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Role of Cytochrome P450 Hydroxylase in the Decreased Accumulation of Vitamin E in Muscle from Turkeys Compared to that from Chickens

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Abstract

Turkeys and chickens reared to 5 weeks of age and fed diets with feedstuffs low in endogenous tocopherols were examined. Treatments included feed supplemented with *RRR* (natural source vitamin E) alpha tocopheryl acetate (AcT, 35 mg/kg feed) and all-racemic (synthetic vitamin E) AcT (10 and 58 mg/kg feed). Alpha tocopherol hydroxylase activity was greater in liver microsomes prepared from turkeys compared to that from chickens ($p < 0.01$). Alpha and gamma tocopherol metabolites were higher in turkey bile than in chicken when assessing the *RRR* AcT diet and the all-racemic AcT diet at 58 mg/kg feed ($p < 0.01$). Turkey cytochrome P450 2C29 was increased relative to its chicken ortholog on the basis of RNA-Seq transcript abundance ($p < 0.001$) and activity-based protein profiling ($p < 0.01$) of liver tissue. Alpha tocopherol concentrations in plasma, liver, and muscle from turkey were lower than the respective tissues from chicken ($p < 0.05$). Lipid oxidation was greater in turkey thigh than in chicken ($p < 0.05$). These results suggest that elevated tocopherol metabolism by cytochrome P450 hydroxylase(s) in turkeys contributes to the decreased accumulation of alpha tocopherol in turkey tissues compared to that of chickens.

Keywords

vitamin E; poultry; lipid oxidation; metabolites; rancidity

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Notes

The authors declare no competing financial interest.

INTRODUCTION

Vitamin E consists of four isomers, alpha, beta, gamma, and delta in both tocopherols and tocotrienols. The synthetic vitamin E source is dl-alpha tocopheryl acetate which consists of eight different stereoisomers; in equal amounts, which are referred to as *RRR*, *RRS*, *RSR*, *RSS*, *SRR*, *SRS*, *SSR*, and *SSS*. The natural vitamin source is *d*-alpha tocopheryl acetate, which consists of one stereoisomer known as *RRR*. Vitamin E can protect tissues from protein and lipid oxidation through the process of free radical scavenging.¹ Vitamin E embedded in cellular membranes can also counteract effects from lysolipids.² Chickens more effectively accumulate dietary vitamin E into muscle tissues than turkeys.³ A possible explanation for the ability of chickens to accumulate vitamin E more effectively in tissues than turkeys may be related to the catabolism of vitamin E in the liver.

In birds, vitamin E is directly transported to the liver from the intestine via portomicrons in the portal blood supply.⁴ Once in the liver, vitamin E can be packed into very low density lipoproteins (VLDL) by alpha tocopherol transfer protein.⁵ Vitamin E that is not transported to the blood supply can be degraded by cytochrome P450 enzymes in the liver.⁶ The elimination pathway of tocopherol involves cytochrome P450 mediated hydroxylation of the tocopherol phytyl side chain. After hydroxylation, the tocopherol molecule undergoes several beta-oxidations yielding carboxychromanol metabolites.⁶ Specifically, the cytochrome P450 4F family isoform 2 enzyme (CYP4F2) has exhibited appreciable tocopherol-omega-hydroxylation activity.⁷ Following omega-hydroxylation, the vitamin E molecule can be glucuronidated and excreted.⁸ Tocopherol glucuronides were present in the intestinal flow of turkeys at greater concentrations than those of chickens.⁹

The main objective of this work was to examine the concentrations of vitamin E metabolites in bile as a means to examine the cause for differences in tissue accumulations of vitamin E when comparing turkeys and chickens. Another objective was to utilize RNA-Seq and activity-based protein profiling of cytochrome P450 enzymes in liver tissue to investigate which tocopherol metabolizing enzymes may contribute to the decreased accumulation of tocopherols in turkey tissues.

MATERIALS AND METHODS

Chemicals

Chloroform preserved with ethanol (0.75%) was used for lipid extractions (Fisher Scientific, Waltham, MA). Alpha tocopheryl acetate (AcT), alpha tocopherol (AT), gamma tocopherol (GT), and a mixed tocopherol standard (comprising alpha, beta, gamma, and delta tocopherols) were used for standard curves and chromatograph identification (Sigma-Aldrich, St. Louis, MO). Distilled, deionized water was used, and all other chemicals were reagent- or technical-grade.

Dietary Treatments, Housing, and Collection of Tissues

Day-old, male turkeys (*Meleagris galapovo*, hybrid converter strain) and chickens (*Gallus gallus*, Ross708) were obtained from commercial hatcheries and were housed in a single facility at the Archer Daniels Midland Intense Research Center (Mendon, IL). All birds were

housed in 56 cm × 112 cm × 56 cm pens with raised plastic flooring. Four birds occupied each pen, with a total of 28 birds in each treatment (7 pens per treatment). Turkeys and chickens were fed one of three dietary treatments, which differed in the quantity and type of AcT (*RRR* versus all-racemic) that was supplemented in the diet. The dietary treatments included 10 IU/kg all-racemic AcT (designated as “low”), 50 IU/kg *RRR* AcT, or 50 IU/kg all-racemic AcT (designated as “high”). All birds were fed ad libitum for 5 weeks, until the final day of the trial when they were fasted 4–6 h before exsanguination and harvesting of tissues. Feed intake (g/day) and weight gain (g/day) were carefully monitored so that gain to feed ratio could be determined. Birds were stunned in a CO₂ chamber for 3 min and bled by severing the carotid artery. Blood was collected in a 50 mL polypropylene tube containing an anticoagulant (30 units saline heparin/mL collected blood). Bile was collected via a syringe with needle and stored in cryovials at –80 °C. Liver, breast muscle (pectoralis major), and thigh muscle (nondifferentiated) were removed from each bird, placed in bags (3 mil barrier), stored on ice for 8 h, vacuum packaged, and stored at –80 °C until analyses.

Lipid Extraction of Feed, Liver, Breast, and Thigh Muscle for Analysis of AcT, AT, and GT

Lipids were extracted from tissues as previously described¹⁰ with some modifications. Liver, muscle, and feed (approximately 10 g each) were homogenized with a PT 10-35 Polytron (Brinkmann Instruments, Westbury NY) in 20 volumes of a 2:1 mixture chloroform/methanol (v/v) and filtered using Whatman 40 filter paper into a separatory funnel. A final 10 volumes of chloroform/methanol was used to further extract residual lipid remaining in the filter paper. Fifteen volumes of 0.8% KCl were then added to the filtrate with shaking, and the separatory funnels were stored at 4 °C for a few hours to allow phase separation. The lower chloroform layer was collected, and lipid was isolated by vacuum distillation in a 55 °C water bath using a Buchi Rotovap model B-490 (Flawil, Switzerland).

