Influences of biotin deficiency and dietary *trans*-fatty acids on tissue lipids in chickens

BY BRUCE A. WATKINS

Department of Poultry Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

(*Received 16 December 1987 – Accepted 5 September 1988*)

1. The combined effects of feeding hydrogenated fats and varying the levels of biotin and linoleate (18:2ω6) on polyunsaturated fatty acids were studied in the chicken.

2. Biotin deficiency signs were not exacerbated by feeding hydrogenated fats or by diets low in linoleate for 21 d.

3. Biotin deficiency resulted in proportionately higher levels of 18:2ω6 and γ-linolenate (18:3ω6) in liver triglycerides, and lower levels of dihomo-γ-linolenate (20:3ω6) in liver and heart phospholipids irrespective of the 18:2ω6 level in the diet.

4. Biotin deficiency did not alter arachidonate (20:4ω6) levels in tissue lipids at 21 d.

5. Feeding high levels of *trans*-18:1 isomers with adequate biotin led to reduced 20:3ω6 and 20:4ω6 levels in liver and heart phospholipids with compensatory increases in ω3 fatty acids.

6. The *trans*-isomers of 18:1 were incorporated into several tissues of the chick. Incorporation was dependent on the levels fed. Very small amounts were incorporated into brain compared with other tissues when dietary *trans*-isomer levels were high, but were similar when dietary *trans*-isomer levels were low. The *trans*-18:1 isomers appear to be preferentially incorporated into phospholipids as opposed to triglycerides in heart and liver.

Suboptimal biotin nutriture coupled with other nutritional and environmental stresses has been implicated in fatty liver and kidney syndrome (FLKS) which can cause high mortality in commercial flocks of meat-type chickens (Hood *et al.* 1976). The symptoms of FLKS differ from classical biotin deficiency in that the onset is sudden and death is rapid (within 24 h), but this condition is responsive to biotin supplementation (Bannister, 1976). Bannister *et al.* (1983) suggested that FLKS results from feeding biotin-deficient, low-protein rations. Classical biotin deficiency symptoms arise when protein levels are adequate.

A second condition involving acute death (sudden death syndrome, SDS) in chickens is thought by some (Hulan *et al.* 1980), but not by others (Steele *et al.* 1982), to be related to biotin status. SDS or 'flip-over' is characterized by unexpected cardiac arrest in fast-growing, apparently healthy chickens (primarily male) of about 3 weeks of age. The most pronounced pathological changes found in these chickens are pulmonary congestion and vascular lesions (Ononiwu *et al.* 1979).

Because of biotin's role in the formation of malonyl-CoA, which is essential for *de novo* fatty acid synthesis and elongation of long-chain polyunsaturated fatty acids, certain alterations in tissue fatty acid composition are seen during biotin deficiency. Watkins & Kratzer (1987*a*) reported higher levels of 16:1, 17:0, 18:1 and 18:2ω6 (linoleate) and lower levels of 20:3ω6 (dihomo-γ-linolenate) in the liver of biotin-deficient chicks compared with biotin-adequate counterparts. These changes have also been reported in livers of rats fed on biotin-deficient diets (Puddu *et al.* 1967; Kramer *et al.* 1984).

A critical step in essential fatty acid metabolism involving biotin may be the conversion of γ-linolenate to dihomo-γ-linolenate. A depression in dihomo-γ-linolenate results during biotin deficiency and this may lead to alterations in prostaglandin biosynthesis which could interfere with the regulation of cardiac function. Watkins & Kratzer (1987*b*) reported decreased levels of prostaglandin E_2 in heart tissue of biotin-deficient chicks.

Giving certain dietary lipids may complicate the effects of biotin deficiency and contribute to the development of FLKS and SDS. Today's poultry diets often contain spent restaurant greases and hydrogenated fats. Prolonged heating and catalytic hydrogenation result in the formation of positional and geometrical isomers of unsaturated fatty acids, particularly of 18:1. Studies with rats have shown that some of these isomers inhibit the Δ6- and Δ5-desaturase enzymes which are necessary for the conversion of linoleate to arachidonate (Mahfouz *et al.* 1980; Hill *et al.* 1982; Lawson *et al.* 1985). In addition, hydrogenated fats often contain low levels of linoleate (Thomassen *et al.* 1984). The presence of *trans*-18:1 in poultry diets, and the possibility of reduced desaturation of linoleate, might further jeopardize the synthesis of prostaglandins by reducing the substrate.

The present study was designed to examine the combined effects of biotin deficiency and the feeding of diets containing hydrogenated fats or low levels of linoleate to young chickens. The primary objectives were: (1) to determine whether biotin deficiency symptoms were exacerbated by feeding hydrogenated fats or low linoleate; (2) to measure the incorporation of geometrical isomers of 18:1 into chick tissues; (3) to determine the effects of the dietary treatments on fatty acid profiles in heart, liver, brain, abdominal fat pads and lungs.

MATERIALS AND METHODS

Diets, animals and tissues

Twelve experimental diets were prepared from the basal diet which was adequate in all nutrients for chickens (National Research Council, 1984) except biotin and linoleate (Table 1). Each diet contained added biotin at 0, 200 or 400 μg/kg diet and 50 g/kg of one of the following lipid sources: maize oil (MO); partially hydrogenated soya-bean oil (HSBO); a mixture of triolein, tripalmitin and tristearin (30:30:40, by wt; OPS); or spent restaurant grease (SRG). The purified triglycerides were purchased from the United States Biochemical Corporation (Cleveland, OH). Dried egg albumen was added to the diets as a source of avidin which binds biotin. All diets contained 14·2 MJ metabolizable energy and 246 g crude protein (nitrogen × 6·25)/kg.

