ELSEVIER

Journal of Nutritional Biochemistry 12 (2001) 492–496

THE JOURNAL OF Nutritional Biochemistry

The effect of a *Tropaeolum speciosum* oil supplement on the nervonic acid content of sphingomyelin in rat tissues

William J. Bettger*, Mandy L. McCorquodale, Clarke B. Blackadar

Department of Human Biology and Nutritional Sciences, College of Biological Science, University of Guelph, Guelph, Ontario, Canada

Received 3 August 2000; received in revised form 24 January 2001; accepted 3 April 2001

Abstract

The lipids of *Tropaeolum speciosum* (*T. speciosum*) are a rich source of naturally occurring nervonic acid (24:1n-9). We report that adding a *T. speciosum* oil supplement to a semi-purified diet significantly increased the amount of 24:1n-9 in liver and heart, but not brain, sphingomyelin (SM) of young rats. The bioavailability of 24:1n-9 from the lipids of *T. speciosum* was similar to that of 24:1n-9 ethyl ester in this rat bioassay.

Keywords: Sphingomyelin; Nervonic acid; Very long chain fatty acids; Rat; Diet

1. Introduction

The nervonic (24:1n-9) and/or lignoceric (24:0) acid content of various tissues has been reported to be altered in peroxisomal disorders [1–3], diabetes [4], alcoholism [5], undernourishment [6], cataracts [7,8], cystic fibrosis [9], multiple sclerosis [10] and other conditions [11–15]. Nervonic acid supplementation has been proposed to prolong functionality in both children and adults with demyelinating diseases [10] and to enhance neurodevelopment in formula-fed and in premature infants [16]. However, the dietary and physiological factors which control the nervonic acid content of tissue, and regulate its molecular form and function, have not been defined.

Previously we have reported that dietary 18:1n-9, 22:1n-9 and 24:1n-9 influence the nervonic acid content and the ratio of 24:1 to 24:0 fatty acids in rat liver sphingomyelin based on testing with 21 common fats and oils as a source of dietary fat [17]. The results of this study indicated that dietary 24:1n-9 was the most potent effector of 24:1n-9 in rat liver. Subsequently, Cook et al. [18] reported that weanling mice fed diets enriched in 22:1n-9 and 24:1n-9 (*Lunaria biennis* oil supplement) had significant increases in 24:1n-9 in liver and erythrocytes.

The lipids of *Tropeaolum speciosum (T. speciosum)*, commonly named the "perennial flame flower", are the richest known natural source of 24:1n-9 and 26:1n-9 in triglyceride form [19] with 24:1 n-9 located primarily in the 1,3 position of glycerol. In this study we investigate the effect of a *T. speciosum* oil supplement on the content of 24:1n-9 and the 24:1/24:0 ratio in the sphingomyelin fraction of liver, heart and brain of growing rats. In order to establish the bioavailability of the *T. speciosum* lipids relative to purified fatty acid standards, we fed additional groups of rats the basal diet supplemented with 20:0, 20:1n-9, 22:0, 22:1n-9, 24:0 or 24:1n-9 in ethyl ester form and analyzed the 24:1n-9 content of SM in rat liver and heart.

2. Materials and methods

Male weanling Wistar rats (45–50g) were fed, for 14 days, a semipurified diet consisting of vitamin-free casein (20%), DL-methionine (0.3%), glucose hydrate (65%), olive oil (5%), cellulose (5%), choline bitartrate (0.2%), vitamins and minerals as previously described [20], but providing 30 mg Zn/Kg diet. The rats were housed under controlled conditions of temperature (22C), humidity (50%), and lighting (12 h day/night) and were provided with food and distilled water ad libitum.

In the first experiment the rats (n = 4) were fed diets containing either 5 wt% olive oil (control) or a diet containing 4.875 wt% olive oil with 0.125 wt% *T. speciosum*

Abbreviations: Sphingomyelin (SM); Tropaeolum speciosum (T. speciosum); Very long chain fatty acids (VLCFA).

