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Constituents of Platypus and Echidna Milk, with Particular Reference to the Fatty Acid Complement of the Triglycerides

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Abstract

The mature milk of platypuses (*Ornithorhynchus anatinus*) exhibit 39·1 g/100 g solids, of which crude lipid accounts for 22·2% crude protein 8·2%, hexose 3·3% and sialic acid 0·43%; iron is in high concentration, 21·1 mg/l. Echidna (*Tachyglossus aculeatus*) milk is more concentrated, containing 48·9% solids, with lipid. protein, hexose and sialic acid accounting for 31·0, 12·4, 1·6 and 0·70% respectively; it is even richer in iron, 33·3 mg/l.

The fatty acid complement of milk triglyceride from platypuses living in their natural habitat is different from that of the echidna living in the wild: platypus milk triglyceride contains 17·6% palmitic, 25·2% oleic and *c.* 30% long-chain polyunsaturated acids, whereas that of echidnas exhibits 15·9% palmitic, 61·2% oleic and only *c.* 7% polyunsaturated acids. The fatty acid complement of echidna milk triglyceride can be changed by altering the fatty acid complement of the dietary lipid.

Introduction

Levels of the proximate constituents, i.e. total solids, crude lipid, crude protein, carbohydrates and minerals, were determined in samples of platypus and echidna milk. The results complement and extend previous research (Griffiths 1965, 1968, 1978, 1983; Messer and Kerry 1973; Griffiths *et al.* 1969; Griffiths *et al.* 1973; Messer *et al.* 1983). Along with determination of the proximate constituents, the fatty acid complements of the triglycerides in echidna and platypus milk fat were determined for comparison with one another and with those of other mammals.

Materials and Methods

Animals and Sampling of the Milk

Milk samples were collected from platypuses, *Ornithorhynchus anatinus*, taken from two localities in New South Wales: the waters of the upper Shoalhaven River, and the upper Murrumbidgee River and its tributaries. The animals were trapped and milked by the methods described by Griffiths *et al.* (1973), Grant and Carrick (1974), and by Grant *et al.* (1983). Milking was carried out within 5 h of capture. The ages of the sucklings were unknown.

Lactating echidnas, *Tachyglossus aculeatus*, were caught opportunely, four at Kangaroo Island, S.A., and one in Boxall State Forest, N.S.W. The latter animal and two from Kangaroo Island were kept in captivity in metal bins on a substratum of bran. Two of these were fed on canned dog meat, Tuckerbox, at 200–300 g/day, homogenized in sufficient water to make a slurry. The third animal was fed daily on a custard made from 30 g Digestelac (powdered milk of low lactose content), two eggs and 200 ml water. This was homogenized and cooked for a few minutes in a boiling water bath. The liquid custard was offered while still warm. Water was offered *ad lib.* The animals were milked as described by Griffiths *et al.* (1969), except that oxytocin (Sandoz) was injected at 1·5 i.u./kg liveweight. Four samples of milk were taken from three of the echidnas at the time of their capture in the bush; 11 more samples were taken from the three animals kept in captivity.

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The ages of the sucklings, estimated from the growth-curve data of Griffiths (1978) and from regressions of growth curves observed in this present work, ranged from 37 to 99 days.

Chemical Analyses

Total solids and crude lipid were determined gravimetrically by the methods of Green *et al.* (1980); crude protein levels were calculated from the nitrogen content determined by the Kjeldahl method. The fatty acid complements of the triglyceride fraction of the lipid isolated on Florisil columns were determined by gas–liquid chromatographic methods described by Griffiths *et al.* (1969) and Griffiths *et al.* (1972). Total fatty acids and triglyceride fatty acids of the canned dog food and custard were also determined by GLC as described by Griffiths *et al.* (1972); both complements were practically identical in the two types of food. The results of both kinds of determinations are expressed as the mean fatty acid complements of the diets. Total hexose was determined by the anthrone method and sialic acid by the thiobarbituric acid method, as described by Messer *et al.* (1983). The levels of minerals were determined by standard techniques: copper, zinc and phosphorus by arc spectrography, iron by flame photometry, sodium, potassium, calcium and magnesium by atomic absorption spectrophotometry.

Statistical comparisons were made by Student's *t*-test, and probability values $<0\cdot05$ were taken to indicate statistical significance.