Male broilers (Hubbard strain; 1-d-old) were randomly assigned to the twelve treatments with two replicate pens per treatment and eight chickens per pen. The chickens were maintained in battery brooders with a daily cycle of 18 h light–6 h dark (06·00–24·00 hours light). Feed and water were provided *ad lib.* for the duration of the 21 d experiment. Total feed consumptions and weight gains were determined at the completion of the experiment for calculation of feed efficiency (total gain/total feed) on a pen basis.

On day 21, chickens were weighed and scored for twisted leg and dermatitis on a scale of 0–4 (Watkins & Kratzer, 1987*a*). Chickens were killed by cervical dislocation. Tissues were surgically removed and frozen at −20° for subsequent analyses for biotin content in liver and heart, and fatty acid compositions of liver and heart phospholipids and triglycerides. The total *trans*-18:1 fatty acids were measured in brain, abdominal fat pads, heart, liver and lungs and in phospholipids and triglycerides of heart and liver.

Analytical procedures

Biotin was measured by the radiochemical method of Hood (1977) in liver and heart from chicks fed on MO. The number of chick tissues assayed varied with treatment.

Total lipids from brain, fat pads, heart, liver and lungs were extracted in chloroform–methanol (2:1, v/v) by the method of Bligh & Dyer (1959). In addition, triglycerides (TG) and phospholipids (PL) were separated in lipid extracts from hearts and livers using thin-layer chromatography (TLC), with a developing cocktail of light

Table 1. *Composition of the basal diet (g/kg diet)*

Ingredients	
Soya-bean protein*	250·0
Maize starch	557·5
Lipid†	50·0
Dried egg albumen‡	30·0
Mineral mix§	30·0
Cellulose	30·0
$CaHPO_4 . 2H_2O$	25·0
$CaCO_3$	15·0
Vitamin premix‖	5·0
DL-Methionine	7·5

* Ralston Purina Assay Protein, RP-101, purchased from Ralston Purina Company, Richland, IN, contained the following (g/kg): moisture 55, crude protein (nitrogen × 6·25) 903, ash 38, fat 3, fibre 1.

† One of the following lipid sources: maize oil (MO), hydrogenated soya-bean oil (HSBO), triolein–tripalmitin–tristearin mixture (30:30:40, by wt; OPS) or spent restaurant grease (SRG).

‡ Dried egg albumen supplied by Henningsen Foods, Omaha, NE, contained the following (g/kg): moisture 80, protein 800, analysed avidin binding was 5·86 units/g (1 unit of avidin binds 1 μg biotin).

§ Contained (mg/kg diet): $CoCl_2 . 6H_2O$ 5, $CuSO_4 . 5H_2O$ 60, $FeSO_4 . 7H_2O$ 500, KCl 1500, K_2HPO_4 6000, KIO_3 6, $MgSO_4 . 7H_2O$ 6000, $MnSO_4 . H_2O$ 300, NaCl 6000, $Na_2MoO_4 . 2H_2O$ 10, Na_2SeO_3 0·43, ZnO 90.

‖ Contained (mg/kg diet): retinol 1·35, cholecalciferol 0·1125, DL-α-tocopheryl acetate 50, menadione 1·5, thiamin 15, riboflavin 15, calcium pantothenate 15, niacin 50, pyridoxine 6, folic acid 6, cyanocobalamin 0·02, choline chloride 1000, butylated hydroxytoluene 200.

petroleum (b.p. 35–60°)–diethyl ether–glacial acetic acid (80:20:1, by vol.). The fatty acids in TG and PL were methylated (Metcalfe *et al.* 1966) and fatty acid profiles were determined with an HP 5890A gas–liquid chromatograph equipped with a flame-ionization detector and a Nelson Analytical Data System (Hewlett Packard Co., Sunnyvale, CA). A DB225 fused silica column (0·25 mm i.d. × 30 m) purchased from J & W Scientific Co. (Folsom, CA) was used with helium as the carrier gas and temperature programming. Fatty acid standards were purchased from Nu-Chek-Prep, Inc. (Elysian, MN). Proportions of fatty acids present in each sample were determined as area %. Eight chicks per treatment were used except where insufficient tissue was available.

Fatty acid analysis was also done for each dietary lipid source, and the *cis*-:*trans*-isomer ratios of 16:1 and 18:1 in HSBO and SRG were determined in triplicate by combined argentation-TLC (Dudley & Anderson, 1975) and gas–liquid chromatography (as described previously). Silver nitrate-impregnated TLC plates were prepared by immersing precoated plates into a saturated methanolic solution of silver nitrate. Methylated samples of each lipid were applied, and the plates were developed twice in hexane–diethyl ether–glacial acetic acid (94:4:2, by vol.). Methyl nonadecanoate was added as an internal standard to the recovered fractions which were then analysed by gas–liquid chromatography. *Cis*-:*trans*-isomer ratios in tissue lipids (brain, fat pads, heart, liver, lungs, and heart and liver TG and PL) were also determined for eight chicks fed on diets containing HSBO and SRG with 400 μg of added biotin/kg diet.

