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Distribution of α-tocopherol stereoisomers in liver and thigh of chickens

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The effect of supplementation with different levels of *all-rac*-α-tocopheryl acetate and the inclusion of different dietary contents of PUFA on the deposition of α-tocopherol stereoisomers in liver and thigh of chickens was evaluated. Ninety-six 1-d-old Ross female broiler chickens were randomly distributed into eight experimental treatments (three replicates each) resulting from four levels of α-tocopheryl acetate without supplementation and supplemented with 100, 200 and 400 mg α-tocopheryl acetate/kg and two levels of dietary PUFA (15 and 61 g/kg). The feeds supplemented with α-tocopheryl acetate contained a similar proportion of each stereoisomer. The diets without α-tocopheryl acetate had the following α-tocopherol stereoisomers (%): *RRR* 35·1, *RRS* 24·5, *RSR* 25·3, *RSS* 13·9 and total 2*S* forms 1·3. Consumption of different levels of α-tocopheryl acetate did not lead to statistical differences in α-tocopherol stereoisomer proportion in the liver and thigh. In general, the stereoisomer profiles in the tissues studied were similar, responding to the stereoisomer profile of the diet. Both tissues preferentially accumulated 2*R* stereoisomer (69–100 %). However, when α-tocopheryl acetate was used the discrimination was not specific for the *RRR* α-tocopherol form. Furthermore, the 2*R*:2*S* ratio had a tendency to increase as the polyunsaturation level of the diet increased.

α-Tocopherol stereoisomers: Dietary polyunsaturation: Thigh: Liver: Chicken

Vitamin E cannot be synthesized by man and animals. Therefore, it must be ingested in the diet. Vitamin E includes four tocopherols (α, β, γ and δ) and four tocotrienols (α, β, γ and δ), which differ in their biological and antioxidant activity. α-Tocopherol (αToc) is the tocopherol with the highest biological and antioxidant activity *in vivo* (Burton *et al.* 1993). αToc has three chiral C in its phytyl tail (2, 4′ and 8′), making eight stereoisomeric forms possible: *RRR*, *RRS*, *RSR*, *RSS*, *SSS*, *SSR*, *SRS* and *SRR*. The natural form of αToc present in foods and feeds is *RRR* (2,5,7,8-tetramethyl-2*R*-(4′*R*, 8′*R*, 12-trimethyltridecyl)-6-chromanol), while the most common vitamin E supplement, *all-rac*-α-tocopheryl acetate (αTA) (2,5,7,8-tetramethyl-2*RS*-(4′*RS*, 8′*RS*, 12-trimethyltridecyl)-6-chromanol), consists of an equimolar mixture of all eight stereoisomers (ST).

The relative biological activity of αToc ST is different according to the rat resorption–gestation test. In general, 2*R* αToc forms (*RRR* + *RRS* + *RSR* + *RSS*) have been shown to have higher biological activity than 2*S* αToc forms (*SSS* + *SSR* + *SRS* + *SRR*). The relative biopotencies of αTA ST in rat resorption assay were (%): *RRR* 100, *RRS* 90, *RSS* 73, *RSR* 57, *SSS* 60, *SRS* 37, *SRR* 31 and *SSR* 21 (Weiser & Vecchi, 1982). The configuration of the C_2 on the chromanol ring may be the most important asymmetrical C with regard to determining the biological activity of αToc (Machlin *et al.* 1982) and also has an important role on ST biodiscrimination (Weiser *et al.* 1996). Thus, 2*R* αToc ST are preferentially found in the tissues of human subjects (Traber *et al.* 1990), rats (Nitta *et al.* 1993; Ueda *et al.* 1993; Kiyose *et al.* 1995; Weiser *et al.* 1996) and pigs (Lauridsen *et al.* 2002), as well as in eggs (Piironen *et al.* 1991). However, very little data are available on the transfer and deposit of the different αToc ST in tissues of chickens consuming different sources of vitamin E.

αToc is also the most important antioxidant *in vivo*. High levels of PUFA in the diet cause an increase in the susceptibility of tissues to lipid oxidation, increasing the requirements for vitamin E (Dutta-Roy *et al.* 1994; Muggli, 1994). Although the biological activity of the different ST has been reported, no information is available, to the best of our knowledge, about how the oxidative status of the chick may affect ST distribution among its tissues. In this sense, Ingold *et al.* (1990) suggested that the 2*R* configuration of αToc provides the maximum antioxidant activity *in vivo*. Therefore, the different antioxidant activities of αToc ST may affect their deposition in chicken tissues. Thus, the objective of the present investigation was to evaluate the effect of supplementation with

Abbreviations: αTA, α-tocopheryl acetate; αToc, α-tocopherol; ST, stereoisomer.

