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Chestnut tannin and chestnut tannin-soy protein isolate complex differently influence fecal *Escherichia coli* count, immune system, caecum fermentation and meat oxidative susceptibility in broiler chickens

Tanin kestena i kompleks tanina kestena i proteinskog izolata soje imaju različit utjecaj na broj *Escherichia coli*, imunosni sustav, fermentaciju u cekumu i oksidaciju u tkivima pilića brojlera

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ABSTRACT

The present study aimed to compare chestnut tannin with the complex of chestnut tannin and soy protein isolate as possible natural growth promoters. The dietary treatments were as follows: Control, without supplementation, FAR, added chestnut tannin, and CPS, added chestnut tannin-soy protein isolate complex. Tannin supplementation positively influenced the final body weight without any additional influence of the tannin source. Cecal fermentation was modulated by tannin supplementation, and the concentrations of short-chain fatty acids were even further increased in the CPS group compared to the FAR group. The number of fecal *Escherichia coli* (*E. coli*) was reduced by tannin, and the CPS group had lower concentrations compared to the FAR group. The mRNA levels of proinflammatory cytokines (IL-6, TNF-α) were decreased only in the CPS group, while TLR4 was decreased in both tannin-supplemented groups compared to the control group. Lipid oxidation was decreased after tannin supplementation in the fresh and frozen meat, with CPS providing some additional improvement in the frozen meat. Tannin also improved n3 fatty acid preservation in the fresh and frozen meat. As with lipid oxidation, the CPS group also showed better results in docosahexaenoic acid concentrations than the FAR group in the frozen meat. It can be concluded that supplementation with tannins has a positive effect on the monitored parameters. Further studies are needed to clarify the differences between the modes of action of these two forms of tannin and to evaluate the efficacy and appropriate dosage of the tannin-soy protein isolate complex in practice.

Keywords: antioxidant, oxidative susceptibility, n3 fatty acids, tannin, tannin-soy complex, broiler chicken

SAŽETAK

Cilj ovog rada bio je usporediti tanin kestena s kompleksom tanina kestena i izolata proteina soje kao mogućih prirodnih promotora rasta. Hranidbeni tretmani su bili: kontrola, bez dodatka, FAR, dodan tanin kestena i CPS, dodan kompleks tanina i proteinskog izolata soje. Dodatak oba tanina pozitivno je utjecao na konačnu tjelesnu masu pilića. Fermentacija u slijepom crijeva bila je promijenjena dodatkom tanina, a porast koncentracije kratkolančanih masnih kiselina bio je veći kod CPS skupine u usporedbi s FAR skupinom. Broj *Escherichia coli* (*E. coli*) u izmetu smanjen je dodatkom oba tanina, a CPS skupina imala je niže koncentracije u usporedbi s FAR skupinom. Razine mRNA proupalnih citokina (IL-6, TNF-α) smanjene su samo u CPS skupini u odnosu na kontrolu, dok je TLR4 smanjen u obje skupine s taninom u usporedbi s kontrolnom skupinom. Oksidacija lipida smanjena je nakon dodatka tanina u svježem i smrznutom mesu, pri čemu je kod CPS skupine primijećeno i dodatno smanjenje oksidacije u smrznutom mesu. Dodavanje tanina također je očuvalo koncentraciju n3 masnih kiselina u svježem i smrznutom mesu. Kao i kod oksidacije lipida, CPS skupina je pokazala bolje rezultate u očuvanju koncentracija dokozaheksaenske kiseline u smrznutom mesu u odnosu na FAR skupinu. Može se zaključiti kako dodavanje tanina u hranu pozitivno utječe na ispitivane pokazatelje. Potrebna su daljnja istraživanja kako bi se razjasnile razlike između načina djelovanja ova dva oblika tanina i procijenila učinkovitost i odgovarajuće doziranje u farmskim uvjetima uzgoja.

Ključne riječi: antioksidativna svojstva, podložnost oksidaciji, n3 masne kiseline, tanin, kompleks tanina i proteinskog izolata soje, tovni pilići

INTRODUCTION

Since the ban on antibiotic growth promotors (AGP) in the European Union in 2006, there is an increasing interest in effective alternatives (Mehdi et al., 2018). The European Parliament and the Council of the European Union adopted Regulation 1831/2003 stating that antibiotics, with the exception of coccidiostats and histomonostats, may only be marketed and used as feed additives until 31 December 2005. Anticoccidials such as ionophoric antibiotics have been banned as feed additives since 2013. Since then, medicinal products in animal feed have been restricted to therapeutic use on the basis of a veterinary prescription (European Parliament, Council of the European Union. 2021. Consolidated text: Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 (2021). One of the possibilities is tannins, which are complex polyphenolic compounds produced by plants as secondary metabolites and deterrents to herbivorous animals. Tannins can be broadly classified into three main categories: condensed tannins and hydrolysable tannins in terrestrial plants and phlorotannins in marine brown algae (Huang et al., 2018). Condensed tannins are oligomers or polymers of flavonoids with flavan-3-ol units (catechin, epicatechin, gallocatechin and epigallocatechin). Their structure is more complex compared to the hydrolysable tannins, and the molecular weight is higher (1,000 to 20,000 Da) (Kemppainen et al., 2014). Hydrolysable tannins contain a polyol as a central core (mainly glucose), which is esterified with phenolic groups (gallic acid and ellagic acid) (Das et al., 2020). The relative molecular weight of hydrolysable tannins is 500–3,000 Da, and they can be hydrolysed (thermal processing, esterification, and acid or base treatment) to yield free gallic acid and ellagic acid (Xu et al., 2023).