Statistical analyses

Values for body-weights, feed efficiencies, and twisted leg and dermatitis scores were subjected to an analysis of variance (Snedecor & Cochran, 1974) with a 4 × 3 factorial arrangement of treatments (four dietary lipids with three levels of biotin). Values for biotin levels in liver and heart were subjected to a one-way design of analysis of variance (Snedecor & Cochran, 1974). Fatty acid values were evaluated as a 4 × 2 factorial

Table 2. *Fatty acid composition (area %) of dietary lipids*
(Values are means of four analyses)

Fatty acid	Dietary lipid source			
	MO	HSBO	OPS	SRG
14:0	3·32	0·00	5·47	4·13
14:1	0·00	0·00	0·27	0·72
15:0	0·00	0·00	0·10	0·69
16:0	12·53	11·42	31·55	28·15
t-16:1	0·00	0·00	0·00	0·18
c-16:1	0·11	0·00	1·60	2·76
17:0	0·08	0·12	0·05	1·60
18:0	2·34	8·04	35·10	16·77
t-18:1	0·00	17·16	0·00	6·37
c-18:1	26·37	23·53	20·68	30·05
t-18:2	0·00	1·01	0·10	0·36
18:2ω6	53·29	31·67	2·18	3·64
18:3ω3	0·84	4·19	0·02	0·13
20:0	0·43	0·31	0·25	0·14

MO, maize oil; HSBO, hydrogenated soya-bean oil; OPS, triolein–tripalmitin–tristearin mixture (30:30:40, by wt.); SRG, spent restaurant grease; *t*, *trans*-isomer; *c*, *cis*-isomer.

Table 3. *Body-weights (at 21 d), feed efficiencies (total gain/total feed intake), twisted leg and dermatitis scores of chicks fed on the experimental diets**
(Values are means for the number of chicks shown)

	Biotin level (μg/kg)	Average body-wt (g)	*n*	Feed efficiency	Twisted leg	Dermatitis
MO	0	245^{d}	16	$0·58^{e}$	$2·69^{b}$	$3·88^{a}$
	200	440^{b}	15	$0·79^{a}$	$0·53^{c}$	$0·23^{bcd}$
	400	501^{a}	15	$0·77^{ab}$	$0·37^{c}$	$0·07^{cd}$
HSBO	0	236^{d}	15	$0·54^{f}$	$3·27^{a}$	$3·87^{a}$
	200	510^{a}	16	$0·76^{abc}$	$0·12^{c}$	$0·32^{bcd}$
	400	503^{a}	15	$0·75^{abc}$	$0·37^{c}$	$0·07^{cd}$
OPS	0	237^{d}	14	$0·53^{f}$	$2·29^{b}$	$4·00^{a}$
	200	385^{c}	15	$0·71^{bcd}$	$0·13^{c}$	$0·40^{b}$
	400	412^{bc}	14	$0·68^{d}$	$0·00^{c}$	$0·07^{cd}$
SRG	0	268^{d}	15	$0·58^{e}$	$2·53^{b}$	$3·93^{a}$
	200	400^{bc}	15	$0·75^{abc}$	$0·00^{c}$	$0·33^{bc}$
	400	439^{b}	13	$0·70^{cd}$	$0·04^{c}$	$0·00^{d}$
Pooled SD		65		0·03	0·76	0·39
Significant effects		Biotin, lipid		Biotin, lipid	Biotin, lipid	Biotin

MO, maize oil; HSBO, hydrogenated soya-bean oil; OPS, triolein–tripalmitin–tristearin mixture (30:30:40, by wt); SRG, spent restaurant grease.

[a–f] Means within a column with different superscript letters were significantly different, as determined by Duncan's multiple-range test ($P < 0·01$).

* For details, see Tables 1 and 2.

Table 4. *Biotin levels in liver and heart of chicks fed on maize oil**

(Values are means with their standard errors for the number of chicks shown)

Dietary biotin (μg/kg)	Biotin (ng/g wet tissue)					
	Liver			Heart		
	Mean	SEM	*n*	Mean	SEM	*n*
0	271[c]	32	13	< 50[c]	—	9
200	831[b]	68	9	122[b]	18	9
400	1274[a]	78	15	196[a]	20	15

[a, b, c] Means within a column with different superscript letters were significantly different, as determined by Bonferroni's multiple-comparison post-priori test ($P < 0.05$).

* For details of diet, see Tables 1 and 2.

