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Polyphenol Content of Plasma and Litter after the Oral Administration of Green Tea and Tea Polyphenols in Chickens

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ABSTRACT: Metabolic profiles of broiler chickens were examined after the ingestion of green tea, tea polyphenols, and (−)-epigallocatechin-3-gallate (EGCG). Solid-phase extraction of serum and litters yielded free catechins and their metabolites, which were then identified and quantified by liquid chromatography−tandem mass spectrometry. In plasma samples, (−)-gallocatechin, (+)-catechin, and EGCG were detected in the green tea group; pyrogallol acid, (epi)catechin-*O*-sulfate, 4′-*O*-methyl-(epi)gallocatechin-*O*-glucuronide, and (epi)catechin-3′-*O*-glucuronide were detected in the tea polyphenols group; and EGCG, (−)-gallocatechin gallate (GCG), and 4′-*O*-methyl-(epi)gallocatechin-*O*-glucuronides were detected in the EGCG group. In litters, gallic acid, EGCG, GCG, and ECG were detected in the green tea and tea polyphenols groups; EGCG and ECG were detected in the EGCG group. The conjugated metabolites, 4′-*O*-methyl-(epi)gallocatechin-*O*-glucuronide, (epi)catechin-3′-glucuronide, and 4′-*O*-methyl-(epi)catechin-*O*-sulfate, were identified in the green tea group; 4′-*O*-methyl-(epi)catechin-*O*-sulfate and 4′-*O*-methyl-(epi)gallocatechin-*O*-sulfate were identified in the tea polyphenols group; only 4′-*O*-methyl-(epi)gallocatechin-*O*-sulfate was detected in the EGCG group. The excretion of tea catechins was 95.8, 87.7, and 97.7% for the green tea, tea polyphenols, and EGCG groups, respectively.

KEYWORDS: *green tea, tea polyphenols, EGCG, absorption, metabolites, broilers*

INTRODUCTION

Tea polyphenols are a family of bioactive compounds present in green tea. During the past decade, green tea has attracted considerable attention for specific health claims regarding its antioxidant properties, which are due to the presence of functional polyphenols known as tea catechins. Tea catechins include the main flavonoids, (−)-epigallocatechin-3-gallate (EGCG), (epi)gallocatechin (EGC), (epi)catechin-3-gallate (ECG), and epicatechin (EC).[1] These four major catechins account for up to 40% of the dry weight of green tea, and purified EGCG can account for 50−80% of the total catechins.[2,3] Catechin structures are usually characterized by di- or trihydroxyl group substitutions on the B ring and *meta*-5,7-dihydroxyl on the A ring.[4]

Green tea added to animal feeds has the potential to improve the quality of animal-derived products. Cho et al.[5] added 5% of various green teas to the feed of juvenile olive flounder to improve growth, body composition, and blood chemistry. The supplementation of feedstuffs with 1−4% (w/w) of green tea powders for 2 weeks did not affect the performance of broiler chickens but reduced the level of lipids in meat.[6] Feeding chickens with a diet containing 200 mg/kg of tea catechins reduced lipid oxidation in the meat, liver, and heart.[7] The supplementation of chicken feed with 50−300 mg/kg of tea catechins inhibited the lipid oxidation of breast and thigh meat stored at −20 °C for long periods of time.[8] Recently, Hassanpour et al.[9] evaluated the effects of adding 2−4% green tea to feed on the intestinal morphology and nitric oxide production of broiler chickens. Many reports in the literature have focused mainly on understanding the pharmacokinetics, absorption and distribution properties, metabolism, and excretion of green tea, tea polyphenols, and their monomers in studies on humans,[4,10−12] mice,[4,13] and dogs.[14] However, few studies have reported the bioavailability of green tea and tea polyphenols in chickens.

This present study aimed to investigate the absorption and metabolism of tea polyphenols in broiler chickens after the oral administration of 500 mg/kg green tea, 100 mg/kg tea polyphenols, and 50 mg/kg EGCG. Tea polyphenols and their metabolites were analyzed in plasma and litter samples collected at three time points within 8 h after intake. Catechin metabolites were identified by solid-phase extraction (SPE) and liquid chromatography−tandem mass spectrometry (LC-MS/MS). Thus, the purpose of this study was to provide an insight into the metabolic changes observable after the administration of tea catechins, which may improve our understanding of the actions played by tea catechins in broiler chicken nutrition.

MATERIALS AND METHODS

Reagents and Materials. Tea polyphenols (lot no.: 200807011, QB2154-95) were purchased from Anhui Redstar Pharmaceutical Co., Ltd. (Anhui Province, China), and these contained (w/w) 45.5% of EGCG, 16.3% of EGC, 10.4% of ECG, 7.4% of EC, 8.0% of (−)-gallocatechin gallate (GCG), and 7.3% (w/w) of (+)-catechin. Green tea was a gift from Anhui Redstar Pharmaceutical Co., Ltd., and the main composition in the green tea was determined according to the method described by Wu et al.[15] with slight modifications; this was as follows (w/w): moisture, 4.58%; EGCG, 16.10%; EGC, 5.83%; ECG, 2.08%; EC, 1.76%; GCG, 1.03%; (+)-catechin, 0.86% (w/w);

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and caffeine, 10.51%. Commercial standards (purity >98%) of EGCG, EGC, ECG, EC, and their corresponding epimers, (−)-gallocatechin (GC), (+)-catechin, GCG, (−)-gallic acid (GA), and ethyl gallate (EG), were purchased from Sigma-Aldrich (a subsidiary company in Shanghai, China). EG (used as an internal standard), L-ascorbic acid, ethylenediaminetetraacetic acid disodium salt dehydrate (Na_2-EDTA), *N*,*N*-dimethylformamide (DMF), and high-performance liquid chromatography (HPLC)-grade acetonitrile, methanol, phosphoric acid (85%), and ethyl acetate were also obtained from Sigma-Aldrich. Water was purified using a Milli-Q system (Millipore, Bedford, MA). All other reagents were of analytical or guaranteed reagent grade from Zhihong Biotech Co., Ltd. (Hefei, China). SPE cartridges were Oasis HLB (30 mg/mL) and Sep-PakC (100 mg/mL) from Zhihong Biotech Co., Ltd.