AT, GT, and AcT Determination in Feed, Liver, Breast, and Thigh

Alpha tocopherol (AT), gamma tocopherol (GT), and alpha tocopheryl acetate (AcT) contents were determined by liquid chromatography with fluorescence detection.^{11,12} Extracted lipids were weighed and dissolved in mobile phase (99:1 hexane: 2-propanol). The lipid in mobile phase (approximately 100 mg/mL) was then filtered (0.45 µm PTFE filters) into amber vials. AT, GT, and AcT contents were quantified by a high-pressure liquid chromatography (HPLC) system, Agilent 1100 series HPLC with an autosampler, using an Alltima silica 5 µm (4.6 × 250 mm²) column (Alltech Associates, Inc., Deerfield, IL), with fluorescence detection and a diode array detector (DAD). The UV–vis spectra (major peak at 210 nm with shoulder at 225 nm and lesser peak at 295 nm) were analyzed to ensure that the signal from each peak in the chromatogram was a known vitamin E isomer. The flow rate of the mobile phase was 1 mL/min. Lipid (20 µL) in mobile phase was injected. The effluent was monitored by fluorescence (excitation 295 nm and emission 325 nm) and DAD detection at 295 nm. AT, GT, and AcT concentrations were determined from peak areas using a standard curve prepared from each standard.

Preparation of Plasma for AT and GT Analysis

A portion of the whole, anticoagulated blood (5 mL) was transferred to a 15 mL polypropylene conical tube. Samples were centrifuged for 10 min at $700 \times g$ (4°C) in J-6B centrifuge (Beckman Instruments Inc., Palo Alto, CA). An aliquot of plasma was placed in a cryovial (Nalgene, Rochester, NY) and stored at -80°C until analysis.

AT and GT Determination in Plasma

Vitamin E isomers in plasma were quantified using GC-MS.⁶ Briefly, a Hewlett-Packard 6890 gas chromatograph coupled to a Hewlett-Packard 5872 massselective detector was used for analyses. The gas chromatograph was fitted with a Hewlett-Packard HP-1 methylsiloxane capillary column (30 m $\times$ 0.25 mm) and operated in split-injection mode using helium as a carrier gas. Deuterated (d_9) alpha tocopherol was used as an internal standard.

AT and GT Metabolites Determination in Bile

Bile samples were treated with beta-glucuronidase Type IX A (800 U) and sulfatase Type IV (0.5 U) at 37°C for 2 h, acidified to pH 2 with 3 N HCl, and extracted with ethyl acetate. The residue was evaporated to dryness, and tocopherol metabolites were quantified by gas chromatography–mass spectrometry (GC-MS), using d_9 -alpha-carboxy ethyl hydroxychroman as an internal standard.¹³ For short-chain tocopherol metabolite analyses, the oven was programmed to ramp from 200°C (2 min hold) to 250°C at $7^{\circ}\text{C}/\text{min}$, followed by a 6 min hold at 250°C , then ramped to 280°C at $25^{\circ}\text{C}/\text{min}$, with a final hold at 280°C for 9 min. Long-chain metabolites and parent substrates were resolved isothermally at 280°C for 10 min. The metabolite values represent a summation of metabolites carboxylated at the 3' and 5' ends of the molecules.

Tocopherol Hydroxylase Activity in Liver Microsomes

Liver microsome preparation and tocopherol hydroxylase activity in liver microsomes were determined as described previously.⁶ The reaction conditions were $150\text{ }\mu\text{M}$ RRR-alpha tocopherol (BSA complex), 1.0 mM NADPH, and 45 min incubation at 37°C . Data were reported as nanomoles of product (13-OH-alpha tocopherol) per milligram of microsomal protein.

RNA Extraction and cDNA Preparation and Sequencing

Methods and quality control procedures used to prepare liver samples for RNA extraction and cDNA preparation and sequencing (RNA-Seq) and all downstream computational analyses were as described in Garic et al.¹⁴ The RNA-Seq module in CLC Genomic Workbench 5.5 (CLC Bio, Cambridge, MD) was used to map the filtered single-end reads (turkey and chicken) to protein-coding genes (known or predicted) in Galgal4 of the *G. gallus* genome (Ensembl Release version 71). Turkey filtered single-end reads were also mapped to protein-coding genes (known or predicted) in UMD2, Turkey_2.01 of the *M. gallapovo* genome (Ensembl Release version 71). Assemblies were matched to the reference genome, individually requiring at least 90% of each read to exhibit 80% or greater alignment similarity. Only reads that mapped uniquely to the reference were included in subsequent

analyses. DESeq (v1.10.1) was used to normalize raw counts of mappings and to test for differential gene expression.¹⁵

Activity-Based Protein Profiling of Turkey and Chicken Livers

Activity-based protein profiling (ABPP) utilizes chemical activity-based probes (ABPs) developed from mechanism-based inhibitors (“suicide substrates”) of P450 enzymes to report directly upon the activity of P450 enzymes. We used a multiplexed mixture of two P450 ABPs, an arylalkyne [“2EN-ABP”]¹⁶ and an aliphatic alkyne [probe “5”]¹⁷ containing chemical probe. Arylalkyne and aliphatic alkynes are known mechanism-based inhibitors of P450 enzymes.¹⁸ The ABPs are oxidized directly by P450 enzymes in a NADPH-dependent manner to yield a reactive ketene moiety on the probe, which subsequently reacts with a nucleophilic amino acid residue within the target P450 enzymes.¹⁶ Following protein labeling, a copper-catalyzed azide–alkyne cycloaddition (CuAAC) is employed to append fluorescent reporters for gel imaging or biotin for enrichment and LC-MS analyses.¹⁶ Because of broad substrate specificity of many P450 enzymes, particularly those associated with detoxification and phase I metabolism, the utilization of two probes provides broad measurement coverage of P450 activity.