Table 5. *Fatty acid composition of liver triglycerides in the chick at 21 d of age**

(Values are means and pooled standard deviations for individual fatty acids)

Fatty acid†	Dietary biotin (μg/kg)	Fatty acids (area %)				Pooled SD	Statistically significant effects
		MO	HSBO	OPS	SRG		
16:0	0	27·61[c]	29·13[bc]	30·59[bc]	32·86[abc]	5·02	Biotin
	400	38·33[a]	35·22[ab]	37·36[a]	35·41[ab]		
16:1	0	8·88[ab]	6·63[bc]	12·74[a]	10·39[ab]	3·36	Biotin, lipid
	400	3·61[c]	6·17[bc]	6·46[bc]	4·32[c]		
17:0	0	0·24[b]	0·23[b]	0·26[b]	0·56[a]	0·18	Lipid
	400	0·15[b]	0·29[b]	0·24[b]	0·68[a]		
18:0	0	6·14[d]	8·21[cd]	7·97[cd]	5·76[d]	4·37	Biotin
	400	17·24[ab]	12·71[abc]	12·00[bc]	17·85[a]		
18:1	0	25·20[b]	29·67[ab]	34·94[a]	34·14[a]	5·15	Lipid
	400	28·55[ab]	30·28[ab]	32·10[ab]	30·23[ab]		
18:2ω6	0	23·28[a]	16·46[b]	5·72[e]	7·20[c]	2·15	Biotin–lipid interaction
	400	7·26[c]	5·20[cd]	2·87[d]	4·74[cd]		
18:3ω3	0	0·39[bc]	0·84[a]	0·46[b]	0·19[cd]	0·19	Biotin–lipid interaction
	400	0·13[d]	0·40[bc]	0·17[cd]	0·24[bcd]		
18:3ω6	0	0·65[a]	0·58[a]	0·74[a]	0·28[b]	0·23	Biotin
	400	0·17[b]	0·09[b]	0·09[b]	0·02[b]		
20:3ω6	0	0·18	0·19	0·30	0·15	0·08	—
	400	0·23	0·20	0·12	0·19		
20:4ω6	0	1·91[a]	1·30[ab]	1·10[ab]	1·00[ab]	0·78	Biotin
	400	0·72[b]	0·94[ab]	0·35[b]	0·80[b]		

MO, maize oil; HSBO, hydrogenated soya-bean oil; OPS, triolein–tripalmitin–tristearin mixture (30:30:40, by wt); SRG, spent restaurant grease.

[a–d] For individual fatty acids, values not sharing a common superscript letter were significantly different ($P < 0.05$).

* Total chicks sampled for 0 and 400 μg biotin respectively, MO, *n* 5, 8; HSBO, *n* 4, 6; OPS, *n* 6, 5; SRG, *n* 6, 7. Details of extraction and separation are described on pp. 100–101.

† Only the major fatty acids are listed.

Table 6. *Fatty acid composition of heart triglycerides in the chick at 21 d of age**

(Values are means and pooled standard deviations for individual fatty acids)

Fatty acid†	Dietary biotin (μg/kg)	Fatty acids (area %) MO	HSBO	OPS	SRG	Pooled SD	Statistically significant effects
16:0	0	23·81^{b}	25·34^{ab}	25·81^{ab}	26·20^{ab}	2·46	Lipid
	400	24·57^{ab}	24·84^{ab}	27·43^{a}	26·77^{ab}		
16:1	0	4·60^{b}	5·06^{b}	9·04^{a}	7·14^{ab}	2·63	Lipid
	400	4·61^{b}	5·34^{b}	7·47^{ab}	6·26^{ab}		
17:0	0	0·80^{a}	0·59^{ab}	0·40^{bcd}	0·57^{abc}	0·23	Biotin, lipid
	400	0·23^{d}	0·29^{cd}	0·19^{d}	0·56^{abc}		
18:0	0	17·09	15·94	10·86	9·09	5·43	—
	400	10·27	10·77	9·96	11·52		
18:1	0	21·09^{e}	27·49^{d}	37·47^{ab}	39·41^{ab}	5·39	Biotin, lipid
	400	30·06^{cd}	34·83^{bc}	41·63^{a}	39·22^{ab}		
18:2ω6	0	16·03^{b}	14·36^{b}	7·19^{c}	8·83^{c}	2·81	Lipid interaction
	400	22·71^{a}	14·44^{b}	5·93^{c}	6·76^{c}		
18:3ω3	0	0·61^{bc}	1·02^{ab}	0·37^{c}	0·36^{c}	0·41	Biotin, lipid
	400	0·74^{bc}	1·39^{a}	0·42^{c}	0·82^{bc}		
18:3ω6	0	0·12	0·13	0·12	0·10	0·08	—
	400	0·19	0·10	0·07	0·05		
20:3ω6	0	0·36^{b}	0·22^{b}	0·27^{b}	0·30^{b}	0·14	Lipid
	400	0·57^{a}	0·34^{b}	0·24^{b}	0·20^{b}		
20:4ω6	0	1·88^{a}	1·31^{ab}	0·43^{bc}	0·67^{bc}	0·69	Biotin, lipid
	400	0·91^{bc}	0·73^{bc}	0·43^{bc}	0·27^{c}		

MO, maize oil; HSBO, hydrogenated soya-bean oil; OPS, triolein–tripalmitin–tristearin mixture (30:30:40, by wt); SRG, spent restaurant grease.

$^{a-e}$ For individual fatty acids, values not sharing a common superscript letter were significantly different ($P < 0·05$).

* Total chicks samples for 0 and 400 μg biotin respectively, MO, *n* 5, 8; HSBO, *n* 7, 8; OPS, *n* 5, 6; SRG, *n* 6, 7. Details of extraction and separation are described on pp. 100–101.

