Chestnut tannins and their relationship in promoting broiler growth by altering the metabolic phenotype in the cecum.

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Abstract

Background

As the demand for alternatives to antibiotic growth promoters (AGPs) increases in food animal production, phytobiotic compounds gain in popularity due to their ability to mimic the desirable bioactive properties of AGPs. Chestnut tannins (ChT) are one of many phytobiotic compounds utilized as feed additives, particularly in South America, for broiler chickens due to its favorable antimicrobial and growth promotion capabilities. While studies have observed the microbiological and immunological effects of ChT, there is a lack of studies evaluating the metabolic function of ChT in the host. Therefore, the objective of this study was to characterize the cecal metabolic changes induced by ChT inclusion and how they relate to growth promotion.

Results

A total of 500 day-of-hatch Cobb 500 chicks were separated into five feed treatment groups: control, 1% ChT, 0.2% ChT, 0.08% ChT, and 0.03% ChT. The cecas from all the chicks in the treatment groups were collected on days 2, 4, 6, 8, and 10 post-hatch. The cytokine mRNA qRT-PCR was determined using TaqMan gene expression assays for IL-1B, IL-6, IL-8, IL-10, and IFN-γ quantification. The cytokine expression showed highly significant increased expressions of IL-6 and IL-10 on days 2 and 6, while the other pro-inflammatory cytokines did not have significantly increased expression. The results from the kinome array demonstrated that the cecas from birds fed with 1% ChT had significant ($p < 0.05$) metabolic alterations based on the number of peptides when compared to the control group across all days tested.

Conclusions

Based on the results, the increased expression of IL-6 appeared to be strongly indicative of altered metabolism while the increased expression of IL-10 indicated the regulatory effect against other pro-inflammatory cytokines other than IL-6. The ChTs initiate a metabolic mechanism during the first ten days in the broiler. For the first time, we show that a phytobiotic product initially modulates metabolism rather than immunity while also potentially supporting growth and feed efficiency downstream. In conclusion, an IL6-mediated immunometabolic phenotypic alteration in the ceca of chickens fed ChT may indicate the importance in the gut of enhanced broiler health.

Background

By moving away from antibiotics in livestock production, there is a growing interest from the poultry industry to find plant-based alternatives to replace antibiotics in feed. There has been increased interest in utilizing plant-based compounds or phytobiotics as antibiotic growth promoter (AGP) alternatives, including chestnut tannins (*Castanea sativa*) [1, 2]. Plant-based tannins can be categorized into two major groups: condensed tannins or hydrolyzable tannins [3]. Tannins can be found in many plant

species, mostly in the inedible portions of the plant such as the bark or wood [4, 5]. Due to enhanced research, the previous knowledge that tannins possess anti-nutritional effects in livestock species is outdated and the benefits vary on poultry species and dosages in feed [6]. The bioactive compounds, polyphenols, found in tannins allow them to be effective immunomodulatory additives that promotes human and animal health [5]. While the mechanism of action is still not widely understood, the benefits of phytobiotics in livestock animals include antioxidation properties, stabilization of intestinal microbiome, and improvement of immune system through immunomodulatory effects [7]. Extensive studies done in swine species exhibit strong evidence of phytobiotics as likely alternatives to antibiotics to improve growth performance and health [8, 9, 10]. Ruminant producers have increased interest in growth promotors because of the efficient utilization of energy during rumen fermentation [11]. The usage of tannins in the ruminant industry has been of major interest because of the tannins' ability to play a role in rumen protein utilization to improve feed efficiency [12].

Previous studies evaluated the functionality of different tannin species against pathogenic infections across livestock species, showing anti-microbial activity in concentrations ranging between 0.5-1 kg/ton [7, 9, 13]. Importantly, tannins in general have also been shown to improve feed efficiency, growth performance, and intestinal health, as seen when up to 0.2% chestnut tannins (ChT) are added into feed [14, 15]. With all these studies conducted, the usage of tannins in production have shown potential to be efficient as growth promoter alternatives, given the quality and concentration provided [15].