Over the last decades, condensed and hydrolysable tannins have been extensively investigated as feed additives in animal nutrition. In contrast to ruminant animals, tannins were first recognized as anti-nutritional factors in simple-stomached animals (Redondo et al., 2014). There is a vast number of scientific studies that have shown undesirable effects of tannins in poultry diets because of decreased feed intake and decreased protein digestibility (Barroga et al., 1985; Longstaff andMcNab, 1991; Hidayat et al., 2021). Nevertheless, it is obvious that the effects of tannins in poultry diets are dose-dependent (Choi andKim 2020), and that low to moderate dosages are able to improve animal performance and health (Redondo et al., 2014). These optimal dosages have been shown to improve productive performance as

well as antimicrobial, antioxidative and anti-inflammatory functions (Schiavone et al., 2008; Starčević et al., 2015; Cengiz et al., 2017; Tonda et al., 2018).

While specific effects of different tannins have been extensively studied, the potential of tannin-soy protein isolate complexes as alternative poultry growth promoters remains largely unexplored, particularly concerning their impact on poultry production, fermentation, immune response, and lipid oxidation. Based on this gap, the hypothesis guiding the present study is that the tannin-soy protein isolate complex may exert distinct and potentially superior effects compared to isolated chestnut tannin alone, thereby serving as a more effective natural growth promoter in poultry.

MATERIALS AND METHODS

Animal study, experimental design and experimental diets

All procedures have been carried out in accordance with the European Union Directive on the protection of animals used for scientific purposes according to European Parliament, Council of the European Union (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, 2010). The research was approved by the Institutional Ethics Committee (University of Zagreb, Faculty of Veterinary Medicine, September 22nd, 2022, 640-01/21-02/09, 251-61-01/139-21-66)

During the 42-day trial, 120 male broilers of the Ross 308 strain were used. All birds were raised on a solid floor with wood shavings (3 kg/m^2) and provided free access to feed and water. The temperature was set at 33 °C at the age of 1 to 7 days and then reduced by 3 °C per week to a final temperature of around 24 °C. The humidity was set at 60 to 65% at the age of 1 to 7d and then 50 to 60%. The birds were vaccinated according to the normal immunization program (Newcastle disease and infectious bronchitis). In a three-phase feeding program, the starter, grower and finisher were offered during days 1–21, 22–35 and 36–42, respectively. Feed and water were offered for ad libitum consumption. The diets were offered to the birds in the meal form, and the chemical composition of the diets is shown in Table 1. Samples of the feed mixture were ground and analysed according to AOAC procedures (AOAC, 1995). There were three treatments, and each treatment had four replicates with ten birds per replicate pen. The treatments differed in the supplement added to the feed: control (CON group), 0.75 g/kg feed of chestnut tannin (Farmatan, Sevnica, Slovenia, FAR group) and 0.75 g/kg feed of chestnut tannin-soy protein isolate complex (CPS, Sevnica, Slovenia, CPS group).

On the 42nd day, the birds were exsanguinated after electrical stunning. The tissue samples of the breast muscle (*m. pectoralis superficialis*) and liver were harvested immediately after slaughter, frozen in liquid nitrogen for 90 seconds, individually wrapped in aluminium foil and transported to the laboratory.

Animal growth performance

Body weight (BW) was recorded weekly for all birds. Body weight gain, feed consumption, and feed conversion (g feed/g of gain) were calculated pen-wise for the respective periods.

Short-chain fatty acids determination

Caecal digesta from both caeca were carefully squeezed into a sterile tube and frozen at −80 °C until analysis (20 samples per group were analysed). To determine the short-chain fatty acids (SCFA), approximately 1 g of the thawed digesta was diluted with 1 mL ultrapure water and centrifuged. After centrifugation (10 minutes at 5000 × g), the supernatant was transferred to another tube. The sample was placed in an ice bath to allow the protein to settle completely. Finally, the samples were centrifuged (10 minutes at 5000 × g), and the clear supernatant was analyzed.

