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Variation in the Milk Macronutrient and Fatty Acid Composition of Captive Tree Shrews (*Tupaia belangeri*) during Different Lactation Periods

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The tree shrew (*Tupaia belangeri*) is an increasingly valuable model animal for research purposes. However, the lactation biology of the tree shrew remains underexplored, hindering progress in their nutritional management during laboratory domestication. Milk samples from tree shrews in captivity at postnatal days 0, 2, 8, 14, 20, 26, and 32 were analyzed, through microanalysis for macronutrient determination and gas chromatography for fatty acid composition. At the midlactation stage, tree shrew milk averaged 44.75% dry matter, 26.35% fat, 11.93% protein, 1.63% sugars, 1.34% ash, and 1323.99 kJ/100 g energy. As lactation progressed, significant increases were observed in dry matter, sugar, and ash content. The fatty acid profile was predominated by C16:0, C14:0, C18:2n6, and C18:1n9. Levels of most saturated fatty acids increased steadily during lactation, while the majority of unsaturated fatty acids exhibited an opposite pattern. In brief, our results suggest that the macronutrient composition and fatty acid profile are influenced by the lactation stage. These findings may aid in formulating milk products that closely approximate the maternal milk of tree shrews, thereby enhancing breeding and domestication efforts for research purposes.

Abbreviations and Acronyms: CAS, Chinese Academy of Sciences; DM, dry matter; FA, fatty acid; KIZ, Kunming Institute of Zoology; LCFA, long-chain fatty acid; MCFA, medium-chain fatty acid; MUFA, monounsaturated fatty acid; MY, milk yield; OCFA, odd-chain fatty acid; P0, postnatal day 0; P2, postnatal day 2; P8, postnatal day 8; P14, postnatal day 14; P20, postnatal day 20; P26, postnatal day 26; P32, postnatal day 32; PUFA, polyunsaturated fatty acid; SCFA, short-chain fatty acid; SFA, saturated fatty acid; UFA, unsaturated fatty acid; VLCFA, very-long-chain fatty acid

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Introduction

The tree shrew (*Tupaia belangeri*), a small mammal classified under the order Scandentia, has recently attracted attention as a promising animal model in biomedical research.[37,50] Despite its classification outside the primate order, growing evidence indicates that the tree shrew shares genetic,[11,12,49,51] structural,[33,54] and physiologic similarities with primates,[6,55] making it a suitable alternative to other laboratory animals in certain research fields. For this reason, the tree shrew has been employed as a model for various diseases such as human viral infection,[25,26,44,46,47] central nervous system disorders,[24] eye diseases,[19] cancers,[28,52] and other human diseases over the last few decades.[16,27,29,50] The establishment and application of these tree shrew models may enhance our understanding of human disease mechanisms and improve strategies for diagnosis, treatment, and prevention of various health issues.[50]

Captive breeding is a fundamental component in the laboratory domestication of wild animals having scientific value, ensuring a stable and sustainable supply of high-quality animals for research. However, sometimes, captive tree shrews fail to produce the milk that meets the demand of their young in quality and quantity, resulting in poor growth, developmental delay, and low survival rate of the pups. In addition, the absence of normal maternal behaviors further contributes to early mortality in the young. These issues become especially pronounced when attempting to establish inbred lines, where insufficient milk production and inadequate maternal care are exacerbated.[3,50] Such challenges have posed significant obstacles to the laboratory domestication of tree shrews.

Practical nutritional management strategies, such as artificial rearing technologies, can be beneficial in addressing these issues. In our earlier research, we discovered that Wombaroo milk replacer for guinea pigs can be effectively used in hand-rearing practices to enhance the survival rates of the tree shrew pups.[3] However, the daily milk intake and the pattern of body weight gain were not identical to those of pups reared by their mothers.[3] These disparities emphasize the necessity of characterizing the milk composition in tree shrews and investigating how it differs across various stages of lactation, as it forms the basis for the nutritional management of tree shrew mothers and their young, particularly in developing milk replacers for hand-rearing of tree shrew pups.

Despite the rising significance of this species in research, data on the composition of tree shrew milk remains sparse, especially concerning how it changes across different lactation periods.