While the previously mentioned studies provide information about microbiological, immunological, and performance data [7, 13, 15], there is a lack of knowledge about the mechanism of the host metabolic interactions in the intestine, specifically the metabolic reaction to dietary ChT. Tannins exert pro-inflammatory immune responses but for a short-lived period and the anti-inflammatory responses follow soon after [16]. Tannins can also exert a synergistic effect with AGP alternatives to promote gut health [15]. Currently, there are proposed mechanisms for immunomodulatory activity based on different types of feed additives [17, 18]. While gene expression provides important information regarding pathogen interaction, there are disadvantages to genetic approaches including its inability to accurately predict the cellular phenotypes [19].

Studying phosphorylation events provides information on the mechanism of post-translational modification, which offers insight in cellular and tissue phenotypes [20, 21]. Peptide arrays for kinomic analysis have already been widely utilized across scientific disciplines [21, 22, 23]. The kinome array provides functional phenotype data, indicating changes within tissue metabolism and immune response to an infection [24]. By understanding the kinase activity, there are increased insights into identifying specific biomarkers to provide future therapeutic targets [21]. Recent advancements have provided a species-specific peptide array for livestock species, including poultry [21, 25]. This integrated array demonstrates the importance of combined immunity and metabolic data on the animal's overall health and growth performance [25].

Therefore, the objective of this study was to determine the metabolic phenotype changes affected by ChT in the ceca. By using gene expression and kinome array, we were able to identify global metabolic phosphorylation-based events in the cecas of birds fed ChT at high doses compared to birds fed regular starter diets.

Materials And Methods

Experimental Animals, Housing, and Treatments

All experiments conducted were in accordance with guidelines set by the United States Department of Agriculture Animal Care and Use Committee (USDA ACUC #2019001), which meets all federal requirements as defined in the Animal Welfare Act, and the Human Care and Use of Laboratory Animals. A total of 500 male by-product day-of-hatch broiler chicks were obtained from a local commercial hatchery and assigned to five treatment groups with two replicate pens per treatment, totaling 50 chicks per pen. The treatments were as follows, per replicate pen: (1) control feed – normal starter feed (n = 50); (2) 1% ChT inclusion feed (n = 50); (3) 0.2% ChT inclusion feed (n = 50); (4) 0.08% ChT inclusion feed (n = 50); and (5) 0.03% ChT inclusion feed (n = 50). Chicks were randomly distributed into each group in pens with fresh pine shavings, water, and starter diet *ad libitum*. They were maintained in BSL-2 units under 24 h of light from start to finish of the study. For the purposes of this manuscript, only the results for the 1% ChT group versus control group were reported; all other treatments will be analyzed in a separate publication. All treatments were fed a corn/soybean-based crumble diet, and they differ in AGP or tannins inclusion. The diets were formulated to meet or exceed broilers requirements (Table 1). The hydrolyzable chestnut tannin additive (Silvateam s.p.a©., Buenos Aires, Argentina) contained 75% tannin content, supplemented with 94% dry matter, lignin and sugars. The experimental process lasted 10 days.

Table 1
Calculated composition of starter diets. The total basal diet contained 1,365 kcal/lb.

Ingredients	**%**
Corn	59.81
SBM 48%	33.84
Monocalcium phosphate 21	1.56
Soy oil	2.09
Choline chloride	0.10
Limestone	1.56
Salt	0.33
L-lysine HCL	0.19
DL-methionine	0.28
Vitamin premix	0.13
Mineral premix	0.05
L-Threonine	0.05
Calculated nutrients, %	99.99
Protein	22.00
Calcium	0.90
Available phosphorus	0.45
AMEn (Kcal/lb)	1,365.00
Digestible methionine	0.59
Digestible total sulfa amino acid	0.88
Digestible threonine	0.77
Digestible lysine	1.18
Choline	1,256.87
Sodium	0.16
Potassium	0.84
Chloride	0.20

Sample collection and processing

On each necropsy day, 10 birds/group were euthanized via cervical dislocation and necropsied on days 2, 4, 6, 8, and 10 of each replicate experiment. Both cecas were removed, flushed with PBS, and flash frozen in liquid nitrogen in order to preserve the kinase enzymatic activity. The frozen tissues were moved into a -80^{O}C storage freezer until further processing.

Real-time quantitative RT-PCR

The immune portion was quantitated by gene expression studies, specifically through a TaqMan based assay adapted from Eldaghayes et al. [26]. Total RNA was extracted using a Qiagen RNeasy® Plus kit (Germantown, MD, USA) and evaluated with a NanoDrop™ 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Cecas stored in RNALater were used for RNA isolation with the Qiagen Rneasy Plus Kit. The cecas were cut longitudinally to expose the lumen and any remaining fecal matter was gently removed with forceps as to not disturb the mucosal layer. For each group, there were 10 cecas processed per replicate experiment for qRT-PCR.

