effect of dietary astaxanthin on immunity indices of duodenal and jejunal intestinal mucosa of Pekin ducks.

Item	Groups[1]					SEM	*P*-value		
	LDG	MDG	HDG	CON	ALG		Treatment	Linear	Quadratic
mIgM, g/L									
Duodenum	3.34^{b}	3.55^{a}	3.54^{a}	3.29^{b}	3.24^{b}	0.058	0.003	0.028	0.002
Jejunum	3.21^{b}	3.78^{a}	3.71^{a}	3.37^{b}	3.63^{a}	0.083	<0.001	0.124	0.010
mIgG, g/L									
Duodenum	6.46^{ab}	6.62^{a}	6.78^{a}	5.67^{c}	6.10^{b}	0.122	<0.001	0.000	0.112
Jejunum	6.47^{b}	7.36^{a}	7.06^{a}	5.79^{c}	6.28^{b}	0.131	<0.001	0.000	0.002
sIgA, μg/mL									
Duodenum	16.75^{a}	17.32^{a}	17.43^{a}	13.22^{c}	15.24^{b}	0.299	<0.001	<0.001	0.217
Jejunum	17.38^{a}	17.89^{a}	17.93^{a}	13.85^{c}	15.77^{b}	0.179	<0.001	<0.001	0.065

LDG = low dose group; MDG = medium dose group; HDG = high dose group; CON = control group; ALG = ad libitum feeding group; SEM = standard error of the mean; mIgM = mucosal immunoglobulin M; mIgG = mucosal immunoglobulin G; sIgA = secretory immunoglobulin A.
[a-c] Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1] Data are expressed as the mean of 5 replicates of 6 birds each.

of these antioxidant genes ($P < 0.05$). Significant variations existed for AST groups in the expression of *GCLC* in both duodenum and jejunum ($P < 0.05$), while none existed for *Nrf2* and *GSTs* in both duodenum and jejunum ($P > 0.05$). The LDG recorded lower values for *GCLC* in both duodenum and jejunum, while MDG and HDG maintained a higher expression level.

4. Discussion

The current study demonstrated that overfeeding induces high nutrient metabolism and oxidative stress, leading to intestinal dysfunction, and reduces performance. Our findings support the hypothesis that dietary astaxanthin can mitigate damage to gut morphology, lipid peroxidation, and apoptotic and inflammatory responses in the intestinal mucosa, thereby improving the growth performance of Pekin ducks under stressors such as overfeeding. Although there is limited information on Pekin ducks exposed to overfeeding, studies on force-feeding which are closely related will be referenced in the discussion to provide further context for these findings.

Our results showed that enhanced growth performance during the overfeeding period aligns with previous studies that reported weight gain in ducks after overfeeding (Saez et al., 2009; Wen et al., 2015). In the current study, the significant effect of AST supplementation on weight gain during the overfeeding period was markedly substantial. Our findings affirm the study of Ao and Kim. (2019), which reported that AST enhanced weight gain and feed efficiency in ducks linearly, indicating efficient metabolism of AST by the animals and its growth-promoting effect. However, the study by Wen et al. (2015) reported that overfeeding beyond 420 g/day did not improve growth performance and reduced the apparent digestibility of nutrients. In our study, providing 400 g of feed daily to the ducks during the overfeeding period led to weight gain, aligning with earlier findings. Additionally, the lack of an effect of AST on the growth performance of broiler chickens, as reported by Perenlei et al. (2014), highlights those discrepancies in the outcomes that may be due to differences in the dosage of the supplement or the physiological status of the animals. Notably, there is a close link between growth performance and gut health, with overfeeding inducing oxidative stress that compromises intestinal stability, a critical factor affecting both growth and overall performance.

In the current study, high serum levels of TG and TC in the control group and lower lipid profiles of the ALG, suggest the impact of overfeeding. Pekin ducks are known for lower lipid catabolism (Hérault et al., 2019), and similar results have been observed in geese, where overfeeding increased liver fat content (Wei et al., 2020). The study by Chen et al. (2019) demonstrated that lipid metabolism imbalance leads to accumulation of free fatty acids, which accelerates ROS synthesis, causing mitochondria dysfunction and cellular toxicity. These alterations may imply lipid metabolism disruptions and hepatic dysfunction, potentially triggering gut inflammation. Nevertheless, AST exerted a hypolipidemic effect, mitigating lipid accumulation and oxidative stress during overfeeding, and are consistent with studies in mice (Jia et al., 2016) and fish (Xie et al., 2020) on high-fat oxidized diets. However, AST had no effect on the lipid profile of animals under a regular diet (Xia et al., 2020) suggesting that its efficacy may be more pronounced under stress conditions. Overfeeding-induced fat accumulation can lead to excessive ROS generation and lipid peroxidation, all detrimental to the intestinal function.