Standard Solutions. Standard samples were prepared using a method developed by Unno et al.[10] with slight modifications. Stock solutions of individual standard samples (50 μg) were prepared in 0.1 M sodium phosphate buffer containing 0.5 mM Na_2-EDTA (pH 3.5) and stored at −20 °C until use. Working stock solutions were made using the same buffer. The stock solution of the internal standard (50 μg/mL) was also diluted using the same buffer to give working solutions containing 0.001, 0.01, and 1 μg/mL of EG. The prepared solutions were used to determine the recovery ratio and the retention times of the individual and standard sample mixture during LC and the retention times of individual standard samples during LC-MS/MS.

Animal Handling and Sample Collection. Thirty-six 5 week old commercial broiler chickens (1.2−1.3 kg body weight, Ross 308) were used. After 3 days of acclimatization, the animals were randomly divided into four groups. Each group consisted of three replicates, and the birds were caged at room temperature. Feed was removed 12 h prior to blood and litters collection. Then, chickens in the control group were orally administered 10 mL of deionized water only by the injector, while the other groups were administered 500 mg of green tea powder/body weight (equivalent to 137.85 mg of total catechins body weight), 100 mg of tea polyphenols body weight (equivalent to 93.9 mg of total tea catechins body weight), and 50 mg of EGCG body weight (equivalent to 49 mg of pure EGCG body weight) with the fiber container; chickens in each experimental group were then fed 10 mL of deionized water with the injector, respectively. After administering the treatment, blood was taken from the left wing vein at 1.5, 4, and 8 h using vacutainer tubes containing ascorbic acid (0.4 M NaH_2PO_4 buffer supplemented with 20% vitamin C−0.1% EDTA, pH 3.6). Plasma was isolated by centrifugation at 1500*g* for 10 min at 4 °C, and 1 mL aliquots of the supernatant were frozen at −20 °C for later analysis.[16] Feces (litter samples) were collected after the administration of green tea, tea polyphenols, or EGCG between 0 and 1.5, 1.5 and 4, and 4 and 8 h, but the feces from control birds were collected between 0 and 1.5 h, as nothing was excreted after 1.5 h in these birds. The litters were cooled and lyophilized for analysis.

Plasma Analysis. Plasma was treated according to the method described by Mata-Bilbao et al.[14] with slight modifications. Briefly, 200 μL of the thawed plasma sample containing 10 μL of 10% (w/v) L-ascorbic acid was added to 10 μL of 0.01 μg/mL internal standard. Plasma samples were diluted by adding 0.7 mL of 0.1 M phosphate buffer containing 0.5 mM Na_2-EDTA (pH 3.5), and then, the samples were applied directly to Oasis HLB cartridges. These cartridges had been preconditioned with 1 mL of water, followed by 1 mL of 70% (v/v) DMF containing 0.1% (v/v) phosphoric acid, and finally 1 mL of water. After this was allowed to run through, the cartridge was washed with 2 mL of water and 1 mL of 30% (v/v) methanol. Then, tea catechins were eluted with 0.7 mL of 70% (v/v) DMF containing 0.1% (v/v) phosphoric acid. After filtration through a 4 mm 0.45 μm PTFE filter (Waters Corp., United States), 20 μL of sample was injected into the LC system.

Handling of Litter Samples. The litter samples collected from the smooth plastic under the cages were lyophilized and crushed to a fine powder using a mortar and pestle. The litters were pre pared based on the procedure reported by Breinholt et al.[13] with slight modifications. To three aliquots containing 1 mg of litter was added 0.5 mL of 90% aqueous methanol and 1 μg/mL of internal standard (suspended in 200 μL of DMSO). Samples were vortexed and extracted overnight in the dark under continuous agitation. After this time, the samples were centrifuged at 3000*g* for 10 min at 4 °C, and the supernatant was transferred to a new vial. This extraction procedure was repeated between two and four times. Then, SPE was performed as for the plasma samples above until >95% of the internal standard had been extracted, which was verified by HPLC analysis of the extract (see below). The supernatants were combined, evaporated in vacuo, and passed through a 4 mm 0.45 μm PTFE filter for LC analysis.

Separation and Identification of Metabolites Using LC and LC-MS/MS. Catechins and their metabolites in the plasma and litter samples were quantified and identified using LC and LC-MS/MS analyses according to slightly modified methods of those described by Mata-Bilbao et al.[14] LC analysis was performed using a Perkin-Elmer series 200 machine (Norwalk, United States) equipped with a quaternary pump and a refrigerated autosampler. A Phenomenex Luna 5 μm C18 (2) RP column [100 Å, 250 mm × 4.6 mm (internal diameter)] was used at 35 °C with an injection volume of 20 μL. Detection was carried out simultaneously at 280 and 369 nm. Solvent A was 0.2% acetic acid, and solvent B was 100% acetonitrile. The following gradient was run at a flow rate of 1 mL/min: 0−32 min, 92% A and 8% B (v/v); 32−50 min: 50% B and 50% A (v/v); 50−58 min, 100% B and 0% A (v/v); and 58−60 min, 8% B and 92% A (v/v). A triple quadrupole mass spectrometer (API 3000; Applied Biosystems, PE Sciex, Concord, Ontario, Canada) equipped with a Turbo Ion spray source was used to obtain the MS and MS/MS data. Prior to its use, the instrument was adjusted to meet the acceptance specifications defined by the manufacturer. Turbo ion spray source settings were as follows: capillary voltage, 3500 V; nebulizer gas (N_2), 10 arbitrary units (AU); curtain gas (N_2), 12 AU; collision gas (N_2), 4 AU; focusing potential, 200 V; entrance potential, 10 V; and drying gas (N_2) heated to 400 °C; and the sample was introduced at a flow rate of 8000 cm^3/min. The declustering potential and the collision energy were optimized for each compound in infusion experiments: individual standard solutions (0.1 to 1 μg/mL) dissolved in 80:20 mobile phase were infused at a constant flow rate of 5 μL/min into the mass spectrometer using a model syringe pump (Harvard Apparatus, Holliston, MA). Full scan data were acquired by scanning from m/z 100 to 1000 in profile mode, using a cycle time of 2 s with a step size of 0.1 U. For MS/MS, a product ion scan utilizing a cycle time of 2 s was used. MS/MS product ions were produced by collision-activated dissociation of the selected precursor ions in the collision cell of the triple-quadrupole mass spectrometer, and the mass was analyzed using the second analyzer of the instrument. Multiple reaction monitoring (MRM), the method of choice due to its high selectivity and sensitivity in quantitative LC-MS/MS, monitored several transitions for each analysis. Both quadrupoles (Q1 and Q3) were operated at unit resolution. The criteria for identifying free catechins and their metabolites were based on three parameters: comparison of the retention time with those available, MRM metabolite transition, and product ion spectra.