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αTA at different levels and the inclusion of different dietary contents of PUFA on the deposition of different αToc ST in the liver and thigh of chickens.

Materials and methods

Animals and diets

The experiment received approval from the Animal Protocol Review Committee of the Universitat Autònoma de Barcelona, and all animal housing and husbandry conformed to European Union guidelines.

Ninety-six 1-d-old Ross female broiler chickens (Granja Solé, Tarragona, Spain) were randomly distributed into eight dietary treatments with three replicates each. The animals were housed in groups of four in twenty-four cages under conditions of standard temperature, humidity and ventilation. The wheat, soyabean and barley-based diet was formulated to meet or exceed the National Research Council (1994) recommendations (Table 1). The experimental treatments resulted from the combination of two levels of dietary PUFA (15 (PU15) or 61 (PU61) g PUFA/kg feed) and four levels of αTA (Rovimix® E-50 Adsorbate; F Hoffmann-La Roche Ltd, Basel, Switzerland) (no supplementation (E0) and supplemented (ES) with 100 (E1), 200 (E2) or 400 (E4) mg αTA/kg feed) (Table 2). To obtain the desired levels of PUFA, 90 g tallow (PU15) or linseed–fish oil (4:1, w/w) (PU61)/kg was added to the basal diet. Since no synthetic αToc was added to the basal diet, the unsupplemented treatment is also denoted as natural source.

Tallow and linseed oil were obtained from Cailà-Parés, S. A. (Barcelona, Spain), fish oil was kindly supplied by Agrupación de Fabricantes de Aceites Marinos, S.A. (Vigo, Spain), and αTA was donated by F. Hoffmann-La Roche Ltd.

Feed and water were provided *ad libitum*. Body weight and food consumption were measured during the experimental period. Feed samples were taken during the experiment for αToc content and ST profile.

Sample collection

At the end of the experimental period (44 d of age), two animals (2319 (SD 110·0) g body weight) per cage were randomly selected and killed in a commercial slaughterhouse. Thighs and livers were removed and weighed individually. Thighs were deboned and ground with skin. Tissue samples were freeze-dried, ground and stored at −20°C until further analyses.

α-Tocopherol analysis

αToc from feed, liver and thigh were saponified and extracted into heptane as described by Jensen *et al.* (1999). The sample weight ranged from 0·5 to 4·0 g feed and from 50 to 200 mg freeze-dried liver and thigh in order to obtain about 10 μg αToc.

α-Tocopherol stereoisomer analysis

The remaining heptane extract containing about 10 μg αToc in 10 ml heptane was evaporated to exact dryness under a N_2 stream. The αToc extracted was derivatized to its methyl ether following the method described by Drotleff & Ternes (2001). The methyl ether derivative was extracted twice with 500 μl heptane. The pooled heptane phases were then used for HPLC determination. Chromatographic separation was achieved on a Chiralcel OD-H column (250 × 4·6 mm, 5 μm particle size, cellulose tris(3,5-dimethylphenylcarbamate); Daicel Chemical Industries Ltd, Tokyo, Japan). The heptane modified with 2-propanol (99·95:0·05, v/v) and degassed with He constituted the mobile phase. HPLC determination was performed according to the conditions described by Drotleff & Ternes (2001). Fluorescence detection was performed

Table 1. Ingredients and composition of the diets*

Diet	g/kg
Ingredients	
Wheat	393·0
Soyabean meal†	340·9
Barley	133·9
Added fat‡	90·0
Dicalcium phosphate	21·7
Calcium carbonate	9·8
Salt	4·5
Vitamin–mineral mix§	4·0
DL-Methionine	2·8
L-Lysine	0·4
Chemical analysis	
DM	907·8
Crude protein (N × 6·25)	229·8
Crude fat	101·7
Crude fibre	34·7
Ash content	60·8
Crude energy (MJ/kg)	18·75
Metabolizable energy (MJ/kg)‖	12·97