Antioxidant biomarkers such as SOD, CAT, GSH-Px, T-AOC, and MDA are crucial for maintaining redox equilibrium and protecting cell structures against oxidative stress-induced damage. Oxidant markers such as ROS and MDA, are key indicators of oxidative stress and are closely linked to the activity of antioxidant enzymes like

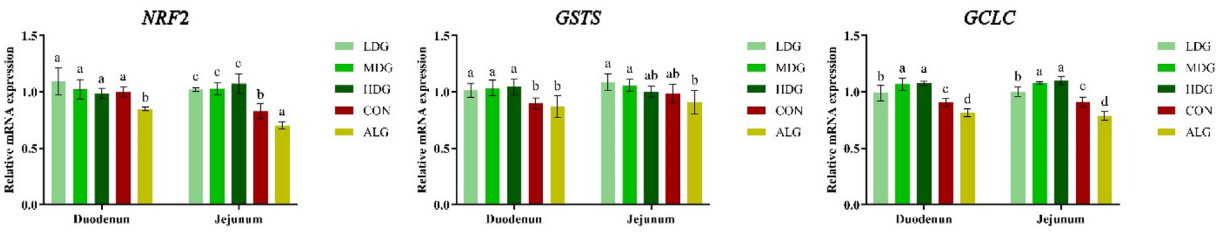

Fig. 3. Effect of dietary astaxanthin on mRNA expression levels of antioxidant genes in the duodenal and jejunal intestinal mucosa of Pekin ducks. *Nrf2* = nuclear factor erythroid 2-related factor 2; *GSTs* = glutathione S-transferases; *GCLC* = glutamate-cysteine ligase catalytic subunit; LDG = low dose group; MDG = medium dose group; HDG = high dose group; CON = control group; ALG = ad libitum feeding group. [a-d] Values within groups with no common superscripts differ significantly ($P < 0.05$). The error bars mean standard error of the mean (SEM).

SOD, CAT, and GSH-Px (Wang et al., 2022). Oxidative stress is reflected in reduced activities of antioxidant enzymes and increased levels of ROS and MDA (He et al., 2016). In this study, overfeeding resulted in a decrease of antioxidant enzyme activities and an increase in oxidative biomarkers in the duodenal and jejunal mucosa. The absence of significant increases in oxidative cues such as ROS, ISP, and MDA levels in the ALG suggests that oxidative stress was induced by overfeeding. In contrast, the control group showed a substantial increase in oxidative biomarkers and a decrease in antioxidant enzyme activity, underscoring the need for supplemental natural antioxidants. These findings are consistent with previous studies in force-fed ducks (Liu et al., 2018) and overfed geese (Wei et al., 2020), which also reported reduced antioxidant enzyme activities and elevated MDA levels. Together, these results demonstrate that nutritional regimens such as overfeeding can cause oxidative damage to the duodenum and jejunum, leading to inflammation and compromised intestinal integrity.

Increased activities of antioxidant enzymes, including SOD, GSH-Px, and CAT, are critical to the antioxidant system by scavenging free radicals and inhibiting lipid peroxidation through a chain reaction mechanism (Shirani et al., 2019). Interestingly, dietary AST significantly upregulated antioxidant enzymes and reduced the oxidative cues in ducks exposed to overfeeding, enhancing the antioxidant defense mechanism in a dose-dependent manner and thus highlighting the inherent antioxidant property of AST in scavenging these radicals and protecting cells and tissues against oxidative damage.

In affirmation of our results, AST has been shown to improve the antioxidant defense system in ducks under normal conditions (Ao and Kim, 2019), as well as in rats (Jia et al., 2016; Wang et al., 2022) and fish (Xie et al., 2020) exposed to nutrition-induced oxidative stress. Astaxanthin also upregulated the mRNA expression of genes related to antioxidant stress response and lipid metabolism, thus mitigating oxidative stress in laying hens and broilers exposed to heat stress (Tolba et al., 2020). Furthermore, in vitro studies demonstrated that AST (in powdered form) effectively suppressed hypoglycaemia-induced ROS production (Manabe et al., 2008) and inhibited ISP generation (Yoshihisa et al., 2014), while its nanoparticle form significantly reduced ROS production (Zhang et al., 2023). However, dietary AST exerted no antioxidant effect on broiler birds fed oxidized fat (Takimoto et al., 2007), possibly due to variations in dosage or supplement source. Additionally, the scavenging capacity of AST may contribute to maintaining redox balance by activating the *Nrf2* signaling pathway.