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Previous studies on tree shrew milk composition have provided some insights into macronutrient and mineral profiles in tree shrew milk, yet methodological limitations exist.[4,48] D'Souza and Martin's work lacked detailed sampling protocols and analysis methods used,[4] making it difficult to assess potential biases, whereas analysis by Yang and colleagues,[48] which relied on pooled gastric contents from nursing pups, raised concerns about contamination from digestive juices and animal welfare issues. In addition, none of these studies examined the changes in macronutrient and fatty acid (FA) profiles across lactation stages, a crucial gap given the importance of FAs in infant growth and development.[14]

In the present study, we initiated an investigation into milk proximate and FA composition in tree shrews over the course of lactation using microanalysis and gas chromatography methods. Our objectives were 2-fold: 1) to further explore the proximate composition of tree shrew milk and its change across lactation, and 2) to characterize the FA profile and assess the influence of lactation stages on this profile. We hypothesized that the tree shrew produces concentrated milk, with variations in macronutrient and FA composition occurring as lactation progresses, thereby better supporting the growth and developmental needs of their pups. Meanwhile, we recorded the weights of pups aged 2 to 26 d and monitored the amount of milk they consumed to evaluate pup growth and maternal milk yield (MY) with corresponding milk composition data. This work may provide an updated version of milk composition in captive tree shrews and assist in formulating milk substitutes for tree shrew pups, which will aid in the laboratory domestication of this species.

Materials and Methods

Animals. All animal care and experiment procedures were conducted in accordance with the regulations of the *Guide for the Care and Use of Laboratory Animals* and approved by the IACUC in Kunming Institute of Zoology (KIZ), Chinese Academy of Sciences (CAS).

From April to October 2022, 75 lactating tree shrews (*Tupaia belangeri*) were chosen randomly from the breeding colony in KIZ, CAS. Among them, 27 dams that were unable to nurse their neonates at delivery and 30 mothers that behaved normally at delivery but failed to nurse their pups on the third day postpartum were designated to provide milk samples on postnatal days 0 and 2 (P0 and P2), respectively; in addition, 18 dams that displayed normal behaviors were grouped for milk sampling on postnatal days 8, 14, 20, 26, and 32 (P8, P14, P20, P26, and P32). Meanwhile, the offspring of these normal mothers were also selected as the objects to evaluate MY of the dams and growth of the young.

To facilitate a comparative analysis of milk components between tree shrews and other species, we defined the early, middle, and late lactation stages for tree shrews as follows: early lactation as less than 10 d, mid-lactation as 10 to 26 d, and late lactation as more than 26 d, following Oftedal and Iverson's method.[34]

Husbandry. Each adult animal was housed in a spacious cage measuring 600 × 600 × 800 mm. A litter of pups under 26 d old was kept in a box (350 × 200 × 186 mm, attached to the cage) where they suckled maternal milk for 15 min every 2 d. They were cohoused with their mother from P26 (after milk collection) until they reached the weaning age of approximately 40 d. The pups attacked or abandoned by their mothers were hand-reared following our feeding procedures reported previously.[3]

All animals capable of consuming solid food had ad libitum access to feed powders (nutrient composition shown in Table S1) and water, and they received a piece of fresh apple once a day.

Environmental conditions were carefully controlled, maintaining an ambient temperature of 24 ± 4 °C and a relative humidity of 55% ± 15%, with a 12-h light/12-h dark cycle.

Milk collection. The sample collection was performed at 0800 in accordance with the experimental scheme (Figure 1A). Prior to milking, the mother and her young were separated for 12 h (on P32) or 48 h to facilitate milk accumulation in the mammary glands.

Each dam received intramuscular injections of ketamine (150 mg/kg; Ketaset injection CP, Jiangsu Pocon Pharmaceutical Industries, Taizhou, China) and oxytocin (12.5 IU/kg; oxytocin CP, Shanghai Harvest, Shanghai, China). The fur around the teats was shaved and the area was cleaned with warm water. Milk was extracted using a homemade milking device (Figure 1B), focusing primarily on the bottom and the middle teats.

Each milk sample was combined with twice its weight of pure water,[18] and immediately stored in a refrigerator at −80 °C until analysis.

Chemical analysis of milk. Milk samples were collected from the tree shrews at various time points during lactation. However, milking attempts were not always successful, with failures occurring for 6 samples at P0, 2 at P2, 1 at P26, and 5 at P32 because of low milk production. In addition, 10 samples at P0 and 9 at P2 were discarded due to small volume (less than 350 µL). The ones contaminated by blood (2 at P0 and 1 at P2) were also discarded. Ultimately, a total of 111 milk samples were available for further analysis. All samples were assayed in duplicate except for one sample on postnatal day 26, which was only analyzed for its gross composition due to insufficient quantity.