Cytokine mRNA expression levels were ascertained using RT-PCR with 28S as the reference gene. The RNAs were stored at -80^{O}C until plate setup. The cytokines IL-1B, IL-6, IL-8, IL-10, and IFN-γ were quantified utilizing the Eldaghayes et al. method [26]. Primer and probe sequences (Table 2) for amplification have been described previously by Kogut et al. [27] and Kaiser et al. [28]. The plates were run in the Applied Biosystems ABI StepOne Plus PCR system (ThermoFisher Scientific, Waltham, MA, USA) with the previously stated TaqMan Assay under the following conditions: one cycle of 48 °C for 30 min, 95 °C for 20 s, and 40 cycles of 95 °C for 3 s and 60 °C for 30 s. Results were calculated with the corrected 40- C_t method, as described in Eldaghayes et al. [26] and expressed in fold change values. Each sample was run in triplicate for technical replication.

Table 2. Real-time quantitative RT-PCR primer and probe sequences.

RNA target		*Probe/primer sequence*	*Exon boundary*	*Accession no.*
28S	Probe	5′-(FAM)-AGGACCGCTACGGACCTCCACCA-(TAMRA)-3′		X59733
	F	5′-GGCGAAGCCAGAGGAAACT-3′		
	R	5′-GACGACCGATTGCACGTC-3′		
IL-1β	Probe	5′-(FAM)-CCACACTGCAGCTGGAGGAAGCC-(TAMRA)-3′	5/6	AJ245728
	F	5′-GCTCTACATGTCGTGTGTGATGAG-3′		
	R	5′-TGTCGATGTCCCGCATGA-3′		
IL-6	Probe	5′-(FAM)-AGGAGAAATGCCTGACGAAGCTCTCCA-(TAMRA)-3′	3/4	AJ250838
	F	5′-GCTCGCCGGCTTCGA-3′		
	R	5′-GGTAGGTCTGAAAGGCGAACAG-3′		
IL-8	Probe	5′-(FAM)-CTTTACCAGCGCGTCCTACCTTGCGACA-(TAMRA)-3′	1/2	AJ009800
	F	5′-GCCCTCCTCCTGGTTTCAG-3′		
	R	5′-TGGCACCGCCAGCTCATT-3′		
IL-10	Probe	5′-(FAM)-CGACGATGCGGCGCTGTCA-(TAMRA)-3′	3/4	AJ621614
	F	5′-CATGCTGCTGGGCCTGAA-3′		
	R	5′-CGTCTCCTTGATCTGCTTGATG-3′		
IFN-γ	Probe	5′-(FAM)-TGGCCAAGCTCCCGATGAACGA-(TAMRA)-3′	3/4	Y07922
	F	5′-GTGAAGAAGGTGAAAGATATATCATGGA-3′		
	R	5′-GCTTTGCGCTGGATTCTCA-3′		

Statistical analysis for qRT-PCR

Cytokine mRNA expression for control and treated cecas from days 2, 4, 6, 8, and 10 were quantitated using a method described by Kaiser et al. [28] and Moody et al. [29]. The 40-C_t values were calculated for each sample with averaged triplicates per sample. Statistical analysis was performed with SAS 9.4 (Cary, NC, USA) based on the data collected from each trial for the qRT-PCR data. Each time frame had samples comparing 1% ChT-treated birds versus control-fed birds. The Shapiro-Wilks test for normality was used to determine if the fold change within each group was parametric or non-parametric, with an alpha of 0.05. For all analyses, statistical significance was considered if $P \leq 0.05$.

All data were found to be non-parametric and were summarized as median values. An ad-hoc analysis using the Kruskal-Wallis test was conducted to determine where the statistical differences lie between the control and 1% ChT. The ceca samples for the IL-1B, IL-6, IL-8, IL-10, and IFN-γ were quantified using the 40-C_t method, as outlined by Eldaghayes et al. [26]. The results were reported in fold change values.