Results

Platypus

Milk collected from 10 platypuses living in the same area of the Shoalhaven River, during 7 December 1981 to 16 March 1982 contained (mean ±s.d., g/100 g): $39\cdot1 \pm 4\cdot6$ total solids, $22\cdot2 \pm 3\cdot6$ crude lipid, $8\cdot2 \pm 1\cdot3$ crude protein, $3\cdot3 \pm 0\cdot9$ total hexose, $0\cdot43 \pm 0\cdot12$ sialic acid and 0·50 minerals. The sum of the constituents measured (34·63 g/100 g) accounted for 87% of the total solids. The mean ±s.d. value of total solids was $39\cdot1 \pm 4\cdot6$ g/100 g milk and the range was 29·7–41·4 g/100 g. These values are similar to data obtained from platypuses living in the Murrumbidgee River and its tributaries, where total solids in samples of milk from 10 platypuses averaged $37\cdot2 \pm 7\cdot5$ g/100 g milk, with the range being 19·8–45·1 g/100 g. Nine of the samples from the Shoalhaven population were collected between 7 and 10 December 1981; the sample collected 16 March 1982 exhibited the highest crude lipid content (27·0 g/100 g milk) and the lowest hexose level (2·0 g/100 ml),* solids being 41·5 g/100 g. However, a sample collected from another platypus on 10 December 1981 also had a very low hexose content, 2·2 g/100 ml milk, total solids being 37·3 g and crude lipid 21·5 g/100 g respectively.

The component fatty acids of the triglycerides of the milks are shown in Table 1. The major fatty acids are palmitic, $C_{16:0}$ (17·6%); stearic, $C_{18:0}$ (4·0%); palmitoleic, $C_{16:1}$ (11·6%); oleic, $C_{18:1}$ (25·2%); linoleic, $C_{18:2}$ (6·1%); linolenic, $C_{18:3}$ (9·5%) and arachidonic, $C_{20:4}$ (3·1%), these accounting for 77% of the total fatty acids. Other long-chain polyunsaturated fatty acids contributed *c.* 10% of the total triglycerides.

The average levels of various minerals in platypus milks are shown in Table 2. The sodium content was negatively correlated with the amount of total solids ($P < 0\cdot05$), but there was no significant relationship between potassium content and total solids.

Echidna

The mean ±s.d. concentration of total solids in 15 samples of milk from the five echidnas was $47\cdot7 \pm 8\cdot3$ g/100 g. Milk samples from three of the echidnas contained (mean ±s.d., g/100 g): $48\cdot9 \pm 4\cdot6$ total solids, $31\cdot0 \pm 4\cdot2$ .crude lipid, $12\cdot4 \pm 3\cdot2$ crude protein, $1\cdot6 \pm 0\cdot04$ hexose, $0\cdot7 \pm 0\cdot08$ sialic acid and 0·5 minerals. The sum of the three constituents measured (46·2 g/100 g) accounted for 94% of the total solids.

*The specific gravity of echidna and platypus milk is very close to unity.

Table 1. Component fatty acids of the triglyceride fraction of the milks of platypuses, three wild and three captive echidnas, and the fatty acids in the diets of captive echidnas, and in two items of the diet of wild echidnas, *Nasutitermes exitiosus* and *Iridomymex detectus*

Values in parentheses are standard deviations; —, not detectable; tr, trace

	Saturated (g/100 g)							Unsaturated (g/100 g)								Others, (g/100 g)
	C_{12}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{20}	$C_{14:1}$	$C_{15:1}$	$C_{16:1}$	$C_{17:1}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:4}$	
Fatty acids in diet																
Canned meat	0·2	2·5	0·7	21·6	1·8	20·0	0·5	0·6	—	4·7	—	36·1	4·7	1·6	0·2	5·3
(n = 4)	(0·1)	(0·5)	(0·1)	(1·4)	(0·2)	(2·1)	(0·2)	(0·3)		(0·6)		(0·8)	(0·3)	(0·4)	(0·3)	(0·8)
Custard (n = 2)	1·0	6·4	0·3	28·8	0·7	8·2	—	0·9	—	5·9	—	39·9	6·7	0·5	0·4	0·3
N. exitiosus	—	3·6	—	15·8	0·2	8·2	—	7·4	—	6·9	—	52·1	4·2	0·2	—	1·4
I. detectus	0·2	0·6	—	24·1	—	2·7	—	0·1	—	2·5	—	64·8	4·5	—	—	0·2
Fatty acids in milk triglycerides																
Captive echidnas																
Fed canned	tr	0·7	0·4	19·1	1·5	13·0	1·8	0·4	—	5·7	—	42·1	7·8	2·0	0·2	5·2
meat (n = 6)		(0·2)	(0·1)	(0·6)	(0·2)	(2·1)	(0·6)	(0·2)		(1·1)		(1·4)	(1·5)	(0·8)		(1·0)
Fed custard	0·5	6·1	0·2	28·4	0·3	7·3	—	0·5	—	3·6	—	42·9	7·7	0·5	0·3	1·7
(n = 3)	(0·5)	(1·1)	(0·4)	(0·7)	(0·5)	(0·8)		(0·4)		(0·6)		(0·6)	(2·2)	(0·0)	(0·1)	(1·4)
Wild Echidnas	1·0	1·4	0·2	15·9	0·5	3·9	0·3	0·4	—	6·2	—	61·2	5·1	0·8	0·5	2·7
(n = 4)	(0·7)	(0·5)	(0·1)	(0·4)	(0·0)	(0·1)	(0·3)	(0·1)		(0·7)		(1·9)	(0·8)	(0·3)	(0·2)	(0·7)
Platypuses	0·8	1·9	0·8	17·6	1·4	4·0	1·0	1·3	0·5	11·6	2·3	25·2	6·1	9·5	3·1	12·9
(n = 10)	(0·5)	(0·4)	(0·1)	(0·6)	(0·3)	(0·9)	(0·5)	(0·2)	(0·1)	(0·9)	(0·2)	(2·6)	(0·8)	(2·8)	(0·5)	(2·3)