Milk samples were thawed at 40 to 50 °C and homogenized before analysis.[8] Total nitrogen was estimated using the micro-Kjeldahl method with a nitrogen-to-protein conversion factor of 6.38.[7] Dry matter (DM), ash, sugars, and lipids were determined following the protocol well suited for small mammals.[18] The milk energy was calculated by summing the contributions from lipids (3,812 kJ/100 g), protein (2,452 kJ/100 g), and sugars (1,653 kJ/100 g).[35]

The analysis of FA composition was performed by Wuhan Anachro Technologies (Wuhan, China) on milk aliquots shipped with dry ice. The assay procedure was briefly described as follows: 50 µL of milk and 1 mL of *n*-ethane was mixed, sonicated for 20 min, and centrifuged at 13,000 rpm for 5 min. The supernatant was dried under nitrogen, mixed with 2 mL of 5% concentrated sulfuric acid methanol solution and 25 µL of 0.2% butyl hydroxytoluene methanol solution. Methylation was performed in a water bath at 90 °C for 90 min. After cooling, the reaction solution was combined with 2 mL of saturated saline and 1 mL of *n*-hexane, followed by a centrifugation (4 °C, 3,500 rpm, 5 min). Subsequently, 60 µL of extracted *n*-hexane was analyzed by gas chromatography using an Agilent 7890A network gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with an Agilent capillary column (10 m × 0.1 mm × 0.1 µm; DB-225, Agilent Technologies, Palo Alto, CA). A 1-µL injection was made with a split ratio of 15:1, using helium (99.9999%) as the carrier gas at a flow rate of 0.3 mL/min.

The temperature of both the injector and detector was set at 250 °C, and the column temperature was programmed from 55 °C (held for 1 min) to 205 °C (held for 1 min) at 30 °C/min, then to 230 °C (maintained for 3 min) at 5 °C/min.

Determination of MY of dams MY and body weight of pups. After milking, the dams were returned to their home cages and