* Corresponding author. Tel.: +519-824-4120 ext 3747; fax: +519-763-5902.-

0955-2863/01/$ – see front matter
PII: S0955-2863(01)00166-8

Table 1
Fatty acid composition of *Tropaeolum speciosum* seed oil

16:0	2.0
16:1	tr.
18:0	tr.
18:1	29.9
18:2	3.3
18:3	1.4
20:0	tr.
20:1	0.5
22:0	tr.
22:1	13.3
24:0	0.5
24:1	38.7
26:1	9.3

Values are means of n = 3, expressed as mol%.
Tr. < 0.5 mol%.

lipids. Seeds from *T. speciosum* were purchased from Thompson and Morgan Inc. (Jackson, New Jersey). The seeds were ground to a fine powder and lipids were extracted by the method of Folch et al. [21]. The final lipid phase was prepared by extensive evaporation of residual chloroform under a stream of nitrogen. The fatty acid composition of the lipid extract is given in Table 1; the composition was found to be comparable to that reported previously for *T. speciosum* [21]. The *T. speciosum* lipid extract was mixed with the dietary fat (olive oil) overnight prior to incorporation in the diets. Fatty acid ethyl esters (99% pure) were purchased from Nu-Check-Prep. Inc. (Elysian, Mn) and pre-mixed with the dietary fat in a similar fashion.

In the second experiment the rats (n = 3) were fed diets containing either 5 wt% olive oil or diets containing 4.9 wt% olive oil plus 0.1 wt% 20:0, 20:1n-9, 22:0, 22:1n-9, 24:0 or 24:1n-9 ethyl esters.

The fatty acid composition of tissue sphingomyelin was analyzed by gas liquid chromatography as previously described [22]. Briefly tissues were homogenized in chloroform:methanol (2:1), then washed with 0.88% KCl in H_2O, spotted on Merck silica gel 60 thin layer chromatography plates (EM Science, Gibbstown, NJ), which were developed in chloroform:methanol:aceticacid:H_2O (50:37.5:3.5:2), sprayed with 0.1% 8-anilinonaphthalene-1-sulfonic acid (ANS), visualized with UV light, scraped, transmethylated with 6% H_2SO_4 in methanol, extracted with hexane, dried under a stream of N_2, resuspended in carbon disulfide and analyzed on a Hewlett-Packard 5890 GC with a flame ionization detector. Data were analyzed by general linear modeling followed by a t-test or Tukey's test as indicated in the table footnotes.

3. Results

At the end of the two-week feeding trials all rats appeared healthy. Body weight gain was not significantly different between the different groups of rats.

The results of experiment 1 are shown in Table 2. Supplementation of the diet with *T. speciosum* lipids had no effect on brain SM fatty acid composition. However, the rats fed diets containing *T. speciosum* lipids had significantly decreased 20:0, 22:0, 23:0 and 24:0 levels in SM of liver and heart. This dietary group also had a significant increase in 24:1n-9 SM, and in the ratio of 24:1 to 24:0 SM of both the heart and liver. The fatty acid 26:1n-9, which is contained in the *T. speciosum* lipid extracts at a level of 9.3 mol%, did not accumulate in the SM of either brain, liver or heart.

The results of experiment 2 on the liver are shown in Table 3. We found that the rats supplemented with the 20:0 ethyl ester had a significantly higher level of 20:0 and 22:0 species of SM. Those supplemented with 22:0 had signifi-

Table 2
Sphingomyelin composition of liver heart and brain of rats fed *Tropaeolum speciosum*

	Liver		Heart		Brain	
	Control	*T. speciosum*	Control	*T. speciosum*	Control	*T. speciosum*
16:0	23.5	25.1	20.0	21.0	3.6	3.8
18:0	10.2	9.9	19.1	19.0	75.9	75.8
18:1	0.5	0.5	0.7	1.2	tr.	0.8
18:2	tr.	0.6	tr.	tr.	tr.	tr.
20:0	2.8[a]	2.4	17.9[a]	15.2	4.5	4.6
22:0	10.9[1]	6.0	13.8[a]	11.6	3.0	3.0
22:1	tr.	tr.	0.5	tr.	ND	ND
23:0	6.7[a]	2.8	4.1[a]	2.7	0.6	tr.
iso 24:0	4.3	3.2	2.2[a]	1.6	ND	ND
24:0	23.9[a]	13.3	12.0[a]	8.3	3.5	3.2
24:1	16.6[a]	35.8	9.4[a]	18.8	8.5	8.2
Total	100.0	100.0	100.0	100.0	100.0	100.0
24:1/24:0	0.70[a]	2.70	0.79[a]	2.27	2.42	2.53

Values are means of n = 4, except for the liver control which has n = 7. All values are means expressed as mol%. Significant differences are indicated by a as indicated by the t-test (P > 0.05). 26:1 was less than 0.5 mol%. Tr. < 0.5 mol%. ND < 0.1 mol%.