* Values are means of eight dietary treatments: result of a 2 × 4 factorial design with two different fat sources and four different levels of dietary supplementation with α-tocopheryl acetate (0, 100, 200 and 400 mg/kg).
† 480 g crude protein (N × 6·25)/kg.
‡ 100 % Tallow was used to obtain 15 g PUFA/kg feed (PU15) or linseed oil–fish oil (4:1, w/w) to obtain 61 g PUFA/kg of feed (PU61).
§ Vitamin and mineral mix (per kg feed); retinol 3·6 mg, cholecalciferol 60 μg, menadione 3 mg, thiamin 2·2 mg, riboflavin 8 mg, pyridoxine 5 mg, cyanocobalamin 11 μg, folic acid 1·5 mg, biotin 150 μg, calcium pantothenate 25 mg, nicotinic acid 65 mg, Mn 60 mg, Zn 40 mg, I 0·33 mg, Fe 80 mg, Cu 8 mg, Se 0·15 mg.
‖ Estimated value.

Table 2. Factorial design of the experiment

Dietary treatment	Dietary PUFA (g/kg)	Dietary supplementation with αTA (mg/kg)
PU15 + E0	15	0
PU15 + E1	15	100
PU15 + E2	15	200
PU15 + E4	15	400
PU61 + E0	61	0
PU61 + E1	61	100
PU61 + E2	61	200
PU61 + E4	61	400

αTA, α-tocopheryl acetate.

with an excitation wavelength of 295 nm and an emission wavelength of 330 nm.

For the evaluation of the methodology, *all-rac*-αTA was derivatized to *all-rac*-α-tocopheryl methyl ether. This should result in one peak with 50 % of the total area for all 2*S* ST and 12·5 % for the other four peaks with 2*R* ST. Sixteen injections led to a mean value of 49·4 (SD 2·9) % for peak with 2*S* ST and mean values 11·6–13·4 (SD 0·8–1·4) % for the other peaks.

Statistical analyses

ANOVA with repeated measures, containing the cage variable as random factor, was performed to determine whether dietary PUFA and αTA level affected the productive variables (*n* 96), the αToc content and ST profile in liver (*n* 48) and thigh (*n* 48) samples of chickens. Since no differences were observed for the ST profile of liver and thigh between 100, 200 and 400 mg αTA/kg, these data were pooled to determine whether αToc source (natural and synthetic) and dietary PUFA affected the ST profile of liver and thigh. Data were treated using the PROC MIXED procedure of SAS package (2000; SAS Institute Inc., Cary, NC, USA). Differences between treatment means were evaluated using Tukey's correction for multiple comparisons. Interactions higher than second order were not taken into consideration. In all cases, P values $\leq 0{\cdot}05$ were considered significant.

Results and discussion

Diet composition

αToc content of the experimental diets is shown in Table 3. The fish and linseed oils used contained 11 and 19 mg αToc/kg respectively, whereas no αToc was found in the tallow. Supplementation with 100, 200 and 400 mg/kg αTA resulted in dietary levels of αToc that matched the amounts added.

The source of dietary αToc clearly affected the profile of the different ST (Table 4). Thus, the non-supplemented diets, which depended on the αToc provided by the feed ingredients, were predominant in 2*R* forms (98·9 %), mainly *RRR* (35·1 %), while the diets supplemented with αTA had similar proportion of the different ST. With the analytical methodology used it was not possible to separate the four 2*S* ST, but we assume, based on data from other research, that the four 2*S* ST are present in similar proportions (Scott *et al.* 1982; Weiser & Vechi, 1982; Riss *et al.* 1994).

It is generally accepted that only the *RRR* ST of αToc is present in nature. However, in the E0 + PU15 diet, in which the tallow used did not have any detectable amounts of αToc, other ST (mainly 2*R* forms) were found. We did not have a definitive explanation for this finding. It may be caused by unknown contamination of the feedstuffs used.

Productive variables

Neither source of dietary αToc nor different levels of dietary supplementation with αTA had a significant effect on performances (results not shown). The average daily feed intake in PU15 treatments was higher than in PU61 treatments (111·7 *v.* 104·6 g/bird per d, $P \leq 0{\cdot}05$). There were no differences in the daily weight gain (60·1 g/bird per d) between the more and the least saturated diets; thus, the feed efficiency was higher in the animals fed the more saturated diet (1·87 *v.* 1·75, $P \leq 0{\cdot}05$). The reduction in the voluntary intake with the inclusion of PUFA has been observed in poultry by other authors (Atteh & Leeson, 1985; Scaife *et al.* 1994) and can be attributed to the better intestinal uptake of PUFA.