Catechins Extraction Efficiencies. The efficiency of the extraction of catechins was evaluated by spiking 200 μL blank samples from the control group with 0.01 μg/mL for plasma or 1 μg/mL for litters of internal standards, EGCG, EGC, ECG, EC, GC, (+)-catechin, GCG, and GA. The spiked samples were extracted as described above and analyzed in triplicate by LC. The percentage recoveries of the individual catechins and the internal standard (EG) are presented in Table 1. The recovery of each catechin is also expressed as a ratio relative to the recovery of EG. Subsequently, this ratio was used in combination with the recovery of the EG internal standard to assess the recoveries of free catechins and their metabolites from plasma and litter samples.

RESULTS

Identification of Catechins and Their Metabolites in Plasma and Litters. Plasma and litter samples were collected after the administration of 500 mg/kg green tea, 100 mg/kg tea

Table 1. Recoveries of Free Catechins Spiked in Blank Plasma and Fecal Samples

			plasma		feces	
peak no.	standard sample	R_t (min)	recovery (%)[a]	ratio[b]	recovery (%)	ratio
1	GA	5.77	87.45 ± 3.69	1.09	84.52 ± 2.75	1.13
2	GC	9.35	75.34 ± 2.54	1.27	73.78 ± 3.39	1.29
3	(−)-epigallocatechin	17.27	90.19 ± 6.32	1.06	88.82 ± 4.21	1.07
4	(+)-catechin	20.38	89.52 ± 5.89	1.07	87.64 ± 4.09	1.09
5	(−)-epicatechin	26.39	85.68 ± 7.98	1.11	82.77 ± 6.26	1.14
6	(−)-epigallocatechin gallate	27.47	82.94 ± 4.09	1.01	90.62 ± 5.16	1.05
7	GCG	29.87	82.69 ± 6.67	1.15	80.38 ± 7.01	1.19
8	(−)-epicatechin gallate	37.10	79.44 ± 4.76	1.20	66.75 ± 5.29	1.43
I.S.	EG	38.57	95.35 ± 5.29	1.00	95.76 ± 6.34	1.00

[a]Data are expressed as means ± standard errors (n = 9). [b]Data are expressed as a ratio relative to the recovery of EG. R_t, retention time.

polyphenols, and 50 mg/kg EGCG. The samples were analyzed by LC (Figures 1 and 2), and then, the composition of each single peak was identified by LC-MS/MS (Table 2). Four out of the eight free catechins, namely, GC, (+)-catechin, EGCG, and GCG, were detected in the plasma. For the litter samples, four out of the eight free catechins, namely, GA, EGCG, GCG, and ECG, were detected in litters. No metabolites of GA, EGC, EC, and ECG were detected in plasma, and no metabolites of GC, EGC, (+)-catechin, and EC were detected in litters. The identification of the complex array of glucuronide, sulfate, and methylation metabolites of (epi)catechin and EGCs is summarized in Table 2.