Table 3
Sphingomyelin composition of livers of rats fed various fatty acid ethyl esters

Dietary FA	20:1	22:1	24:1	20:0	22:0	24:0
Tissue FA						
16:0	31.5	22.6	20.5	26.3	24.9	25.9
18:0	6.7	6.3	6.0	9.7	4.6	9.0
18:1	$1.4^{a,b}$	$1.8^{a,b}$	$1.8^{a,b}$	0.7^{b}	$3.0^{a,b}$	4.4^{a}
18:2	2.3	2.0	1.1	2.7	1.4	2.2
20:0	1.7^{b}	1.6^{b}	1.5^{b}	3.2^{a}	1.5^{b}	1.4^{b}
22:0	7.5^{c}	7.5^{c}	5.1^{c}	12.8^{b}	17.6^{a}	4.6^{c}
22:1	2.0	0.8	1.2	0.6	tr.	1.2
23:0	5.7	5.6	4.2	5.7	4.5	3.4
iso 24:0	6.0	3.5	2.2	2.4	2.0	2.5
24:0	18.8^{b}	$20.3^{a,b}$	15.5^{b}	$21.6^{a,b}$	$25.8^{a,b}$	31.8^{a}
24:1	16.6^{c}	28.3	40.9^{a}	14.4^{c}	14.3^{c}	10.1^{c}
Total	100.0	100.0	100.0	100.0	100.0	100.0
24:1/24:0	0.92^{c}	1.40^{b}	2.64^{a}	$0.67^{c,d}$	$0.56^{c,d}$	0.32^{d}

All values arc means of n = 3. All values are means expressed as mol%. Significant differences are indicated by different letters according to Tukey's ($P > 0.05$). 26:1 was less than 0.5 mol%. Tr. < 0.5 mol%. ND < 0.1 mol%.

cantly higher levels of 22:0 SM. Those supplemented with 24:0 had a significantly higher level of 24:0 SM. Those supplemented with 24:1n-9 had significantly higher levels of 24:1n-9 SM. The 23:0 species of SM was significantly higher in both the 24:0 and 24:1 supplemented diets. The ratio of 24:1n-9 to 24:0 SM was the highest in the group supplemented with 24:1n-9 and lowest in the group supplemented with 24:0. The 16:0, 18:0, 18:2n-6 and iso 24:0 species of SM did not vary significantly with any dietary treatment.

The results of experiment 2 on the heart are shown in Table 4. Rats supplemented with 20:0 had a significantly higher level of 20:0 SM. The group which was supplemented with 22:0 had significantly higher levels of 22:0 SM. The group which received 22:1n-9 had significantly higher levels of 22:1n-9 and 24:1n-9 SM. Those supplemented with 24:0 had significantly higher levels of 24:0 SM. The group supplemented with 24:1n-9 had a significantly higher level of the 24:1n-9 species of SM. The ratio of 24:1n-9 to 24:0 SM was the highest in the 24:1n-9 supplemented group and lowest in the 24:0 supplemented group. The 16:0, 18:0, 18:2n-6, 22:1n-9, 23:0 and iso 24:0 species of SM did not vary significantly with any dietary treatment.

A summary of the data generated in experiments 1 and 2 is shown in Table 5. The effect of feeding a diet supplemented with *T. speciosum* lipid extract closely resembled the effect of feeding a diet containing a 24:1n-9 ethyl ester supplement in both heart and liver.

4. Discussion

Dietary very long chain fatty acids [(VLCFA) - 22 or more carbons chain length], when saturated or monounsat-

Table 4
Sphingomyelin composition of hearts of rats fed various fatty acid ethyl esters

