The composition of polyunsaturated fatty acids in mare's blood and foal's blood plasma at birth and the first days after parturition

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Summary

Eight mares and their foals were included in this study. Samples of blood plasma and mare milk were obtained before the first milk intake (day 0) and on day 1, 2 and 10 after parturition. The fatty acid composition of the total lipid fraction of plasma and milk was analyzed by gas chromatography. Immediatly after birth the concentration of linoleic acid (18:2n-6; LA) of foal blood plasma was very low compared to mare blood plasma. There was an increase in LA of foal blood plasma during the sampling period. This was probably due to the high content of LA in mare milk (20–25 % of total fatty acids). In contrast to LA, dihomogammalinolenic acid (20:3n-6; DGLA) and arachidonic acid (20:4n-6; AA) were markedly higher in plasma from newborn foals than in mare plasma. The content of DGLA and AA in foal plasma decreased during the sampling period.

Keywords: fatty acids, delivery, blood plasma lipids, milk

Zusammensetzung mehrfach ungesättigter Fettsäuren im Blut von Stuten und Fohlen bei der Geburt und postnatal

An dieser Untersuchung waren acht Warmblutstuten mit ihren Fohlen beteiligt. Blutplasma- und Stutenmilchproben wurden vor der ersten Kolostralmilchaufnahme (Tag 0), sowie an den Tagen 1, 2 und 10 post partum entnommen. Die Fettsäurenzusammensetzung der Gesamtlipidfraktion des Plasmas und der Milch wurde durch eine gaschromatographische Untersuchung ermittelt. Unmittelbar nach der Geburt war der Linolsäuregehalt (18:2n-6; LA) im Fohlenblutplasma verglichen zur Stute sehr niedrig. Während der Probenentnahmeperiode kam es zu einem Anstieg der LA im Fohlenplasma. Dies ist wahrscheinlich auf den hohen LA-Gehalt (20–25 % der gesamten Fettsäuren) in der Stutenmilch zurückzuführen. Die Dihomogammalinolensäure- (20:3n-6; DGLA) sowie die Arachidonsäurewerte (20:4n-6; AA) sind, anders als die LA, signifikant höher im Plasma frisch geborener Fohlen als im Stutenblutplasma. AA und DGLA im Fohlenplasma nahmen während des Untersuchungszeitraumes ab.

Schlüsselwörter: Fettsäure, Geburt, Plasmafette, Milch

Introduction

Polyunsaturated fatty acids are required during foetal development for the synthesis of eicosanoids and for the formation of membrane structures in all tissues. Evidence is growing that special long-chain polyunsaturated fatty acids are essential for normal development of brain and retina (*Crawford* et al. 1989; *Hoffman* et al. 1993).
The composition of polyunsaturated fatty acids in plasma lipids in neonates differs from the fatty acid composition in the maternal plasma in humans (*Hull 1979*) and in horses (*Stammers* et al. 1991). In the neonates the content of long-chain polyunsaturated fatty acids is higher, whereas the concentration of linoleic acid (18:2n-6; LA) is lower compared to maternal plasma. During the first days of life the fatty acid composition in blood plasma of human neonates (*Hull 1979*; *Pita* et al.,1990) and piglets (*Innis* et al. 1989) changes rapidly. LA rises, whereas arachidonic acid (20:4n-6; AA) and other long-chain polyunsaturated fatty acids decrease. These changes are modulated by the type of diet and postnatal age (*Hull 1979*).

The aim of this experiment was to study the composition of polyunsaturated fatty acids in mare and foal blood plasma at birth and the first days after parturition.