† Only the major fatty acids are listed.

arrangement of treatments (four dietary lipids with two levels of biotin). The statistical models did consider variation between chicks within pens and between pens for all values except feed efficiences. Bonnferroni's multiple-comparison post-priori test or Duncan's multiple-range test were employed when significant differences existed (Neter & Wasserman, 1974). The standard error of the mean was calculated for liver biotin levels. Variation between means for performance values, deficiency symptoms and fatty acid values was expressed as a pooled standard deviation (the square root of the mean square for error) since sample sizes were unequal. Data were presented to indicate individual treatment means and the significant main effects and interactions. The statistical models used revealed no significant pen effect on the measurements taken.

RESULTS

A fatty acid compositional analysis of the four dietary lipid sources is given in Table 2. The MO diets contained high levels of linoleate and no *trans*-isomers of 18:1. The diets containing HSBO had adequate amounts of linoleate (15·8 g/kg diet) and 42% of the 18:1 fatty acids were *trans*-isomers. Diets containing SRG had limiting amounts of linoleate (1·8 g/kg diet) and 17% of the 18:1 fatty acids were *trans*-isomers. When diets contained OPS, no unnatural positional or geometrical isomers of 18:1 were present and linoleate

Table 7. *Fatty acid composition of liver phospholipids in the chick at 21 d of age**

(Values are means and pooled standard deviations for individual fatty acids)

Fatty acid†	Dietary biotin (μg/kg)	Fatty acids (area %) MO	HSBO	OPS	SRG	Pooled SD	Statistically significant effects
16:0	0	21·56	17·14	19·89	19·21	2·46	—
	400	18·53	18·00	18·40	18·98		
16:1	0	2·41^{b}	1·88^{b}	6·58^{a}	3·63^{a}	2·02	Biotin, lipid
	400	1·05^{b}	1·64^{b}	2·98^{ab}	1·73^{b}		
17:0	0	0·27^{bc}	0·19^{cd}	0·19^{cd}	0·48^{a}	0·05	Biotin, lipid
	400	0·08^{d}	0·08^{d}	0·11^{d}	0·32^{b}		
18:0	0	19·69^{b}	20·74^{b}	17·83^{b}	19·25^{b}	3·64	Biotin
	400	25·55^{a}	23·65^{a}	23·20^{a}	23·83^{a}		
18:1	0	7·36^{c}	7·92^{c}	17·03^{a}	15·22^{ab}	3·68	Biotin, lipid
	400	9·71^{bc}	14·10^{abc}	16·37^{ab}	13·75^{abc}		
18:2ω6	0	22·48^{a}	20·93^{ab}	11·92^{e}	14·72^{d}	1·38	Biotin–lipid interaction
	400	18·81^{bc}	17·84^{c}	11·93^{e}	14·48de		
18:3ω3	0	0·07^{b}	0·26^{a}	0·21^{ab}	0·15^{ab}	0·08	Lipid
	400	0·06^{b}	0·27^{a}	0·14^{ab}	0·17^{ab}		
18:3ω6	0	0·62^{ab}	0·50^{ab}	0·38^{b}	1·00^{a}	0·25	Biotin, lipid
	400	0·25^{b}	0·19^{b}	0·24^{b}	0·29^{b}		
20:3ω6	0	0·85^{d}	1·02^{d}	1·82^{c}	1·40^{cd}	0·33	Biotin
	400	2·63^{a}	2·49^{ab}	2·34^{ab}	2·11^{ab}		
20:4ω6	0	14·73^{a}	15·30^{a}	10·66^{ab}	12·24^{ab}	2·73	Biotin–lipid interaction
	400	14·62^{a}	9·53^{b}	9·72^{b}	11·62^{ab}		
20:5ω3	0	0·10^{c}	0·53^{b}	0·62^{ab}	0·60^{ab}	0·15	Biotin, lipid
	400	0·14^{c}	0·87^{a}	0·72^{ab}	0·81^{ab}		

MO, maize oil; HSBO, hydrogenated soya-bean oil; OPS, triolein–tripalmitin–tristearin mixture (30:30:40, by wt); SRG, spent restaurant grease.

$^{a-e}$ For individual fatty acids, values not sharing a common superscript letter were significantly different ($P < 0·05$).

* Total chicks sampled for 0 and 400 μg biotin respectively, MO, *n* 5, 8; HSBO, *n* 4, 6; OPS, *n* 6, 5; SRG, *n* 6, 7. Details of extraction and separation are described on pp. 100–101.

† Only the major fatty acids are listed.

was only 1 g/kg diet. The linoleate requirement for the chick has been established at 10 g/kg diet (National Research Council. 1984).

Body-weight, feed efficiency, and twisted leg and dermatitis values are presented in Table 3. Average body-weights were dependent on the level of biotin and the lipid source fed. There were no differences among body-weights of chicks given 0 μg added biotin, indicating no confounding effects of fat source on growth depression caused by biotin deficiency. Chicks fed on 400 μg added biotin and OPS or SRG weighed less than those fed on diets containing the same amount of added biotin with MO or HSBO. Chicks fed on HSBO grew most rapidly. The incidence of twisted leg was dependent on the biotin level and lipid source fed, while the dermatitis response was due only to biotin deficiency. Feed efficiency improved with the addition of biotin at 200 μg/kg diet for all lipid sources fed.

Biotin levels in liver and heart tissues were dependent on the amount of biotin fed (Table 4). Chicks receiving 0 μg biotin had the lowest levels of this vitamin in both tissues. Levels of biotin in heart were much lower than those in liver.