Chicken-specific kinome (Peptide) array

For the phenotype readout, a peptide array was utilized to provide tissue immunometabolism information from the host. At three of the time points (days 4, 6 and 10), three whole cecas from three randomly selected birds – stored in -80^{o}C – were defrosted for analysis (27 samples total for all three days). Each sample was weighed to obtain a consistent 40 mg sample for the array. The samples were homogenized by the Omni International Bead Ruptor Elite (Kennesaw, GA, USA) in 100 uL of lysis buffer (20 mM Tris–HCl pH 7.5, 150 mM NaCl,1 mM EDTA, 1 mM ethylene glycol tetraacetic acid (EGTA), 1% Triton X-100,

2.5 mM sodium pyrophosphate, 1 mM Na3VO4, 1 mM NaF, 1 µg/mL leupeptin, 1 g/mL aprotinin and 1 mM phenylmethylsulphonyl fluoride). All products were obtained from Sigma Aldrich (St. Louis, MO, USA), unless indicated. Following homogenization, the peptide array protocol was carried out as per Jalal et al. [30] with alterations described in Arsenault et al. [19]. The resulting tissue lysates were applied onto the PepStar peptide microarrays customized by JPT Peptide Technologies GmbH (Berlin, Germany).

Data Analysis: Kinome Array

Data normalization was performed for the kinome array, based on Li et al. [31] using the PIIKA2 online platform (http://saphire.usask.ca/saphire/piika/index.html), a tool designed for *in silico* analysis of phosphorylation sites [32]. The array data were analyzed by conducting variance stabilization normalization, and then performing t-test, clustering and pathway analysis for statistical data. Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed by uploading the statistically significant peptide lists to the Search Tool for the Retrieval of Interacting Genes (STRING) [33].

Results

Real-time quantitative RT-PCR results

Across both replicate experiments, only IL-6 and IL-10 were found to have statistically significant increases in mRNA expression on days 2 and 6 across all days tested in the experiment. Figure 1 shows the mRNA expression levels of all tested days (days 2, 4, 6, 8, and 10) for IL-6. The results demonstrated that the expression levels of IL-6 were significantly increased following treatment with 1% ChT on day 2 and day 6 when compared to controls. There were no significant differences when compared to controls on days 4, 8 and 10. Figure 2 shows the mRNA expression levels of all tested days (day 2, 4, 6, 8, and 10) for IL-10. The results also demonstrated that the expression levels of IL-10 were significantly increased following treatment with 1% ChT on day 2 and day 6. However, there were no significant differences when compared to controls on days 4, 8 and 10.

Kinome results

Using STRING-db [33], the resulting analysis of the kinome data showed distinct metabolic differences between the 1% ChT group compared to the control group. Each day is representative of three birds normalized and combined into representative datasets. The biological process (BP) terms generated from Gene Ontology (GO) for each dataset include sets of molecular events with a defined beginning and end that pertain to the functioning of the integrated living units [34]. Based on the false discovery rate (FDR) of the analysis, values $P \leq 0.01$ were listed and considered statistically significant. The analysis of the kinome data showed distinct differences in the observed BP between 1% CT fed birds compared to the control birds. Table 3 summarizes the top 15 GO STRING-generated biological pathways of metabolic processes and the number of differentially phosphorylated peptides associated with them. Day 6 samples have the greatest number of primary biological processes related to metabolic pathways out of

the three days, indicated by the number of significant peptides. Day 4 had the most amount of fatty acid metabolic process present, but it decreased by day 6 and was not present by day 10.

Additionally, the top metabolic KEGG pathways [35] were obtained from the STRING-db, as summarized in Table 4, for 1% ChT-fed group compared to the control group. The pathways of interest were those that showed significant changes at multiple time points. Day 6 samples have the greatest number of altered immune and metabolic pathways out of the three days, indicated by the number of peptides and number of pathways shown in the tables. By day 10, the number of peptides is decreased, as well as the number of pathways.

Table 3
The top 15 Gene ontology (GO) metabolic biological processes (BP) identified at days 4, 6, and 10 comparing 1% ChT-fed birds against control birds. The hyphens indicate non-significant BPs based on the FDR.