Material and methods

Eight german warmbloodmares, oldenburg brand, aged between 3 and 10 years, and their foals were included in this study. All mares were housed for at least 6 weeks prior to delivery. They were fed hay and cornsilage, oats and vitaminized pellets at their needs. Water was supplied *ad libitum*. Blood plasma was taken from the jugular vein and collected into heparin vacuum tubes. Milk samples were obtained by handmilking. Samples of blood plasma and mare milk were collected before the first milk intake (at birth) and on day 1, 2 and 10 after parturition. The samples were stored in small plastic tubes at –20 °C on the farm, and at –80 °C in the laboratory until processed.
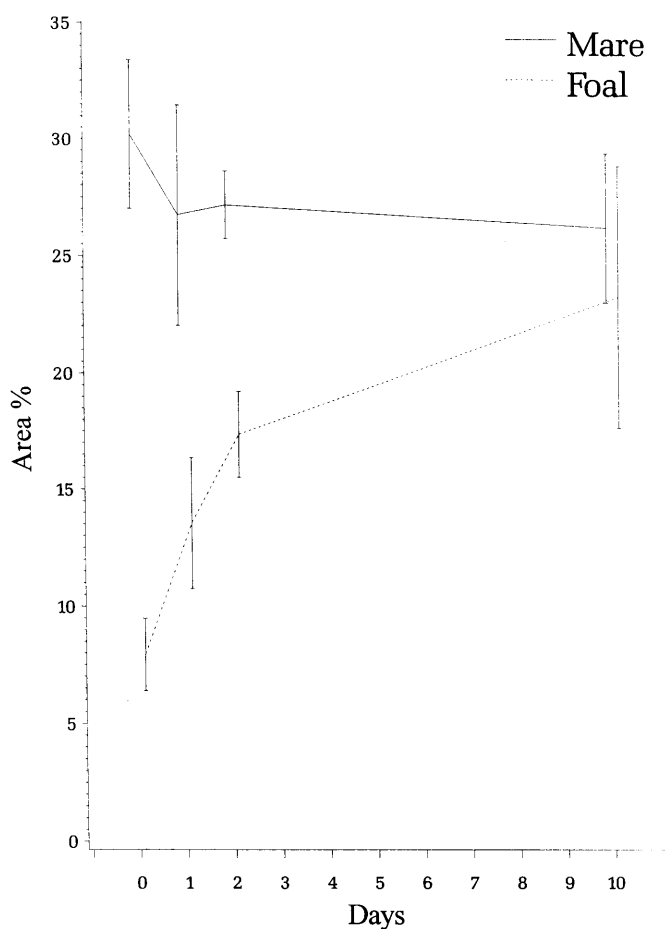
Fig. 1: The composition (area %) of linoleic acid (18:2n-6; LA) in mare's blood and foal's blood plasma at birth and the first days after parturition. The bars indicate 2SEM.

Anteil (%) der Linolsäure (18:2n-6; LA) im Blut der Stuten und Blutplasma der Fohlen zur Geburt und in den ersten Tagen p.p. (Balken: 2s)
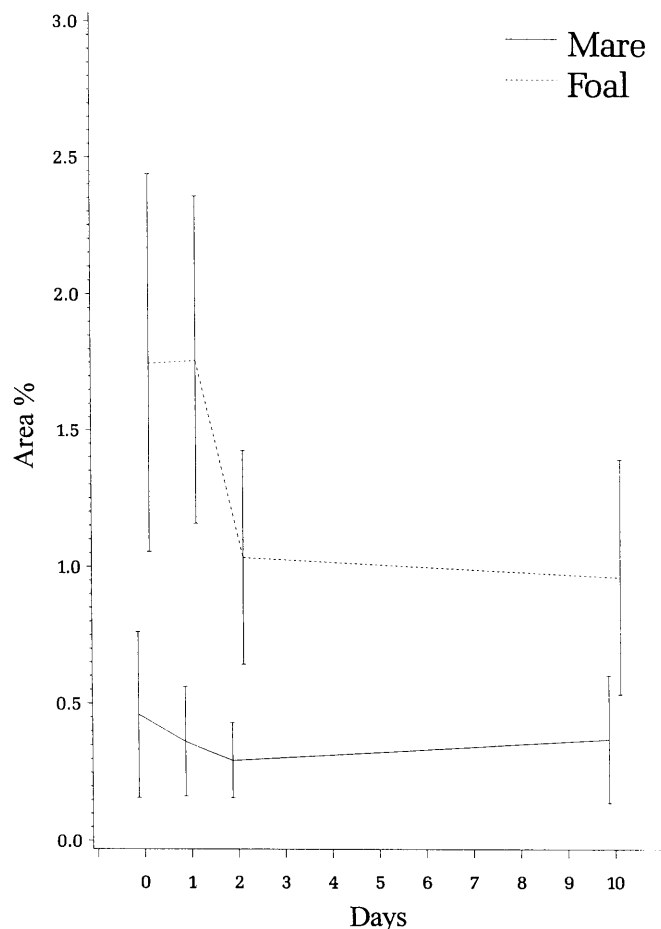

Fig. 2: The composition (area %) of arachidonic acid (20:4n-6; AA) in mare's blood and foal's blood plasma at birth and the first days after parturition. The bars indicate 2SEM.

Anteil (%) der Arachidonsäure (20:4n-6; AA) im Blut der Stuten und Blutplasma der Fohlen zur Geburt und in den ersten Tagen p.p. (Balken: 2s)

Tab. 1: The fatty acid composition (area %) of the total lipid fraction of foal blood plasma. Mean (SD).

