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Tea Polyphenol Nanoparticles Promote Intestinal Development and Barrier Integrity in Pullets by Activating the PI3K-AKT Signaling Pathway

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Abstract

The rearing period is critical for intestinal maturation in laying hens, yet antibiotic restrictions pose significant challenges to gut health. A total of 180 one-day-old female chicks were randomly allocated to three groups: control (NC, regular drinking water), free tea polyphenols (TP, 200 mg/L), and sodium alginate-coated iron-based tea polyphenol nanoparticles (NaTP, 200 mg/L). The NaTP group significantly increased the retention of major catechins (EGCG, ECG, EGC, and EC) across the digestive tract. NaTP increased body weight at day 56 and reduced the feed-to-gain ratio compared with TP. No significant differences in growth performance were detected between NaTP and the control. It also enhanced relative organ weights and lengths of the duodenum and jejunum, and promoted intestinal morphology. NaTP strengthened systemic and jejunal mucosal antioxidant capacity by reducing MDA levels and elevating SOD, GSH-Px, and T-AOC activities. Transcriptome and Western blot analyses revealed that NaTP activated the PI3K-Akt signaling pathway, contributing to these protective effects. This is the first report of an iron-based metal–polyphenol network nano-system designed for poultry drinking water applications. These findings demonstrate that NaTP effectively overcomes the limitations of conventional tea polyphenols, providing a novel green strategy to support intestinal health and antibiotic-free poultry production.

Keywords: tea polyphenol; nanoparticle; breast; antioxidant

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1. Introduction

The brooding and rearing period represents a critical developmental window for intestinal maturation in laying hens [1]. During early post-hatch development, the gastrointestinal tract undergoes rapid maturation, enhancing intestinal structure, digestive function, and mucosal immunity. Adequate intestinal development may help meet the nutritional and metabolic demands of sexual maturation and egg production [2]. Therefore, early intestinal health may contribute to the long-term physiological fitness

and productive potential of laying hens. An intact intestinal barrier encompassing physical, chemical, immunological, and microbial components maintains epithelial integrity, immune homeostasis, and feed utilization efficiency, ultimately determining body weight gain, feed conversion ratio, and egg quality [3]. However, with the full implementation of comprehensive antibiotic bans and restrictions, pre-laying rearing of laying hens is confronted with prominent challenges, including oxidative stress, tight junction disruption, and villus atrophy [4]. These insults result in increased intestinal permeability, growth retardation, reduced feed efficiency, and heightened pathogen susceptibility [5]. Studies have shown that, in relevant stress models during the brooding and rearing period of laying hens, such damage further exacerbates intestinal inflammation and systemic immune dysregulation, thereby severely constraining the sustainable development of the poultry industry [6]. Consequently, maintaining intestinal health is not only central to the growth and development of chicks during the brooding and rearing phase but also the key to achieving antibiotic-free poultry production [7]. Developing safe, efficient, and environmentally sustainable natural intervention strategies to protect intestinal health has thus become an urgent challenge that must be addressed within the industry.

Tea polyphenols (TPs), as natural bioactive compounds, have attracted increasing attention as potential alternatives to antibiotics for improving intestinal health in poultry [8]. Tea polyphenols, primarily comprising epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG), exhibit antioxidant properties [9]. Studies in poultry have suggested that tea-derived polyphenols may improve antioxidant status and intestinal health, although their effectiveness varies with supplementation levels, experimental conditions, and the physiological status of birds [10]. In addition, evidence from other animal models and in vitro studies has demonstrated that tea polyphenols can regulate antioxidant defenses, improve intestinal epithelial integrity, and alleviate oxidative stress [11]. For instance, studies in broiler chickens have reported that dietary supplementation with green tea extracts can alleviate intestinal damage and modulate inflammatory responses under experimental disease challenges [12]. However, these findings may not be directly applicable to healthy pullets during early rearing, as differences in age, physiological status, and experimental conditions may influence the biological responses to tea polyphenols. Moreover, conventional tea polyphenols remain susceptible to oxidative degradation under varying environmental conditions, including light, oxygen, and pH fluctuations [13]. Their relatively low bioavailability and rapid gastrointestinal metabolism may further limit their intestinal retention and biological efficacy [14]. Consequently, the protective effects of free tea polyphenols in poultry may vary across experimental conditions, highlighting the need for improved delivery strategies to enhance their stability and intestinal availability [15].

To address the critical bottlenecks of free tea polyphenols in terms of chemical stability and intestinal targeted delivery, nano-carrier technology has emerged as a cutting-edge strategy for overcoming their application limitations [16]. Among various metal ions, Fe^{3+} is particularly attractive for constructing metal–polyphenol networks (MPNs) because of its ability to coordinate with the phenolic groups of tea polyphenols. Coordination of tea polyphenols with metal ions to form nanoparticles has been explored as a strategy to improve their chemical stability, antioxidant properties, and delivery efficiency in different experimental systems [17]. Existing studies have shown that carriers such as liposomes, polymeric nanoparticles, nanoemulsions, metal–organic frameworks, and mesoporous silica effectively shield tea polyphenols from gastric acid, oxidative degradation, and enzymatic breakdown, achieving encapsulation efficiencies of over 80–90% and significantly prolonging intestinal retention time [18]. For instance, in oxidative

stress models, nano-formulated tea polyphenols elicit pronounced elevations in antioxidant enzyme activities and reductions in MDA levels even at extremely low concentrations, demonstrating superior protective efficacy compared with their free counterparts [19]. Sodium alginate was selected for its hydrophilicity, film-forming ability, and potential to protect bioactive compounds during gastrointestinal transit. It may enhance nanoparticle dispersibility and protect the iron–polyphenol core. Furthermore, alginate-based systems have been investigated for their potential to regulate polyphenol release and gastrointestinal availability [20]. Nevertheless, much of the existing evidence regarding nano-formulated tea polyphenols has been obtained from in vitro investigations, non-poultry models, or food preservation applications [21]. Consequently, evidence supporting the effectiveness of these delivery systems in livestock and poultry, particularly through drinking water supplementation, remains limited [22]. In particular, there is a notable lack of iron-based metal–polyphenol network (MPN) self-assembly systems that serve as the core architecture while simultaneously delivering comprehensive improvements in animal intestinal health [23]. Therefore, the development of an iron-based tea polyphenol nanoparticle formulation suitable for drinking water addition represents a key research direction [24]. Such a system would not only resolve the practical application challenges of conventional tea polyphenols but also provide an efficient and targeted intervention strategy for safeguarding intestinal health in chicks during the brooding and rearing period.

The present study aimed to develop an iron-based tea polyphenol nanoparticle formulation coated with sodium alginate and evaluate its effects on intestinal health in pullets during the early rearing period. Specifically, the formulation was administered through drinking water at a concentration of 200 mg/L, and its effects on catechin retention, intestinal morphology, antioxidant capacity, and related signaling pathways were systematically investigated. By comparing the nanoparticle formulation with free tea polyphenols at the same nominal supplementation concentration, this study sought to assess the potential advantages of the iron-based MPN delivery system for improving intestinal health. These findings may provide a basis for the further development of tea polyphenol-based drinking water additives for poultry production.