The *Nrf2* signaling pathway regulates the expression of antioxidant enzymes and the transcriptional activity of antioxidant cues by binding to the antioxidant response elements. In our study, AST upregulated the *Nrf2* pathway and expression of its downstream target genes *GST* and *GCLC*, consolidating the antioxidant system against overfeeding-induced oxidative stress. These results are in tandem with previous reports from in vivo and in vitro studies showing that AST downregulates the NF-κB pathway through modulation of *Nrf2*-dependent and independent mechanisms, thereby exerting antioxidant and anti-inflammatory effects (Farruggia et al., 2018). In another study, AST has been shown to protect cells from oxidative stress by activating the *Nrf2-ARE*-mediated expression of antioxidant enzymes (Saw et al., 2013). The upregulation of the *Nrf2* pathway promotes antioxidant enzyme activity (Mo et al., 2014) and mediates an anti-inflammatory response by inhibiting the *NF-κB* pathway (Lai et al., 2014; Zhang and Tsao, 2016). Consequently, AST supplementation boosted the antioxidant defense system and immunity through *Nrf2* activation, likely contributing to reduced intestinal mucosal inflammation.

Mucosal immunity plays a critical role in maintaining intestinal health and gut barrier functions through both specific and non-specific immune activities (Wu et al., 2021; Zuo et al., 2020).

Secretory immunoglobulin A and immunoglobulins IgM and IgG are key regulators of mucosal immunity and serve as critical indicators of gut health (Mountzouris et al., 2010; Zhao et al., 2020). The current study demonstrated that overfeeding reduced immunoglobulin the levels, impairing the immunity status of Pekin ducks. However, dietary AST boosted the immunity status by promoting the secretion of immunoglobulins, which are primary components of the immunity index. This effect is likely attributed to the activation of *Nrf2*, which boosts intestinal antioxidant capacity, facilitates immunoglobulin secretion, and may influence inflammatory responses in the intestinal mucosa.

The immune response of the intestinal mucosa is mainly mediated through cytokine secretion, which reflects the mucosal immune function (Diefenbach et al., 2020). Cytokines produced during the effector phase of innate and specific immunity regulate both immunological and inflammatory responses (Millman et al., 2021). Thus, pro-inflammatory (IL-1β, IL6, TNF-α, and IFN-γ) and anti-inflammatory (IL-4 and TGF-β) cytokines are commonly used to profile immune responses. For instance, IL-6 plays a key role in immunoglobulin production (IgM, IgA, and IgM), while IFN-γ regulates lymphocytes and monocyte activation.

Current findings indicated that overfeeding induced inflammation in the intestinal mucosa, evidenced by elevated pro-inflammatory cytokines and a reduction in anti-inflammatory cytokines levels. These results align with previous research showing increased IL-2 and IFN-γ levels in the duodenum and jejunum, along with elevated IL-10, IL-17, and TNF-α levels in the duodenum of force-fed ducks (Liu et al., 2018). However, the efficacy of AST supplementation in mitigating the inflammatory response was evident through its enhanced effect on expression of anti-inflammatory cytokine and inhibition of pro-inflammatory cytokine expression in the intestinal mucosa. The anti-inflammatory effects of AST are well-documented, including its ability to protect immune cells and inhibit inflammatory responses (Chen et al., 2017). Recent studies have also highlighted AST's efficacy in alleviating intestinal inflammatory diseases, further supporting its role in reducing inflammation in the gut (Chang and Xiong, 2020). These findings accentuate the potential role of AST as a dietary intervention to restore intestinal immune balance and protect mucosal health.

The anti-inflammatory effects of dietary AST during oxidative stress have been demonstrated in various studies. For instance, AST alleviated acute intestinal injury in mice by reducing pro-inflammatory factors, thereby improving structure and function of the intestinal mucosa (Yang et al., 2019). In rats, dietary AST reduced TNF-α and IL-1β expression (Song et al., 2022), while in laying hens, it decreased TNF-α expression (Gao et al., 2020). Dietary AST also modulated cytokine expression in fish fed a high-fat oxidized diet (Xie et al., 2020). In ducks under normal conditions, AST increased IL-6 expression but had no effect on TNF-α and IFN-γ levels (Ao and Kim, 2019). However, in broilers challenged with lipopolysaccharide (LPS), AST showed no anti-inflammatory effect at an early growth stage (Takahashi et al., 2011). Overall, AST demonstrates significant anti-inflammatory and antioxidant effects across various species, offering potential benefits for intestinal health and immune function. While its impact on inflammatory cytokine expression is well-documented in animals like mice, rats, and hens, further research is needed to fully understand its effects in overfed Pekin ducks. The antioxidant and anti-inflammatory effects of AST may reduce the apoptotic cell rate in intestinal mucosal cells.