Biological Processes	**Day 4**	**Day 6**	**Day 10**
phosphate-containing compound metabolic process	100	132	79
primary metabolic process	145	187	122
protein metabolic process	99	136	83
cellular metabolic process	142	184	125
fatty acid metabolic process	90	19	-
glucose metabolic process	11	14	9
glycerolipid metabolic process	18	28	16
cellular lipid metabolic process	37	48	24
glycogen metabolic process	8	9	6
ATP metabolic process	13	11	10
lipid metabolic process	38	50	26
carbohydrate metabolic process	21	25	18
hexose metabolic process	13	15	-
NAD metabolic process	8	11	8
pyruvate metabolic process	10	11	9

Table 4

Summarized table of Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathways at days 4, 6, and 10 comparing 1% ChT-fed birds and control birds. The hyphens indicate less than 10 peptides and not significant for our analysis.

Metabolic Pathways						
	Day 4		Day 6		Day 10	
Identified pathways	**Number of peptides**	**p-value**	**Number of peptides**	**p-value**	**Number of peptides**	**p-value**
PI3K-Akt signaling pathway	34	1.15×10^{-22}	44	4.28×10^{-29}	24	1.11×10^{-13}
MAPK signaling pathway	30	1.75×10^{-20}	49	2.32×10^{-37}	22	2.44×10^{-13}
Metabolic pathway	28	1.39×10^{-05}	32	4.67×10^{-05}	-	-
Insulin signaling pathway	27	5.29×10^{-25}	27	1.30×10^{-22}	20	7.77×10^{-17}
AMPK signaling pathway	21	1.19×10^{-18}	20	7.95×10^{-16}	16	3.16×10^{-13}
HIF-1 signaling pathway	18	1.82×10^{-16}	18	5.60×10^{-15}	12	6.91×10^{-10}
mTOR signaling pathway	18	6.72×10^{-14}	18	2.23×10^{-12}	18	5.53×10^{-14}
cAMP signaling pathway	17	2.85×10^{-11}	13	1.79×10^{-06}	-	-
Glucagon signaling pathway	14	8.32×10^{-12}	15	1.22×10^{-11}	11	9.36×10^{-09}

Metabolic Pathways						
VEGF signaling pathway	12	7.40×10^{-12}	17	7.06×10^{-17}	-	-
Calcium signaling pathway	11	2.42×10^{-06}	10	0.00012	-	-
Glycolysis/Gluconeogenesis	10	5.39×10^{-09}	10	4.75×10^{-08}	-	-
GnRH signaling pathway	10	4.43×10^{-08}	14	3.03×10^{-11}	-	-
Adipocytokine signaling pathway	-	-	18	3.60×10^{-17}	-	-

Discussion

The current study objective was to provide metabolic information on the effects of ChT when incorporated into the broiler feed. Tannins are already known to improve livestock health and growth performance, but the mechanism is still widely unknown [7]. To date, these results are the first of its kind to provide mechanistic information on how host intestine responds to tannins inclusion in the feed. These observed metabolic changes may be related with the improved performance in broilers fed tannins. With the qRT-PCR and kinome data, we were able to provide global post-translational perspectives on how intestine metabolic function is correlated in a tannins-fed diet.

Based on the qRT-PCR data, the cytokines of interest based on our results are the elevated expressions of IL-6 and IL-10 by day 6. A previous experiment by Ferro et al. [36] concludes that IL-6 may need to work in tandem with other immunomodulatory cytokines (such as IL-1 family cytokines) to stimulate immune responses. In fact, McGeachy et al. [37] discovered that IL-10 is upregulated by IL-6, revealing a dependent relationship between the two cytokines during an immune response. This supports what we found in our results since only IL-6 and IL-10 were significantly upregulated, compared to the other cytokines tested, providing evidence of the IL-10 immunoregulatory properties. As the gene expression results show, the elevated IL-6 upregulation may be the peak time of modulation in the bird (day 6), with IL-10 regulating this pro-inflammatory response. What is important to note is the temporary peak of IL-6 expression: by day 10, the high level of expression is no longer present. This is beneficial for the birds because the chestnut tannins stimulate a response to prime the birds' immunity. While IL-6 is known to have pro- and anti-inflammatory properties based on the stimulus [36], long-lasting IL-6 presence may contribute to chronic inflammation and tissue damage, which is undesirable for the birds and producers

[38]. There is also strong evidence of IL-6 involved in metabolic function, rather than just immune response. Flint et al. [39] found that IL-6 induction stimulated a metabolic reprogramming which directly induced an immune suppression in mice. They predicted that this metabolic pathway of IL-6 helps prevent immunological damage due to inflammation. Reviews by Pavlov and Tracey [40] and Ghanemi and St Amand [41] outline further proof of IL-6 and its direct linkage to metabolic pathways, especially affecting insulin pathways and fatty acid synthesis. These reviews discuss how IL-6 should be considered beyond its immunological function due to its effects on lipids, protein, and glucose metabolism.