The component fatty acids of the triglycerides in the milk and in the dietary items of echidnas are shown in Table 1. The canned meat diet contained five major fatty acids, palmitic, stearic, palmitoleic, oleic and linoleic, which accounted for about 87% of the total. These acids accounted for 87·8% of the fatty acids in the triglycerides of the milk of the echidnas fed on that diet. However, in the milk there is less stearic and more linoleic acid (Table 1).

The fatty acid complement of the lipid of the custard contained more myristic ($C_{14:0}$) and palmitic acids and much less stearic acid than the canned meat. The triglyceride fatty acid complement of the milk of the echidnas fed on the custard closely resembled fatty acid intake (Table 1) and therefore differed from the milks of the echidnas fed on canned meat.

The fatty acid complements of the milk of the captive echidnas on either diet differed significantly from that of free-living echidnas feeding on ants and termites; this was particularly so for stearic ($P < 0 \cdot 001$) and oleic acids ($P < 0 \cdot 001$) in milk from echidnas eating canned meat (Table 1); and for myristic acid ($P < 0 \cdot 001$) in milk from the echidna eating custard. Table 1 also shows the component fatty acids in the lipids of a termite *Nasutitermes exitiosus* (various castes) and of an ant *Iridomyrmex detectus* (virgin queens); both of these species are eaten by wild echidnas during the lactation season (Griffiths and Simpson 1966; Griffiths 1968, 1978).

Average levels of minerals found in the mature milk of captive and wild echidnas are shown in Table 2. The levels of sodium in the milk were negatively correlated ($P < 0 \cdot 001$) with the total solid contents of the milk, and levels of potassium showed a similar relationship ($P < 0 \cdot 05$).

Table 2. Levels (mg/100 ml, mean ±s.d.) of minerals found in the milk of the echidna and platypus

	Sodium	Potassium	Calcium	Magnesium	Phosphorus	Iron	Copper	Zinc
Echidna	90 ± 29 (n = 15)	97 ± 18 (n = 14)	117 ± 50 (n = 5)	16 ± 3 (n = 5)	285 (n = 2)	3·33 ± 1·28 (n = 11)	0·38 (n = 2)	1·51 (n = 2)
Platypus	93 ± 20 (n = 10)	98 ± 15 (n = 10)	191 ± 43 (n = 10)	16 ± 3 (n = 10)	133 (n = 1)	2·11 ± 0·78 (n = 7)	0·10 (n = 1)	1·92 (n = 1)

Discussion

The range of 29·0–41·4% total solids found for the milk of the Shoalhaven platypuses is similar to that found for the Murrumbidgee River population (19·8–45·1%). The milk with only 19·8% solids was obtained on 6 November, which is early in the lactation season (Grant *et al.* 1983); such a relatively dilute milk may indicate that the female was suckling young which had only recently hatched, since Griffiths (1978) found that a sample of echidna milk taken 1 day after hatching contained only 12% solids, whereas mature milk contains *c.* 47%. This marked increase in total solids resembles the changes found in the milk of marsupials during lactation. In all marsupials examined the milk at birth and for a few days afterwards is a pellucid fluid; early milk in *Macropus eugenii*, for example, contains 8–12% solids (B. Green and M. Griffiths, unpublished data) and, as has been shown (see Green 1983), the milk of this species becomes progressively more concentrated during lactation, culminating in a milk containing 35–40% solids. Echidna lactation also resembles that of macropodid marsupials (see Griffiths *et al.* 1972; Green *et al.* 1983) in that the oleic acid concentration of milk triglycerides up to 2 weeks after hatching and birth is low (26 and 17% for echidnas and macropodids respectively) and increases to 61 and 50–53% respectively in mature milk.