The supernatants were analyzed to determine SCFA concentrations using a Shimadzu GC2010Plus gas chromatograph (Shimadzu, Japan) equipped with a model AOC 20i autoinjector, a flame ionization detector (FID) and a NukolTM column (30 m × 0.25 mm × 0.25 µm)

(Supelco, Bellefonte, PA, USA). The chromatographic conditions were helium as carrier gas, split ratio 1:100, injection volume 1 µl, injector temperature 220 °C, detector temperature 230 °C, oven temperature program with initial 100 °C and increase by 20 °C/min to 140 °C and 8 °C/min to 200 °C. The internal standard used was crotonic acid.

Fecal Escherichia coli quantification

For the quantification of *Escherichia coli* (*E. coli*), fresh faecal droppings of chickens in the research facilities were collected (45 g of faeces per replicate) and prepared for further analyses. Each sample was diluted 1:2 with sterile saline solution from which serial dilutions 10^3 - 10^8 were prepared for culturing. An amount of 100 µl of each dilution was transferred to a Brilliance UTI Clarity chromogenic agar plate (Oxoid, Basingstoke, UK) and streaked with a sterile 10 µl loop (Golias, Ljubljana, Slovenia). All plates were incubated in aerobic conditions at 37 °C for 24 hours. As Brilliance UTI Clarity agar is a non-selective, differential growth medium, *E. coli* colonies were easily differentiated from other bacterial species due to their specific macroscopic features. The plates were photographed, and the number of bacterial colonies was counted using IC Measure software v1.0. The plates with a total count of 20 - 400 bacterial colonies were

Table 1. Ingredients and nutrient content of the diets fed during the trial

Ingredients and nutrient content (g/kg< unless otherwise stated)	Starter (1st to 21st day)	Grower (22nd to 35th day)	Finisher (35th to 42nd day)
Ingredients			
Corn	455	600	688
Wheat bran	125	30	–
Soybean meal	215	125	180
Alfalfa meal	–	–	30
Yeast	50	50	–
Sunflower meal	100	145	45
Vegetable oil	5.0	–	7.0
Mineral and vitamin mixture*	50	50	50
Calculated nutrient content			
Crude protein	221.5	209.2	195.2
Crude fat	69.0	70.5	71.3
Crude fibre	33.1	32.1	32.0
Calcium	10.3	10.1	10.6
Phosphorus	6.6	6.3	6.2
Lysine	14.2	12.7	11.5
Methionine	6.0	5.8	5.9
Metabolisable energy (MJ)	12.4	13.0	13.3

* Comprising per kg of mineral and vitamin mixture: Ca, 180 g; P, 50 g; Na, 23 g; methionine, 50000 mg; lysine, 24000 mg; retinol acetate, 90 mg; cholecalciferol, 1mg; DL-α-tocopheryl acetate, 600 mg; menadione, 40 mg; thiamine hydrochloride, 20 mg; riboflavin sodium phosphate, 120 mg; pyridoxine, 40 mg; cyanocobalamine, 300 µg; C, 300 mg; niacin, 800 mg; calcium pantothenate, 240 mg; folic acid, 10 mg, biotin, 2 mg; choline chloride, 10 g; Fe, 1200 mg; I, 1200 mg; Cu, 100 mg; Mn, 1600 mg; Co, 3 mg; Zn, 1000 mg; Se, 3 mg; BHT antioxidant, 3000 mg.

selected as representative (O'Toole, 2016). For each replicate, the colony-forming unit (CFU) per gram of feces was calculated as a final result.

Determination of fatty acid profile by gas chromatography

Half of the samples of *m. pectoralis superficialis* were analysed fresh immediately after the slaughter, and the other half was analysed after 6 months (these samples were frozen at -20 °C for six months to imitate normal meat storage procedures). For fatty acid analyses, 18 samples per group were analysed as fresh and 18 samples as frozen. Tissue was homogenized on ice, and total lipids were extracted using a chloroform/methanol mixture (2:1, v/v) (Folch et al., 1957). The lipids were dried under N2, dissolved in the same mixture (150 µL) with the addition of 0.3 mg/mL BHT, and stored at −80 °C. Fatty acid methyl esters were obtained by base-catalyzed transmethylation (2M KOH in methanol) in the presence of internal standard (C19:0). The obtained fatty acid methyl esters solution was analyzed by gas chromatography (Gas Chromatograph GC 2010 Plus; Shimadzu, Kyoto, Japan) using a BPX70 capillary column (0.25 mm internal diameter, 0.25 µm film thickness, 30 m long, SGE, Austin, TX, USA) and adopting previously established conditions (Starčević et al., 2015). The FAMEs were identified by comparing the retention times to those of a standard FAME mixture (Sigma–Aldrich, Steinheim, Germany). Fatty acid composition was calculated as a percentage of each individual fatty acid relative to total fatty acids.