On the basis of the chromatogram peaks in Figures 1 and 2, peak 1 (R_t = 5.77 min) was found only in litters from groups receiving green tea and tea polyphenols. This peak had a negatively charged molecular ion ($[m - H]^-$) at m/z 169, which on MS^2 did not give a fragment ion. Peak 1 was identified as GA according to the retention time on the chromatogram and LC-MS/MS of the GA standard. Peak 2 (R_t = 9.35 min) and peak 4 (R_t = 20.38 min) occurred only in plasma from the green tea and tea polyphenols groups, and these peaks gave $[m - H]^-$ ions at m/z 305 and m/z 289, respectively. However, neither of these ions gave fragment ions on MS^2. The compounds were identified as GC (m/z 305) and (+)-catechin (m/z 289) based on the retention times on the chromatogram and LC-MS/MS of GC and (+)-catechin standards. Peak 3 (R_t = 17.27 min) and peak 5 (R_t = 26.39 min) in the chromatogram of the standard sample (Figure 1A) were not detected in plasma or litter samples. Peak 6 (R_t = 27.47 min) and peak 7 (R_t = 29.89 min), which were detected in plasma and litters, respectively, gave an $[m - H]^-$ ion at m/z 457 and MS^2 ions at m/z 331, 305, 193, and 169. The presence of EGCG or GCG was further confirmed based on the LC-MS/MS of standard solutions of EGCG or GCG and retention times on the chromatogram. Peak 8 (R_t = 37.10 min) was detected in litters but not plasma; it had an $[m - H]^-$ ion at m/z 441 and MS^2 ions at m/z 331, 289, 271, and 169, confirming that the peak corresponded to ECG. Peak 9 (R_t = 8.37 min), which only occurred in plasma and had an $[m - H]^-$ at m/z 125, gave no fragment ions on MS^2 but was identified as pyrogallol acid (PA).[17] Peak 10 (R_t = 12.55 min) was detected in plasma and litters and had an $[m - H]^-$ ion at m/z 369, which yielded an MS^2 ion at m/z 289. This 80 amu loss indicated the cleavage of a SO_3^- unit, which confirms that peak 10 was (epi)catechin-*O*-sulfate. Peak 11 (R_t = 39.94 min) was detected in plasma and litters, and this gave an $[m - H]^-$ ion at m/z 495 and an MS^2 ion at m/z 319. This 176 amu loss indicated the cleavage of a glucuronyl unit. Thus, peak 11 was confirmed as 4′-*O*-methyl-(epi)gallocatechin-*O*-glucuronide.[18] Peak 12 (R_t = 42.15 min), which was detected in plasma but not litters, had an $[m - H]^-$ ion at m/z 465, and this yielded yielded an m/z 289 fragment after MS^2 that indicated the cleavage of a glucuronyl unit. Peak 12 was identified as (epi)-catechin-3′-*O*-glucuronide, which is consistent with the results reported by Stalmach et al.[18] Peak 13 (R_t = 19.57 min) was detected in litters and had an $[m - H]^-$ ion at m/z 383, which on loss of 80 amu (cleavage of a SO_3^- unit) yielded an MS^2 ion at m/z 303. Peak 13 was identified as 4′-*O*-methyl-(epi)-catechin-*O*-sulfate.[17,19] Peak 14 (R_t = 24.01 min) and was detected only in litters. Peak 14 gave an $[m - H]^-$ ion at m/z 399, which on MS^2 produced a fragment at m/z 319, indicative of the cleavage of a SO_3^- unit, and so peak 14 was attributed to 4′-*O*-methyl-(epi)gallocatechin-*O*-sulfate.[18]

Analysis of Catechins and Their Metabolites in Plasma. Figure 1A shows the chromatogram of the mixed standard sample including GA, GC, EGC, (+)-catechin, EC, EGCG, GCG, ECG, and the EG internal standard (each at 0.001 μg/mL). The respective retention times of the standard sample mixture can be obtained from the chromatogram to identify the free catechins in the plasma and litters.

Plasma samples were collected at 1.5, 4, and 8 h after the administration of 10 mL of deionized water, 500 mg/kg green tea, 100 mg/kg tea polyphenols, and 50 mg/kg EGCG. The plasma samples were analyzed by LC, and the metabolites in the plasma are shown in Figure 1B–D and Table 3. Free catechins and their metabolites were absent from the control group (Figure 1A), except for the peak of the internal standard EG. GA, EGC, EC, and ECG were not detected in plasma (Figure 1B–D). It is possible that these compounds were not absorbed in the gut tract of the broiler, or they might have been cleaved completely after absorption. The methyl and sulfate derivatives of (epi)catechin and EGC [4′-*O*-methyl-(epi)-catechin-*O*-sulfate and 4′-*O*-methyl-(epi)gallocatechin-*O*-sulfate], respectively, were also not found in plasma. These results were inconsistent with data reported for humans,[10−12] mice,[4,13] and dogs.[14] Thus, the mechanisms were implicated in the absorption of such components in birds.

GC (peak 2) and (+)-catechin (peak 4) were detected in the green tea and tea polyphenols groups, but these peaks were absent from the samples derived from the EGCG group (Figure 1b). The greatest contents of GC and (+)-catechin in plasma occurred at 4 h, and their levels were 15.21 and 56.69 ng/mL for the green tea group and 21.35 and 9.25 ng/mL for the tea polyphenols group, respectively. Levels of GC and (+)-catechin then reduced rapidly at 8 h. The level of EGCG slightly increased for the green tea group over time, but