Chicken and turkey livers were processed to obtain the microsomes for ABPP analyses as described previously for mouse liver microsomes.¹⁶ Livers were rinsed with ice-cold 1.5% KCl, finely diced with a razor blade, and dounce homogenized in 250 mM sucrose (5 mL per g liver) in PBS buffer. EDTA was not included in the buffer because of incompatibility with subsequent click chemistry reactions. Liver homogenates were treated with a tissue tearor for five pulses. Highspeed centrifugation was used to remove the heavy membrane components and ultimately to isolate microsomes: 10 000 × g (25 min, pellet = heavy membrane fraction) to obtain the S9 fraction and 100 000 × g (90 min, pellet = microsome fraction). Microsomes were resuspended in 250 mM sucrose in PBS buffer with minimal dounce homogenization. All microsomes were stored at –80 °C until use. Microsomes were subjected to no more than two freeze–thaw cycles. Microsomal proteome (1 mg/mL protein concentration; 350 μ L total volume for chicken and 1.5 mL total volume for turkey) samples were treated with a mixture of the two ABPs. NADPH (1 mM) was added in each probed sample. For control samples, probes were added without NADPH. Following ABP incubation, the samples were reacted with an azido-biotin tag under click-chemistry conditions.¹⁹ ABP-labeled proteins were enriched on streptavidin resin, reduced with TCEP, and alkylated with iodoacetamide.¹⁹ Proteins were digested on-resin with trypsin, and the resulting peptides were collected and analyzed on a LTQ instrument by LC-MS as described previously.²⁰ Generated MS/MS spectra were searched using the MSGF+ algorithm²¹ against the publicly available *G. gallus* or *M. gallopavo* translated genome sequences, and rescored using the MS-GF approach.²¹ Identified peptides of at least six amino acids in length having MS-GF scores $> 1 \times 10^{-10}$, corresponding to an estimated false discovery rate (FDR) <1% at the peptide level, were used for analysis. To obtain tabulated spectral counts, each MS-based experimental observation of a peptide unique to a single protein is counted. Highly active P450s labeled by probes results in higher spectral counts. To mitigate false positives, we required the following in identifying a protein as specifically labeled by the ABPs: (i) at least 2 spectral counts for each P450 and (ii) the protein exhibits a 2-fold

higher spectral count in the ABP-labeled + NADPH sample relative to the –NADPH controls.

Lipid Oxidation in Thigh Muscle

Thigh muscle was deboned from one leg of each turkey and chicken. Thigh muscle from an individual bird was used as the experimental unit. Each thigh was individually ground through a 5 mm plate using a KS M90 mincer (Kitchen-Aid Inc. St. Joseph, MI), salted (1% NaCl), and placed into an air-permeable polyethylene bag, sealed, and stored at –4 °C for 8 weeks.

Thiobarbituric Acid Reactive Substances in Thigh Muscle

Thiobarbituric acid reactive substances (TBARS) were determined as previously described previously.²² Briefly, muscle is homogenized in 6 vol of trichloroacetic acid followed by filtration using Whatman 1 filter paper. The filtrate is then mixed with an equal volume of thiobarbituric acid reactive substances, heated at 100 °C for 40 min, and optical density recorded at 532 nm. A standard curve was constructed using tetraethoxypropane as a precursor of malonaldehyde.

Statistical Evaluations

Comparisons were made between turkey and chicken because the focus of the work was to assess comparative differences between the two bird species. Furthermore, the fact that *RRR* AcT was supplemented at 33–38 mg/kg feed versus 56–60 mg/kg feed of all-racemic AcT limited the usefulness of making statistical comparisons between these diets. Student's *t* test was used for making comparisons between turkey and chicken.²³ The quantity of analyte in tissue from each individual animal was used as the source of variation for the statistical analyses except in the cases of assessing feed intake, weight gain, gain/feed ratio, and milligrams of AcT intake per kilograms of body weight when the value from each pen was used as the source of variation. For assessment of lipid oxidation during frozen storage, Student's *t* test was employed at each time point for values in chicken thigh compared to that of turkey.

RESULTS

Analysis of AT and GT Content in Feeds and Intake of Supplemented AcT

The AT, GT, and AcT concentrations in the chicken and turkey feeds were determined. AT and GT are contained in the feedstuffs (corn starch, soy protein isolate, and wheat middlings), whereas AcT is added to the feeds as a supplement. Mean values for AT were 0.47 and 0.91 mg/kg feed in the chicken and turkey feed, respectively (Table 1). The elevated levels of AT in turkey feed compared to that in chicken feed ($p < 0.01$) may have been due to the higher amount of soy protein isolate included in the turkey feed to meet the elevated protein requirement of turkeys (Table 2). The fat content in the protein isolate that was used was 1%, which may contain some residual tocopherols. Mean values for GT were 5.2 and 5.6 mg/kg feed in the chicken and turkey feed, respectively (Table 1). A typical commercial poultry ration using feedstuffs of milled corn and soybean meal diet contains

concentrations of AT and GT 5- to 10-fold greater than those in the feedstuffs used in our study.²⁴

For a given diet, feeds for the chickens and turkeys were formulated to contain the same concentration of supplemented alpha tocopheryl acetate (AcT). Analysis of the feeds indicated similar concentrations of AcT for the low and high levels of allracemic AcT (Table 1). However, *RRR* AcT in the turkey feed was significantly higher (1.13-fold) than that in chicken feed (Table 1). The weight gain per pen, feed intake per pen, and AcT content of each feed was used to determine the milligrams of AcT intake per kilograms of body weight for chickens and turkeys on each of the diets during the 5 week grow out. Chicken AcT intake was 1.2- to 1.3-fold greater than that for turkeys ($p < 0.001$) when the all-racemic AcT diets were fed (Table 1). AcT intake per kilograms of body weight was similar between turkeys and chickens when the *RRR* AcT diet was fed (Table 1).

Feed Intake, Weight Gain, and Gain/Feed Ratio in Chickens and Turkeys

Intake (grams per day) was 1.72- to 1.81-fold higher on average in chickens than in turkeys when examining the three diets, whereas gain (grams per day) was 1.58- to 1.65-fold higher in chickens than in turkeys (Table 3). These findings caused the gain/feed ratio (g gain per kg feed) in turkeys to be 1.08- to 1.10-fold higher than that in chickens in all three diets (Table 3). These differences between turkeys and chickens in intake, gain, and gain to feed ratio were statistically significant for each of the three diets (Table 3).

Metabolites of AT and GT in Chicken and Turkey Bile

AT and GT metabolites that originate in the liver because of the action of specific cytochrome P450 hydroxylases remain to be identified definitively in avian species. After hydroxylation of the tail portion of the tocopherol molecule, a series of β -oxidation steps ensue. These tocopherol metabolites then accumulate in the bile. AT and GT metabolites were measured in bile rather than liver because of more uniform quantification of the metabolites occurs when assessing bile.

AT metabolites were 8.3-fold greater in turkey bile than in chicken bile ($p < 0.001$) when the *RRR* AcT diet was examined (Table 4). AT metabolites were 2.2-fold greater in turkey bile than in chicken bile ($p < 0.01$) when the high level of dietary all-racemic AcT (ca. 58 mg/kg diet) was examined (Table 4). At the low level of all-racemic AcT (ca. 10 mg/kg diet), a high degree of variability in AT metabolites was observed in both turkey and chicken bile (Table 3). Mean values of AT metabolites were 2.2-fold elevated in the turkey bile, but the large variability caused there to be no significant difference between turkeys and chickens receiving the low all-racemic AcT diet.