Stereoisomers in liver and thigh

The αToc content of liver and thigh, expressed as mg/kg tissue, is shown in Table 5. αToc content of tissues significantly increased with the dietary supplementation with αTA. Our present results are consistent with other reports that αToc content in chicken tissues increases linearly with dietary supplementation (Jensen *et al.* 1999; Flachowsky *et al.* 2002; Cortinas *et al.* 2002). However, to our knowledge, the effect of different levels of dietary supplementation with αTA on the ST profile of different chicken tissues has not been evaluated.

αToc ST profiles in liver and thigh are shown in Tables 6 and 7 respectively. Consumption of different levels of synthetic αToc did not alter αToc ST proportion in these tissues (results not shown). Thus, data from treatments supplemented with different levels of αTA were grouped to compare natural (E0) and synthetic form (ES) of supplementation with αToc (Table 4). Contrary to our present results, Nitta *et al.* (1993) found a reduction in the proportion of 2*R*

Table 3. α-Tocopherol content of the experimental diets (mg/kg)*
(Mean values and standard deviations)

			ES					
	E0		E1		E2		E4	
Dietary polyunsaturation	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PU15	5	0·3	135	7·3	252	13·3	441	32·4
PU61	5	0·6	135	6·3	219	7·9	436	47·1

E0, no supplementation with α-tocopheryl acetate; ES, supplemented with 100 (E1), 200 (E2) and 400 (E4) mg/kg *all-rac*-α-tocopheryl acetate; PU15, 15 g PUFA/kg feed; PU61, 61 g PUFA/kg feed.
* For details of composition of diets, see Table 1.

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Table 4. α-Tocopherol stereoisomer profile of the experimental diets* (%)

	E0		ES	
Stereoisomer	PU15	PU61	PU15	PU61
RRR	32·5	37·6	14·1	14·6
RRS	25·6	23·4	15·2	14·9
RSR	27·9	22·6	11·6	12·1
RSS	12·8	15·0	11·4	11·1
Total 2*R* forms†	98·9	98·6	52·2	52·6
Total 2*S* forms‡	1·1	1·4	47·8	47·4
2*R*:2*S* ratio	89·9	70·4	1·1	1·1

E0, without α-tocopheryl acetate supplementation; ES, supplemented with 100, 200 or 400 mg/kg *all-rac*-α-tocopheryl acetate; PU15, 15 g PUFA/kg feed; PU61, 61 g PUFA/kg feed.
* For details of the composition of the diets, see Table 1.
† Total 2*R* forms: *RRR* + *RRS* + *RSR* + *RSS*.
‡ Total 2*S* forms: *SSS* + *SSR* + *SRS* + *SRR*.

ST in liver from 93·0 to 78·4 % when dietary supplementation with αTA was increased from 10 to 100 mg/kg. This difference may be caused by the higher dietary doses of αTA used in our present study, which dilutes the *RRR* αToc content in the basal diet.

Furthermore, dietary polyunsaturation also affected αToc deposition in the studied tissues (Table 5). As such, the αToc content of liver and thigh was higher and the increase caused by supplementing diets with different levels of αTA was more marked in the diet with lower PUFA content. As the degree of dietary polyunsaturation increased, the PUFA content of tissues increased and the saturated fatty acids and MUFA contents decreased (Table 5), creating higher oxidative pressure (Husveth *et al.* 2000; Cortinas *et al.* 2001; Grau *et al.* 2001). The higher lipid oxidation rate can lead to higher consumption of αToc and consequently there is less αToc available to be deposited into the tissues. Ingold *et al.* (1990) reported that the chiral configuration of αToc is important for its antioxidant activity. Therefore, the oxidative pressure in the tissues of the animal may affect the pattern of deposition of the different ST, depending on their antioxidant activities. This is why the ST profiles of liver and thigh were studied in the present work, since how dietary fatty acids affect the fatty acid profile and lipid oxidation is understood well.