Determination of malondialdehyde (thiobarbituric acid-reactive substances)

Malonaldehyde (MDA) content was measured as thiobarbituric acid reactive substances (TBARS) (Agarwal and Chase, 2002). Samples were injected onto a Shimadzu LC-2010HT with an Inert-Sustain C18 column (4.6 mm x 150 mm, 5 µm particle size; GL Sciences, Tokyo, Japan). The standard curve was prepared using 1,1,3,3-tetraethoxypropane. Thiobarbituric acid-reacting substances (TBARS) were expressed as mmol per gram of wet tissue. Similar to the fatty acids, half of the samples for MDA analyses were analysed fresh after the slaughter, and the other half was analyzed after 6 months (the samples were frozen at -20 °C for six months, 18 samples per group were analysed as fresh and 18 samples as frozen).

Quantitative PCR

The hepatic tissue samples were frozen -80 °C until the analysis. The isolation of total RNA from 30 mg of liver tissue was performed with an SV Total RNA Isolation System (Promega GMBH, Mannheim, Germany), according to the manufacturer's instructions (12 samples per group were analysed). Total RNA quantity and purity were measured by spectrophotometry using BioDrop (BioDrop, LITE, BioDrop, Cambridge, UK). Reverse transcription and quantification of the isolated RNA were performed by One-Step SYBR PrimeScript RT-PCR Kit II (Perfect Real Time, TaKaRa Bio Inc. Shiga, Otsu, Japan) according to the manufacturer's manual, using a Stratagene MxPro3005 (Agilent Technologies, US and Canada) thermocycler.

The oligonucleotides used for quantitative PCR were:

IL-6 F, 5′-caaggtgacggaggaggac-3′, IL-6 R, 5′-tggcgaggagggatttct-3′, TLR4 F, 5′-gtctctccttccttacctgctgttc-3′, TLR4 R, 5′-aggaggagaaagacagggtaggtg-3′, TNF-α R, 5′- agcgttgtctgctctgtagc -3′, TNF-α F, 5′- cctgctgggggaatgctagg -3′, β-Actin F, 5′- caacacagtgctgtctggtgg -3′, β-Actin R, 5′- atcgtactcctgcttgctgat -3′, GAPDH F, 5′- actttggcattgtggagggt -3′, GAPDH R, 5′-ggacgctgggatgatgttct -3′

(Lu et al., 2009, Mountzouris et al., 2020, Rodríguez et al., 2023).

Gene expression was relatively quantified using the ΔΔCt method (2-ΔΔCt) after it was normalized to the expression level of the housekeeping genes (Livak and Schmittgen, 2001). Data are presented as the fold change in gene expression relative to the corresponding control group.

Statistical analyses

Data were analysed using GraphPad Prism 8 v. 8.4.3. software (GraphPad Software Inc., San Diego, CA, USA). All data were expressed as mean ± standard deviation (SD). The normality of the distribution was tested using the Shapiro-Wilks test. ANOVA and the post-hoc Tukey test were used to determine statistical differences between group means. The standard deviations of fold changes for the tested gene expression were determined according to the established procedure (Livak and Schmittgen, 2001). Significant differences were considered at $P<0.05$.

RESULTS

Growth performance

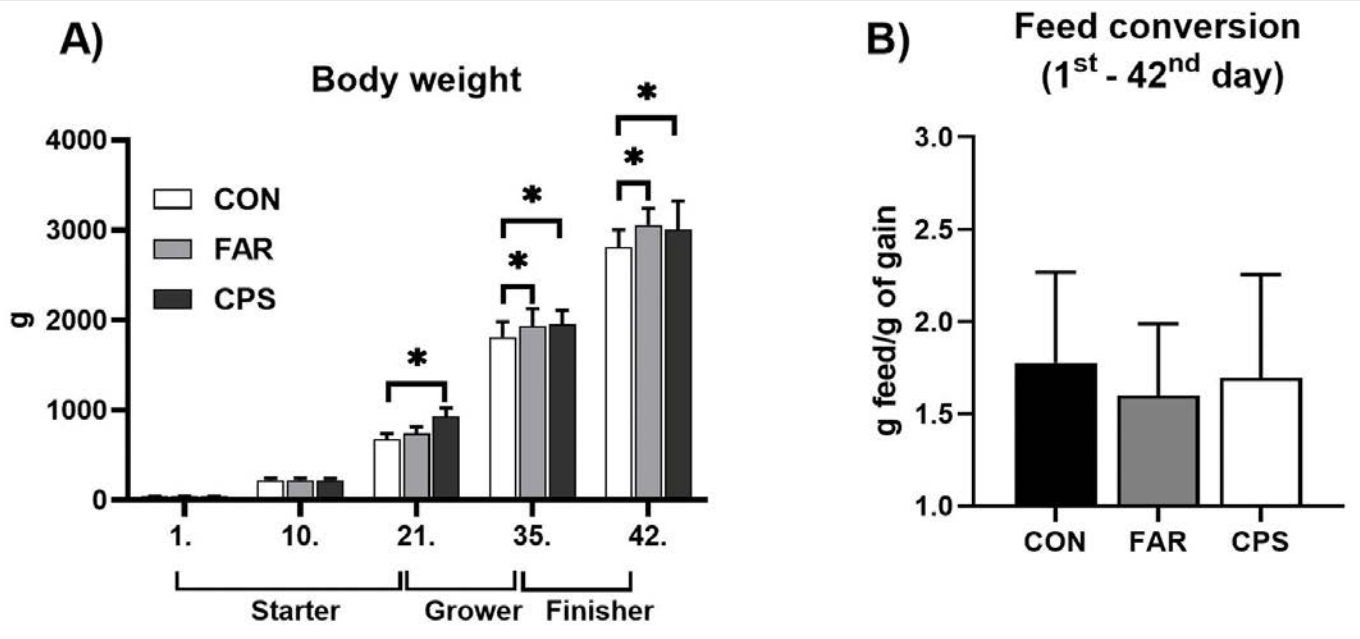
The data on growth performance and feed conversion of broiler chickens fed the experimental diets are shown in Figures 1A and B. The tannin groups had higher final body weight compared to the control group (+8% and +6% for the FAR and CPS groups compared to the control group). Despite these positive trends in the tannin-supplemented groups, we did not find any significant differences in feed conversion between the experimental groups.