The level of crude lipid in echidna milk is higher than that of platypus milk but, relative to the milk of many eutherians, both platypus and echidna milk contain far more total solids and lipid. In the echidna this is related to infrequency of suckling: pouch young and sucklings may not take in milk for 3 or 4 days at a time (Griffiths 1968, 1978; B. Green, K. Newgrain and M. Griffiths, unpublished data), but they are capable of taking in an amount of milk equal to 20% of their liveweight at a single nursing. Nothing is known of suckling behaviour in platypuses but, since their milk is almost as rich as that of the echidna, the interval between feeds for platypus young may also be as long as 2–3 days. The solids content of eutherian milks is related to suckling regimen (Ben Shaul, 1962; Jenness and Sloan 1970).

The contribution of the seven major fatty acids to the total in the triglycerides in platypus milk fat (77·0%) is almost identical to that found for 11 samples of milk (77·5%) by Griffiths *et al.* (1973). The five major fatty acids in the echidna milk triglycerides account for 92% of the total, a figure in agreement with the 96% found for samples of milk from nine wild echidnas by Griffiths *et al.* (1973). The proportions of all the fatty acids found in that study are similar to those found in this present work.

This study also confirms a previous finding (Griffiths *et al.* 1973) that the milk triglyceride fatty acid complement of platypuses living in their natural habitat and feeding on their summer diet of larvae of Trichoptera, Odonata, Diptera and Megaloptera (Faragher *et al.* 1979) is quite different from that of echidnas feeding on their natural diet of ants and termites. That the fatty acid complement of echidna milk can be changed, as occurs in other mammals, by altering the proportion of $C_{18:1}$ in the dietary lipid (Griffiths *et al.* 1973; Griffiths 1978) is supported by the results of the present work. The finding that the milk triglycerides of wild echidnas contain over 60% oleic acid is also confirmed. This high oleic acid content is probably due to a large dietary intake of oleic acid, since these two items of the diet of wild echidnas, the ant and the termite, have lipid of very high oleic acid content (64·8% and 52·1% respectively). It is possible, then, that the difference of platypus milk triglycerides from those of wild echidnas could be attributed to differences in diet, but the fact remains that, although the sucklings of the two species are anatomically similar, they are raised on very dissimilar milks.

The fact that $C_{18:2}$ levels in the milk of the echidnas fed on canned meat were much higher than in the diet is not readily explained since in eutherian mammals $C_{18:2}$ and $C_{18:3}$ are essential fatty acids which cannot be synthesized and must be supplied in the diet. Perhaps these two echidnas stored $C_{18:2}$ in their depot fat and later passed it into the milk, or perhaps echidnas can synthesize $C_{18:2}$ and $C_{18:3}$ from short chain precursors.

The triglycerides of echidna milk are different from those of eutherians and marsupials in that they are largely symmetrical, with the major saturated fatty acids $C_{16:0}$ and $C_{18:0}$ almost equally distributed, in the glycerol moiety, between the sn-1 and the sn-3 positions while the unsaturated acids $C_{18:1}$, $C_{18:2}$ and $C_{18:3}$ are preferentially esterified at the sn-2 position (Grigor 1980; Parodi 1982). Surprisingly, the distribution pattern of the fatty acids in the triglycerides of platypus milk is like that of eutherian and marsupial milks (Parodi and Griffiths 1983). Thus, the type of distribution in echidna triglycerides is unique, not having been observed in the milk triglycerides of any other mammal, but it is akin to the fatty acid distribution found in the vegetable oils.

Although the amount of hexose in the echidna milk was less than that in platypus milk, the sialic acid content of the former was significantly higher ($P < 0 \cdot 001$). This is in agreement with the finding that the major oligosaccharide of echidna milk is sialyllactose, whereas it is difucosyllactose in platypus milk (Messer *et al.* 1983).

The levels of calcium and magnesium in echidna and platypus milk are similar to those in milks of eutherians, but are much lower than those found in marsupials (Green 1984). Conversely, the concentrations of iron and copper in monotreme milk are similar to those of marsupials and much higher than levels in eutherian milk. However, newborn eutherians have enough iron in their livers to support them until they can ingest iron in their definitive diets. The marsupial neonate and the monotreme hatchling, however, are minute and their tiny livers cannot store enough iron to support them for a prolonged period on milk; hence their milks are rich in iron.