The current study is the first to demonstrate metabolic importance associated with ChTs, as indicated by the number of post-translational modifications and significant p-values. Based on its known antimicrobial effects, ChTs and other phytobiotics make ideal candidates for growth promotion and health improvements based its ability to retain bacterial diversity in the gut but reducing drug-resistant bacteria [2, 42]. Chestnut tannins have already been known to affect bifidobacteria in the ceca of mammals and chickens [2], which have been shown to alter carbohydrate metabolism and other metabolic processes downstream in the host by modifying enzymes and sugar transport pathways [43]. Our research supports this through the direct alteration of the carbohydrate and primary metabolic pathways (Table 3). The main difference in bacterial taxa between control and tannins-fed birds was the increase in bacterial diversity over time within the tannins-fed group, especially with *Lactobacillus* and *Enterococcus* species [2]. These bacterial species utilize sugar metabolism (such as hexose and glucose metabolism) and carbohydrate metabolism which supports our findings in our tannins-fed group [44]. Another study found older birds treated with tannins consistently had increased populations of order Clostridiales and family Ruminococcaceae, which have been of interest in the poultry industry as potential probiotic options [2]. This is noteworthy evidence of how tannins-fed chickens could have increased members of the family Ruminococcaceae to alter the SCFAs profile in the cecum towards butyrate production. The increased presence of these butyrate-producing communities would indicate the usage of carbohydrate metabolism, fatty acid metabolism, glycerolipid metabolism downstream to ultimately benefit host physiology [44, 45].

An interesting note by Diaz Carrasco et al. [2] was the sensitivity effect that dietary tannins had on Gram-positive bacteria, as also noted in rat studies with increased Gram-negative bacteria in the gastrointestinal tract [46]. Molino et al. [47] have shown preliminary data on the importance of tannins in gut microbial fermentation and the nutritional importance in human application. This study provides additional new information on affected pathways in the intestine with the introduction of ChTs in early broiler growth. Although the microbiota of young birds are more prone to fluctuating gut microbial communities, these results support the relevance of chestnut tannins in the feed to not only promote growth but to also provide more evidence as an alternative to antibiotics. Future studies in our group will look at growth promotion effects in depth by investigating the individual pathways that were altered with the introduction of ChT in the feed. Another future study will also test the other treatment groups (0.2%, 0.08% and 0.03% ChT inclusion groups) for metabolic differences.

Therefore, with the current trend of removing antibiotics in feed, tannins are one of the promising feed additives due to its ability to stimulate immunoregulatory effects and host modulation to prevent resistance, which is ideal as an alternative to antibiotics. This aims to reduce the immune response while redirecting energy towards growth [25]. Ideal alternatives would promote health without risking loss of growth promotion or antibiotic resistance [7]. Chestnut tannnins have already shown applicable results in reducing incidence of necrotic enteritis and *Salmonella* in livestock, although the effects on growth improvements varied based on dosages [2, 13, 48]. The results from this current study are providing further proof to what is currently in the literature of ChTs as a potential alternative to antibiotics. The strong metabolic connection found in our study shows the promising nature of utilizing ChTs as an antibiotic alternative because there would be promotion of growth on top of promotion of health. Our group has already conducted studies on the host-pathogen interactions of chickens using the kinome array [34, 49, 50], which shows the importance of viewing immunity and metabolism comprehensively instead of as individual units. With the increasing population, there will be a growing demand for poultry products especially cost-effective feed additives [7]. Therefore, identifying an alternative to AGPs will be crucial in the future to keep up with increased demands for safe poultry products. Tannins might be a promising alternative to AGPs due to the improvements in performance and microbiota, as seen in previous studies, and the current data support its metabolic modulation in the intestine.