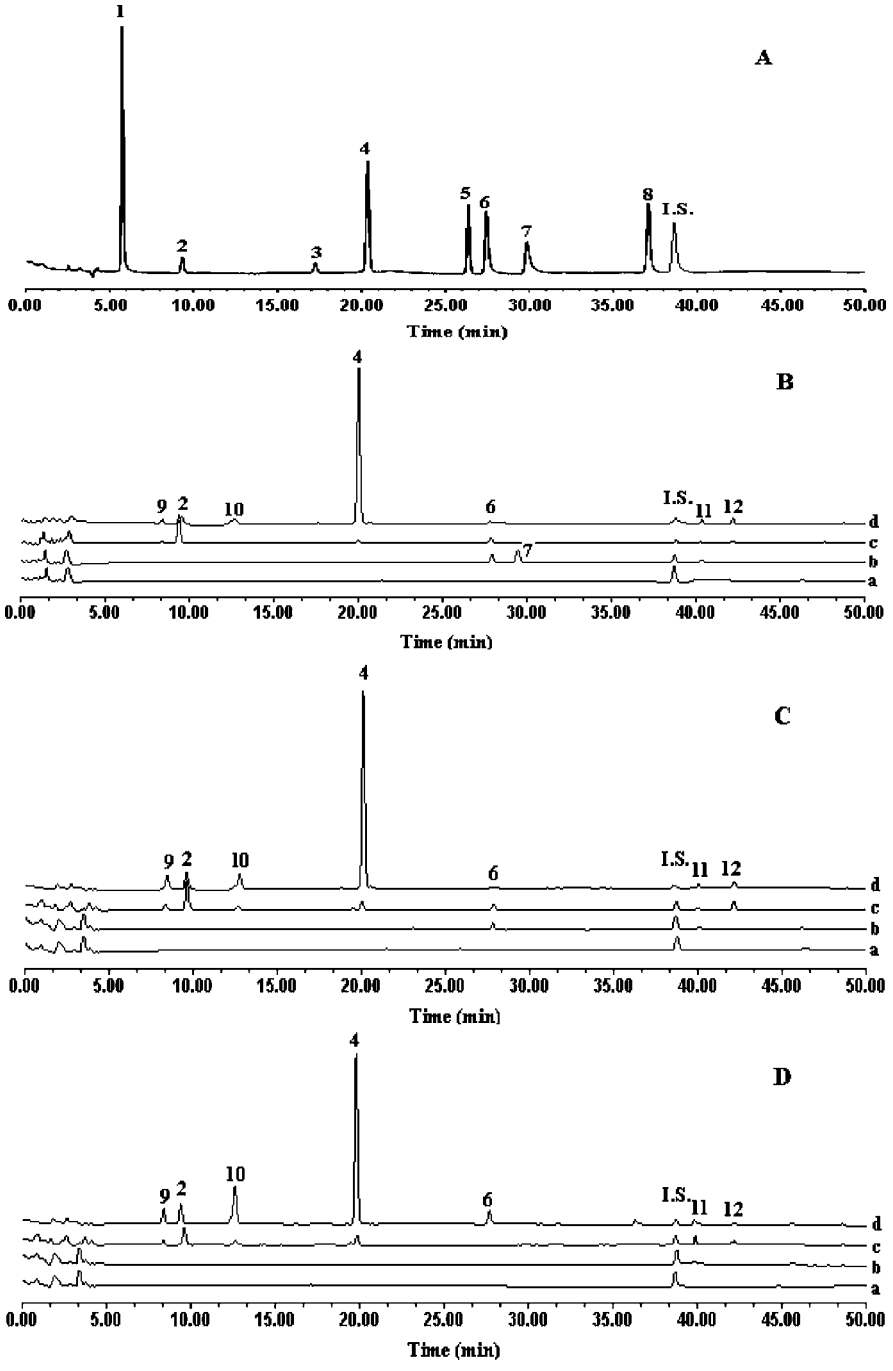

Figure 1. LC quantification of tea catechins and metabolites in plasma. (A) Standard sample mixture, (B) 1.5 h plasma samples, (C) 4 h plasma samples, and (D) 8 h plasma samples. (a) Control group, (b) administration of 50 mg/kg EGCG, (c) administration of 100 mg/kg tea polyphenols, and (d) administration of 500 mg/kg green tea. Peak annotation: 1, GA; 2, GC; 3, (−)-epigallocatechin; 4, (+)-catechin; 5, (−)-epicatechin; 6, (−)-epigallocatechin gallate; 7, GCG; 8, (−)-epicatechin gallate; 9, pyrogallol acid; 10, (epi)catechin-*O*-sulfate; 11, 4′-*O*-methyl-(epi)gallocatechin-*O*-glucuronide; 12, (epi)catechin-3′-*O*-glucuronide; and I.S., internal standard.

EGCG levels decreased gradually for both the tea polyphenol groups and the EGCG group between 1.5 and 4 h post-administration; EGCG was not detectable at 8 h. GCG in plasma was detected only in the EGCG group at 1.5 h, but this was not detected at all in the green tea and tea polyphenols groups. PA as an epimer was detected in the plasma from the green tea and tea polyphenols groups only. The maximum level of PA was detected at 4 h, but then, levels decreased. PA was not found in plasma from the EGCG group, which indicated that PA might come from the cleavage of or be a derivative of catechins other than EGCG. The sulfated derivative of (epi)-catechin was found in plasma samples from the green tea and tea polyphenols groups but not the EGCG group. The maximum concentration of (epi)catechin-*O*-sulfate occurred at 4 h and then decreased rapidly. EGC in plasma existed in methylated and glucuronic acid derivatives. The levels of 4′-*O*-methyl-(epi)gallocatechin-*O*-glucuronide gradually reduced in the green tea group, and the greatest concentrations in the tea polyphenols and EGCG groups occurred at 4 h, before decreasing at 8 h. In plasma, (epi)catechin-3′-*O*-glucuronide was detected

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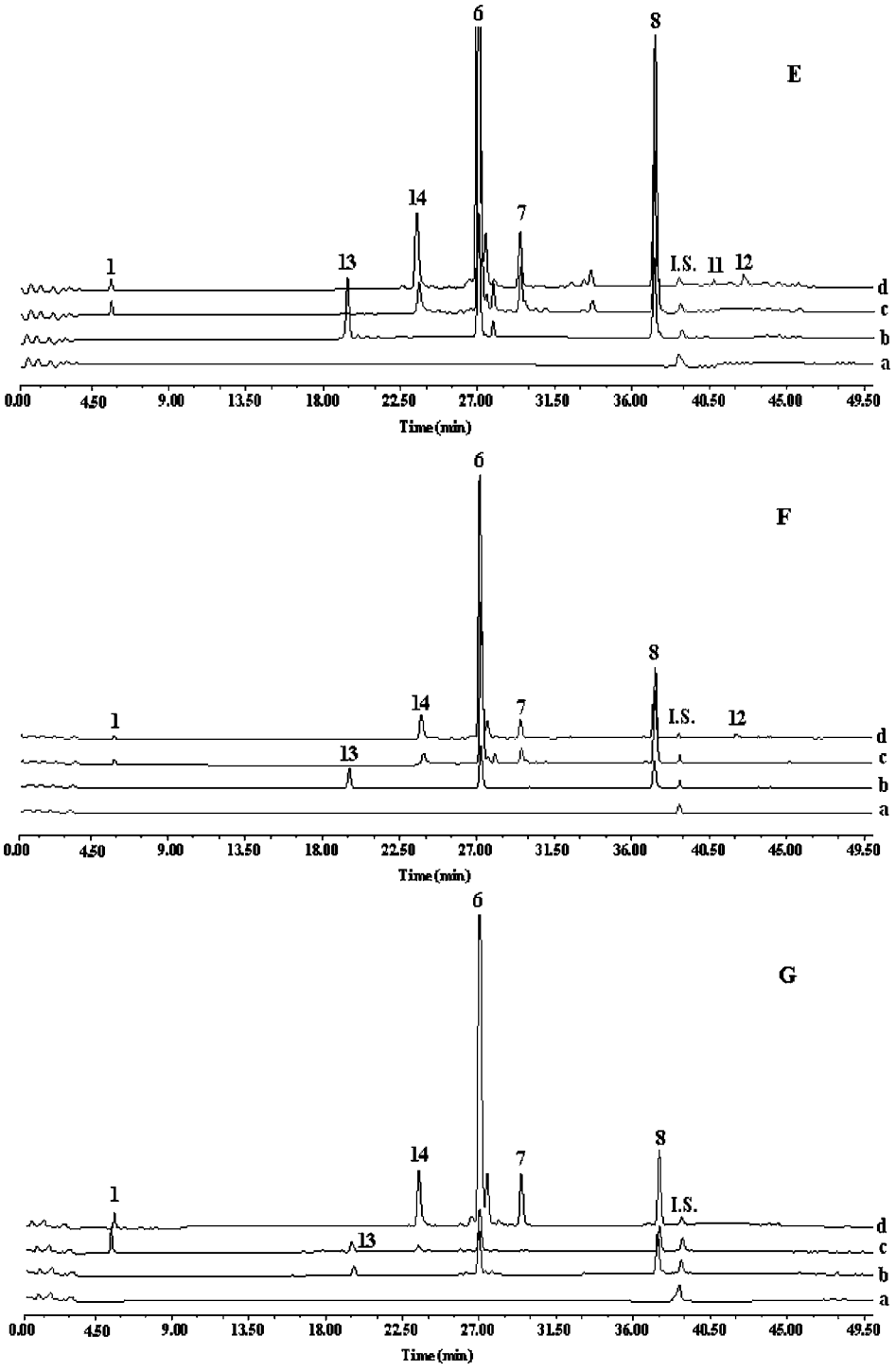