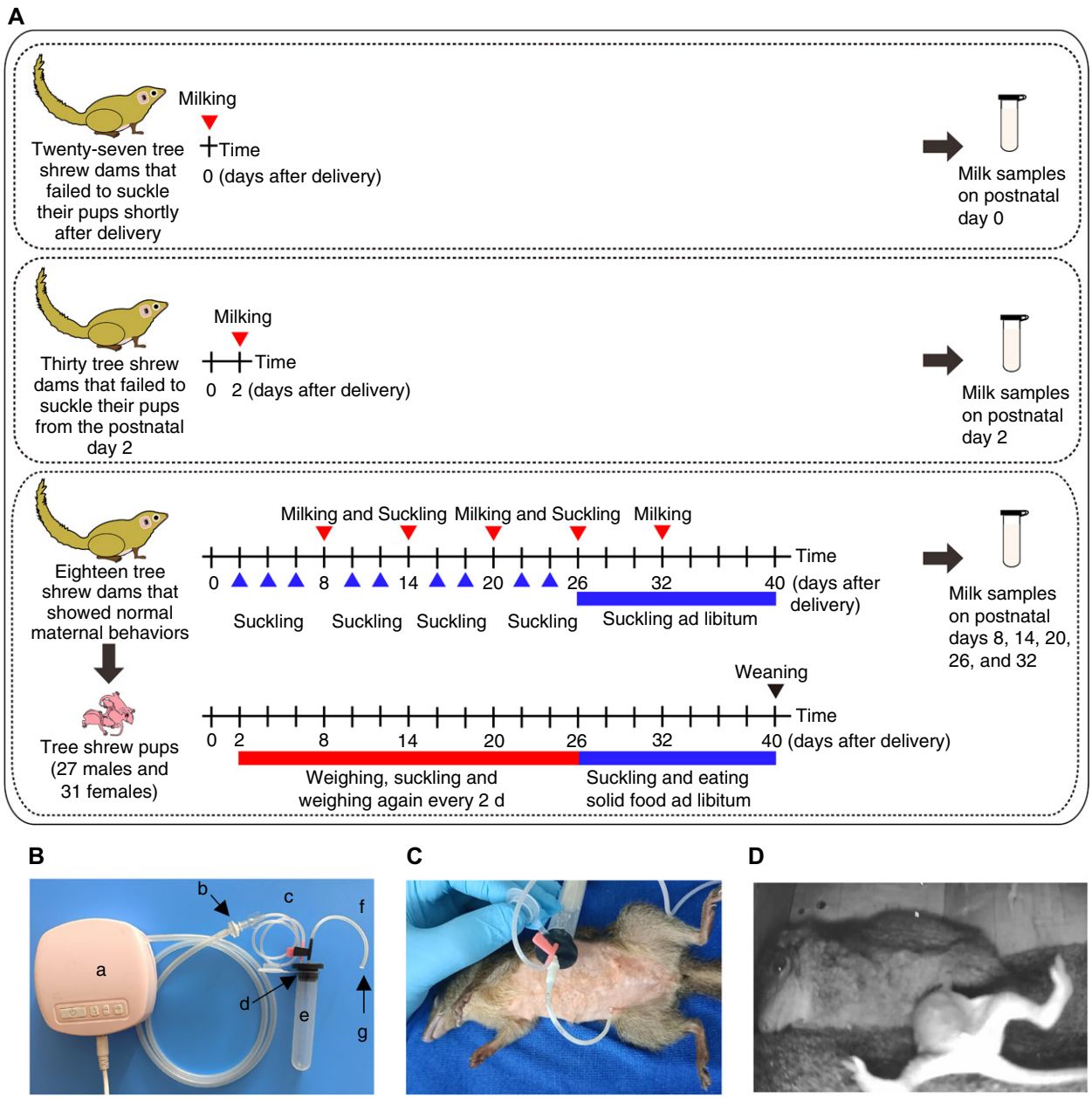

Figure 1. Experimental scheme and milking procedures. (A) To investigate the milk composition of tree shrews and its changes during lactation, milk samples on postnatal days 0 (P0), 2 (P2), 8, 14, 20, 26 and 32 were collected for chemical analysis. The P0 and P2 samples were from the captive dams that failed to nurse the young from the first or the third day after delivery, and the others were from the ones with normal maternal behaviors. At the same time, the offspring of dams with normal maternal behavior were also included in the experiment to evaluate the milk yield of dams and growth of the young during the early and mid-lactation. (B) Apparatus for milking tree shrews in the present study. a, Electric breast pump; b, filter of disposable infusion set; c, disposable infusion needle used as the pulse tubing; d, rubber stopper; e, centrifuge tube used as a collection container; f, part of disposable infusion needle used as milk tubing; g, enlarged, smooth incision at the end of pipe used as a teat cap. (C) Lactating mother being milked using the homemade milk apparatus for tree shrews. (D) Mother being suckled by her pup after being milked and waking up from anesthesia.

monitored until fully recovered from anesthesia. At 1300, dams that did not show abnormal maternal behaviors in the process of delivery, were placed in the box attached to the cages to nurse their offspring. Pups were weighed individually before and after nursing until they reached 26 d old. The MY was calculated as the total amount of milk consumed by pups from a litter.

Statistical analysis. Summary values are expressed as the mean ± SD. The Shapiro–Wilk test and Brown–Forsythe test were performed for normality and homoscedasticity, respectively. For normally distributed data, one-way ANOVA with a Bonferroni test or Welch ANOVA with a Tamhane T2 test, depending on the homogeneity of the data variance, was conducted to test for constituent differences. When the data violated the normality assumption, the nonparametric Kruskal–Wallis test and Dunn test were applied. The Spearman rank correlation test was used to analyze the potential relationships between nutrient content and lactation, as well as between the MY of the mothers and the growth of their pups. All analyses above were performed on GraphPad Prism 8 (GraphPad Software, San Diego, CA). The relationship between FAs was performed on the Hiplot Pro (https://hiplot.com.cn/) with R software (v4.2.2) package corrplot (v0.92) and ggplot2 (v3.4.2),[40,41] using the Spearman rank correlation test. The significance level was set to 0.05.

Results

Proximate composition of tree shrew milk. The proximate analysis of the tree shrew milk is shown in Table 1. The sum of moisture (100% − DM), lipids, protein, sugars, and ash accounted for 96.73% ± 2.90% of each fresh sample, indicating minimal analytic errors. At mid-lactation, the milk contained 44.75% ± 3.43% DM, 11.93% ± 1.34% protein, 1.63% ± 0.21% sugars, 26.35% ± 1.79% lipids, 1.34% ± 0.23% ash, and 1,323.99 ± 77.46 kJ/100 g energy. Lipids contributed the majority of energy (75.86% ± 2.13%), followed by protein (22.10% ± 2.15%), while the contribution from sugars was negligible (2.04% ± 0.30%). With the extension of lactation, levels of DM, sugars, and ash gently increased (Table 1; Figure 2A, D, and E). The energy from lipids decreased (Figure 2G), while the energy from sugars increased slightly (Figure 2I).

FA composition of tree shrew milk. A total of 31 types of FAs were identified (Table 2), with palmitic acid (C16:0, 26.82%) and myristic acid (C14:0, 14.65%), followed by linoleic acid (C18:2n6c, 13.55%) and oleic acid (C18:1n9c, 13.33%) as the predominant FAs. C16:0, C18:1n9, and C18:2n6c also were the most abundant FAs in their respective categories, accounting for 39.33%, 87.18%, and 76.76% of the total concentration of saturated FAs (SFAs), monounsaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs) at mid-lactation, respectively.