Conclusions

In conclusion, it was observed that the influence of ChT in the diet alters gut immunometabolism of broilers. By focusing on the ChT-fed group compared to the control group, the results from these two replicate trials demonstrated the metabolic outcome of when ChTs are introduced into the diet. These are the first data in the literature demonstrating pathway data that supports growth and health promotion with ChT. The objective of this study was to provide a global overview of chestnut tannins' effect on the metabolism, providing mechanism information that is currently lacking in the literature. These data offer ChT not only as an immunoregulator but also a potential host-directed therapy option in disease studies. Phytobiotics can offer promising results since they can target metabolic and immunological pathways of the host and may affect the pathogen. With the growing restriction of antibiotics in the feed, this study offers further evidence of ChT as an important alternative to antibiotics.

Declarations

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AUTHORS' CONTRIBUTION

AL and MHK conceived and designed the experiment. AL, GDP, MBF performed the necropsies and collected the experimental data. SJ performed the statistical analysis. AL and MHK interpreted the data. All authors contributed in reviewing the manuscript and gave final approval for the final publishable version.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All experiments conducted were in accordance with guidelines set by the United States Department of Agriculture Animal Care and Use Committee (USDA ACUC #2019001), which meets all federal requirements as defined in the Animal Welfare Act, and the Human Care and Use of Laboratory Animals.

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AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONSENT FOR PUBLICATION

Not applicable

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest.

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Figures

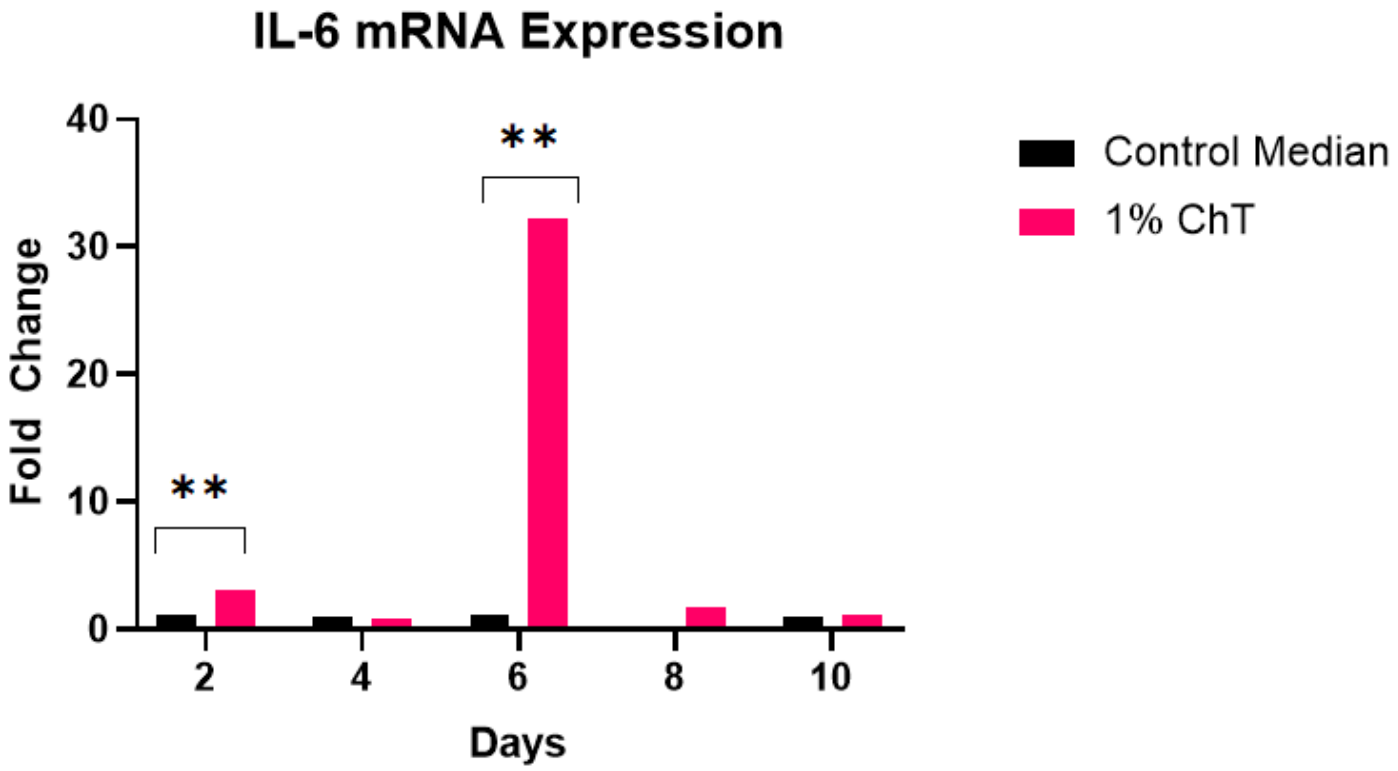

Figure 1

The fold change values based on days tested of the mRNA expression assay for IL-6. These data reflect the averaged replicate experiments (N=200). The asterisks denote significant differences between the control and 1% ChT group.