2. Materials and Methods

2.1. Animals and Experimental Design

A total of 180 one-day-old healthy female Wannan yellow chicks were obtained from Anhui Agricultural Reclamation Group Company Limited. Before the experiment, the chicks were examined for general health status and randomly allocated to three treatment groups, with six replicates per treatment and 10 chicks per replicate. The treatment groups were as follows: control group (NC), supplied with regular drinking water; free tea polyphenol group (TP), supplied with drinking water supplemented with 200 mg/L free tea polyphenols; and sodium alginate-coated iron-based tea polyphenol nanoparticle formulation group (NaTP), supplied with drinking water supplemented with 200 mg/L of the nanoparticle formulation. The tea polyphenols used in the experiment (purity 30%) were provided by Jiangsu Aomai Bio-Technology Co., Ltd. (Nanjing, China). The sodium alginate-coated iron-based tea polyphenol nanoparticle formulation was synthesized by the National Center for Nanoscience and Technology, Chinese Academy of Sciences. Chicks were housed in a well-ventilated poultry facility with a temperature maintained at 32–34 °C during the first week, gradually reduced by 2–3 °C per week until reaching 22–24 °C. Relative humidity was maintained at 50–65%, and adequate ventilation was provided throughout the experiment to ensure air quality. A lighting program of 23 h light and 1 h darkness was applied during the first week, followed by 16 h light and 8 h darkness for the remainder of the trial. The experimental period lasted 8 weeks.

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Throughout the trial, all chicks had access to feed and drinking water, the composition and nutrient levels of the basal diet are presented in Table S1. On day 56, one chick of average body weight was randomly selected from each replicate for slaughter and sample collection. Serum samples were stored at −20 °C, and intestinal epithelial mucosa and intestinal digesta samples were immediately snap-frozen in liquid nitrogen after collection and subsequently stored at −80 °C until further analysis.

2.2. Preparation of Iron-Based Tea Polyphenol Nanoparticle Formulation

Tea polyphenols (primarily composed of EGCG, ECG, EGC, EC, etc.) were dissolved in deionized water to prepare a 2–5 mg/mL solution. An aqueous solution of $FeCl_3 \cdot 6H_2O$ ($FeCl_3$ mass corresponding to 1–4% of the tea polyphenol mass) was added slowly. The mixture was reacted for 1 h under magnetic stirring at 600 rpm in a 30–60 °C water bath to form the iron–tea polyphenol coordination core. The pH of the reaction system was adjusted to 8.0–10.0 with 0.5 mol/L NaOH solution, and stirring was continued for 12 h to promote complete self-assembly of the MPN. Subsequently, a sodium alginate solution (sodium alginate mass corresponding to 5–10% of the tea polyphenol mass) was added, and the mixture was stirred for an additional 2 h to enable coating. The nanoparticles were collected by centrifugation (8000 rpm, 10 min) and washed three times to remove unreacted ions and impurities. Finally, the product was freeze-dried to yield a light gray powdery iron-based tea polyphenol nanoparticle formulation. The theoretical tea polyphenol content in the initial formulation was approximately 87.7–94.3% of the total solid ingredients. Tea polyphenols were supplied by Jiangsu Aomai Biotechnology Co., Ltd. (Nanjing, China), and the preparation of NaTP was assisted by the National Center for Nanoscience and Technology, Chinese Academy of Sciences (Beijing, China). The experimentally measured contents of individual catechins in the tea polyphenol material are presented in Table S2.

2.3. Nanoparticle Characterization

A 1 mg/mL aqueous dispersion of the NaTP formulation was prepared. Zeta potential was measured using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., Malvern, UK). The size and morphology of the NaTP particles were characterized by Tecnai G2 20 S-TWIN (FEI Company, Eindhoven, Netherland) and scanning electron microscopy (SEM; SU8020, Hitachi High-Technologies Corporation, Tokyo, Japan). Two hundred randomly selected NaTP particles were analyzed, and the average particle size was calculated using Image J software (Version 1.54u). Functional groups of the NaTP were identified by Fourier-transform infrared spectroscopy (PerkinElmer, Inc., Waltham, MA, USA).

2.4. Growth Performance Measurement

The initial and final body weights of the chicks in each treatment were recorded. Average daily gain (ADG, g/day) was calculated as the difference between the final body weight on day 56 and the initial body weight divided by the number of experimental days. Feed consumption was recorded weekly throughout the experimental period, and the average daily feed intake (ADFI, g/day) for the entire period was calculated. The feed-to-gain ratio (F/G) was determined by dividing ADFI by ADG.

2.5. Organ Index Measurement

After slaughter, the heart, liver, spleen, pancreas, bursa of Fabricius, duodenum, jejunum, and ileum were collected and weighed. The relative organ weight (g/kg BW) of each organ was calculated based on the live body weight of the chicks. The lengths of the duodenum, jejunum, and ileum were accurately measured, and the relative length (cm/kg BW) was calculated according to the live body weight of the chicks.

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2.6. Catechin Content in the Digestive Tract Measurement

Digesta samples were collected from the crop, proventriculus, gizzard, duodenum, jejunum, ileum, and cecum of chicks in the TP and NaTP groups, along with fresh excreta collected within 10 min of excretion. Briefly, 0.5 g of each sample was mixed with 5 mL of 70% methanol, vortexed, and ultrasonically extracted for 30 min. The extracts were centrifuged at 12,000× *g* for 10 min at 4 °C, and the supernatants were filtered through a 0.22 μm membrane before HPLC-MS analysis. Chromatographic separation was performed using an Agilent Pursuit XRs C18 reversed-phase column (4.6 × 150 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA) on an Agilent 1260 Infinity II LC system (Agilent Technologies, Santa Clara, CA, USA). The mobile phases consisted of 0.1% formic acid in water (A) and acetonitrile (B), with gradient elution. Mass spectrometric detection was performed using an InfinityLab LC/MSD XT mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray ionization source operating in negative mode (ESI^-). Selected ion monitoring (SIM) was used to monitor the characteristic ions at *m*/*z* 457, 441, 305, and 289 for EGCG, ECG, EGC, and EC, respectively. Catechins (EGCG, ECG, EGC, and EC) were identified based on the retention times and characteristic ions of reference standards and quantified using the external standard method. The sources of catechin standards and corresponding calibration curves are presented in Table S3.

2.7. Antioxidant Capacity

The malondialdehyde (MDA) content and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), as well as total antioxidant capacity (T-AOC) in serum and jejunal epithelial mucosa were determined strictly according to the manufacturer's instructions of the commercial assay kits from Nanjing Jiancheng Bio. Ins. (MDA: A003-1-2; SOD: A001-2-2; GSH-Px: A005-1-2; CAT: A007-1-1; T-AOC: A015-2-1). For the jejunal mucosal samples, the final values of the relevant indices were normalized to total protein concentration using a bicinchoninic acid (BCA) protein assay kit (A045-4-2).

2.8. Intestinal Morphometric Parameters

Jejunal tissue samples fixed in 4% paraformaldehyde were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Villus height (VH) and crypt depth (CD) were measured under a light microscope on the cross-sections of jejunal slices, and the villus-to-crypt ratio (VH/CD) was subsequently calculated. In each cross-section, goblet cells on 10 adjacent villi were identified and counted using Image-Pro Plus software (Version 6.0).

2.9. RT-qPCR Analysis

Jejunal tissue samples collected on day 56 were homogenized in pre-chilled trizol reagent at a weight-to-volume ratio (*w*/*v*) of 1:10. Total RNA was extracted following homogenization and centrifugation. The concentration and quality of the extracted RNA were determined using a nucleic acid analyzer. RNA concentrations were then normalized by the addition of trizol prior to reverse transcription. Reagents were prepared according to the manufacturer's instructions of the fluorescent quantitative PCR kit, and the cycle threshold (Ct) values for each gene were measured on a real-time quantitative PCR instrument. The primer sequences of the genes used in the experiment are listed in Table S4. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with β-actin serving as the internal reference gene.