Tree shrew milk contained high levels of SFAs rather than unsaturated FAs (UFAs). Both the majority of individual SFAs and the total SFAs exhibited a progressive rise (Table 2; Figure 3A, E, G, and N; Figure 4G), while most individual UFAs combined with the total UFAs, MUFAs, and PUFAs progressed through an opposite pattern (Table 2; Figure 3H, L, O–Q, S, and T; Figure 4H–J). This opposing trend resulted in an increasing ratio of SFAs/PUFAs (Figure 4K). In addition, the concentration of n3-FAs and n6-FAs simultaneously decreased (Figure 4L and M), leading to a stable n6/n3 ratio of around 7 (Table 2).

Based on the chain length, long-chain FAs (LCFAs, 13 to 21 carbons)[32] were the most abundant (72.88%) in tree shrew milk. Medium-chain FAs (MCFAs, 6 to 12 carbons)[32] followed, accounting for 20.71%. The very-long-chain FAs (VLCFAs, 22 or more carbons)[32] presented in scarce amounts (1.35%), and short-chain FAs (SCFAs, 5 or fewer carbons)[32] were not detected. Moreover, odd-chain FAs (OCFAs) were also identified at a low level (0.62%). As lactation advanced, the contents of LCFAs, VLCFAs, and OCFAs gradually declined (Table 2; Figure 4O–Q), whereas the content of MCFAs increased steadily (Table 2; Figure 4N).

The correlation between FAs in milk from tree shrews is illustrated in Figure 5. All FAs identified, except C6:0, exhibited significant relationships with each other. Notably, one FA was often associated with multiple others, either positively or negatively. For instance, all MCFAs, except C11:0, exhibited positive correlations with each other but negative correlations with other FAs (Figure 5A), primarily PUFAs. Consequently, a negative correlation was found between the total MCFAs and the total LCFAs, n3-FAs, or other FA categories (Figure 5B).

MY of lactating tree shrews and the body weight of pups. Figure 6 illustrates the MY of dams that exhibited normal maternal behaviors and the growth pattern of their young before the introduction of solid food. As the lactation continued, MY gradually increased from 6.79 to 12.09 g on average every 2 d between P2 and P8, peaking at more than 20 g every 2 d around P20, before slowly declining. In the early stages (P2 to P8) pups experienced slow growth. The average weight of pups ranged from 11.90 to 18.06 g, and the litter weight averaged

Table 1. Gross composition of tree shrew milk

Item	Postnatal day 0 (n = 9)	Postnatal day 2 (n = 18)	Postnatal day 8 (n = 18)	Postnatal day 14 (n = 18)	Postnatal day 20 (n = 18)	Postnatal day 26 (n = 17)	Postnatal day 32 (n = 13)	Mid-lactation (n = 53)
Dry matter (g/100 g)	41.51 ± 2.36	42.80 ± 3.07	42.71 ± 2.92	43.22 ± 3.39	44.94 ± 3.29	46.16 ± 3.11	44.56 ± 4.25	44.75 ± 3.43
Lipids (g/100 g)	27.17 ± 1.70	26.12 ± 2.41	25.29 ± 2.13	26.27 ± 2.18	26.63 ± 1.51	26.16 ± 1.68	25.66 ± 1.68	26.35 ± 1.79
Protein (g/100 g)	11.83 ± 0.68	11.44 ± 1.44	11.24 ± 1.19	11.28 ± 0.90	12.07 ± 1.10	12.47 ± 1.69	11.62 ± 1.23	11.93 ± 1.34
Sugars (g/100 g)	0.84 ± 0.12^{Aa}	1.13 ± 0.17^{ABb}	1.46 ± 0.18^{Bb}	1.51 ± 0.14^{Bb}	1.68 ± 0.17^{Bb}	1.69 ± 0.26^{Bb}	1.73 ± 0.42^{Bb}	1.63 ± 0.21
Ash (g/100 g)	1.07 ± 0.14	1.12 ± 0.21	1.18 ± 0.22	1.25 ± 0.24	1.36 ± 0.20	1.41 ± 0.24	1.34 ± 0.23	1.34 ± 0.23
Energy (kJ/100 g)	1,339.52 ± 67.15	1,294.63 ± 105.17	1,263.72 ± 89.60	1,302.69 ± 87.45	1,338.86 ± 68.78	1,330.79 ± 74.43	1,292.02 ± 64.29	1,323.99 ± 77.46

Eighteen lactating tree shrews provided milk samples on postnatal days 8, 14, 20, 26, and 32, respectively, with 1 dam and 5 dams that failed due to a decreased lactation on postnatal days 26 and 32, respectively. A total of 27 dams that did not suckle the newborn on the day of delivery provided milk samples on postnatal day 0 (P0), and 6 of them failed because of inadequate lactation. Twenty-eight of the 30 dams that did not suckle their pups from the third day after delivery successfully offered milk samples on postnatal day 2 (P2). Two samples at P0 and 1 sample at P2, respectively, contaminated by blood were excluded. Ten samples at P0 and 9 samples at P2 were also not included due to small volume that was insufficient for analysis. Values are given as mean ± SD in the whole milk. Differences in each macronutrient content at different postnatal days were analyzed using one-way ANOVA followed by a Bonferroni test or Welch ANOVA followed by a Tamhane T2 test if the Brown–Forsythe test failed to verify the homogeneity of the data variance. Within a row, values with no lowercase or uppercase letters in common in the same row are significantly different at α = 5% or α = 1%. The values at mid-lactation (from 10 to 26 d postpartum) were not included in the comparison.