Fatty acid compositions of liver TG are shown in Table 5 (only major fatty acids are

Table 8. *Fatty acid composition of heart phospholipids in the chick at 21 d of age**

(Values are means and pooled standard deviations for individual fatty acids)

Fatty acid†	Dietary biotin (μg/kg)	Fatty acid (area %) MO	HSBO	OPS	SRG	Pooled SD	Statistically significant effects
16:0	0	14·48	15·85	14·95	14·54	1·56	—
	400	15·71	14·72	16·49	14·66		
16:1	0	0·72^{bc}	1·19^{abc}	1·84^{a}	1·53^{ab}	0·51	Biotin, lipid
	400	0·47^{c}	0·72^{bc}	1·44^{abc}	1·07^{abc}		
17:0	0	0·19^{c}	0·24^{bc}	0·20^{bc}	0·42^{a}	0·05	Biotin, lipid
	400	0·12^{c}	0·14^{c}	0·13^{c}	0·32^{ab}		
18:0	0	16·33^{b}	19·65^{ab}	21·51^{ab}	20·12^{ab}	3·13	—
	400	22·75^{a}	20·77^{ab}	20·13^{ab}	20·98^{ab}		
18:1	0	7·19^{c}	13·03^{b}	14·13^{b}	13·74^{b}	1·36	Biotin, lipid
	400	9·06^{c}	13·40^{b}	17·56^{a}	15·57^{ab}		
18:2ω6	0	24·81^{ab}	23·49^{ab}	22·97^{ab}	25·53^{ab}	2·24	Lipid
	400	24·91^{ab}	26·68^{a}	22·09^{b}	22·86^{ab}		
18:3ω3	0	0·07	0·19	0·12	0·11	0·12	—
	400	0·07	0·19	0·14	0·23		
18:3ω6	0	0·06^{bc}	0·08^{abc}	0·08^{abc}	0·13^{a}	0·03	Biotin–lipid interaction
	400	0·01^{c}	0·06^{bc}	0·11^{ab}	0·07^{abc}		
20:3ω6	0	0·57^{e}	0·76de	1·43^{bc}	1·34^{c}	0·24	Biotin, lipid
	400	1·21^{cd}	1·49^{bc}	1·93^{a}	1·75^{ab}		
20:4ω6	0	21·03^{a}	16·09^{ab}	12·65^{cd}	12·85^{cd}	2·64	Biotin, lipid
	400	15·87^{ab}	12·76^{cd}	7·95^{d}	10·63^{c}		
20:5ω3	0	0·06^{c}	0·34^{b}	0·42^{ab}	0·50^{ab}	0·10	Biotin, lipid
	400	0·06^{c}	0·49^{ab}	0·56^{a}	0·60^{a}		

MO, maize oil; HSBO, hydrogenated soya-bean oil; OPS, triolein–tripalmitin–tristearin mixture (30:30:40, by wt); SRG, spent restaurant grease.

$^{a-e}$ For individual fatty acids, values not sharing a common superscript letter were significantly different ($P < 0·05$).

* Total chicks sampled for 0 and 400 μg biotin respectively, MO, *n* 5, 8; HSBO, *n* 7, 8; OPS, *n* 5, 6; SRG, *n* 6, 7. Details of extraction and separation are described on pp. 100–101.

† Only the major fatty acids are listed.

presented so the totals are not 100%). Chicks receiving 0 μg added biotin and adequate linoleate (MO) had lower levels of 16:0 and 18:0, but higher levels of 16:1, 18:2ω6, 18:3ω3, 18:3ω6 and 20:4ω6 (Table 5). Biotin-deficient chicks fed on HSBO and OPS also had higher levels of 18:2ω6, 18:3ω3 and 18:3ω6 compared with chicks receiving 400 μg biotin. Higher levels of linoleate, α-linolenate (18:3ω3) and 18:3ω6 occurred during biotin deficiency independent of the linoleate levels fed. The changes in fatty acids observed in chicks fed on SRG were not great enough to be significant.

Heart TG (Table 6) contained higher levels of 17:0 and 20:4ω6 and lower levels of 18:1, 18:2ω6 and 20:3ω6 in biotin-deficient chicks fed on MO compared with chicks receiving 400 μg biotin. Chicks fed on OPS and SRG tended to have higher levels of 16:1 and 18:1 in liver and heart TG compared with chicks fed on MO and HSBO. This may be due to the higher levels of 16:0 and 18:0 in OPS and SRG (Table 2) and to the chick's ability to desaturase these fatty acids.

Fatty acid values in liver and heart PL are presented in Tables 7 and 8 respectively (only major fatty acids are presented so the totals are not 100%). Observations in liver PL due to biotin deficiency included increased values for 18:2ω6 in the MO and HSBO treatments but values for 18:0 and 20:3ω6 were decreased in all lipid treatments. Also with biotin

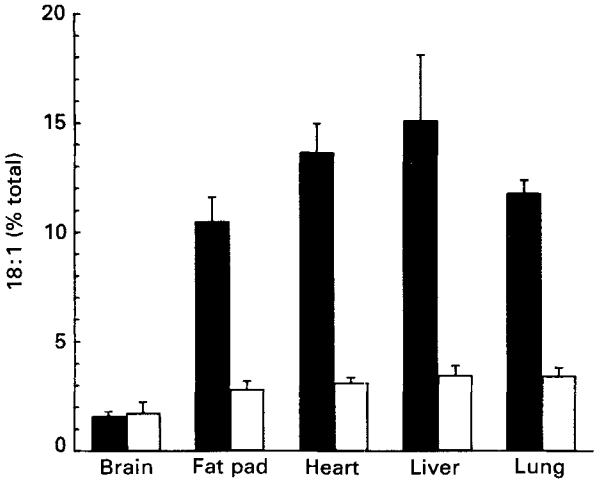

Fig. 1 Incorporation of *trans*-18:1 fatty acids into tissues of chicks receiving 400 μg added biotin/kg diet. Values are means with their standard errors represented by vertical bars for eight chicks fed on hydrogenated soya-bean oil (■) or spent restaurant grease (□).