Figure 2. LC quantification of tea catechins and metabolites in litters. (E) 1.5 h litter samples, (F) 4 h litter samples, and (G) 8 h litter samples. (a) Control group, (b) administration of 50 mg/kg EGCG, (c) administration of 100 mg/kg tea polyphenols, and (d) administration of 500 mg/kg green tea. Peak annotation: 1, GA; 6, (−)-epigallocatechin gallate; 7, GCG; 8, (−)-epicatechin gallate; 11, 4′-*O*-methyl-(epi)gallocatechin-*O*-glucuronide; 12, (epi)catechin-3′-*O*-glucuronide; 13, 4′-*O*-methyl-(epi)catechin-*O*-sulfate; 14, 4′-*O*-methyl-(epi)gallocatechin; and I.S., internal standard.

in the green tea and tea polyphenols groups but not the EGCG group. The (epi)catechin-3′-*O*-glucuronide levels decreased gradually in the green tea and in tea polyphenols groups, with maximum levels occurring at 4 h.

From the quantitative analyses of plasma, most of free catechins and their metabolites occurred at 4 h. (+)-Catechin levels were greatest among all of the compounds detected after administration of green tea, followed by GC. However, GC levels were greatest after the administration of tea polyphenols, followed by (+)-catechin.

Analysis of Catechins and Their Metabolites in Litters. Litter samples were collected for the periods 0−1.5, 1.5−4, and 4−8 h after administration of deionized water, green tea, tea polyphenols, and EGCG. Quantitative estimates of the main

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Table 2. MS/MS Identification and Location of Free Catechins and Their Metabolites

peak no.	compd	$[M - H]^-$ (m/z)	MS^2 fragments (m/z)	location
1	GA	169		feces
2	GC	305		plasma
4	(+)-catechin	289		plasma
6	EGCG	457	331, 305, 169, 193	plasma, feces
7	GCG	457	331, 305, 169, 193	plasma, feces
8	(−)-epicatechin-3-gallate	441	289, 331, 271, 169	feces
9	pyrogallol acid	125		plasma
10	(epi)catechin-O-sulfate	369	289	plasma, feces
11	4′-*O*-methyl-(epi) gallocatechin-*O*-glucuronides	495	319	plasma, feces
12	(epi)catechin-3′-*O*-glucuronide	465	289	plasma
13	4′-*O*-methyl-(epi)catechin-*O*-sulfate	383	303	feces
14	4′-*O*-methyl-(epi) gallocatechin-*O*-sulfate	399	319	feces

free catechins and their metabolites excreted in the litters are presented in Table 4. Figure 2 shows that no compounds were detected in the control group (Figure 2a), except for the peak of the internal standard. GC, EGC, (+)-catechin, EC, PA, (epi)catechin-*O*-sulfate, and *O*-methyl-(epi)catechin-*O*-sulfate were not found in litter samples. Besides the unlabeled peaks in Figure 2 that were not identified, seven identified compounds were excreted in the green tea group (Figure 2d), five compounds were excreted in the tea polyphenols group (Figure 2c), while only three excretion compounds were detected in the litters of the EGCG group (Figure 2B).

GA levels in the green tea and tea polyphenols groups increased gradually as time increased, but GA was not detected in the EGCG group. Maximum levels of EGCG and ECG were reached during the at 1.5−4 h period for each experimental group. During this time, EGCG and ECG levels were 29956 and 13691 μg for the green tea group, 19467 and 9937 μg for the tea polyphenols group, and 9007 and 7577 μg for the EGCG group, respectively. EGCG and ECG levels were significantly higher in the litters than in the plasma. Regarding derivatives of EGC, 4′-*O*-methyl-(epi)gallocatechin-*O*-glucuronide (937 μg) was detected only in the green tea group at 1.5 h, and this was not found in the tea polyphenols and EGCG groups. (Epi)catechin-3′-*O*-glucuronide was only detected in the green tea group at 0−1.5 and 1.5−4 h. Furthermore, 4′-*O*-methylation-(epi)catechin-*O*-sulfate was detected in the EGCG group, and the maximum level occurred at 4 h (4579 μg). In the tea polyphenols group, 4′-*O*-methylation-(epi)catechin-*O*-sulfate was excreted only during 4−8 h. 4′-*O*-Methyl-(epi)-gallocatechin-*O*-sulfate was detected in the green tea and tea polyphenols groups but not in the EGCG group. Maximum levels of 4′-*O*-methyl-(epi)gallocatechin-*O*-sulfate occurred at 1.5−4 h in the green tea and tea polyphenols groups and reached 7584 and 2426 μg, respectively. Table 4 shows that the total excretion of catechins and their metabolites reached more 95.8% (equivalent to 4.2% of the green tea absorbed) for the green tea group, 87.7% (equivalent to 12.3% of the tea polyphenols absorbed) for the tea polyphenols group, and