Table 5. α-Tocopherol (αToc) content (mg/kg) and fatty acid (FA) content (g/kg) of liver and thigh*

(Least square mean values obtained from ANOVA (*n* 6) with their pooled standard errors)

	Liver			Thigh		
	PU15	PU61	SE	PU15	PU61	SE
αToc content†						
E0	1·1^{c}	0·2^{c}	4·41	2·6^{ef}	0·1^{f}	2·88
E1	17·3^{bc}	6·1^{c}		21·0^{cd}	10·8^{def}	
E2	33·3^{ab}	8·9^{c}		36·8^{b}	14·8de	
E4	44·9^{a}	17·0^{bc}		53·2^{a}	29·3^{bc}	
FA content						
TFA‡	35·8	35·1	0·98	141·2	116·8	4·93
SFA‡	14·2	13·5	0·33	46·8	27·1	1·56
MUFA§	10·2	7·2	0·42	76·4	34·0	2·25
PUFA§	11·4	14·4	0·48	17·9	55·7	2·21

PU15: 15 g PUFA/kg feed; PU61, 61 g PUFA/kg feed; E0, without supplementation with α-tocopheryl acetate; E1, supplemented with 100 mg *all-rac*-α-tocopheryl acetate/kg; E2, supplemented with 200 mg *all-rac*-α-tocopheryl acetate/kg; E4, supplemented with 400 mg *all-rac*-α-tocopheryl acetate/kg; TFA, total fatty acids; SFA, total saturated fatty acids.
^{a,b,c,d,e,f} Mean values with unlike superscript letters were significantly different (dietary PUFA × αToc supplementation, $P<0·05$).
* For details of diets and procedures, see Tables 1–3 and p. 296.
† Effect of dietary PUFA level and α-tocopheryl acetate supplementation and the interaction between both factors significant at $P\leq0·01$ for both tissues.
‡ Effect of dietary PUFA level significant at $P\leq0·001$ for thigh.
§ Effect of dietary PUFA level significant at $P\leq0·001$ for liver and thigh.

Liver and thigh both responded to the ST profile of the diet. When no αTA was supplemented, and only the αToc from feed ingredients (with 98·8 % 2*R* αToc) was available, the ST profile differed between tissues depending on the polyunsaturation level of the diet (Tables 6 and 7). In liver at the highest dietary polyunsaturation level (PU61), only *RRR*-αToc was detected. This may be because the concentration of ST forms other than *RRR* αToc was very low and below the detection limit of this method. Alternatively, at the low dietary polyunsaturation level (PU15), although the *RRR* was the predominant form, the other 2*R* and 2*S* forms were also present. There was an increase in *RRR* level with respect to the diet (liver:diet ratio 1·2), a reduction in *RRS* and *RSR*, and an increase in the proportion of *RSS* and 2*S* (liver:diet ratio 6·4). In thigh, the ST profile of the animals from PU15 treatment was similar to that found in liver, with enrichment in *RRR* (thigh:diet ratio 1·6). In birds from the PU61 treatment, *RRR* was the main ST and the rest of 2*R* and 2*S* forms were present in this tissue.

When the synthetic source is used, with equal amount of the eight ST, the ST profile of liver and thigh tissues is similar. In general, both tissues predominantly accumulated the 2*R* ST (from 52·4 % in diet to 73·8 % in liver and 70·4 % in thigh). The 2*R*:2*S* ratio showed a tendency to increase as the polyunsaturation level of the diet increased, from 2·4 to 3·5 in liver ($P=0·089$) and from 2·3 to 2·6 in thigh ($P=0·070$). That indicated that as the polyunsaturation level increased, the deposit of 2*R* forms was favoured at the expense of 2*S* forms. These results are in agreement those of Weiser *et al.* (1996), who studied the ST profile in different tissues of rats fed diets with synthetic αToc and found a similar ST pattern. Similarly, Ueda *et al.* (1993) found a similar accumulation of 2*R* ST in the liver, brain, adrenal glands and adipose tissue of rats fed a diet containing 100 mg αTA/kg.