Dietary FA	20:1	22:1	24:1	20:0	22:0	24:0
Tissue FA						
16:0	22.2	16.5	22.2	14.3	17.3	20.2
18:0	15.8	18.0	15.9	13.6	12.8	17.4
18:1	2.5	1.5	1.3	1.3	1.6	6.3
18:2	3.4	0.7	1.8	0.7	1.4	4.9
20:0	$14.8^{a,b}$	$18.3^{a,b}$	$15.5^{a,b}$	25.0^{a}	10.2^{b}	10.5^{b}
22:0	$12.9^{b,c}$	$13.7^{b,c}$	$11.1^{b,c}$	16.2^{b}	29.8^{a}	6.8^{c}
22:1	$tr.^{b}$	1.2^{a}	$tr.^{b}$	$tr.^{b}$	$tr.^{b}$	$tr.^{b}$
23:0	5.3	5.5	5.1	4.4	3.4	5.2
iso 24:0	1.9	1.5	1.9	3.0	1.3	2.3
24:0	$9.6^{b,c}$	$9.0^{b,c}$	7.1^{c}	$11.2^{b,c}$	12.2^{b}	19.2^{a}
24:1	$11.4^{b,c}$	$14.2^{a,b}$	17.9^{a}	$10.0^{b,c}$	$9.9^{b,c}$	7.0^{c}
Total	100.0	100.0	100.0	100.0	100.0	100.0
24:1/24:0	1.19^{c}	1.57^{b}	2.54^{a}	0.91^{d}	0.81^{d}	0.36^{e}

All values are means of n = 3. All values are means expressed as mol%. Significant differences are indicated by different letters according to Tukey's ($P > 0.05$). 26:1 was less than 0.5 mol%. Tr. < 0.5 mol%.

Table 5
Sphingomyelin composition of livers and hearts of rats fed various fatty acid ethyl esters or *Tropaeolum speciosum*

Dietary Fat	Olive	22:1	24:1	*T. speciosum*
Tissue FA		Liver		
24:1	15.8[a]	28.3[a,b]	40.9[c]	35.8[b,c]
24:1/24:0	0.68[a]	1.40[b]	2.64[c]	2.70[c]
		Heart		
24:1	9.3[a]	14.2[b]	17.9[c]	18.8[c]
24:1/24:0	0.79[a]	1.57[b]	2.54[c]	2.27[d]

Values are means of n = 3 for dietary 22:1 and 24:1 dietary ethyl ester, n = 4 for olive and *T. speciosum*. (Ethyl ester supplemented diets contained 4.9% olive oil plus 0.1% ethyl ester; *T. speciosum* supplemented diet contained 4.875% olive oil plus 0.125% *T. speciosum* seed extract.) All values are means expressed as mol%. Significant differences are indicated by different letters according to Tukeys ($P > 0.05\%$).

urated, are potent modifiers of SM fatty acid composition in some tissues [11,18,23–29]. The results of these experiments demonstrate that the nervonic acid found in the *T. speciosum* lipid extract directly influences the 24:1n-9 content and the 24:1/24:0 ratio in liver SM, consistent with our previous work on other dietary fats and oils in this rat bioassay [17]. These data demonstrate that the heart has a similar sensitivity to dietary 24:1n-9; however, the brain, at least under the conditions of this bioassay, is not sensitive to dietary VLCFA. This lack of effect on brain is consistent with a report from Cook et al. [18] that brain SM fatty acid composition is not altered in weanling mice fed very high levels of VLCFA.

Previously [17], we had reported that the ratio of 24:1 to 24:0 SM of liver, in a two week feeding trial of weanling, male Wistar rats, is directly related to their intake of VLCFA according to the equation: 24:1/24:0 SM = 1.88(mol%24:1) − 1.49(mol% 24:0) + 0.21(mol% 22:1) + 0.01 (mol% 18:1) + 0.26, ($r^2 = 0.95$, $P < 0.0001$). This formula was obtained by stepwise linear regression analysis of the composition of 21 dietary fats and oils, and the resulting 24:1 to 24:0 ratio of liver SM. When the dietary fatty acid composition of the *T. speciosum* diet is fitted into this equation, the 24:1 to 24:0 ratio of liver SM are very close to predicted values (2.70 vs. 2.72) further indicating the predictive value of this equation when a bioavailable form of 24:1n-9 is provided at less than or equal to 1 g 24:1n-9/kilogram diet. Data from this report, combined with previously generated data [30] and unpublished results, suggest that the equation, which was generated for rat liver in a specific bioassay, may give some predictive value for effect of VLCFA on the heart (2.27 observed vs. 2.72 predicted). The equation holds no predictive capacity for the fatty acid composition of brain SM.