2.10. Western Blot Analysis

An appropriate amount of sample was homogenized in RIPA lysis buffer at a 1:10 (mass:volume) ratio. After homogenization, the lysates were centrifuged at 4 °C for 10 min, and the supernatant was collected. Protein concentration was determined using a BCA assay kit and normalized to 5 μg/μL with additional RIPA lysis buffer, and the samples from 2 chickens were pooled into 1 replicate ($n = 3$). The normalized supernatant was mixed with 5× loading buffer at a 4:1 ratio and denatured in a boiling water bath. SDS-PAGE gels were prepared according to the manufacturer's instructions. 10 μL of protein sample was loaded per well and electrophoresed at a constant voltage of 80 V. After electrophoresis, proteins were transferred onto PVDF membranes using transfer buffer containing an appropriate methanol concentration at a constant current of 200 mA. The membranes were then blocked with 5% skim milk for 2 h, washed thoroughly with 1× TBST, incubated with primary antibody overnight at 4 °C (18 h), and then with secondary antibody for 2 h. The suppliers and dilution ratios of the antibodies are presented in Table S5. Protein bands were visualized using an ultra-sensitive chemiluminescence imaging system. Gray values were quantified with Image J software (Version 1.54u), and relative protein expression levels were calculated using Tubulin as the internal reference.

2.11. Transcriptome Sequencing

Library preparation and sequencing. Total RNA was extracted from jejunal mucosal samples using TRIzol reagent. RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). After RNA extraction, mRNA was enriched using oligo(dT) magnetic beads and fragmented into short fragments. The fragmented mRNA was reverse-transcribed into cDNA, followed by end repair, A-tailing, adapter ligation, and PCR amplification. The resulting libraries were purified using AMPure XP beads and subjected to quality assessment before sequencing on an Illumina NovaSeq 6000 platform.

Transcriptomic data processing. Raw sequencing reads were filtered using fastp (v0.18.0) to remove adapter sequences and low-quality reads. Clean reads were aligned to the chicken reference genome using HISAT2 2.2.2, and transcripts were assembled using StringTie (v1.3.1). Gene expression levels were quantified using RSEM v1.3.3 and expressed as FPKM values. Differential expression analysis was performed using DESeq2 1.52.0, with adjusted $p < 0.05$ and $|\log_2 FC| > 1$ as the thresholds for identifying differentially expressed genes (DEGs). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed to investigate the biological functions and pathways associated with DEGs.

2.12. Statistical Analysis

The experimental unit was the replicate ($n = 6$ per treatment). For individual measurements, one representative chick was sampled from each replicate. Western blot analysis included three pooled biological replicates per treatment ($n = 3$). Data were analyzed using one-way ANOVA in SPSS 26.0 after assessing normality and homogeneity of variance using the Shapiro–Wilk and Levene's tests, respectively. Differences among treatments were evaluated using Duncan's multiple range test. Data are presented as mean ± standard error (SE), and statistical significance was set at $p < 0.05$.

3. Results

3.1. Characterization of Iron-Based Tea Polyphenol Nanoparticles

As shown in Figure 1, NaTP appeared as a light gray powder and formed a visually dispersed suspension in water (Figure 1A). FTIR analysis revealed characteristic functional groups associated with tea polyphenols and sodium alginate (Figure 1B). SEM and TEM observations showed a relatively uniform granular morphology (Figure 1C,D). ImageJ analysis indicated that most particles were distributed within the 100–200 nm range (Figure 1E). Zeta potential measurements indicated a negatively charged particle surface (Figure 1F).

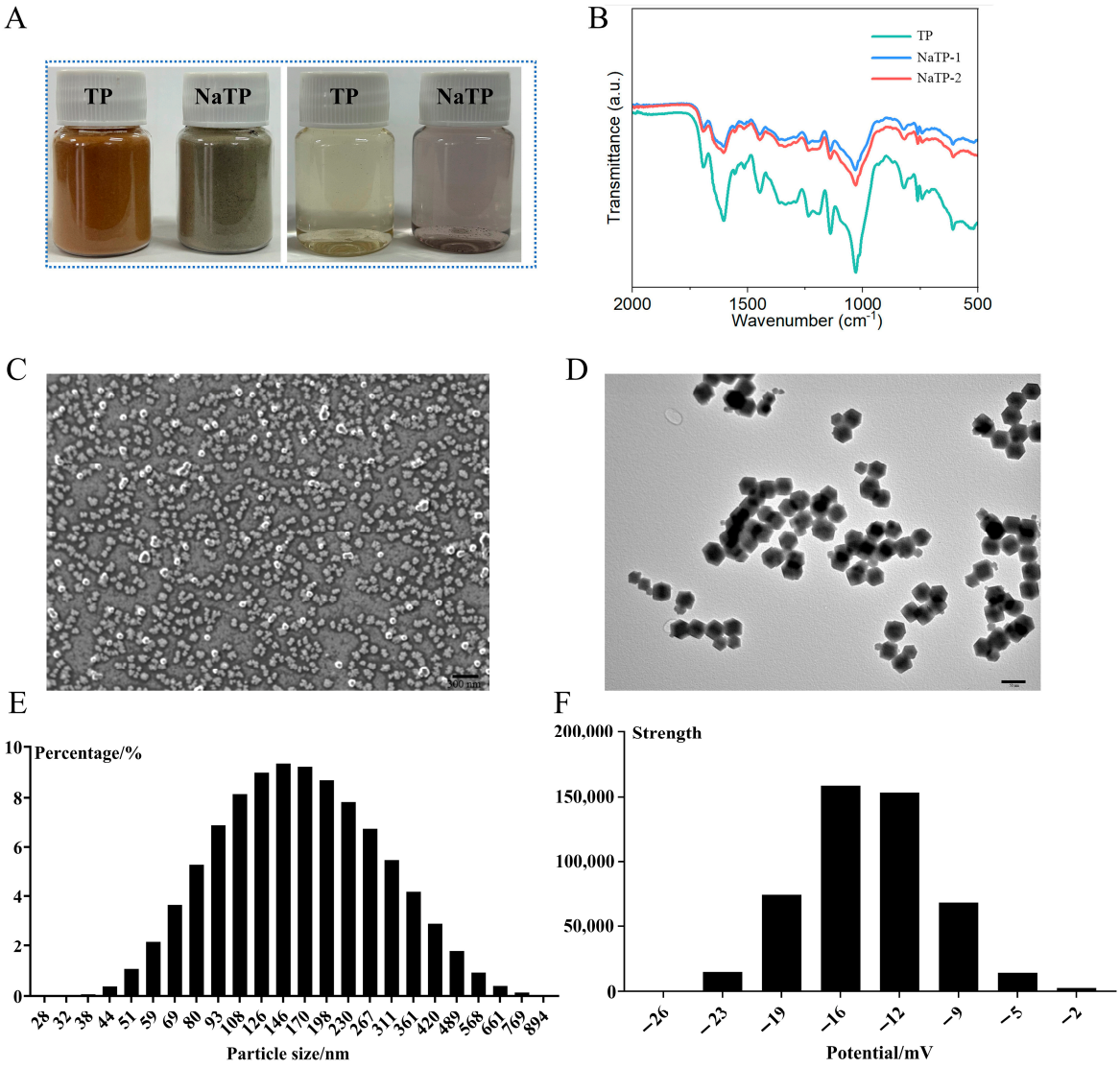


Figure 1. Characterization of TP and NaTP. (**A**) Differences in solid-state appearance and aqueous solubility between TP and NaTP. (**B**) Fourier-transform infrared (FTIR) absorption spectra of TP and NaTP. (**C**) Scanning electron microscopy (SEM) morphology of TP and NaTP. (**D**) Transmission electron microscopy (TEM) morphology of TP and NaTP. (**E**) Particle size distribution of TP and NaTP. (**F**) Zeta potential characteristics of TP and NaTP.