Globally, our present results show that a bias in favour of 2*R* ST exists in the chicken when equal proportions of the eight ST were supplied, since the 2*R*:2*S* ratio was >2 in both tissues examined. These results are in accordance with other studies that have found that after the administration of αTA to rats, tissues were enriched preferentially with 2*R* forms (2*R*:2*S* ratio in livers ranging from 2·3 to 5·6; Nitta *et al.* 1993; Ueda *et al.* 1993; Weiser *et al.* 1996). Among the different 2*R* ST, the *RRR* and *RRS* forms were the predominant ST. The liver of the animals fed with αTA responded in the following order: $RRR = RRS > RSR = RSS$. Alternatively, the response in thighs depended on the dietary polyunsaturation level, but it can be summarized as $RRS \geq RRR > RSR > RSS$. Both liver and thigh from birds fed diets supplemented with αTA

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Table 6. Effect of supplementation with *all-rac*-α-tocopheryl acetate and dietary polyunsaturation on α-tocopherol stereoisomer profile in liver (%)*

(Least square mean values obtained from ANOVA (*n* 6) with their pooled standard errors)

	E0		ES		Statistical significance of variance (*P*)			
Stereoisomer	PU15	PU61	PU15	PU61	PUFA	α-Toc source	PUFA × α-Toc source	SE
RRR	$39{\cdot}2^{bx}$	$100{\cdot}0^{ay}$	$18{\cdot}7^{cy}$	$21{\cdot}1^{cy}$	0·001	0·001	0·001	1·18
RRS	$20{\cdot}3^{aby}$	$0{\cdot}0^{cz}$	$18{\cdot}4^{by}$	$20{\cdot}9^{ay}$	0·001	0·001	0·001	0·69
RSR	$13{\cdot}7^{bz}$	$0{\cdot}0^{cz}$	$16{\cdot}2^{abz}$	$17{\cdot}5^{az}$	0·001	0·001	0·001	1·10
RSS	$19{\cdot}9^{ay}$	$0{\cdot}0^{cz}$	$16{\cdot}6^{bz}$	$17{\cdot}6^{bz}$	0·001	0·001	0·001	0·87
Total 2*R* forms†	93·1	100·0	69·9	77·1	0·001	0·001	0·927	1·56
Total 2*S* forms‡	7·0	0·0	30·1	22·9	0·001	0·001	0·927	1·02
2*R*:2*S* ratio	14·4	–	2·4	3·5	0·089	0·001	–	0·47

E0, without α-tocopheryl acetate supplementation (natural source); ES, supplemented with 100, 200 and 400 mg *all-rac*-α-tocopheryl acetate/kg; PU15, 15 g PUFA/kg feed; PU61, 61 g PUFA/kg feed; αToc, α-tocopherol.

[a,b,c] Mean values in the same row with unlike superscript letters were significantly different (dietary PUFA × αToc source; $P<0{\cdot}05$).

[x,y,z] Mean values in the same column with unlike superscript letters were significantly different ($P<0{\cdot}05$).

* For details of diets and procedures, see Tables 1–3 and p. 296.

† Total 2*R* forms: *RRR* + *RRS* + *RSR* + *RSS*.

‡ Total 2*S* forms: *SSS* + *SSR* + *SRS* + *SRR*.

showed a similar discrimination towards *RRR*-αToc. Thus, the tissue *RRR*:diet *RRR* ratio was 1·4, showing the preferential enrichment of *RRR*-αToc in the tissues studied.

Among the four 2*R* ST, *RRR* and *RRS* were in higher proportion in liver and thigh among diets supplemented with αTA. Although differences between *RRR* + *RRS* and *RSR* and *RSS* are small, they are significant, showing that there is a preferential deposition for *RRR* and *RRS*. That observation is in concordance with the observations of Weiser & Vecchi (1982) that, in general, among 2*R* forms, biological activities of 4*R* (*RRR* and *RRS*) are higher than those of the 4*S* ST (*RSR* and *RSS*). Contrary to our present results, Weiser *et al.* (1996) observed that after the treatment of rats with *all-rac*-αTA, the presence of the different 2*R* ST was similar in different tissues, and concluded that the storage capacity of the tissues was almost the same for all 2*R* ST.

The biodiscrimination of 2*R* ST has been described previously in rats, human subjects and pigs (Traber *et al.* 1990; Nitta *et al.* 1993; Ueda *et al.* 1993; Kiyose *et al.* 1995; Weiser *et al.* 1996; Lauridsen *et al.* 2002), and confirms that the configuration at C_2 of the αToc molecule has a major impact on ST biodiscrimination (Weiser *et al.* 1996). Piironen *et al.* (1991) found that when laying hens were fed diets supplemented with αTA, the enantiomeric pair *RRR* + *SSS* was transferred to eggs in a greater proportion than the other ST, suggesting that the transfer efficiencies of different ST from feed to eggs, and probably to other tissues, are proportional to their biological activities. Taking the relative concentration of ST as an indicator of its biological activity, we may assume that the biological activity of the 2*R* ST is more than twice the activity of the 2*S* (Weiser & Vecchi, 1982).