The results from feeding purified VLCFA ethyl esters to rats, while suggesting that nervonic acid from *T. speciosum* oil has a bioavailability similar to that of 24:1n- 9 ethyl ester (Table 5), provide some additional information about the effect of dietary fatty acids on SM fatty acid composition [17]. The fatty acid 20:1n-9 was not detectable (<0.1 mole%) in SM in liver or heart even when it is fed as ethyl esters at 0.1 wt% of the diet (data not shown). The fatty acid 22:1n-9 is not a major fatty acid in heart or liver SM, even when it is fed at levels of 1g/kg diet. Dietary 22:1n-9 does cause a small, but statistically significant, increase in rat heart SM when fed at these test levels [1g fatty acid/kg diet]. The fatty acid 20:0 in the diet resulted in elevated 20:0 and 22:0 in liver and, to a lesser extent, in heart SM. Dietary 22:0 ethyl ester increased the 22:0 content of SM in heart and liver.

The fate of the dietary 26:1n-9 from the *T. speciosum* lipid is more difficult to discern. It did not accumulate in liver, heart or brain SM. Neither did it accumulate in liver, heart or brain triglycerides (data not shown). We found it remained a trace component (<0.5%) of the fatty acids of SM and triglycerides in these tissues.

In conclusion, these results demonstrate that *T. speciosum* lipids are a rich source of the trace nutrient 24:1n-9 for the young rat. The 24:1n-9 of *T. speciosum* lipids has a similar bioavailability compared to the 24:1n-9 in common dietary oils [17]. Under these assay conditions, brain SM fatty acid composition is not influenced by dietary nervonic acid. The bioavailability of dietary 26:1n-9 from *T. speciosum* lipids could not be determined as the 26:1n-9 content of tissue SM did not rise with dietary intake of 26:1n-9.

References

[1] A.B. Moser, D.S. Jones, G.V. Raymond, H.W. Moser, Plasma and red blood cell fatty acids in peroxisomal disorders, Neurochem Res. 24, (1999) 187–97.
[2] A.B. Moser, N. Kreiter, L. Bezman, S. Lu, G.V. Raymond, S. Naidu, H.W. Moser, Plasma very long chain fatty acids in 3,000 peroxisome disease patients and 29,000 controls, Ann Neurol. 45, (1999) 100–10.
[3] R.J. Wanders, P.G. Barth, R.B. Schutgens, J.M. Tager, Clinical and biochemical characteristics of peroxisome disorders: an update, Eur J Pediatr. 153 (Suppl 1), (1994) S44–S48.
[4] M. Seigneur, G. Freyburger, H. Gin, M. Claverie, D. Lardeau, G. Lacape, F. Le Moigne, R. Crockett, M.R. Boisseau, Serum fatty acid profiles in type I and type II diabetes: metabolic alterations of fatty acids of the main serum lipids, Diabetes Res Clin Pract. 23, (1994) 169–77.
[5] J. Adachi, A. Miwa, Y. Ueno, M. Asano, T. Naito, H. Imamichi, Y. Tatsuno, Abnormality of very long-chain fatty acids of erythrocyte membrane in alcoholic patients, Alcohol Clin Exp Res. 22, (1998) 103S–107S.
[6] Y.Y. Yeh, Long chain fatty acid deficits in brain myelin sphingolipids of undernourished rat pups, Lipids, 23, (1988) 1114–8.
[7] R.T. Swindell, H. Harris, L. Buchanan, C. Bell, B. Albers-Jackson, Ganglioside composition in human cataractous nuclei, Ophthalmic Res. 20, (1988) 232–236.
[8] R.V.P. Tao, E. Cotlier, Ceramides of human normal and cataractous lens, Biochim et Biophys Acta, 409, (1975) 329–341.
[9] A. Slomiany, B.L. Slomiany, H. Witas, M. Aono, L.J. Newman, Isolation of fatty acids covalently bound to the gastric mucus glycoprotein of normal and cystic fibrosis patients, Biochem Biophys Res Commun. 113, (1983) 286–93.
[10] J.R. Sargent, K. Coupland, R. Wilson, Nervonic acid and demyelinating disease, Med Hypotheses, 42, (1994) 237–42.