3.2. Catechin Content in the Digestive Tract

As shown in Figure 2, compared with the TP group, the NaTP group significantly increased EGCG content in the proventriculus, gizzard, duodenum, jejunum, ileum, cecum, and feces (Figure 2A). Likewise, ECG content was significantly elevated in the proventriculus, gizzard, duodenum, jejunum, ileum, and cecum (Figure 2B); EGC content was significantly increased in the proventriculus, gizzard, duodenum, jejunum, and ileum (Figure 2C); and EC content was significantly higher in the proventriculus, gizzard, duodenum, and jejunum (Figure 2D).

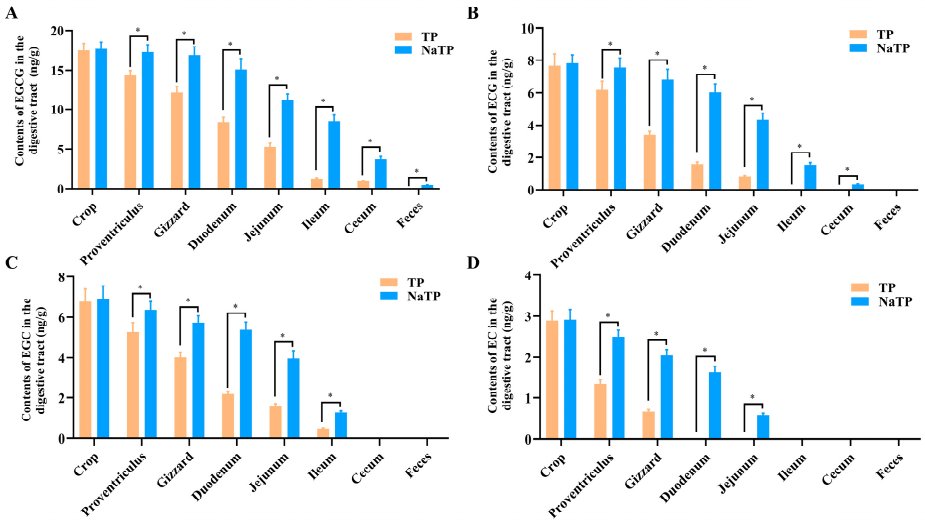


Figure 2. Distribution of catechin contents in the digestive tract and intestine. (**A**) Contents of EGCG in the digestive tract and intestine. (**B**) Contents of ECG in the digestive tract and intestine. (**C**) Contents of EGC in the digestive tract and intestine. (**D**) Contents of EC in the digestive tract and intestine. TP: 200 mg/L free tea polyphenols in drinking water; NaTP: 200 mg/L sodium alginate-coated iron-based tea polyphenol nanoparticles in drinking water. Data are presented as Mean ± SE, $n = 6$. The * indicate significant differences ($p < 0.05$).

3.3. Growth Performance

As shown in Figure 3, there were no significant differences in growth performance between the NaTP group and the NC group (Figure 3A–D). Compared with the TP group, the NaTP group significantly increased the body weight on day 56 (Figure 3A) and decreased the feed-to-gain ratio over the entire experimental period (Figure 3D).

3.4. Organ Index

As shown in Table 1, NaTP treatment significantly increased the relative weights of the pancreas, liver, duodenum, and jejunum compared with the TP and NC groups ($p < 0.05$), whereas the relative liver weight showed no significant difference among treatments ($p = 0.052$). In addition, the relative lengths of the duodenum and jejunum were significantly increased in the NaTP group. Increased relative organ weight does not necessarily indicate improved organ function, as it may reflect changes in organ development or physiological adaptation. Further histological and functional assessments are needed to clarify these changes.

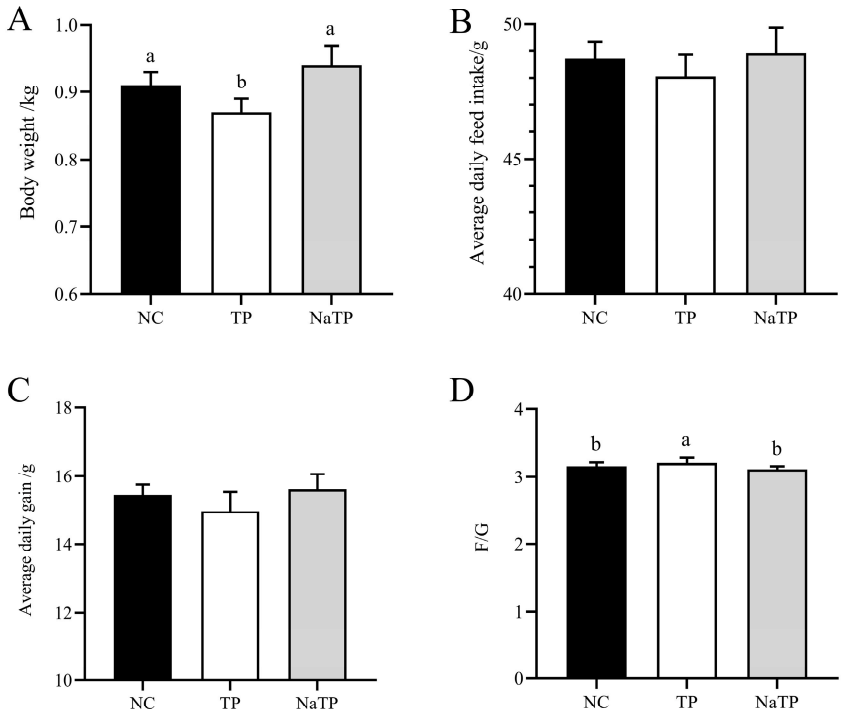


Figure 3. Effects of drinking water supplementation with TP and NaTP on growth performance. (**A**) Body weight. (**B**) Average daily feed intake. (**C**) Average daily gain. (**D**) Feed-to-gain ratio (F/G). NC: control group, regular drinking water; TP: 200 mg/L free tea polyphenols in drinking water; NaTP: 200 mg/L sodium alginate-coated iron-based tea polyphenol nanoparticles in drinking water. Data are presented as Mean ± SE, $n = 6$. Different superscript letters (a, b) indicate significant differences ($p < 0.05$).

Table 1. Effects of TP and NaTP added to drinking water on organ indices in different groups.