Levels of GT metabolites were 5.5-fold greater in turkey bile than in chicken bile ($p < 0.001$) when the *RRR* AcT diet was examined (Table 4). Levels of GT metabolites were 2.4-fold greater in turkey bile than in chicken bile ($p < 0.01$) when the high level of dietary all-racemic AcT (~58 mg/kg diet) was examined (Table 3). At the low level of all-racemic AcT (~10 mg/kg diet), a high degree of variability in GT metabolites was observed in both turkey and chicken bile (Table 3). Mean values of GT metabolites were 1.9-fold elevated in the

turkey bile, but the large variability caused there to be no significant difference between turkeys and chickens on the low all-racemic AcT diet.

Comparative RNA-Seq of Liver Tissue from Chickens and Turkeys

Total RNA was isolated from the liver tissue of chickens and turkeys. The treatments were dietary *RRR* AcT at 33–38 mg/kg feed ($n = 2$ turkeys, $n = 2$ chickens) and all-racemic AcT at 56–60 mg/kg feed ($n = 2$ turkeys, $n = 2$ chickens). A total of 6 and 7 genes were differentially expressed in chicken and turkey liver, respectively, when comparing the diets within bird type (data not shown). Because none of the differentially expressed genes within bird type were related to tocopherol metabolism or transport, livers from each bird type receiving the *RRR* AcT and all-racemic AcT diet were grouped ($n = 4$) for RNA-Seq analysis of CYPs. This grouping of chicken and turkey livers was also used for ABPP of cytochrome P450s as described in the following section. The cytochrome P450 genes are of particular interest in the context of tocopherol metabolism because CYP3A and CYP4F have been shown to metabolize gamma tocopherol in mammals.^{7,25} There were 21 and 29 cytochrome P450 genes identified in the turkey liver samples when mapping to the turkey genome and chicken genome, respectively. Comparative RNA-Seq between turkey and chicken was done, utilizing the chicken genome because of the more extensive annotation of its genome compared to that of the turkey genome. Three CYP450 enzymes were differentially expressed (all up-regulated) in turkey versus chicken liver. The CYP450s that were upregulated in turkey (on the basis of the chicken genome) were 2 C45 (ENSGALG 000 00 005 46 9), 7 A1 (ENS-GALG00000015432), and 51A1 (ENSGALG00000009365). The ortholog of chicken CYP2C45 is CYP2C29 in turkey. CYP2C29 and CYP2C45 have 454 and 494 amino acids, respectively, in which amino acid sequence identity is 77% (381 out of 494 residues). Expression of turkey CYP2C29 transcripts was 16.9-fold greater ($p < 0.001$) than that of its chicken ortholog on the basis of RNA-Seq (Table 4). CYP51A1 (a lanosterol demethylase) and CYP7A1 (hydroxylase of cholesterol at position 7) are known to be involved in cholesterol biosynthesis and bile acids biosynthesis, respectively.²⁶ RNA-Seq indicated statistically similar transcript abundance of the alpha tocopherol transfer protein (ATTP) when comparing the livers from chickens to those from turkeys.

Activity-Based Protein Profiling of Liver Tissue from Chicken and Turkey

The two ABPs used to measure cytochrome P450 activity were broad coverage probes, intended to capture a wide range of active P450 isoforms that may be involved in demethylation, hydroxylation, or other enzymic functions. Thus, these probes were not designed specifically to detect omega-hydroxylation of tocopherols. Microsomal proteins containing bound probe were enriched and digested with trypsin so that the proteins with P450 activity in liver tissue were identified and quantified as described in the Materials and Methods. There were 12 and 24 cytochrome P450 proteins identified in the turkey liver when mapping to the turkey genome and chicken genome, respectively. Turkey CYP2C29, –3A9, and –4F22 were scrutinized compared to their chicken orthologs (CYP2C45, –3A40, and –4F22) to determine if comparisons of LC-MS spectral counts were warranted. The query coverage for turkey 2C29 versus chicken 2C45 was 100%, with 77% amino acid identity (381 out of 494 residues). The query coverage for turkey 3A9 versus chicken 3A40 was 76%, with 61% amino acid identity (307 out of 506 residues). The query coverage for

turkey 4F22 versus chicken 4F22 was 53%, with 83% amino acid identity (159 out of 192 residues). These degrees of homology were considered sufficient to make comparisons of spectral counts between turkey and chicken liver. Spectral counts for turkey P450 2C29 were significantly greater ($p < 0.01$) compared to those of its chicken ortholog (Table 4). Spectral counts for turkey P450 3A9 and 4F22 were significantly greater ($p < 0.05$) compared to those of their chicken orthologs (Table 4).

Tocopherol Concentrations in Thigh and Breast Muscle of Chickens and Turkeys

AT levels in turkey thigh were significantly lower (1.9- to 2.8-fold) than those in chicken thigh for all three dietary treatments (Table 5). The moisture and fat content in breast and thigh muscle from turkey compared to that from chicken was similar (data not shown). Thus, AT results on wet weight and lipid basis yield similar differences between chicken and turkey compared to the units shown in Table 5 ($\mu\text{mol AT/kg}$ on a dry-matter basis). GT in turkey thigh was significantly lower (1.9-fold) than that in chicken thigh only when the low level of all-racemic AcT was fed (Table 5).

The level of AT in turkey breast was significantly lower (2.9- to 4.7-fold) than that in chicken breast in all three diets (Table 5). These findings are consistent with the elevated AT metabolism noted in turkey bile compared to that in chicken (Table 4). GT in turkey thigh was significantly lower (3.4-fold) than that in chicken thigh only when the high level of all-racemic AcT was fed (Table 5). It should be noted that GT levels in thigh and breast muscle were up to 150-fold lower than AT levels, which was partly due to the fact that AT combined with AcT levels in the diets were up to 12-fold greater than GT levels in the diets (Table 1). Also, AT is known to accumulate in tissues preferentially to GT because of the preferential binding and transport of AT by the alpha tocopherol transfer protein (ATTP) in the liver compared to that of GT.²⁷

The occasionally lower level of GT in turkey muscle compared to that of chicken (in 2 of the 6 comparisons) suggests that GT metabolism was elevated in turkey, which is consistent with the elevated GT metabolism in turkey bile relative to that in chicken bile (Table 4). The fact that GT was not always lower in turkey muscle in spite of elevated GT metabolites is likely due to the low level of GT in the diets (Table 1).