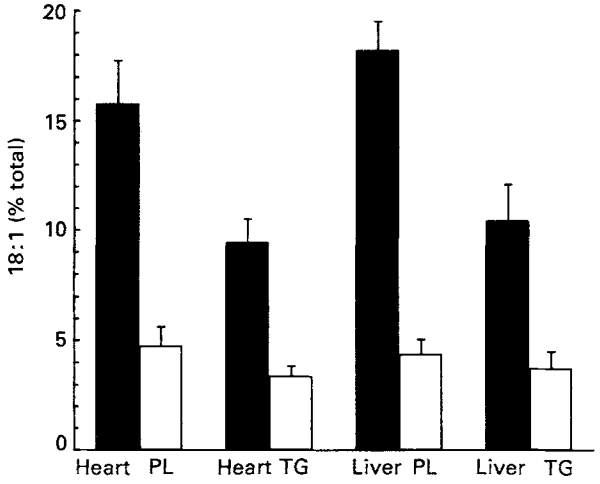

Fig. 2. Distribution of *trans*-18:1 fatty acids in heart and liver phospholipids (PL) and triglycerides (TG) of chicks receiving 400 μg added biotin/kg diet. Values are means with their standard errors represented by vertical bars for eight chicks fed on hydrogenated soya-bean oil (■) or spent restaurant grease (□).

deficiency in liver PL, elevated levels of 17:0 were found in the MO and SRG treatments, levels of 16:1 and 18:3ω6 were higher in the SRG treatment, and in the HSBO treatments, 20:4ω6 was increased while 20:5ω3 was decreased. In heart PL, the value for 20:4ω6 in the HSBO treatment was elevated and values for 20:3ω6 were decreased in all lipid treatments due to biotin deficiency. Values for 18:0 and 18:1 were decreased for the MO and OPS treatments respectively. Levels of linoleate in liver and heart PL were highest in the MO and HSBO treatments but 20:4ω6 levels were lower in the HSBO treatment when adequate biotin was fed. Values for 20:5ω3 in liver and heart PL were lowest in the MO treatment compared with the other lipids. These findings would indicate that biotin deficiency results

in depressed elongation of 18:3ω6 and that significant alterations in polyunsaturated fatty acids occur in liver and heart PL.

Incorporation of *trans*-isomers of 18:1 into the tissues of chicks receiving 400 μg biotin/kg was dependent on the dietary level of *trans*-18:1, and on tissue type and lipid fraction (Figs. 1 and 2). Much less *trans*-isomer was found in tissues of chicks fed on SRG than in chicks fed on HSBO. Brain tissue contained the lowest levels of *trans*-isomers (Fig. 1). Chicks fed on HSBO had about 16% *trans*-isomers in liver and slightly less in heart, lung and fat pads, but those fed on SRG had about 3% in all these tissues. There was a significantly greater incorporation of *trans*-18:1 into PL compared with TG in heart and liver of HSBO-fed chicks (Fig. 2). Lower levels of *trans*-18:1 were found in PL and TG in chicks fed on SRG.

DISCUSSION

In the present study, biotin deficiency did not result in depressed arachidonate levels. For all four dietary lipid sources, tissue levels of arachidonate were either higher or unchanged in the biotin-deficient chicks when compared with the biotin-adequate groups. In an earlier study, Watkins & Kratzer (1987*b*) found arachidonate to be lower in liver total fatty acids of biotin-deficient chicks compared with their biotin-adequate counterparts at 15 d of age but not at 21 d. Perhaps the changes in 20:4ω6 levels due to biotin deficiency are age-related or the amounts of lipids, triglycerides or phospholipids, or all three, change. Research by Gibson *et al.* (1984) suggests that membranes are buffered from extreme changes in fatty acid compositions which might be expected from differences in dietary lipids. Nutritional stresses may first result in reduced levels of total phospholipids rather than in altered fatty acid profiles. Unfortunately, levels of triglycerides and phospholipids could not be quantified in the present experiment.

A consistent observation in the present experiment and in previous studies with biotin-deficient chicks (Watkins & Kratzer, 1987*a*, *b*), is a depression in 20:3ω6 in liver and heart lipids. Based on fatty acid profiles of tissue lipids, it appears that elongation of γ-linoleate is reduced in biotin deficiency. Although arachidonate serves as a major precursor of prostaglandins, the prostaglandins and related compounds biosynthesized from 20:3ω6 may be reduced in the biotin-deficient chicken. The effects of biotin deficiency on 20:3ω6 eicosanoids has not been studied in the chicken.