Caecal SCFA production, fecal E. coli count and immune response

SCFA concentrations in the caeca of birds fed experimental diets are shown in Figure 2A-D. Tannin-containing diets increased the caecal concentrations of acetate, butyrate and total SCFA in the tannin-treated groups compared to the control. In the tannin-treated groups, the CPS groups had higher concentrations of acetic acid and butyric acid and total SCFA compared to the FAR group.

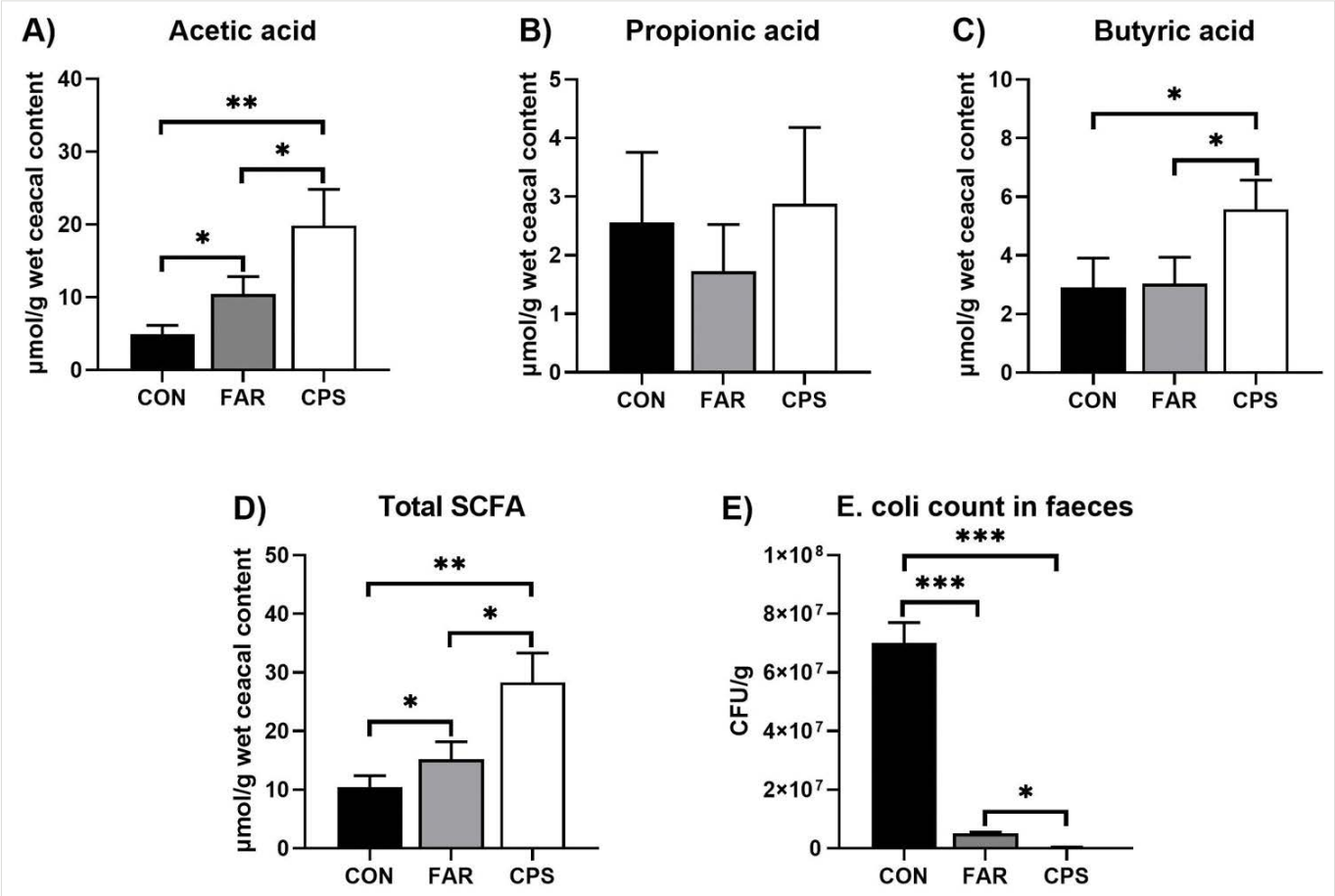
The fecal count of *E. coli* was drastically reduced in the tannin-treated groups, and even further in the CPS group (Figure 2E).