Tocopherol Concentrations in Liver and Plasma of Chickens and Turkeys

Liver and plasma are two separate compartments where dietary tocopherols can localize after intake. Portomicrons that are formed in enterocytes will deliver tocopherols to the liver via the portal vein system. Hepatic tocopherols, including AT and GT, will be transported to the plasma via ATTP in the liver. GT was shown to be bound and transported via ATTP at 9% of the efficiency of AT.²⁸

The level of AT in turkey liver was significantly lower (2.1- to 2.7-fold) compared to that in chicken liver for all three diets (Table 6). GT in turkey liver was significantly lower (1.9- to 2.2-fold) than that in chicken liver when the low and high level of all-racemic AcT was fed but not when *RRR* AcT was fed (Table 6). It should be noted that similar concentrations of AT were found in muscle and liver from chickens fed *RRR* AcT compared to those from

chickens fed all-racemic AcT (Tables 5 and 6) in spite of the fact that *RRR* AcT was fed at 35 mg/kg feed versus 58 mg/kg feed of all-racemic AcT. This indicated that *RRR* AcT accumulated in tissues more efficiently than allracemic AcT, which was expected because the specificity of ATTP for *SRR* AT was shown to be 11% of that of *RRR* AT.²⁸ The all-racemic AcT is an equal mixture of the 8 possible stereoisomers of AT (*RRR*, *RRS*, *RSR*, *RSS*, *SRR*, *SSR*, *SRS*, and *SSS*). AT in turkey thigh was actually significantly higher when turkeys were fed the *RRR* AcT diet versus the all-racemic AcT diet, on the basis of a Student's *t* test (Table 5).

The level of AT in turkey plasma was significantly lower (1.7- to 2.4-fold) than that in chicken plasma for all three diets (Table 6). These results again are consistent with the increased concentration of AT metabolites observed in turkey compared to that in chicken bile (Table 4). GT in turkey plasma was significantly lower (1.9- to 2.2-fold) than that in chicken plasma when the low level of all-racemic AcT was fed but not when *RRR* AcT or the high level of all-racemic AcT was fed (Table 6). Again, the low level of GT in the diet relative to AT from dietary AcT should be noted.

Inferences on Intestinal Absorption Differences between Turkeys and Chickens

Besides differences in tocopherol metabolism by hydroxylases, differences in intestinal absorption of dietary tocopherols when comparing turkeys to chickens is an alternative explanation for the decreased accumulation of tocopherols in plasma, liver, and muscle of turkeys. The comparison of a high and low level of dietary allracemic AcT provides an opportunity to make inferences on intestinal absorption differences between turkeys and chickens. In the event that turkeys had decreased absorption of dietary tocopherols compared to that in chickens accumulation of AT should be lower in turkey tissue (plasma, liver, breast, and thigh) compared to that of the same chicken tissues because the dietary load of AcT is increased fivefold in both bird species. However, the relative increase of AT in turkey tissues was similar to that in chicken tissues as the dietary AcT was increased (Table 7). Thus, intestinal absorption of dietary tocopherols appeared to be similar, on the basis of this indirect metric of absorption.

Lipid Oxidation in Minced and Salted Turkey Thigh Compared to that in Chicken Thigh

Thiobarbituric acid reactive substances (TBARS) were used to measure lipid oxidation in minced and salted turkey thigh compared to that in chicken thigh during -4 °C storage. The *RRR* AcT diet and the high level of all-racemic AcT diet were examined. At weeks 0–7, the statistical comparison was pooled turkey (*n* = 5) versus pooled chicken (*n* = 6) because there was no effect of diet within bird type at those time points. TBARS were higher in turkey thigh than in chicken thigh at 0, 2, 3, 4, 5, 6, and 7 weeks of storage (Figure 1). These findings of slower onset of lipid oxidation in the treatments with elevated concentrations of AT are consistent with the ability of AT to inhibit lipid oxidation during storage.²⁹ TBARS were lower at week 8 of storage in thigh from chickens receiving *RRR* AcT diet versus the allracemic AcT (Figure 1).

Tocopherol Hydroxylase Activity in Isolated Microsomes

Tocopherol hydroxylase activity in microsomes prepared from turkey and chicken liver was examined in a separate grow out of birds that received the *RRR*-alpha tocopheryl acetate diets for 5 weeks. *RRR*-alpha tocopherol was used as the substrate in the liver microsome assay (Table 8). Tocopherol hydroxylase activity was higher in the turkey microsomes than in those from chicken ($p < 0.01$). The alpha tocopherol metabolites content in the accompanying bile was also determined, indicating a level of metabolites in the turkey bile ($p < 0.01$) higher than that in chicken (Table 8). The alpha tocopherol content in the accompanying thigh muscle was also determined, indicating tocopherol levels in the turkey thigh muscle ($p < 0.01$) lower than those in chicken (Table 8).

DISCUSSION

Our observations that levels of AT and GT metabolites were elevated in turkey bile compared to those in chicken suggest that CYP2C29 was the cytochrome P450 in the turkeys that was particularly effective at hydroxylating AT and GT for the following reasons. First, RNA expression of turkey CYP2C29 was 16.8-fold greater than its chicken ortholog (CYP2C45), on the basis of comparative RNA-Seq in liver tissue. The only other CYPs out of the 29 identified by RNA-Seq that were differentially expressed in turkey versus that in chicken were CYP51A1 and CYP7A1, which are a cholesterol and bile acid synthesis genes, respectively. Second, activity-based protein profiling (ABPP) was 2.9-fold greater in turkey CYP2C29 than in chicken CYP2C45. Broad coverage probes (an aryl alkyne and an aliphatic alkyne) were used to maximize the potential of finding CYPs with differential enzyme activity. Third, CYP2C9 (which is in the same family as CYP2C29) and CYP4F2 were both involved in the metabolism of phenprocoumon.³⁰ Mammalian CYP4F2 is known to metabolize GT.⁷ The ability of CYP4F2 to share a substrate for metabolism with CYP2C shows potential for CYP2C to be involved in vitamin E metabolism. Furthermore, CYP2C9 has been shown to facilitate omega-hydroxylation of ibuprofen.³¹ This bears notable resemblance to omega-hydroxylation of gamma tocopherol by mammalian CYP4F2. It should also be noted that CYP4F22 and CYP3A9 were higher in turkey than in chicken on the basis of ABPP (Table 4). CYP3A9 is noted in regard to GT metabolism in mammals.²⁵ Thus, CYP2C29, CYP4F22, and CYP3A9 are candidates that cause increased tocopherol metabolism in turkeys compared to that in chickens. The higher alpha tocopherol hydroxylase activity in turkey microsomes compared to those from chicken (Table 8) was additional evidence that omega-hydroxylation of tocopherols by cytochrome P450 hydroxylase was elevated in turkeys.