Table 3. Quantification of the Major Tea Catechins and Their Metabolites in Plasma at Different Times (ng/mL)[a]

		green tea			tea catechins			EGCG		
peak no.	compd	1.5 h	4 h	8 h	1.5 h	4 h	8 h	1.5 h	4 h	8 h
1	GA	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	GC	11.42 ± 1.34	15.21 ± 2.16	8.50 ± 0.56	20.37 ± 4.25	21.35 ± 2.57	8.42 ± 1.62	ND	ND	ND
3	(−)-epigallocatechin	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	(+)-catechin	50.97 ± 4.36	56.69 ± 3.49	47.93 ± 2.68	6.75 ± 0.57	9.25 ± 0.47	4.82 ± 0.36	ND	ND	ND
5	(−)-epicatechin	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	(−)-epigallocatechin gallate	3.12 ± 0.23	6.32 ± 0.94	6.78 ± 0.48	8.03 ± 1.02	6.12 ± 0.78	ND	9.31 ± 1.24	4.26 ± 1.06	ND
7	GCG	ND	ND	ND	ND	ND	ND	16.98	ND	ND
8	(−)-epicatechin gallate	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	pyrogallol acid	6.43 ± 1.12	11.26 ± 2.69	6.85 ± 1.01	3.09 ± 0.13	5.38 ± 0.26	1.27 ± 0.15	ND	ND	ND
10	(epi)catechin-O-sulfates	11.03 ± 1.79	16.21 ± 2.45	11.32 ± 2.54	ND	6.84 ± 1.02	1.04 ± 0.07	ND	ND	ND
11	4′-*O*-methyl-(epi)gallocatechin-*O*-glucuronide	5.52 ± 0.21	3.25 ± 0.57	1.78 ± 0.42	1.02 ± 0.36	1.61 ± 0.24	1.44 ± 0.20	1.16 ± 0.15	1.96 ± 0.48	0.92 ± 0.03
12	(epi)catechin-3′-*O*-glucuronide	7.02 ± 1.26	6.85 ± 1.61	0.93 ± 0.30	3.15 ± 0.28	8.03 ± 0.56	0.64 ± 0.02	ND	ND	ND
13	4′-*O*-methyl-(epi)catechin-*O*-sulfate	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	4′-*O*-methyl-(epi)gallocatechin-*O*-sulfate	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a]Data are expressed as means ± standard errors (n = 3).

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Table 4. Quantification of the Major Tea Catechins and Metabolites in Feces at Different Time Points (μg/g)

		green tea			tea catechins			EGCG		
peak no.	compd	0–1.5 h	1.5–4 h	4–8 h	0–1.5 h	1.5–4 h	4–8 h	0–1.5 h	1.5–4 h	4–8 h
1	GA	1061 ± 25	1562 ± 43	1780 ± 50	1078 ± 37	1589 ± 34	2368 ± 57	ND	ND	ND
2	GC	ND	ND	ND	ND	ND	ND	ND	ND	ND
3	(−)-epigallocatechin	ND	ND	ND	ND	ND	ND	ND	ND	ND
4	(+)-catechin	ND	ND	ND	ND	ND	ND	ND	ND	ND
5	(−)-epicatechin	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	(−)-epigallocatechin gallate	17050 ± 104	29956 ± 127	14739 ± 123	13967 ± 112	19467 ± 165	7622 ± 73	6705 ± 59	9007 ± 63	5013 ± 48
7	GCG	3055 ± 50	4246 ± 47	4059 ± 62	3687 ± 21	3242 ± 30	351 ± 10	ND	ND	ND
8	(−)-epicatechin gallate	12834 ± 123.	13691 ± 124	7035 ± 56	8521 ± 17	9937 ± 130	3546 ± 52	5395 ± 66	7577 ± 43	3823 ± 20
9	pyrogallol acid	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	(epi)catechin-*O*-sulfate	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	4′-*O*-methyl-(epi) gallocatechin-*O*-glucuronide	937 ± 11	ND	ND	ND	ND	ND	ND	ND	ND
12	(epi)catechin-3′-glucuronide	1548 ± 30	1431 ± 19	ND	ND	ND	ND	ND	ND	ND
13	4′-*O*-methyl-(epi)catechin-*O*-sulfate	ND	ND	ND	ND	ND	1526 ± 24	4434 ± 26	4579 ± 33	1189 ± 21
14	4′-*O*-methyl-(epi) gallocatechin-*O*-sulfate	5108 ± 22	7584 ± 34	4669 ± 36	2037 ± 40	2426 ± 22	938 ± 13	ND	ND	ND
total excretion of catechins (excretory rate, %)[a]	41593 ± 51 (30 ± 3%)	58447 ± 66 (42 ± 4 %)	32282 ± 66 (23 ± 2 %)	29290 ± 46 (31 ± 1 %)	36661 ± 76 (39 ± 2 %)	16351 ± 38 (17 ± 3 %)	16534 ± 50 (33 ± 1 %)	21163 ± 46 (43 ± 2 %)	10025 ± 30 (21 ± 1 %)	

[a]Data in parentheses indicate the amount excreted as a percentage of intake.