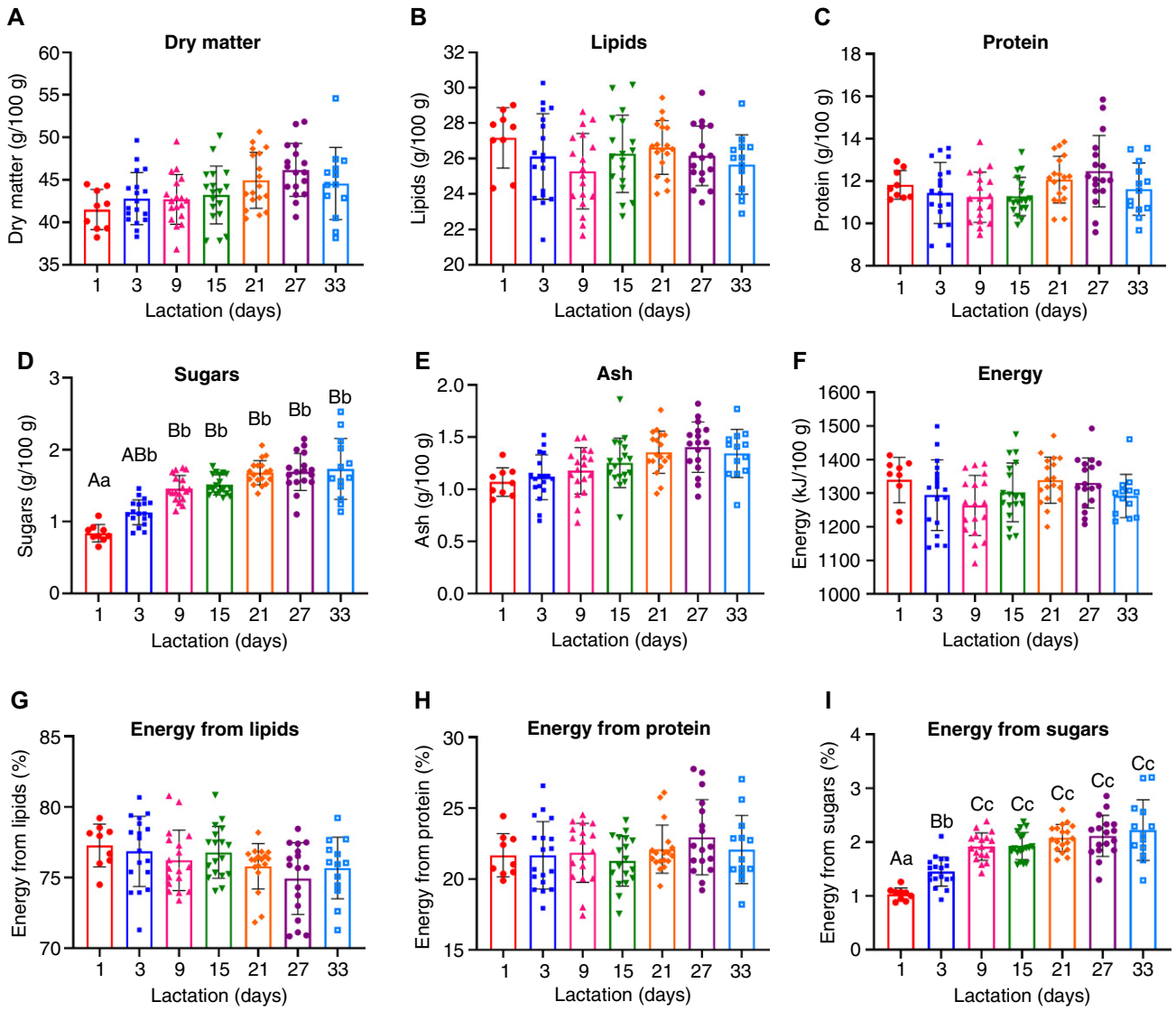

Figure 2. Changes in the (A) day matter, (B) lipids, (C) protein, (D) sugars, (E) ash, (F) energy, (G) energy from lipids, (H) energy from protein, and (I) energy from sugars in milk from captive tree shrews during lactation. Each point represented a single sample. Bars represent the mean ± SD. Means not sharing any lowercase letters are significantly different at the 5% level, while those not sharing any uppercase letters are significantly different at the 1% level. ANOVA with a Bonferroni test was used to analyze the normally distributed data with equal variances, whereas a Welch ANOVA with a Tamhane T2 test was for the normally distributed data with unequal variances. When the data were not in a normal distribution, the nonparametric Kruskal–Wallis test with a Dunn test was run.