Tocopherol metabolism results should be considered in the context of differences in feed intake, weight gain, and gain/feed ratios that were observed between the turkeys and chickens. Turkeys consumed 1.76-fold less feed per day but gained 1.62-fold less weight per day compared to chickens, which resulted in increased gain/feed ratio in the turkeys (Table 3). It would be ideal to have equivalent intake, gain, and gain/feed ratio to make comparative specie assessments, but this could not be achieved with the current experimental design. However, the milligrams of AcT intake per kilograms of body weight was similar between turkeys and chickens when *RRR*-AcT was fed, whereas chicken intake

was about 1.3-fold more AcT per kilograms of body weight when all-racemic AcT was fed. It turned out that the *RRR* AcT turkey diet was inadvertently 1.13-fold higher in AcT compared to the *RRR* AcT chicken diet (Table 1). This appears to be fortuitous because it resulted in statistically similar milligrams of AcT intake per kilograms of body weight when comparing turkeys to chickens (Table 1) that aided assessment of comparative tocopherol metabolism. The largest increase in AT and GT metabolite levels in turkey compared to those in chicken bile (5.5 and 8.3-fold) occurred when the dietary treatment was *RRR*-AcT (Table 4). Even when chicken intake was 1.3-fold more AcT per kilograms of body weight than turkeys in the form of dietary all-racemic AcT, AT and GT metabolites were still significantly higher in turkey than in chicken bile (2.2- and 2.4-fold).

Differences in feed composition between the turkeys and chickens to meet differing nutrient requirements for each of the species may also affect interpretations of tocopherol metabolism results. The dietary protein (amino acid) requirement for turkeys is 28% of the diet compared to 23% for chickens through 5 weeks of age. The energy requirement of broilers for the first 5 weeks of life is 400 kcal (metabolizable energy/kg diet) greater than that for turkeys. Equivalent diet formulations would therefore result in one of the species receiving excesses or deficiencies in the energy or protein requirements, which would potentially stratify differences in growth and feed intake when comparing chickens and turkeys. We chose to meet the requirements for each of the species. Similar body weights were reported when chicks and turkeys were fed feedstuffs containing sorghum and protein soybean meal for 28 days.⁹ Sorghum was 60.8 and 40.7% of the chicken and turkey diets, respectively, whereas the soybean meal was 32.3 and 52.0% of the respective diets. These levels of sorghum and soybean meal may be useful as a starting point to standardize intake, gain, and gain/feed ratio in comparative feeding trials. However, providing different levels of polyphenols that are endogenously present in sorghum could be a confounding factor.

It should be noted that the base feedstuffs in our studies (corn starch, soy protein isolate, and wheat middlings) provided a feed that was lower in endogenous tocopherols than the milled corn and soybean meal that is usually fed to chickens and turkeys in commercial settings. Elevated AT and GT from the milled corn and soybean meal feedstuffs may alter tocopherol-metabolizing enzymes versus our study in which AT and GT from the feedstuffs were kept at low concentration in the feed. For example, elevated AT and GT in the diet could induce expression of hydroxylases to a different degree in turkeys and chickens compared to that in our study feeding with low endogenous tocopherols. We chose to use feedstuffs with low levels of endogenous tocopherols so that effects due to the supplemented AcT could be monitored with a minimized influence from the endogenous tocopherols.

Market weight turkeys are older in age (18–22 weeks) than market weight chickens (5–6 weeks). This type of age difference as well as environmental variables that occur during grow out at commercial facilities has the potential to modulate tocopherol metabolism compared to that in the controlled study described herein. It should be noted that we have also observed increased tocopherol metabolites in bile from market weight turkeys compared to that from market weight chickens that were obtained from commercial growers (data not shown). This indicates that increased tocopherol metabolites in turkeys compared to that in chickens also occurs in commercially relevant settings.

Results of RNA-Seq used to compare gene expression in turkey and chicken livers should be interpreted with caution. One limitation of RNA-Seq is that the procedure measures gene transcript expression (mRNA) and not actual proteins or protein activities. The steady-state concentration of mRNA is determined by the rates of synthesis and decay.³² Half-lives of some mRNA in chickens have been shown to be as short as 3 min.³³ The mRNA of some genes may be degrading before RNA-Seq can measure expression. In regards to our specific analysis, an additional limitation was the species comparisons of turkeys and chickens. Although both species are evolutionarily distinctive, we were able to successfully map the majority of turkey transcripts to the chicken genome (and vice versa), an indication of high sequence [gene] homology between the two species. To date, the genomes of both species are not fully annotated, resulting in reads mapping to the genome but not having a corresponding (known or predicted) gene or protein product. RNA-Seq has been used to compare gene expression in turkeys and chickens by mapping turkey expression to the chicken genome.³⁴ Comparative genetic maps have shown that turkey and chicken genomes are relatively stable through the course of avian evolution,³⁵ but large-scale annotation changes still occur, the most recent of which being the release of version 4 of the *G. gallus* assembly. RNA-Seq has previously been used as a comparative tool for gene expression between humans, chimpanzees, and rhesus monkeys as well as to compare human and mouse platelet transcriptomes.^{36,37} It is noteworthy that CYP2C29 was upregulated in liver compared to its chicken ortholog (CYP2C45) not only on the basis of RNA-Seq but also when using the activity-based proteomic approach, demonstrating consistency at the gene and protein levels of assessment (Table 4).

There are factors other than the relatively low tocopherol content in turkey muscle that could contribute to the increased lipid oxidation in turkey thigh compared to that in chicken (Figure 1). Low-molecular-weight iron content was shown to be 4.5-fold elevated in thigh from turkey compared to that in chicken.³⁸ Low-molecular-weight metals can activate hemoglobin and myoglobin as oxidants.³⁹ We determined no significant differences in total heme protein content (hemoglobin, myoglobin, and cytochrome c) when comparing the thigh muscle of turkeys and chickens used in the current study (data not shown). Fatty acid composition was similar when assessing phospholipids and triacylglycerols in turkey muscle compared to that in chicken.⁴⁰

Increased AT metabolite levels in turkey compared to those in chicken bile corresponded with the decreased accumulation of alpha tocopherol in turkey compared to those in chicken tissues (plasma, muscle, and liver) and the increased lipid oxidation in turkey thigh compared to that in chicken. The increased AT tocopherol hydroxylase activity in liver microsomes from turkey compared to that in chicken liver microsomes further suggests that elevated P450-mediated hydroxylation of AT in the liver of turkeys contributes to the lower content of AT in the turkey thigh muscle. Future work should further characterize the cytochrome P450 hydroxylases that are responsible for metabolism of tocopherols in avian species. This may lead to strategies that improve the ability of turkeys to accumulate dietary tocopherols in their muscle so that oxidative rancidity is delayed during refrigerated and frozen storage.

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Figure 1.

Thiobarbituric acid reactive substances (TBARS) values in thigh muscle from turkeys and chickens fed *RRR* AcT (35 mg/kg feed) and all-racemic AcT (58 mg/kg feed).

Corresponding AT and GT values in the respective thigh muscles are shown in Table 5.