Index		Groups			*p*-Value
		NC	TP	NaTP	
Relative weight g/kg BW	Heart	4.63 ± 0.33	4.67 ± 0.40	4.84 ± 0.46	0.556
	Liver	24.15 ± 1.46 ^{ab}	23.01 ± 1.34 ^{b}	25.82 ± 1.12 ^{a}	0.052
	Spleen	2.45 ± 0.11	2.49 ± 0.25	2.31 ± 0.12	0.448
	Pancreas	4.56 ± 0.15 ^{b}	4.52 ± 0.29 ^{b}	4.89 ± 0.41 ^{a}	0.049
	Bursa of fabricius	5.42 ± 0.39	5.39 ± 0.29	5.34 ± 0.30	0.539
	Duodenum	8.78 ± 0.56 ^{b}	8.42 ± 0.39 ^{b}	9.68 ± 0.33 ^{a}	0.033
	Jejugum	16.78 ± 1.20 ^{b}	18.86 ± 0.98 ^{a}	18.50 ± 0.61 ^{a}	0.035
	Ileum	12.47 ± 0.94	13.35 ± 1.02	13.56 ± 0.96	0.741
Relative length cm/kg BW	Duodenum	21.89 ± 0.95 ^{b}	21.85 ± 1.31 ^{b}	23.86 ± 1.38 ^{a}	0.042
	Jejugum	47.29 ± 2.69 ^{b}	51.64 ± 2.00 ^{a}	52.44 ± 3.42 ^{a}	0.039
	Ileum	45.25 ± 2.15	45.97 ± 3.82	46.13 ± 2.89	0.684

NC: control group, regular drinking water; TP: 200 mg/L free tea polyphenols in drinking water; NaTP: 200 mg/L sodium alginate-coated iron-based tea polyphenol nanoparticles in drinking water. Data are presented as Mean ± SE, $n = 6$. Different superscript letters (a, b) indicate significant differences ($p < 0.05$).

3.5. Small Intestinal Morphometric Parameters

As shown in Figure 4, based on H&E staining (Figure 4A), the NaTP group exhibited significantly greater villus height in both the duodenum and jejunum compared with the

NC group (Figure 4B). In addition, the VH/CD index in the jejunum was markedly increased in the NaTP group (Figure 4D). Furthermore, the NaTP group significantly elevated the number of goblet cells per villus in the jejunum and ileum relative to the NC group, whereas the TP group only increased goblet cell numbers in the jejunum (Figure 4E). These results indicate that tea polyphenols and their nanoparticle formulation primarily exert their effects on the jejunum.

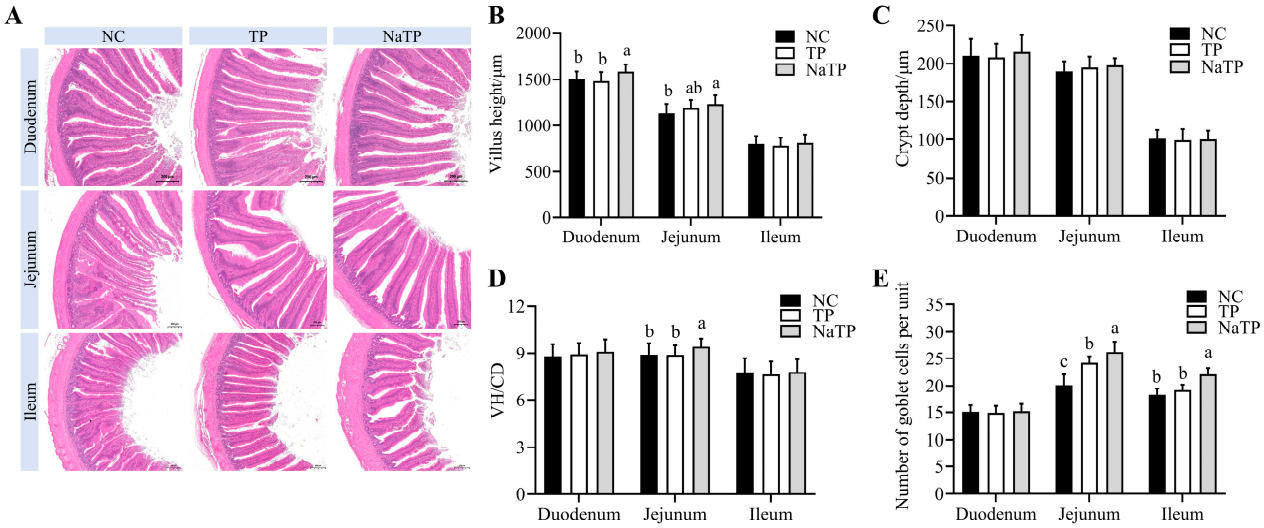


Figure 4. Effects of drinking water supplementation with TP and NaTP on small intestinal structure and morphology. (**A**) Hematoxylin and eosin (H&E) staining of the small intestine, the scale bar is 200μm. (**B**) Villus height of the small intestine. (**C**) Crypt depth of the small intestine. (**D**) Villus-to-crypt ratio of the small intestine. (**E**) Number of goblet cells per unit in the small intestine. NC: control group, regular drinking water; TP: 200 mg/L free tea polyphenols in drinking water; NaTP: 200 mg/L sodium alginate-coated iron-based tea polyphenol nanoparticles in drinking water. Data are presented as Mean ± SE, $n = 6$. Different superscript letters (a–c) indicate significant differences ($p < 0.05$).

3.6. Serum and Jejunal Mucosal Antioxidant Capacity

As shown in Figure 5, both TP and NaTP significantly reduced serum MDA levels and increased SOD activity compared with NC (Figure 5A,B). NaTP also significantly increased serum GSH-Px activity and T-AOC relative to NC (Figure 5C,E). Notably, serum MDA levels were significantly lower in NaTP than in TP, whereas no significant differences were observed between TP and NaTP in serum SOD, GSH-Px, or T-AOC. In jejunal mucosa, both treatments significantly decreased MDA levels and increased SOD activity (Figure 5F,G), whereas NaTP significantly enhanced GSH-Px activity and T-AOC compared with NC (Figure 5H,J). However, only jejunal T-AOC was significantly higher in NaTP than in TP, while the other jejunal antioxidant parameters did not differ significantly between the two treatments. These findings indicate that both formulations improved antioxidant status, while the effects of NaTP varied among individual antioxidant parameters.