between 38.34 and 57.20 g. In contrast, a significant acceleration in growth occurred from the 10th day postpartum, with the pups gaining an average of 1.75 g per day and the litter gaining an average of 5.21 g per day. In addition, the body weight of these pups showed a strong positive correlation with their mothers' MY before they began consuming solid food ($r = 0.895$, $P < 0.0001$).

Discussion

In the present study, we investigated the proximate and FA composition levels in milk from captive tree shrews and their changes across different lactation stages. Our results reveal that the captive tree shrew produces concentrated milk rich in lipids, protein, SFAs, and LCFAs; and the macronutrient concentration and FA composition varied over the course of lactation. To the best of our knowledge, this presents the first report to describe the dynamic changes in the milk proximate composition of captive tree shrews over the course of lactation, as well as the characterization of the FA composition in milk from captive tree shrews and its change as lactation advances. This research may improve captive breeding and management practices in the laboratory domestication of tree shrews. The data on milk composition, particularly on FA profile, and its variation across different lactation stages are crucial for a more comprehensive understanding of the lactational physiology in tree shrews and the nutritional needs of suckling pups of varying ages, and, at a practical level, for developing and optimizing feeding strategies, nutritional interventions, and modeling artificial milk and feed formulas.

Tree shrew milk is highly nutrient-dense, containing significantly higher levels of DM, fat, and protein, compared with most terrestrial species.[34] This composition reflects the tree shrew's specialized lactation strategy, characterized by short lactation lengths and a long suckling interval. In contrast to species with longer lactation lengths, such as elephants (2 to 8 y) and primates,[1,15] tree shrews possess a relatively abbreviated lactation of approximately 5 wk.[56] During this phase, they must allocate a high daily rate of nutrients to their pups, who rely predominantly on maternal milk for rapid growth (Figure 6B) and development. On the other hand, lactating