Thigh muscle was ground, salted (1% NaCl), and stored at -4°C for 8 weeks. Mean values and standard deviations shown represent an average of two or three thighs from individual birds (per treatment) that were ground and stored separately. At weeks 0–7, the statistical comparison was pooled turkey ($n = 5$) versus pooled chicken ($n = 6$) because there was no effect of diet within bird type at those time points. At week 8, the comparisons were pooled turkey ($n = 5$) versus chicken *RRR* AcT ($n = 3$) and pooled turkey ($n = 5$) versus chicken all-racemic AcT ($n = 3$) because there was an effect of diet on TBARS in chicken at week 8 (*, $p < 0.05$; ***, $p < 0.001$).

Table 1

Tocopherol Concentrations in Basal Feedstuff and Experimental Feeds Used for Chickens and Turkeys Grown to 5 Weeks of Age and AcT Intake Per Kilogram of Body Weight for Each of the Diets Fed^a

	chicken	turkey	fold difference (turkey relative to chicken)
AT in feedstuff (mg/kg)	0.47 ± 0.23	0.91 ± 0.22	+1.93**
GT in feedstuff (mg/kg)	5.15 ± 0.40	5.56 ± 0.08	ns
AcT in Experimental Diets (mg/kg)			
RRR AcT diet	33.1 ± 0.6	37.5 ± 1.9	+1.13*
all-racemic AcT (low) diet	11.3 ± 1.0	9.3 ± 1.3	ns
all-racemic AcT (high) diet	60.0 ± 6.2	56.2 ± 5.8	ns
AcT Intake (mg/kg body weight)			
RRR AcT diet	51.7 ± 1.4	53.7 ± 1.7	ns
all-racemic AcT (low) diet	17.8 ± 0.4	13.6 ± 0.2	-1.31***
all-racemic AcT (high) diet	94.4 ± 1.7	80.4 ± 1.7	-1.17***

^a AT, alpha tocopherol; GT, gamma tocopherol; AcT, alpha tocopheryl acetate. Units for AT, GT, and AcT were mg/kg feed on a fed basis. AT and GT concentrations reported are from triplicate extractions from each of the three diets ($n = 9$). AcT concentrations are from triplicate extractions of each diet ($n = 3$). Standard deviations shown for milligrams of AcT intake per kilograms of body weight represents variation by pen for each treatment ($n = 7$ pens). ns- not significantly different between chicken and turkey.

*
, $p < 0.05$;

**
, $p < 0.01$;

, $p < 0.001$.

Table 2

Feed Formulas (Ingredients, Less Vitamin E) as Percentages in the Diets on As-Is Basis for Turkeys and Chickens at Time of Slaughter (5 Weeks of Age)

	turkey	chicken
corn starch	35.59	48.10
soycomil P (soy protein isolate)	35.54	25.15
wheat middlings	20.05	20.05
choice white grease	2.50	2.50
monocalcium phosphate	2.85	1.55
calcium carbonate	2.10	1.50
salt	0.35	0.34
methionine, DL 99.5%	0.35	0.30
lysine, HCl 98%	0.13	0.00
P11901 micro ^a	0.30	0.30
sodium sesquicarbonate	0.08	0.10
choline chloride 60%	0.10	0.04
Coban 90	0.05	0.05
threonine	0.00	0.01

^aThe composition of the vitamin and mineral premix (P11901 micro) was as follows: iodine (420 mg/kg premix), manganese (36.60 g/kg premix), zinc (33.12 g/kg premix), selenium (100 mg/kg premix), vitamin A (2,760,000 IU/kg premix), vitamin D3 (1,380,000 IU/kg premix), vitamin K (1210 mg/kg premix), biotin (55 mg/kg premix), niacin (20.80 g/kg premix), pyridoxine (1,100 mg/kg premix), thiamine (770 mg/kg premix), folic acid (550 mg/kg premix), pantothenic acid (4.28 g/kg premix), riboflavin (2.74 g/kg premix), and vitamin B12 (4.90 mg/kg premix).

Table 3Feed Intake, Weight Gain, and Gain/Feed Ratio for Turkeys and Chickens Grown to 5 Weeks of Age^a

	chicken	turkey	fold difference (turkey relative to chicken)
<i>RRR</i> AcT Diet			
intake (g/day)	109.0 ± 10.7	63.1 ± 5.5	-1.72***
gain (g/day)	69.8 ± 5.9	44.1 ± 3.9	-1.58***
gain/feed (g/kg)	641 ± 18	699 ± 21	+1.09***
All Racemic AcT (Low) Diet			
intake (g/day)	105.0 ± 5.7	60.0 ± 8.4	-1.75***
gain (g/day)	66.7 ± 3.9	41.1 ± 5.8	-1.62***
gain/feed (g/kg)	635 ± 15	684 ± 11	+1.08***
All Racemic AcT (High) Diet			
intake (g/day)	102.3 ± 7.8	56.4 ± 9.8	-1.81***
gain (g/day)	65.0 ± 4.7	39.5 ± 7.4	-1.65***
gain/feed (g/kg)	636 ± 12	700 ± 15	+1.10***

*, $p < 0.05$;**, $p < 0.01$;^a AcT, alpha tocopheryl acetate. Standard deviations represent variation by pen for each treatment ($n = 7$ pens).***, $p < 0.001$.

Table 4

Tocopherol Metabolite Concentrations ($\mu\text{mol/L}$) in Bile from Chickens and Turkeys Grown to 5 Weeks of Age with Accompanying Gene Expression of CYP2C Family and CYP Activity in Liver^a

	chicken	turkey	fold difference (turkey relative to chicken)
<i>RRR</i> -AcT diet			
AT metabolites	19.9 $\pm$ 5.2	165.9 $\pm$ 6.6	+8.3***
GT metabolites	17.6 $\pm$ 3.9	97.4 $\pm$ 8.4	+5.5***
All-Racemic AcT (Low) Diet			
AT metabolites	20.5 $\pm$ 28.0	44.4 $\pm$ 24.0	ns
GT metabolites	42.9 $\pm$ 64.2	83.1 $\pm$ 55.3	ns
All-Racemic AcT (High) Diet			
AT metabolites	39.2 $\pm$ 12.7	85.3 $\pm$ 9.2	+2.2**
GT metabolites	11.6 $\pm$ 2.3	28.2 $\pm$ 4.3	+2.4**
RNA-Seq Counts			
2C45/2C29	892 $\pm$ 629 (2C45)	15058 $\pm$ 2783 (2C29)	+16.8***
ABPP Spectral Counts			
2C45/2C29	3.5 $\pm$ 1.7 (2C45)	10.3 $\pm$ 2.1 (2C29)	+2.9**
3A40/3A9	0.8 $\pm$ 0.5 (3A40)	6.0 $\pm$ 3.7 (3A9)	+7.5*
4F22	0.5 $\pm$ 0.6	1.5 $\pm$ 0.6	+3.0*

^aTissue from each bird was used as an experimental unit for the statistical evaluations ($n = 3$ for metabolite comparisons and $n = 4$ for gene expression and CYP activity comparisons). Units for tocopherol metabolites were μM . Gene transcript expression values (mRNA) from RNA-Seq are normalized counts as described in the Materials and Methods. Units for activity-based protein profiling (ABPP) assay are spectral counts as described in the Materials and Methods. ns, not significantly different between chicken and turkey.