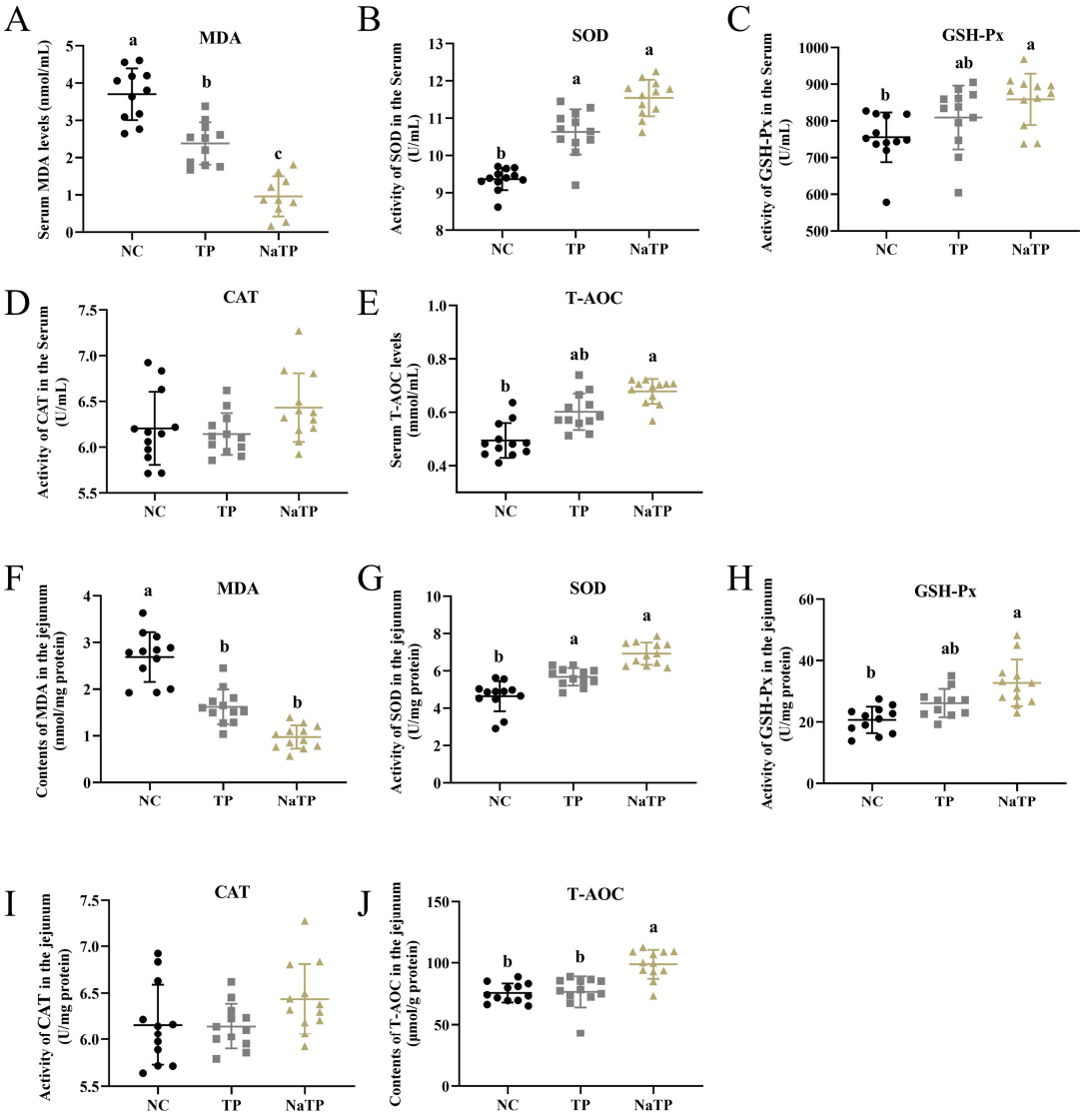


Figure 5. Effects of drinking water supplementation with TP and NaTP on antioxidant capacity in the body. (**A**–**E**) MDA content, T-AOC level, and activities of SOD, GSH-Px, and CAT in serum. (**F**–**J**) MDA content, T-AOC level, and activities of SOD, GSH-Px, and CAT in jejunal mucosa. NC: control group, regular drinking water; TP: 200 mg/L free tea polyphenols in drinking water; NaTP: 200 mg/L sodium alginate-coated iron-based tea polyphenol nanoparticles in drinking water. Data are presented as Mean ± SE, $n = 6$. Different superscript letters (a–c) indicate significant differences ($p < 0.05$).

3.7. Jejunal Mucosa Transcriptome Analysis

Figure 6 illustrates the transcriptome differences among the NC, TP, and NaTP groups. The Venn diagram (Figure 6A) shows that a total of 12,554 genes were detected. Among them, 163 genes were unique to the NC group, 109 to the TP group, and 133 to the NaTP group. The numbers of overlapping genes between each pair of groups were 81, 133, and 154, respectively. Principal component analysis (PCA) (Figure 6B) revealed clear separation of the three groups along the first two principal components (PC1: 73.79%, PC2: 15.78%). Samples from the NC and TP groups formed relatively tight clusters, whereas the NaTP samples exhibited greater dispersion. Differential expression analysis identified a large number of significantly regulated genes (Figure 6C,D). Compared with the NC group, the TP group had 244 upregulated and 246 downregulated genes, while the NaTP

group had 511 upregulated and 318 downregulated genes. Direct comparison between the TP and NaTP groups showed 332 upregulated and 418 downregulated genes in the TP group (adjusted $p < 0.05$, $|\log_2 FC| > 1$). Gene Ontology (GO) enrichment analysis highlighted distinct biological processes and molecular functions associated with the differentially expressed genes across the groups (Figure 6E–G). In the NC vs. TP comparison (Figure 6E), significantly enriched GO terms included RNA-DNA hybrid ribonuclease activity, extracellular region, immune response, and hydrolase activity. In the NC vs. NaTP comparison (Figure 6F), the most highly enriched GO terms primarily included immunoglobulin complex, extracellular region, receptor ligand activity, signal receptor activator activity, as well as various transporter and endopeptidase inhibitor activities. In the TP vs. NaTP comparison (Figure 6G), significantly enriched terms included immunoglobulin complex, RNA–DNA hybrid ribonuclease activity, antigen binding, extracellular region, and immune-related processes such as MHC protein complex and antigen processing/presentation.



Figure 6. Transcriptomic analysis of jejunal mucosa. (**A**) Venn diagram. (**B**) Principal component analysis (PCA). (**C**) Volcano plot. (**D**) Upset plot. (**E–G**) Gene Ontology (GO) enrichment analysis of differentially expressed genes. NC: control group, regular drinking water; TP: 200 mg/L free tea polyphenols in drinking water; NaTP: 200 mg/L sodium alginate-coated iron-based tea polyphenol nanoparticles in drinking water.

3.8. PI3K-Akt Signaling in Jejunal Mucosa

As shown in Figure 7, based on the transcriptome data, the Gene Set Enrichment Analysis (GSEA) revealed significant enrichment of multiple biological pathways across the comparisons (Figure 7A–C). In NC vs. TP (Figure 7A), prominent enriched pathways

included steroid biosynthesis, focal adhesion, ECM–receptor interaction, adherens junction, and PI3K-Akt signaling pathway. In NC vs. NaTP (Figure 7B), the top enriched gene sets were associated with growth hormone synthesis/secretion/action, PI3K-Akt signaling pathway, graft-versus-host disease, and non-small cell lung cancer. The direct comparison between TP and NaTP (Figure 7C) highlighted enrichment in ribosome, ribosome biogenesis, PI3K-Akt signaling pathway, graft-versus-host disease, and viral carcinogenesis. Detailed enrichment plots confirmed consistent positive enrichment of the PI3K-Akt signaling pathway (KEGG: KO04151) across all comparisons (Figure 7D–F). The pathway showed significant normalized enrichment scores (NES) in NC vs. TP (Figure 7D), NC vs. NaTP (Figure 7E), and TP vs. NaTP (Figure 7F). Corresponding heatmaps of leading-edge genes (Figure 7D–F, bottom) illustrated the core contributors driving the enrichment, including key members such as *PIK3CA*, *AKT1*, *PIK3CB*, and *FOXO3*. To validate the transcriptomic findings, qRT-PCR and Western blot analyses were performed on core components of the PI3K-Akt pathway. At the mRNA level (Figure 7G), *PIK3CA*, *PIK3CB*, *AKT1*, and *AKT3* were significantly upregulated in both TP and NaTP groups compared with NC, with NaTP generally showing the highest expression. Protein-level analysis by Western blotting (Figure 7H) further confirmed elevated total PI3K, phosphorylated PI3K (p-PI3K), total AKT, and phosphorylated AKT (p-AKT) in TP and NaTP relative to NC, with NaTP displaying the strongest activation of the pathway.