The changes in linoleate levels in liver TG were particularly dramatic. In the chicks fed on MO and HSBO, there was nearly a threefold increase in 18:2ω6 with biotin deficiency. Since the liver is the site of 90–95% of all *de novo* fatty acid synthesis in the chick (O'Hea & Leveille, 1969), and a site of triglyceride synthesis, the increased levels of 18:2ω6 may result from attenuated *de novo* synthesis of non-essential fatty acids. Also a block in the synthesis of dihomo-γ-linolenate due to reduced elongation of γ-linolenate during biotin deficiency may elevate linoleate (lower flux through the ω6 pathway). The latter may contribute more to higher 18:2ω6 since elongation of γ-linolenate is depressed (lower 20:3ω6) in liver slices of biotin-deficient chicks (B. A. Watkins, unpublished results).

The most significant change in fatty acid profiles resulting from feeding HSBO to chicks, with adequate biotin, was a decrease in arachidonate in liver and heart PL compared with chicks fed on MO (9·53 *v.* 14·62 and 12·76 *v.* 15·87). Levels of ω3 fatty acids (18:3ω3 and 20:5ω3) in liver and heart PL of chicks fed on HSBO were increased compared with chicks fed on MO, as a consequence of the higher levels of 18:3ω3 present in the HSBO. Similar trends were seen in lungs, but the effects were less dramaic (values not shown). Fatty acid profiles in brain and abdominal fat pads (not shown) were practically unchanged by feeding HSBO. These findings support previous results (Rogel & Watkins, 1987) that arachidonate levels in liver microsomes are decreased when chicks are fed on HSBO and adequate biotin

(200 μg added biotin/kg diet). Similar observations were also reported for rats (Lawson *et al.* 1983).

Positional and geometrical isomers of 18:1 have been shown to inhibit liver microsomal desaturation of linoleate and dihomo-γ-linolenate in vitro (Mahfouz *et al.* 1980, 1981). In rats, *trans*-18:1 isomers are more effective than *cis*-18:1 isomers in reducing arachidonate levels. The *cis*-18:1 isomers appears to compete for acylation at the 2-position of liver phosphatidylcholine, while *trans*-18:1 isomers are found mostly in the 1-position of liver phosphatidylcholine and phosphatidylethanolamine (Lawson *et al.* 1985).

In the present experiment *trans*-isomers were incorporated into all five tissues investigated. When HSBO (which contained 42% of 18:1 in the *trans* form) was fed, 10–16% of the tissue 18:1 found in fat pads, heart, liver and lungs was in the *trans* form. Tissue *trans*-isomer levels were much lower, less than 4%, when SRG was fed (SRG contained 17% of the total 18:1 as *trans*-isomers). These results indicate that the dietary level of *trans*-isomers influences the incorporation of *trans*-18:1 into several tissue lipids except brain, which contained 1·7% *trans*-isomers of 18:1. The low level of incorporation of *trans*-fatty acids into brain is thought to be due to slower rates of fatty acid metabolism and turnover in this organ (Dhopeshwarkar & Mead, 1973).

Results similar to those seen here for chicks have been reported previously for other species. *Trans*-isomers were incorporated into various tissues when rats were fed on hydrogenated fats (Egwim & Sgoutas, 1971*a*, *b*; Emken, 1984; Blomstrand *et al.* 1985) and were found in human tissues studied at autopsy (Cook, 1981; Kummerow, 1983).

Values in Fig. 2 show that higher proportions of *trans*-18:1 accumulated in PL of heart and liver than in TG of these two organs. Studies in rats suggest that this may be due to a lowered incorporation of *cis*-18:1 isomers in PL (Wood, 1979). Incorporation of *cis*-18:1 isomers into tissues was not examined in the present study, although fatty acid profiles indicate that positional isomers were present in both *cis* and *trans* forms.

Studies with rats have shown that *trans*-isomers of 18:1 can exaggerate symptoms of essential fatty acid (EFA) deficiency (Privett *et al.* 1977; Hill *et al.* 1979). Unlike the rats studied by Thomassen *et al.* (1984), which showed elevated levels of 20:3ω9 after 3 weeks of feeding EFA-deficient diets, the chicks in the present study fed on SRG or OPS showed no EFA-deficiency signs. Chicks do not generally develop EFA deficiency signs this early (Craig-Schmidt *et al.* 1986), so this was not unexpected. Feeding isomers of 18:1 at the levels chosen for the present experiment were not effective in triggering EFA deficiency symptoms at this time.

Altered linoleate metabolism may contribute to the aetiologies of FLKS and SDS. Biotin deficiency reduced the levels of dihomo-γ-linolenate in the chicken, presumably by depressing elongation of γ-linolenate (B. A. Watkins, unpublished results). On the other hand, feeding *trans*-18:1 fatty acids to chickens appears to lower arachidonate levels in liver. The combined effects of marginal biotin and dietary fats high in *trans*-fatty acids may precipitate SDS in commercial meat-type chickens reared under normal husbandry conditions. Experiments are planned to determine if practical diets containing varying levels of biotin and blended fats can influence the incidence and severity of FLKS and SDS symptoms in chickens reared to market age.

The technical assistance of Anne M. Rogel is appreciated. This research was funded by a USDA Animal Health Grant (no. 85-CRSR–2–2739).

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Printed in Great Britain