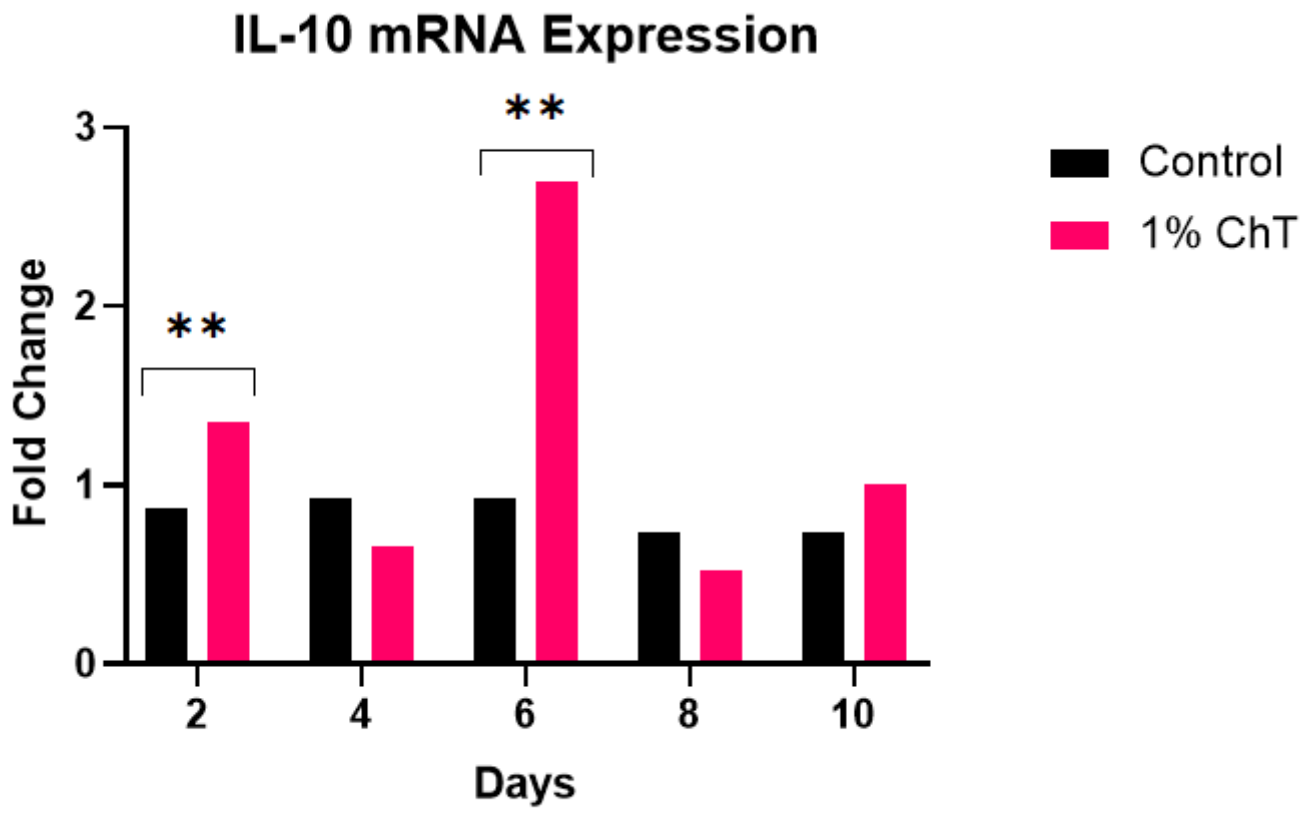

Figure 3

The fold change values based on days tested of the mRNA expression assay for IL-10. These data reflect the averaged replicate experiments (N=200). The asterisks denote significant differences between the control and 1% ChT group.