The mRNA expression of proinflammatory genes was influenced by tannin source (Figure 3). The CPS group had significantly lower expression of TNF-α and IL-6 compared to the Control and FAR groups. The expression of TLR4 was significantly lower in both tannin-supplemented groups compared to the Control.



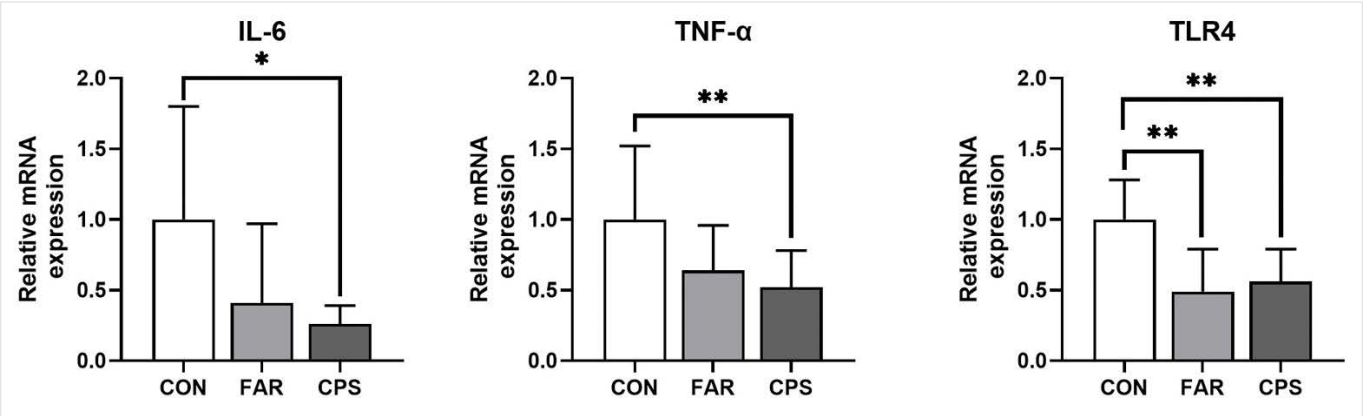
Values are presented as mean values ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Figure 1. Growth performance of experimental birds:
A) Body weight at the 42nd day and
B) Feed conversion (g feed/g of gain) 1st – 42nd day



Values are means ± SD. *P < 0.05, **P < 0.01, ***P < 0.001

Figure 2. Cecal SCFA content determined by gas chromatography:
A) Acetic acid,
B) Propionic acid,
C) Butyric acid and
D) Total SCFA Fecal *E. coli* count (E)