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97.7% (equivalent to 2.3% of the EGCG absorbed) for the EGCG group during the experiments.

DISCUSSION

In the present study, the absorption and excretion of metabolites by broiler chickens were compared after the oral administration of green tea, tea polyphenols, and EGCG. It was found that absorptions in the green tea, tea polyphenols, and EGCG groups were 12.3, 4.2, and 2.3% of total tea polyphenols, respectively. Studies have shown that the bioavailability of green tea flavan-3-ols in humans ranges from 2 to 8.1%.[18,20] The digestive system of birds, besides the oral cavity, muscular stomach, duodenum, jejunum, ileum, cecum, and colon, includes the craw and glandular stomach, and the bird lacks a specific urinary excretion system found in humans and mammals. The differences in the digestive systems might explain the better absorption of catechins by birds. In this present study, prior to quantification and identification, the metabolites of catechins from plasma and litters were not converted into their free forms using β-glucuronidase and sulfatase so as to provide a more accurate data set. Because of the low overall recovery of compounds in these analyses, the measured quantitative values should be regarded as relative and not absolute.

The free catechins GA, EGC, EC, and ECG were not detected in the analysis of the plasma. Previous studies in humans reported that ingested GA[21] and EGC[22] were absorbed easily into the blood. It is possible that differences in the digestive systems of birds and humans caused these apparent differences in absorption of GA and EGC. GCG was identified in the EGCG group at 1.5 h, but this was absent in the samples from the green tea and tea polyphenols groups. The lack of GCG absorption by the chickens in the green tea and tea polyphenols groups could be due to the low amounts of GCG in these ingredients, which was 1.03 and 8% (w/w), respectively. (+)-Catechin levels in the plasma from the green tea group (containing 0.86% catechin) was slightly high and reached 56.69 ng/mL at 4 h. There was a trace of (+)-catechin in the plasma of the tea polyphenols group, but no (+)-catechin was detected in the EGCG group. Some studies have reported that (+)-catechin levels in plasma from humans,[23] rabbits,[24] and rats[25] were relatively high after the ingestion of catechins. The dose of EGCG administered was relatively high in the green tea (16.1%, equivalent to 80.5 mg EGCG), tea polyphenols (45.5%, equivalent to 45.5 mg EGCG), and EGCG groups (98%, 49 mg EGCG), but EGCG levels in the plasma were low (less than 0.001% absorption after ingestion). Only three metabolites were obtained after ingestion of EGCG. This result does not coincide with the absorption and bioavailability of EGCG in humans[22,26] and rats.[27] PA was detected in the plasma of the green tea and tea polyphenols groups but not the EGCG group, as this feed did not contain PA. PA might come from the cleavage of the B ring of EGC or the D ring of ECG but not from EGCG.[4] Most of the tea polyphenols in the plasma and urine from humans and rats are in the form of O-methylated, sulfated, and glucuronide conjugates of (epi)-catechin and EGC,[18,27,28] but in this present study, just three metabolites of these conjugated forms were detected in the green tea and tea polyphenols groups, and only one derivative was detected in the EGCG group. The levels of these derivatives in the plasma were very low. Moreover, we found that the birds in the tea polyphenols group absorbed more catechins than the chickens in the green tea and EGCG groups. These differences in absorption might be caused by the different compounds found in green tea and tea polyphenols. We suggest that among the eight free catechins, (+)-catechin and GC might be more easily absorbed by chickens. Seven compounds were detected and identified after the administration of green tea and tea polyphenols, but only three compounds were found after ingestion of EGCG. It is possible that the mixture of catechins promoted the absorption of each other within the digestive tract of the chickens.

Many previous studies have focused on the excretion of tea catechins and their conjugated metabolites in the urine of humans[4,18] and rats.[27] In this present study, the excretions of birds contain urine and fecal components; thus, the analysis included a mixture of urinary and fecal components, as this bird lacks a specific urinary excretion system. Although the ingested material did not contain GA, as compared with total tea catechins intake, the GA levels in the litters reached 3.2% in the green tea group and 5.4% in the tea polyphenols group. GA was not detected in excreta of the EGCG group. Thus, GA probably came from the cleavage of catechins other than EGCG. Excretion of EGCG was 76.7% for the green tea group, 90.2% for the tea polyphenols group, and 42.3% for the EGCG group when compared with total EGCG ingested. Large excretion of EGCG has also been found in mouse feces.[29,30] The methylation, glucuronide, and sulfate conjugates of (epi)-catechin and EGC were detected at very low levels. Only 4′-*O*-methyl-(epi)gallocatechin-*O*-glucuronide (at 0−1.5 h) and (epi)catechin-3′-glucuronide (at 0−4 h) were detected in the green tea group; these conjugates were not detected in the tea polyphenols and EGCG groups. 4′-*O*-Methyl-(epi)catechin-*O*-sulfate was detected in the EGCG group, and it was also detected in the tea polyphenols group but only at 8 h. 4′-*O*-Methyl-(epi)gallocatechin-*O*-sulfate was detected in the green tea and tea polyphenols groups but not in the EGCG group. Moreover, EGC and EC were found neither in plasma nor in litters. We suggest that these catechins might be spilt or turned into other compounds by enzymes in the digestive tract. During the investigations, the maximum excretion levels occurred at 1.5−4 h after the administration of green tea, tea polyphenols, and EGCG. Combined with the analysis of the catechins and their metabolites in plasma, the best period of metabolism of green tea and tea polyphenols in the broiler chickens took place at approximately 4 h, because the free catechins and their metabolites in plasma reached maximum levels at 4 h, and excretion levels were also greatest at 4−8 h. Baba et al.[31] suggested that chemical features of each compound, such as solubility and lipophilicity, might influence the results of biotransformation and bioavailability studies in animals. In the future, complete concentration versus time profiles for the catechins, including their free forms and complex metabolites in the plasma and litters after ingestion, will be examined to further understand the differences between absorption and excretion of various catechins.

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