Recent studies have shown that liver has a critical role in the biodiscrimination of ST, because of the presence of αToc transfer protein. This protein preferentially selects 2*R* αToc for secretion into plasma (Leonard *et al.* 2002), and although it has been described in rats and human subjects (Sato *et al.* 1991, 1993; Kuhlenkamp *et al.* 1993), its presence in chicken tissues has not been demonstrated. However, our present results, in which enrichment in 2*R* ST was observed after feeding equal amounts of all eight ST may be explained by the presence of the αToc transfer protein in chicken liver. Hosomi *et al.* (1997) have shown

Table 7. Effect of supplementation with *all-rac*-α-tocopheryl acetate and dietary polyunsaturation on α-tocopherol stereoisomer profile in thigh (%)*

(Least square mean values obtained from ANOVA (*n* 6) with their pooled standard errors)

	E0		ES		Statistical significance of variance (*P*)			
Stereoisomer	PU15	PU61	PU15	PU61	PUFA	α-Toc source	PUFA × α-Toc source	SE
RRR	$53{\cdot}1^{by}$	$64{\cdot}2^{ay}$	$19{\cdot}4^{cx}$	$20{\cdot}1^{cx}$	0·018	0·001	0·036	2·62
RRS	$11{\cdot}6^{z}$	$12{\cdot}4^{z}$	$23{\cdot}3^{w}$	$20{\cdot}4^{ax}$	0·497	0·001	0·206	0·99
RSR	$9{\cdot}5^{cz}$	$5{\cdot}5^{cz}$	$14{\cdot}9^{by}$	$17{\cdot}7^{ay}$	0·682	0·001	0·023	0·96
RSS	$19{\cdot}2^{az}$	$5{\cdot}2^{cz}$	$11{\cdot}7^{bz}$	$13{\cdot}5^{bz}$	0·001	0·810	0·001	1·73
Total 2*R* forms†	93·4	87·4	69·2	71·6	0·681	0·001	0·142	2·21
Total 2*S* forms‡	$6{\cdot}6^{b}$	$12{\cdot}6^{b}$	$30{\cdot}8^{a}$	$28{\cdot}4^{a}$	0·384	0·001	0·042	1·36
2*R*:2*S* ratio	$13{\cdot}5^{a}$	$7{\cdot}6^{ab}$	$2{\cdot}3^{b}$	$2{\cdot}6^{b}$	0·070	0·001	0·042	1·90

E0, without α-tocopheryl acetate supplementation (natural source); ES, supplemented with 100, 200 and 400 mg *all-rac*-α-tocopheryl acetate/kg; PU15, 15 g PUFA/kg feed; PU61, 61 g PUFA/kg feed; αToc, α-tocopherol.

[a,b,c] Mean values in the same row with unlike superscript letters were significantly different (dietary PUFA × α-Toc source; $P<0{\cdot}05$).

[w,x,y,z] Mean values in the same column with unlike superscript letters were significantly different ($P<0{\cdot}05$).

* For details of diets and procedures, see Tables 1–3 and p. 296.

† Total 2*R* forms: *RRR* + *RRS* + *RSR* + *RSS*.

‡ Total 2*S* forms: *SSS* + *SSR* + *SRS* + *SRR*.

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that the biological activity of αToc ST is related to its relative affinity for αToc transfer protein. Other authors suggested that the metabolism of 2*S* ST in the liver may be faster than that of 2*R* ST, thereby reducing the presence of 2*S* ST in liver and other tissues (Kiyose *et al.* 1995; Kaneko *et al.* 2000).

In conclusion, consumption of different levels of synthetic αToc did not modify αToc ST profile in liver and thigh. Both tissues had a similar ST pattern depending on ST profile of the diet. When synthetic αTA was used, increasing dietary polyunsaturation favoured the deposition of 2*R* forms. Our present results seem to indicate that chickens have a mechanism to biodiscriminate among different ST forms, in favour of the 2*R* ST. However, it is not clear that this mechanism is specific for *RRR* αToc ST. This biodiscrimination may be caused by the presence of αToc transfer protein in chicken liver.

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