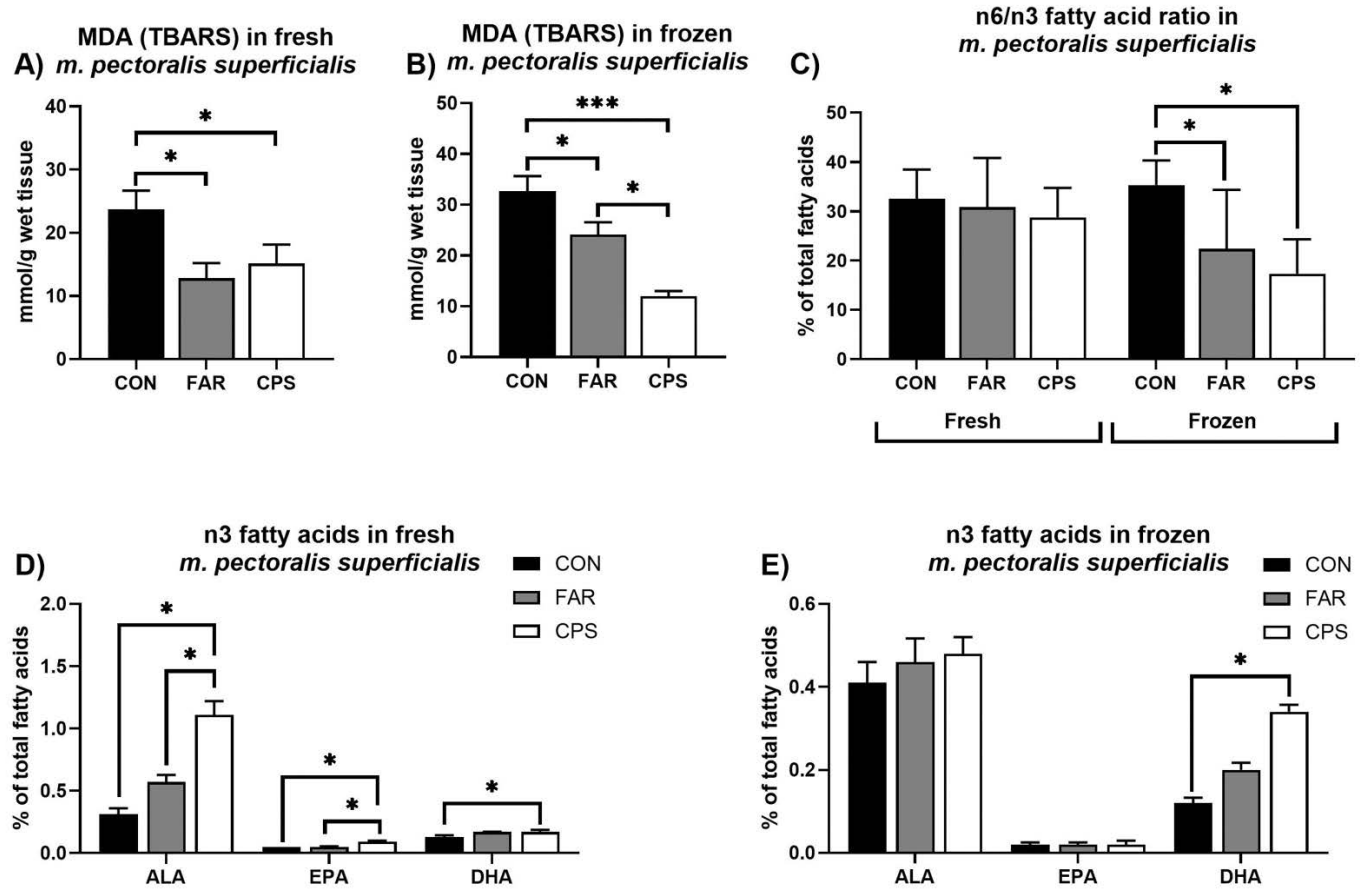
Values are presented as mean values ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 3. The expression of different proinflammatory genes: Interleukin 6 (IL 6) (A), Tumor necrosis factor alpha (TNF-α) (B) and Toll-like receptor 4 (TLR4) (C)

Oxidative susceptibility and fatty acid profile

Lipid peroxidation was measured by malondialdehyde concentration (MDA, TBARS) (Figure 4A, B) on day 42 (fresh tissue) and at day 220 (tissue frozen for 6 months at -20 °C) in breast tissue (*m. pectoralis superficialis*). At day 42 (fresh tissue), MDA levels were significantly lower in the tannin-treated groups than in the control group, with no difference between the tannin groups. A similar trend was also seen at day 220, with a significant difference between the tannin-treated groups compared to the control group, but also with a lower value for the CPS group compared to the FAR group.

Fatty acid composition of the breast (*m. pectoralis superficialis*) tissue is shown in Figure 4C-E. In the fresh breast tissue, tannin supplementation preserved the main n3 fatty acids: ALA, EPA and DHA. The same trend was also partially visible in frozen tissue (DHA). These preserved quantities of the main n3 fatty acids also led to a decreased n6/n3 fatty acid ratio in the frozen breast tissue.



Samples of *m. pectoralis superficialis* were analysed fresh on day 42 of the experiment or frozen at -20 °C for 6 months on day 220. Values are means ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

Figure 4. Malondialdehyde concentration (MDA-TBARS) in fresh *m. pectoralis superficialis* (A) and in frozen condition (B). Ratio of n6 and n3 fatty acids in fresh and frozen *m. pectoralis superficialis* (C). N3 fatty acids (ALA, EPA and DHA) in fresh *m. pectoralis superficialis* (D) and in frozen m. pectoralis superficialis (E)

DISCUSSION

Growth performance

Traditionally, tannins were considered antinutritional factors in the production of poultry and other monogastric animals. That viewpoint was based on decreased feed intake and impaired growth performance observable after tannin application in feed (Ramah et al., 2020). These toxic effects include: binding of proteins, which impair dietary protein digestion and activity of digestive enzymes (Medugu et al., 2012), astringent taste of the feed, which lowers palatability and feed intake (Butler et al., 1984), forming complexes with iron, which impair iron plasma status (Lee et al., 2010) and increase in liver proteolytic activity (Marzo et al., 2002). It is now well established that plant-based feed additives affect broiler growth and feed conversion efficiency. However, this effect strongly depends on dosage. In contrast, using the lower quantities could result in a complete lack of effect. Therefore, determining the effective dosage is of utmost importance in commercializing plant-derived growth promotors. Some authors, therefore, proposed a tentative range of 0.5 g/kg to 5 g/kg of tannic acid (representative for hydrolysable tannins) as an optimal dose in poultry diet that could improve growth rate and gut health (Choi and Kim, 2020). Our dosage was well within that optimal range, which could explain the absence of negative effects.