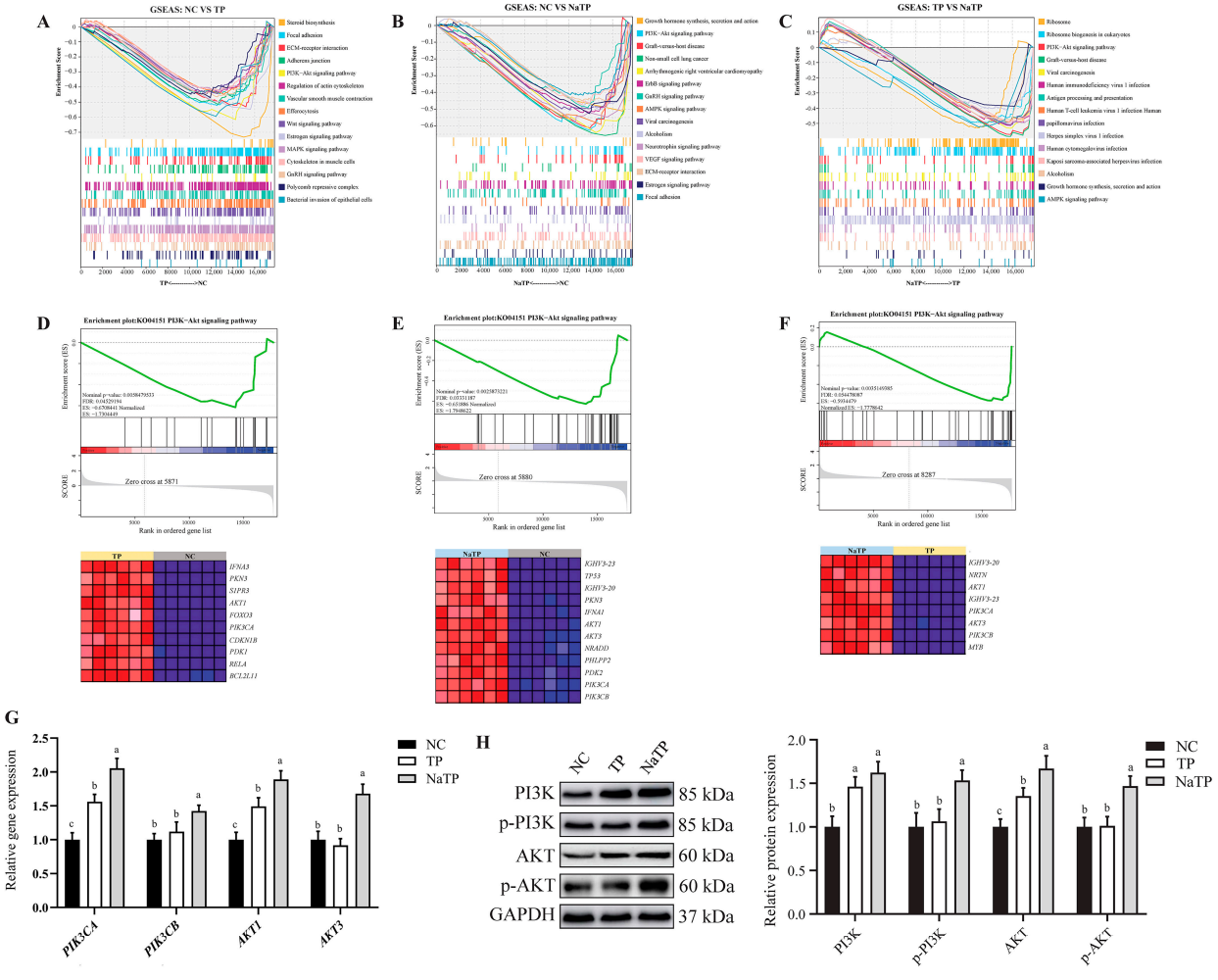


Figure 7. Jejunal mucosal PI3K-Akt signaling pathway expression. (**A–C**): Top 15 enriched pathways from GSEA. (**D–F**): Enrichment plots and leading-edge gene heatmaps of the KEGG PI3K-Akt signaling pathway. (**G**): Relative mRNA expression of key genes in the PI3K-Akt signaling pathway.

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(**H**): Protein expression of key components in the PI3K-Akt signaling pathway (Western blot). NC: Control group, regular drinking water; TP: 200 mg/L free tea polyphenols in drinking water; NaTP: 200 mg/L sodium alginate-coated iron-based tea polyphenol nanoparticles in drinking water. Data are presented as Mean ± SE, $n = 6$. Different superscript letters (a–c) indicate significant differences ($p < 0.05$).

4. Discussion

In the present study, supplementation of 200 mg/L iron-based tea polyphenol nanoparticle formulation in the drinking water of pullets during the brooding and rearing period (0–56 days of age) significantly improved intestinal health and growth performance. Compared with the group receiving the same dose of free tea polyphenols, the NaTP group exhibited significantly higher contents of EGCG, ECG, EGC, and EC in multiple gastrointestinal segments, indicating improved catechin retention. This enhanced retention may contribute to the antioxidant effects observed following NaTP supplementation. Consistent with this possibility, NaTP improved systemic and jejunal mucosal antioxidant status, as evidenced by increased activities of antioxidant enzymes, including SOD and GSH-Px, enhanced T-AOC, and reduced MDA levels. Together with the observed improvements in intestinal morphology, these findings suggest that NaTP may support intestinal development and growth performance through complementary protective effects. This study is the first to demonstrate that the iron-based metal–polyphenol network nanodelivery system can effectively overcome the major limitations of conventional tea polyphenols in drinking water applications, including poor stability, low bioavailability, and insufficient intestinal retention. By providing multidimensional protection during the critical window of rapid intestinal development in pullets, the NaTP formulation lays a solid foundation for subsequent mechanistic investigations.

The NaTP significantly enhanced the stability and application efficacy of tea polyphenols in drinking water systems. The coordination core was formed through the self-assembly of iron ions and tea polyphenols via a metal–polyphenol network, a strategy that effectively improved the chemical stability, antioxidant activity, and intestinal retention of free tea polyphenols [25]. Previous studies have shown that iron-based metal–polyphenol nanoparticles can markedly enhance the antioxidant and antibacterial properties of polyphenols while improving their bioavailability through a one-step self-assembly process [26]. Further coating with sodium alginate, a natural polysaccharide, not only conferred favorable water dispersibility, negative surface potential, and an appropriate particle size (100–200 nm) to the nanoparticles, but also strengthened their mucosal adhesion and controlled-release properties in the intestine [27]. This promoted the uniform distribution of catechins from the proventriculus to the cecum [28], thereby overcoming the common limitations of conventional tea polyphenols, such as susceptibility to oxidation and precipitation in drinking water as well as low bioavailability [29]. Consistent with these potential advantages, NaTP supplementation resulted in higher digestive tract concentrations of major catechins than free TP [30]. The higher catechin concentrations in NaTP digesta may be attributed to iron–polyphenol coordination, which could protect catechins against oxidative degradation, and the sodium alginate coating, which may delay their release during gastrointestinal transit [25,27,29]. These structural features may help preserve catechins along the digestive tract. However, as gastrointestinal release kinetics and catechin stability were not directly evaluated, these explanations remain potential mechanisms requiring further investigation. In addition, the actual catechin doses were not strictly equivalent, and daily water consumption was not reliably recorded. Differences in digesta water content may also have influenced the measured catechin concentrations. Therefore, the higher concentrations observed in NaTP digesta cannot be attributed exclusively to improved formulation stability or intestinal retention.