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References

Ben Shaul, D. M. (1962). The composition of the milk of wild animals. *Int. Zool. Yearb.* **4**, 333–42.

Faragher, R. A., Grant, T. R., and Carrick, F. N. (1979). Food of the platypus (*Ornithorhynchus anatinus*) with notes on the food of brown trout (*Salmo trutta*) in the Shoalhaven River, N.S.W. *Aust. J. Ecol.* **4**, 171–9.

Grant, T. R., and Carrick, F. N. (1974). Capture and marking of the platypus (*Ornithorhynchus anatinus*) in the wild. *Aust. J. Zool.* **18**, 133–5.

Grant, T. R., Griffiths, M., and Leckie, R. M. C. (1983). Aspects of lactation in the platypus (*Ornithorynchus anatinus*) in waters of eastern New South Wales. *Aust. J. Zool.* **31**, 881–9.

Green, B. (1983). Composition of milk and energetics of growth in marsupials. In 'Physiological Strategies in Lactation'. (Eds M. Peaker, R. G. Vernon and C. H. Knight.) Symposium Zool. Soc. London, 1982, No. 51, pp. 369–87.

Green, B., Newgrain, K., and Merchant, J. (1980). Changes in milk composition during lactation in the tammar wallaby (*Macropus eugenii*). *Aust. J. Biol. Sci.* **35**, 145–52.

Green, B., Griffiths, M., and Leckie, R. M. C. (1983). Qualitative and quantitative changes in milk fat during lactation in the tammar wallaby (*Macropus eugenii*). *Aust. J. Biol. Sci.* **36**, 455–61.

Griffiths, M. (1965). Rate of growth and intake of milk in a suckling echidna. *Comp. Biochem. Physiol.* **16**, 383–92.

Griffiths, M. (1968). 'Echidnas.' (Pergamon Press: Oxford, G.B.)

Griffiths, M. (1978). 'Biology of the Monotremes.' (Academic Press: New York.)

Griffiths, M. (1983). Lactation in Monotremata and speculations concerning the nature of lactation in Multituberculata. In 'Second Symposium on Mesozoic Terrestrial Ecosystems'. (Eds Z. Kielan-Jaworowska and H. Osmolska.) *Acta Palaeontol. Pol.* **28**, 93–102.

Griffiths, M., Elliott, M. A., Leckie, R. M. C., and Schoefl, G. I. (1973). Observations of the comparative anatomy and ultrastructure of mammary glands and on the fatty acids of the triglycerides in platypus and echidna milk fats. *J. Zool. London* **169**, 255–79.

Griffiths, M., McIntosh, D. L., and Coles, R. E. A. (1969). The mammary glands of the echidna, *Tachyglossus aculeatus*, with observations on the incubation of the egg and on the newly-hatched young. *J. Zool. London* **158**, 371–86.

Griffiths, M., McIntosh, D. L., and Leckie, R. M. C. (1972). The mammary glands of the red kangaroo with observations on the fatty acid components of the milk triglycerides. *J. Zool. London* **166**, 265–75.

Griffiths, M., and Simpson, K. G. (1966). A seasonal feeding habit of spiny-anteaters. *CSIRO Wildl. Res.* **11**, 137–43.

Grigor, M. (1980). Structure of milk triacylglycerols of five marsupials and one monotreme: evidence for an unusual pattern common to marsupials and eutherians but not found in the echidna, a monotreme. *Comp. Biochem. Physiol.* B **65**, 427–30.

Jenness, R., and Sloan, R. E. (1970). The composition of milk of various species: a review. *Dairy Sci. Abstr.* **32**, 599–612.

Messer, M., and Kerry, K. R. (1973). Milk carbohydrates of the echidna and the platypus. *Science (Wash., D.C.)* **180**, 201–3.

Messer, M., Gadiel, P. A., Ralston, G. B., and Griffiths, M. (1983). Carbohydrates of the milk of the platypus. *Aust. J. Biol. Sci.* **36**, 129–37.

Parodi, P. W. (1982). Positional distribution of fatty acids in triglycerides from milk of several species of mammals. *Lipids* **17**, 437–42.

Parodi, P. W., and Griffiths, M. (1983). A comparison of the positional distribution of fatty acids in milk triglycerides of the extant monotremes platypus (*Ornithorhynchus anatinus*) and echidna (*Tachyglossus aculeatus*). *Lipids* **18**, 845–7.

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