Caecal SCFA production and fecal Escherichia coli count

The concentration of SCFA in chickens is time-dependent and acetate is the first one to be detected in chicken ceca (on day 3), while propionic and butyric acid can be detected between day 12 and 15, with the stabilization of all SCFA concentration around day 15 (van Der Wielen et al., 2000). During the growth period, chickens develop a natural resistance to some pathogenic bacteria due to the establishment of native microflora. This resistance is attributed to sufficient concentrations of SCFA and low pH, which can reduce the number of Enterobacteriaceae in the caeca of broiler chickens from the 2nd week onwards (Barnes et al., 1979; van Der Wielen et al., 2000). Our previous experiment has shown that phenolic compounds affect fermentation in the caecum of broiler chickens differently; tannic and gallic acid increase the concentration of SCFA, and thymol decreases (Mašek et al., 2014). An increase in SCFA concentration after tannin administration was also observed in the caecum of other monogastric animals, e.g. rats (Barszcz et al., 2011).

As already mentioned, an increase in SCFA inhibits Enterobacteriaceae growth in the caeca, which was indicated in our trial with a significant decrease in *E. coli* number. Interestingly, the decrease in *E. coli* number was even higher in the tannin–soy protein isolate complex than in the pure tannin group. Phenolic compounds have significant antimicrobial activity, but with significant differences among them (Dong et al., 2018). The exact mechanism of their antimicrobial action is an active area of research, and it is evident that it involves many sites of action at the cellular level (Sikkema et al., 1995). Studies suggest that the antibacterial activity of most phenolic substances is related to the interactions between the substance and the bacterial cell surface (Bouarab-Chibane et al., 2019). In our trial, pure tannin and tannin soy protein isolate complex had some differences in the inhibitory effects on *E. coli* count. That difference should be further evaluated *in vitro*.

We measured the expression of various proinflammatory genes, as there is some evidence that hydrolysable tannins and their products, ellagitannins and gallotannins, can influence the immune and inflammatory response (Marrone et al., 2023; Xu et al., 2023). Our data showed that the decrease in TNF-α and IL-6 mRNA expression was related to the source of tannin (tannin–soy protein isolate complex vs. pure tannin). Interestingly, the influence of tannin on the immune response is dose-dependent, with positive effects when using a low dose (0.5 g/kg diet), and with negative or no effects when using a high dose of tannin (30 g/kg diet) (Ramah et al., 2020).

Oxidative susceptibility and fatty acid profile

Phenolic compounds are best known for their antioxidant properties (Rice-Evans et al., 1997). The antioxidant capacity of phenolic compounds could be measured in vivo as a reduction in the accumulation of lipid peroxidation products (MDA). Therefore, we measured the MDA concentration in chicken breast tissue and found a significant decrease in lipid peroxidation. These changes were previously observed after dietary supplementation with various tannins and other phenolic compounds (Mašek et al., 2014; Starčević et al., 2015). To further evaluate the possibility of using the phenolic mixture as a natural endogenous antioxidant, we froze the samples for 6 months, and the positive effects of the tannins were visible in the tissue even after prolonged freezing. These results suggest that tannins can positively affect lipid peroxidation in both fresh chicken tissue and tissue frozen at −20 °C for 6 months. We were also interested in whether these effects could also be present in the fatty acid profile, as previous studies have shown that dietary phenols can affect the fatty acid profile of edible tissues (Ebrahim et al., 2015; Starčević et al., 2015). The fatty acids most susceptible to oxidation in edible meat are long-chain PUFAs with two or more double bonds (Santos-Filho et al., 2005). These fatty acids include DHA and EPA, which are nowadays considered very important for human and animal health. Therefore, we measured their concentration in the frozen meat. The results showed that the concentration of EPA and DHA was higher in the fresh meat and in the frozen meat. These results suggest that supplementation with tannin can influence the amount of long-chain n-3 polyunsaturated fatty acids and maintain their amount in frozen meat. Obviously, the total concentrations of DHA and EPA are relatively low, and the preservation effects of tannins do not have a major nutritional impact. However, in animals with higher levels of long-chain n3-PUFAs supplied in the diet (linseed oil, fish oil, etc.), this preservation effect could be much more significant.

CONCLUSIONS

The interest in natural compounds with antimicrobial and antioxidative properties significantly increased after the ban on antibiotics as growth promoters in animal feed, which was a result of a global recognition of antimicrobial resistance as a severe threat. Nowadays, one of the promising and heavily investigated compounds is hydrolysable and condensed tannins. The main finding of our study was that the source of tannin (tannin–soy protein isolate complex vs. pure tannin) may exert differential effects on the health-promoting effects of hydrolysable tannins. However, the limitation of our study is that specific mechanisms underlying the differences in mode of action between pure tannin and the tannin-soy protein isolate complex were not completely elucidated. Therefore, further research is needed to identify optimal interactions, determine appropriate dosages, and develop effective in-field application methods.

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