* , $p < 0.05$;

** , $p < 0.01$;

*** , $p < 0.001$.

Table 5

Tocopherol Concentrations ($\mu\text{mol/kg}$) in Thigh and Breast Muscle from Chickens and Turkeys Grown to 5 Weeks of Age^a

	chicken	turkey	fold difference (turkey relative to chicken)
AT in Thigh			
fed <i>RRR</i> -AcT diet	140.3 $\pm$ 13.5	72.4 $\pm$ 3.9	-1.9**
fed all-racemic AcT (low) diet	38.3 $\pm$ 0.3	13.7 $\pm$ 1.2	-2.8***
fed all-racemic AcT (high) diet	132.2 $\pm$ 11.9	60.8 $\pm$ 5.3	-2.2***
GT in Thigh			
fed <i>RRR</i> -AcT diet	2.57 $\pm$ 0.97	1.56 $\pm$ 0.21	ns
fed all-racemic AcT (low) diet	1.79 $\pm$ 0.01	0.94 $\pm$ 0.14	-1.9***
fed all-racemic AcT (high) diet	1.34 $\pm$ 0.26	1.63 $\pm$ 0.59	ns
AT in Breast			
fed <i>RRR</i> -AcT diet	97.3 $\pm$ 13.0	33.2 $\pm$ 2.6	-2.9**
fed all-racemic AcT (low) diet	24.1 $\pm$ 2.4	8.1 $\pm$ 3.9	-3.0**
fed all-racemic AcT (high) diet	97.0 $\pm$ 7.5	20.5 $\pm$ 9.6	-4.7**
GT in Breast			
fed <i>RRR</i> -AcT diet	3.02 $\pm$ 2.75	1.29 $\pm$ 0.52	ns
fed all-racemic AcT (low) diet	1.95 $\pm$ 0.62	2.06 $\pm$ 1.73	ns
fed all-racemic AcT (high) diet	2.07 $\pm$ 0.60	0.61 $\pm$ 0.11	-3.4*

^aTissue from each bird was used as an experimental unit for the statistical evaluations ($n = 3$). ns, not significantly different between chicken and turkey. Units are μmol tocopherol isomer per kg tissue on a dry-matter basis.

* , $p < 0.05$;

** , $p < 0.01$;

*** , $p < 0.001$.

Table 6

Tocopherol Concentrations in Liver ($\mu\text{mol/kg}$) and Plasma ($\mu\text{mol/L}$) from Chickens and Turkeys Grown to 5 Weeks of Age^a

	chicken	turkey	fold difference (turkey relative to chicken)
AT in Liver			
fed <i>RRR</i> -AcT diet	200.3 $\pm$ 13.0	73.1 $\pm$ 4.9	-2.7**
fed all-racemic AcT (low) diet	57.4 $\pm$ 10.3	22.3 $\pm$ 1.2	-2.6***
fed all-racemic AcT (high) diet	207.6 $\pm$ 27.6	97.5 $\pm$ 24.4	-2.1*
GT in Liver			
fed <i>RRR</i> -AcT diet	3.08 $\pm$ 1.03	1.31 $\pm$ 0.45	ns
fed all-racemic AcT (low) diet	1.55 $\pm$ 0.24	0.70 $\pm$ 1.03	-2.2**
fed all-racemic AcT (high) diet	1.50 $\pm$ 0.21	0.78 $\pm$ 0.20	-1.9*
AT in Plasma			
fed <i>RRR</i> -AcT diet	36.5 $\pm$ 3.9	21.9 $\pm$ 5.4	-1.7*
fed all-racemic AcT (low) diet	10.1 $\pm$ 1.1	4.2 $\pm$ 0.6	-2.4*
fed all-racemic AcT (high) diet	37.1 $\pm$ 7.8	15.8 $\pm$ 2.0	-2.4**
GT in Plasma			
fed <i>RRR</i> -AcT diet	0.86 $\pm$ 0.29	0.44 $\pm$ 0.26	ns
fed all-racemic AcT (low) diet	0.45 $\pm$ 0.08	0.21 $\pm$ 0.03	-2.0**
fed all-racemic AcT (high) diet	0.10 $\pm$ 0.17	0.00 $\pm$ 0.00	ns

^aTissue from each bird was used as an experimental unit for the statistical evaluations ($n = 3$) with the exception of GT in liver from turkeys fed *RRR* AcT ($n = 2$). ns, not significantly different between chicken and turkey. For liver, units are μmol tocopherol isomer per kg tissue on a dry-matter basis.

* , $p < 0.05$;

** , $p < 0.01$;

*** , $p < 0.001$.

Table 7

Effect of Increasing Dietary All-Racemic AcT Fivefold on Accumulation of AT in Plasma and Tissues from Turkey and Chicken^a

	fold increase in tissue due to 5-fold increase of AcT in diet	
	chicken	turkey
plasma	3.7	3.8
liver	3.6	4.4
breast muscle	4.0	2.5
thigh muscle	3.5	4.4
avg $\pm$ std dev	3.7 $\pm$ 0.2	3.8 $\pm$ 0.9

^a AcT, alpha tocopherol acetate. AT, alpha tocopherol.

Table 8

Alpha Tocopherol Hydroxylase Activity in Liver Microsomes from Chickens and Turkeys Grown to 5 Weeks of Age with Accompanying Alpha Tocopherol Metabolite Concentrations in Bile and Alpha Tocopherol Concentrations in the Thigh Muscles^a

<i>RRR</i> -AcT diet	chicken	turkey	fold difference (turkey relative to chicken)
AT hydroxylase activity in liver microsomes	0.87 ± 0.25	1.93 ± 0.15	+2.2 ^{**}
AT metabolites in bile	19.6 ± 10.0	71.5 ± 4.2	+3.7 ^{**}
AT in thigh	124.1 ± 10.8	31.5 ± 17.8	-3.9 ^{**}

Substrate concentration of *RRR*-alpha-tocopherol (AT) in the liver microsome assay was 150 μ M. Incubation time was 45 min at 37 °C. Units for alpha tocopherol metabolites were μ M. Units for alpha tocopherol content in thigh muscle were μ mol alpha tocopherol per kg tissue on a dry-matter basis. Tissue from each bird was used as an experimental unit for the statistical evaluations ($n = 3$).

^aUnits for alpha tocopherol hydroxylase activity were nmol product (13-OH alpha tocopherol) per mg microsomal protein.

^{**}, $p < 0.01$.