[11] H. Iida, Y. Takashima, S. Maeda, T. Sekiya, M. Kawade, M. Kawamura, Y. Okano, Y. Nozawa, Alterations in erythrocyte membrane lipids in abetalipoproteinemia: phospholipid and fatty acyl composition, Biochem Med. 32, (1984) 79–87.
[12] V. Gallai, C. Firenze, Modifications of the erythrocyte membrane phospholipids in a family with cerebellar ataxia, Eur Neurol. 22, (1983) 340–3.
[13] H.H. Goebel, R. Heipertz, W. Scholtz, K. Iqbal, I. Tellez-Nagel, Juevenile Huntington chorea: clinical, ultrastructural and biochemical studies, Neurology, 28, (1978) 23–3.
[14] J. Assies, R. Lieverse, P. Vreken, R.J. Wanders, P.M. Dingemans, D.H. Linszen, Significantly reduced docosahexaenoic acid and docosapentaenoic acid concentrations in erythrocyte membranes from schizophrenic patients compared with carefully matched control group, Biol Psychiatry, 49 (2001) 510–522.
[15] A. Kalofoutis, N. Stratakis, E. Diskakis, A. Koutselinis, Erythrocyte phospholipid fatty acid fluctuations in patients with beta-thalassemia, Clin Biochem. 13 (1980) 273–76.
[16] J. Farquharson, E.C. Jamieson, R.W. Logan, W.J.A. Patrick, A.G. Howatson, F. Cockburn, Docosahexaenoic and nervonic acids in term and preterm infant cerebral white matter, Prenat Neonat Med. 1 (1996) 234–240.
[17] W.J. Bettger, C.B. Blackadar, Dietary very long chain fatty acids directly influence the ratio of tetracosenoic (24:1) to tetracosanoic (24:0) acids of sphingomyelin rat liver, Lipids, 32 (1997) 51–55.
[18] C. Cook, J. Barnett, K. Coupland, J. Sargent, Effects of feeding *Lunaria* oil rich in nervonic and erucic acids on the fatty acid compositions of sphingomyelins from erythrocytes, liver and brain of the quaking mouse mutant, Lipids, 33 (1998) 993–1000.
[19] C. Litchfield, Tropaeolum speciosum seed fat: a rich source of cis-15-tetracosenoic and cis-17-hexacosenoic acids, Lipids, 5 (1970) 144–46.
[20] R.A. Avery, W.J. Bettger, Effect of dietary zinc deficiency and the associated drop in voluntary food intake on rat erythrocyte membrane polyamines, J Nutr. 118 (1988) 987–997.
[21] J. Folch, M. Lees, H.L. Stanley, A simple method for the isolation and purification of total lipides from animal tissues, J Biol Chem. 226 (1957) 497–509.
[22] E.R. Driscoll, W.J. Bettger, Effect of dietary zinc deficiency on the lipid composition of the rat erythrocyte membrane, Lipids, 26 (1991) 459–466.
[23] W.B. Rizzo, R.T. Lishner, A. Odone, A.L. Dammann, D.A. Craft, M.E. Jensen, S.S. Jennings, S. Davis, R. Jartly, J.A. Sgro, Dietary erucic acid therapy for X-linked adrenoleukodystrophy, Neurology, 39 (1989) 1415–1422.
[24] J. Lercef, J.L. Bodin, Effects of high erucic acid diet on sphingomyelin biosynthesis in rat lung microsomes, J Physiol. (Paris), 79 (1984) 345–51.
[25] D.E. Barre, B.J. Holub, The effect of borage oil consumption on the composition of individual phospholipids in human platelets, Lipids, 27 (1992) 315–20.
[26] R.E. Pitas, G.J. Nelson, R.M. Jaffe, R.W. Mahet, Delta 15,18-tetracosadienoic acid content of sphingolipids from platelets and erythrocytes of animals fed diets high in saturated or polyunsaturated fats, Lipids, 13 (1978) 551–56.
[27] J.K.G. Kramer, Comparative studies on composition of cardiac phospholipids in rats fed different vegetable oils, Lipids, 15 (1980) 651–660.
[28] J.K.G. Kramer, E.R. Farnworth, K.M. Johnston, M.S. Wolynetz, H.W. Modler, F.D. Sauer, Myocardial changes in newborn piglets fed sow milk or milk replacer diets containing different levels of erucic acid, Lipids, 25 (1990) 729–737.
[29] J.K. Kramer, F.D. Sauer, E.R. Farnworth, D. Stevenson, G.A. Rock, Hematological and lipid changes in newborn piglets fed milk-replacer diets containing erucic acid, Lipids, 33 (1998) 1–10.
[30] W.J. Bettger, C.B. Blackadar, M.L. McCorquodale, The effect of dietary fat type on the fatty acid composition of sphingomyelin in rat liver and heart, Nutr Res. 16 (1996) 1761–1765.