Fettsäurenzusammensetzung in % des Gesamtfettes im Blutplasma von Fohlen. x(s)

Fatty acid	At birth (Day 0)		Day 1		Day 2		Day 10	
12:0ᵇ†	41.9	(8.5)	31.3	(6.2)	32.1	(4.5)	32.5	(9.1)
14:0	1.12	(0.67)	1.13	(0.59)	1.02	(0.43)	0.80	(0.41)
16:0ᵃ	11.1	(2.0)	15.0	(1.6)	14.5	(1.3)	13.6	(2.0)
16:1n-7ᵃ†	2.3	(0.6)	2.9	(0.4)	2.6	(0.3)	1.8	(0.7)
18:0	9.3	(2.3)	10.1	(0.9)	9.2	(1.2)	9.8	(2.3)
18:1n-9ᵃ†	17.0	(3.9)	18.7	(2.9)	16.1	(1.5)	12.2	(4.8)
18:2n-6ᵇ*	7.9	(2.1)	13.6	(3.7)	17.4	(2.5)	23.2	(7.4)
20:3n-6ᵃ†	2.01	(0.80)	1.89	(0.73)	1.03	(0.38)	0.96	(0.57)
20:4n-6ᵃ†	1.75	(0.92)	1.76	(0.79)	1.03	(0.52)	0.97	(0.53)

ᵃ Foal > Mare; ᵇ Foal < Mare; *Increase during the sampling time.

The lipids from 0.5 ml plasma and milk were extracted by a modification of the procedures of *Folch* et al. (1957), and methylated as described by *Taugbøl* (1993). The methylated fatty acids were extracted with 1 ml hexane and separated on a fused silica wall coated capillary column (Chrompack, WCOT Fused Silica (CP-Sil-5 CB); 50 m˜0.25 mm, film thickness 0.12 mm) in a Dani 86.10 gas chromatograph fitted with a flame-ionization detector and a programmable temperature vaporizer (PTV) injector. Carrier gas used was helium. The injector and detector temperature was 260 °C. The column temperature was kept at 60 °C for 1.2 min, then increased to 200 °C at a rate of 25 °C/min, then up to 250 °C at a rate of 0.8 °C/min (total time 70 min). Identification of major peaks was made by comparing the retention time with those of standard fatty acid-methylesters. The results are reported as relative distribution of fatty acids from the percentage of the total area under the peaks.

All results are given as the mean and standard deviation (SD). The statistical analysis applied was an ANOVA, with two different factores to control for: Mare and foal plasma samples and the time trend within the sampling period. The model checking was carried out by the use of Jack-

Tab. 2: The fatty acid composition (area %) of the total lipid fraction of mare blood plasma. Mean (SD).

Fettsäurenzusammensetzung in % des Gesamtfettes im Blutplasma von Stuten. x(s)

Fatty acid	At birth (Day 0)	Day 1	Day 2	Day 10
12:0	36.0 (8.1)	43.5 (9.9)	45.2 (4.6)	41.4 (10.0)
14:0	0.98 (0.15)	1.10 (0.31)	1.06 (0.48)	1.09 (0.37)
16:0	11.4 (1.6)	8.7 (2.0)	8.2 (0.7)	9.0 (3.5)
16:1n-7	1.08 (0.43)	0.48 (0.13)	0.50 (0.15)	0.77 (0.50)
18:0	9.6 (1.4)	8.8 (1.7)	8.7 (0.8)	8.9 (1.7)
18:1n-9	8.0 (1.0)	6.1 (1.5)	5.0 (0.3)	5.8 (1.7)
18:2n-6†	30.2 (3.6)	26.7 (5.8)	27.2 (1.9)	26.2 (4.2)
20:3n-6	–	–	–	–
20:4n-6	0.46 (0.30)	0.36 (0.20)	0.29 (0.15)	0.37 (0.28)

† Decrease during the sampling time.

Tab. 3: The fatty acid composition (area %) of the total lipid fraction of mare milk. Mean (SD).

Fettsäurenzusammensetzung in % des Gesamtfettes in der Stutenmilch. x(s)

Fatty acid	At birth (Day 0)	Day 1	Day 2	Day 10
8:0	0.85 (0.37)	1.10 (0.58)	2.40 (1.26)	1.87 (0.81)
10:0	4.6 (1.0)	5.2 (2.5)	9.5 (4.6)	8.6 (3.8)
12:0	16.3 (6.3)	10.9 (4.5)	14.8 (5.0)	15.8 (4.8)
14:0	4.3 (0.7)	5.2 (1.3)	5.8 (1.1)	6.7 (1.5)
16:0	21.3 (2.9)	21.9 (1.4)	20.3 (2.3)	21.4 (2.3)
16:1n-7	3.9 (1.6)	4.3 (1.2)	4.4 (1.2)	4.8 (1.2)
18:0	1.56 (0.90)	1.41 (0.50)	1.09 (0.43)	1.07 (0.63)
18:1n-9	20.5 (2.7)	22.8 (4.1)	19.2 (3.4)	18.9 (5.3)

knife residuals and Cook's d. The level of significance was set to 0.05.