Tea polyphenols are primarily derived from the buds and leaves of the tea plant and represent the main bioactive components in the processing of various teas, including green tea and black tea [31]. As the world's largest producer and consumer of tea, China produced 7.4 million tons of tea in 2025, with a tea plantation area exceeding 3.9 million hectares [32]. The continuous expansion of the tea industry provides abundant raw materials for the development and utilization of tea polyphenols [33]. Previous studies have shown that dietary or drinking water supplementation with tea polyphenols can significantly improve growth performance, antioxidant capacity, and intestinal health in livestock and poultry [34]. Previous studies have reported that tea polyphenol supplementation in pigeon diets enhanced growth performance and intestinal barrier function [35]. Similarly, dietary tea polyphenols improved egg production rate and egg quality in laying hens [15]. However, in the present study, during the brooding and rearing period, the free tea polyphenol group showed no significant improvement in growth performance compared with the control group. This lack of effect may be attributed to the immature intestinal development in young pullets, combined with the poor stability of free TP in drinking water and its low bioavailability, which make it difficult to maintain effective concentrations in the intestine for sustained action [36].

Notably, supplementation with tea polyphenol nanoparticles significantly increased body weight at 56 days of age and reduced the F/G. This improvement can be ascribed to the nano-delivery system, which markedly enhanced the intestinal retention and absorption of catechins [18]. At the molecular level, tea polyphenols exert protective effects by activating the PI3K-Akt signaling pathway [37]. Based on the GSEA of the transcriptome, a significant enrichment of this pathway across multiple group comparisons. The mRNA expression of core genes, including *PIK3CA*, *PIK3CB*, *AKT1*, and *AKT3*, was significantly upregulated, and Western blot analysis confirmed elevated protein levels of phosphorylated PI3K and phosphorylated AKT. These findings are consistent with previous reports on the activation of metabolic regulation by green tea polyphenols via the AMPK pathway [38]. Activation of the PI3K-Akt pathway has been implicated in intestinal epithelial cell proliferation and survival, which may contribute to improvements in intestinal morphology, including villus height and the VH/CD ratio [39]. In addition to these potential effects on intestinal development, PI3K-Akt signaling may be involved in the regulation of cellular antioxidant responses. In the present study, the increased activities of SOD and GSH-Px, enhanced T-AOC, and reduced MDA levels observed following NaTP supplementation are consistent with an improvement in antioxidant defense and a reduction in lipid peroxidation. These findings suggest a potential association between PI3K-Akt pathway activation and the antioxidant effects of NaTP, although further mechanistic studies are required to establish a causal relationship. Therefore, the observed improvements in intestinal morphology, antioxidant capacity, and barrier-related indicators should be interpreted as being associated with PI3K–Akt activation rather than definitively caused by it [13].

Intestinal health serves as the cornerstone of growth and development in young livestock and poultry, directly influencing nutrient absorption, immune system maturation, and the realization of subsequent production performance [40]. During the critical brooding and rearing window period, the rapid maturation of the intestinal barrier is particularly important [41]. However, most existing studies on tea polyphenol supplementation via diet or drinking water have primarily focused on the later growth stages or laying period. Systematic evaluations of application during the brooding phase remain relatively limited [8]. Moreover, free tea polyphenols are prone to oxidation and precipitation, and their low bioavailability makes it difficult to maintain effective concentrations throughout the intestinal tract, thereby restricting their protective potential [11]. At the same time, many antibiotic alternatives (such as probiotics and organic acids) suffer from drawbacks

including inactivation by gastric acid, low intestinal delivery efficiency, and unstable biological effects, which limit their efficacy during the early stages of intestinal development [42]. The present study demonstrated that drinking water supplementation with NaTP during the brooding and rearing period of laying hens significantly optimized intestinal morphology, antioxidant capacity, and catechin retention. These findings provide a stable, efficient, and green novel drinking water additive strategy as an alternative to antibiotics in poultry production [12]. Such early improvements in intestinal health are expected to lay a solid foundation for enhanced laying performance and egg quality in later stages [43]. Nevertheless, this study was limited by its small sample size and lack of long-term and laying-period evaluations. Although iron primarily served as a structural component, its accumulation and long-term safety remain unassessed. In this formulation, iron primarily served as a coordination component to improve tea polyphenol stability [25,26]. Since this study focused on tea polyphenol modification rather than iron supplementation, a separate iron-only control was not included. Future studies should evaluate iron exposure, tissue residues, and dose-dependent safety to support practical application. In addition, this study has several limitations, including the evaluation of only a single supplementation dose, a relatively short experimental period (56 days), and the lack of measurements of actual daily catechin intake and digesta water content, which limits the interpretation of measured catechin concentrations. These concentrations reflect catechin levels in gastrointestinal contents rather than intestinal absorption or systemic bioavailability. Future studies should optimize supplementation dosages across different livestock and poultry models, extend the experimental period, assess safety and residue levels, quantify actual water consumption, normalize catechin concentrations to digesta dry matter, and measure circulating catechin levels to better characterize bioavailability.

5. Conclusions

In summary, supplementation with 200 mg/L sodium alginate-coated iron-based tea polyphenol nanoparticles during the early rearing period improved growth performance, intestinal morphology, and antioxidant capacity in pullets. Compared with free tea polyphenols, NaTP increased catechin concentrations in digestive tract samples and was associated with enhanced PI3K–Akt signaling. However, the causal involvement of this pathway in intestinal development and barrier function remains to be established. Furthermore, improvements in intestinal barrier integrity require confirmation through direct measurements of tight junction proteins or intestinal permeability. This work represents the first successful development of a green, facile iron-based metal–polyphenol network nano-formulation. Offering a promising alternative to antibiotics in intensive production systems, and provides an efficient and sustainable strategy for advancing antibiotic-free poultry farming. However, further dose–response studies, long-term laying performance assessments, and comprehensive safety evaluations are necessary before practical applications can be recommended.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/d18110719/s1, Table S1: Composition and nutrient levels of basal diets; Table S2: The measured contents of individual catechins in the tea polyphenol material; Table S3: Standard curves of the catechin standards; Table S4: Real-time PCR primer nucleotide sequence; Table S5: The suppliers and dilution ratios for the primary and secondary antibodies.

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Data Availability Statement: The data generated in this study are publicly available in the National Center for Biotechnology Information (NCBI) BioProject database. The RNA sequencing data are accessible under accession number PRJNA1552876 (https://dataview.ncbi.nlm.nih.gov/object/PRJNA1552876?reviewer=stlkal8ukg410faeeqdv2gvjc, accessed on 30 October 2026).

Conflicts of Interest: Ping Xu and Yibin Zhang are employed by Anhui Agricultural Reclamation Group, Anhui Jiangnan Chahai Poultry Industry Company Limited, they provided chicken coops. The company had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ADFI	Average daily feed intake
ADG	Average daily gain
CAT	Catalase
CD	Crypt depth
EC	Epicatechin
ECG	Epicatechin gallate
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
GSH-Px	Glutathione peroxidase
MDA	Malondialdehyde
MPN	Metal–polyphenol network
SOD	Superoxide dismutase
VH	Villus height

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