Apoptosis, an essential physiological process for cellular homeostasis, can be detrimental when excessive. Overfeeding-induced oxidative stress increases apoptosis, as evidenced by higher apoptotic cell levels in control groups compared to the ALG, suggesting the inability of intestinal cells to cope with increased metabolic demand during overfeeding. In support of our findings, the study by Rémignon et al. (2018); Remifnon and Burgues (2023), demonstrated that force-feeding induced increased apoptosis in the hepatocytes of ducks, highlighting the detrimental effects of overfeeding on cellular integrity. Nevertheless, AST mitigated this effect by inhibiting apoptosis in the intestinal mucosal cells. Astaxanthin has also been shown to repress apoptosis in hepatoma cells of mice (Shao et al., 2016). The anti-apoptotic effect of AST on intestinal mucosal cells is critical for maintaining intestinal integrity, which is essential for nutrient absorption and utilization in animals.

The intestinal tract, which is responsible for nutrient digestion and absorption, is influenced by gut morphometrics such as villus height (VH), crypt depth (CD), and the VH/CD ratio. These morphometrics are used as markers to assess intestinal function and health (Tian et al., 2022). The intestinal architecture is closely linked to nutrient absorption capacity in an organism. A higher villus height indicates mature, functional villi that support efficient nutrient absorption, while a reduced villus height limits the surface area for absorption, limiting nutrient uptake. Crypt depth reflects turnover of epithelial cells. Shallower crypts do not support or maintain intestinal integrity. The VH/CD ratio provides a comprehensive evaluation of digestion function and absorption capacity (Abdel-Kafy et al., 2022; He et al. 2016;). The gastrointestinal tract is highly susceptible to oxidative stress and exposure to overfeeding in Pekin ducks resulted in alterations in the villi function, compromising the gut morphology. Similar disruptions in villous morphology were observed in force-fed ducks (Liu et al., 2018). Whereas, Wei et al. (2021) reported no significant impairment in villus morphology of geese exposed to overfeeding. Additionally, the feeding regime reduced intestinal wall thickness, suggesting increased intestinal permeability, which could contribute to oxidative stress. In contrast, AST supplementation mitigated these detrimental effects, preserving gut integrity and improving villi morphometrics. This improvement in gut structure likely enhanced nutrient absorption and contributed to better growth performance and weight gain. These findings suggest that AST may play a pivotal role in maintaining intestinal health, thereby supporting overall growth and metabolic efficiency.

Taken together, these findings highlight the significant role of AST's antioxidant and anti-inflammatory properties in enhancing intestinal health and growth performance in overfed Pekin ducks. Astaxanthin improved oxidative balance, modulated immune responses and reduced apoptosis, which collectively supported intestinal integrity and function. Antioxidants in the digestive tract play a crucial role in protecting the intestinal mucosa, preventing epithelial cell damage from oxidative stress, and promoting the development and structure of villi, ultimately enhancing nutrient absorption (Lin et al., 2016; Qiu et al., 2020). Moreover, a positive correlation between immune status and antioxidant capacity, as noted by Zhu et al. (2019), reinforces the potential of AST to boost overall health by enhancing immune regulation. These mechanisms, driven by AST's natural antioxidant properties, are likely responsible for its positive effects on growth performance and gut health. Our findings suggest that AST has substantial potential as a feed additive in Pekin duck nutrition. Future research should delve into the genetic and metabolic pathways underlying AST's mechanisms, particularly its interactions at the microbiome and metabolome levels, to optimize its practical applications in animal production.

5. Conclusion

Overfeeding, as a nutritional regimen, impaired gut health via increased oxidative stress, higher pro-inflammatory cytokine levels, elevated apoptosis, and disrupted villous morphology. These adverse effects compromised nutrient metabolism and contributed to poor growth performance. However, the study highlights the beneficial effects of AST at an inclusion level of 80 mg/kg, which significantly improved growth performance and intestinal health, reversing the detrimental impacts of overfeeding in Pekin ducks. The beneficial effects of AST can be attributed to its antioxidant, anti-inflammatory, and anti-apoptotic properties. These functions contributed to improved villus morphology and intestinal integrity, facilitating more efficient nutrient absorption and utilization, which ultimately resulted in increased weight gain and enhanced growth performance. The research study highlights the potential of astaxanthin as a therapeutic agent to mitigate oxidative stress and promote the growth and health of animals in stressful conditions.

Credit Author Statements

Xueze Lv: Methodology, Investigation. **Uchechukwu Edna Obianwuna:** Writing – original draft. **Weifang Yang:** Writing – review & editing. **Ziyue Zhang:** Supervision, Data curation. **Keying An:** Supervision, Data curation. **Bozhi Shi:** Writing – review & editing, Data curation. **Yingchao Dong:** Writing – review & editing. **Shugeng Wu:** Supervision, Funding acquisition. **Zhaofei Xia:** Supervision, Funding acquisition.

Declaration of competing interest

We declare that we have no financial or personal relationships with other people or organizations that might inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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