Results

The fatty acid compositions of foal's and mare's blood plasma are presented in table 1 and 2, respectively. Immediatly after birth the concentration of LA of foal blood plasma was very low compared to mare blood plasma. There was an increase in LA of foal blood plasma during the sampling period, fig. 1. In contrast to LA, dihomogammalinolenic acid (20:3n-6; DGLA) and arachidonic acid AA were markedly higher in plasma from newborn foals than in mare plasma. The content of DGLA and AA in foal plasma decreased during the sampling period. Fig. 2 shows the relative concentration of AA in foal and mare plasma lipids.

The fatty acid composition of mare milk lipids is shown in table 3. Mare milk contained about 20–25 % of LA, but only minor traces of other polyunsaturated fatty acids.

Discussion

In agreement with *Stammers* et al. (1991) we found the percentage of LA markedly lower and of DGLA and AA

markedly higher in newborn foal plasma lipids than in maternal mare plasma lipids. These differences between foetal and maternal plasma lipids are also known from humans (*Hull 1979*; *Ruyle* et al. 1990) and other mammals (*Innis* et al. 1989). In humans the total lipid concentration is lower in cord blood than in maternal blood at delivery (*Ruyle* et al. 1990), and the absolute amount of AA in umbilical plasma is therefore lower compared with maternal values (*Ruyle* et al. 1990; *Al* et al. 1995). In the horse the content of total blood lipids in foetal venous blood is almost as high as in maternal venous blood (*Stammers* et al. 1991). Therefore the relative composition of total plasma fatty acids reflects the absolute fatty acid status in the horse.

The occurrence of polyunsaturated ω6 fatty acids in foetal plasma demonstrates placental transfer, because they cannot be synthesized in mammalian tissues. The higher amounts of DGLA and AA could be due to de novo synthesis by the fetus and the placenta. The evidence for fetal and placental elongation and desaturation of LA is sparse and to some extent conflicting (*Scott* and *Bazan 1989*; *Booth* et al. 1981; *Zimmermann* et al. 1979; *Chambaz* et al. 1985). A prefered transport of DGLA and AA at the expense of LA may also be responsible (*Kuhn* and *Crawford 1986*). Long chain polyunsaturated fatty acids seem to be hidden within a lipid fraction that does not recross the placental barrier. An α-fetoprotein is supposed to carry out this role. This protein is mainly synthesized by the fetal liver and the yolk sac. Although the biological role of α-fetoprotein is not completely elucidated, it binds polyunsaturated fatty acids with high affinity (*Subbiah 1991*). Not only different fractions of plasma lipids, but also the phospholipids of erythrocytes may play a role in the necessary transport of polyunsaturated fatty acids into the fetus. *Ruyle* et al. (1990) found a venous-arterial difference in umbilical erythrocytes for docosahexaenoic acid (22:6n-3; DHA).

Between the first and the second day of life a dramatic decrease in the foal's plasma lipid concentration of DGLA and AA occurred. This is probably due to an expanding plasma volume as a result of higher milk intake, and a decreased dry matter content of colostrum. Immediately after foaling the dry matter content of colostrum is about 25 %, whereas the dry matter content on day 2 differs only slightly from those obtained 5 days after foaling, about 12 % (*Csapó* et al. 1995). The rapid decrease of DGLA and AA could also indicate a placental fatty acid elongation and desaturation activity, which cannot be compensated by biosynthesis of long-chain polyunsaturated fatty acids in the newborn foal.

The main dietary polyunsaturated fatty acid, LA, increased in foal blood plasma from the first milk intake.

In this study we did not find any ω3 polyunsaturated fatty acids, neither in blood plasma nor in milk. *Csapó* et al. (1995) found high amounts of linolenic acid (18:3n-3; LNA) in mare milk, probably due to a pasture of better quality in Hungary than we can expect in North-Germany in the early spring time. *Stammers* et al. (1991) did find

long-chain ω3 fatty acids in both mare and foal blood plasma, whereas these fatty acids were under the detection limit for the analyses carried out in our experiment.

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