Table 2. Fatty acid composition of tree shrew milk

Item	Postnatal day 0 (n = 9)	Postnatal day 2 (n = 18)	Postnatal day 8 (n = 18)	Postnatal day 14 (n = 18)	Postnatal day 20 (n = 18)	Postnatal day 26 (n = 16)	Postnatal day 32 (n = 13)	Mid-lactation (n = 52)
C6:0 (g/100 g fat)	0.57 ± 0.08^{ABa}	0.67 ± 0.09^{ABab}	0.59 ± 0.16^{Aa}	0.81 ± 0.13^{ABCabc}	0.90 ± 0.19^{BCbc}	0.97 ± 0.16^{Cc}	1.01 ± 0.12^{Cc}	0.89 ± 0.17
C8:0 (g/100 g fat)	4.17 ± 0.34^{Aab}	3.64 ± 0.33^{ABabc}	2.37 ± 0.46^{Cd}	2.95 ± 0.58^{BCcd}	3.42 ± 0.63^{ABbc}	4.01 ± 0.81^{ABabc}	5.04 ± 0.95^{Aa}	3.44 ± 0.79
C10:0 (g/100 g fat)	7.59 ± 0.72^{ABab}	7.33 ± 0.76^{ABab}	6.01 ± 0.87^{Ac}	6.98 ± 1.14^{ABac}	7.97 ± 0.97^{Bab}	9.09 ± 1.46^{BCbd}	10.92 ± 1.62^{Cd}	7.97 ± 1.45
C11:0 (g/100 g fat)	0.02 ± 0.01^{ABab}	0.02 ± 0.00^{Aa}	0.01 ± 0.01^{Bb}	0.01 ± 0.01^{Bb}	0.01 ± 0.01^{Bb}	0.01 ± 0.00^{ABab}	0.01 ± 0.00^{ABab}	0.01 ± 0.01
C12:0 (g/100 g fat)	6.65 ± 0.85^{Aa}	7.31 ± 0.89^{Aab}	7.24 ± 0.92^{Aa}	7.79 ± 0.86^{ABabc}	8.46 ± 0.70^{ABbc}	9.02 ± 0.83^{Bc}	8.99 ± 0.65^{Bc}	8.40 ± 0.93
C13:0 (g/100 g fat)	0.00 ± 0.00^{ABab}	0.00 ± 0.00^{Aa}	0.01 ± 0.01^{ABab}	0.02 ± 0.03^{Bb}	0.01 ± 0.01^{ABab}	0.01 ± 0.01^{ABab}	0.01 ± 0.01^{ABab}	0.01 ± 0.02
C14:0 (g/100 g fat)	8.63 ± 1.31^{Aa}	10.75 ± 1.45^{Aa}	13.32 ± 1.56^{Bb}	14.15 ± 0.90^{Bb}	14.77 ± 1.02^{Bb}	15.08 ± 1.01^{Bb}	14.42 ± 1.04^{Bb}	14.65 ± 1.03
C14:1n5 (g/100 g fat)	0.14 ± 0.04^{ABab}	0.14 ± 0.03^{Bb}	0.09 ± 0.02^{ABCac}	0.10 ± 0.02^{ABCabc}	0.09 ± 0.02^{ABCac}	0.08 ± 0.01^{ACc}	0.07 ± 0.01^{Cc}	0.09 ± 0.02
C15:0 (g/100 g fat)	0.23 ± 0.02^{Aa}	0.19 ± 0.03^{ABab}	0.16 ± 0.02^{BCbc}	0.14 ± 0.02^{CDcd}	0.13 ± 0.02^{CDcd}	0.13 ± 0.01^{Dd}	0.12 ± 0.02^{Dd}	0.13 ± 0.02
C15:1n5 (g/100 g fat)	0.00 ± 0.00^{ab}	0.00 ± 0.00^{a}	0.01 ± 0.03^{ab}	0.02 ± 0.02^{ab}	0.02 ± 0.03^{ab}	0.01 ± 0.02^{ab}	0.04 ± 0.04^{b}	0.02 ± 0.03
C16:0 (g/100 g fat)	21.18 ± 1.79^{Aa}	23.21 ± 1.33^{Aab}	28.68 ± 1.78^{Bc}	27.94 ± 2.43^{Bcd}	26.99 ± 2.59^{BCcd}	25.38 ± 2.14^{ABbd}	22.44 ± 2.49^{ACab}	26.82 ± 2.58
C16:1n7 (g/100 g fat)	2.56 ± 0.56^{Aa}	2.05 ± 0.33^{Aa}	1.37 ± 0.25^{Bb}	1.54 ± 0.26^{ABab}	1.36 ± 0.18^{Bb}	1.39 ± 0.22^{Bb}	1.49 ± 0.25^{ABab}	1.43 ± 0.23
C17:0 (g/100 g fat)	0.21 ± 0.02^{Aa}	0.18 ± 0.02^{ABab}	0.18 ± 0.02^{ABCab}	0.15 ± 0.02^{BCbc}	0.15 ± 0.02^{BCbc}	0.14 ± 0.02^{Cc}	0.15 ± 0.02^{BCbc}	0.15 ± 0.02
C17:1n7 (g/100 g fat)	ND	ND	ND	ND	ND	ND	ND	ND
C18:0 (g/100 g fat)	3.52 ± 0.59^{ABab}	3.13 ± 0.30^{Aa}	4.13 ± 0.51^{Bbc}	4.18 ± 0.53^{Bbc}	4.28 ± 0.73^{Bbc}	4.28 ± 0.52^{Bbc}	4.91 ± 0.72^{Bc}	4.24 ± 0.60
C18:1n9t (g/100 g fat)	ND	ND	ND	ND	ND	ND	ND	ND
C18:1n9c (g/100 g fat)	18.40 ± 1.52^{Aa}	17.15 ± 1.46^{Aab}	14.97 ± 1.57^{ABbc}	14.17 ± 1.12^{Bc}	13.11 ± 1.33^{Bc}	12.61 ± 1.64^{Bc}	13.09 ± 1.14^{Bc}	13.33 ± 1.49
C18:2n6t (g/100 g fat)	ND	ND	ND	ND	ND	ND	ND	ND
C18:2n6c (g/100 g fat)	19.50 ± 2.03^{Aa}	18.13 ± 1.96^{Aa}	15.15 ± 2.13^{ABb}	14.02 ± 1.13^{Bb}	13.42 ± 1.12^{Bb}	13.15 ± 0.85^{Bb}	13.06 ± 0.96^{Bb}	13.55 ± 1.09
C18:3n3 (g/100 g fat)	1.45 ± 0.19^{ABab}	1.36 ± 0.14^{Ab}	1.17 ± 0.19^{ABabc}	1.09 ± 0.13^{Bac}	1.05 ± 0.12^{Bc}	1.03 ± 0.09^{Bc}	1.01 ± 0.10^{Bc}	1.06 ± 0.11
C18:3n6 (g/100 g fat)	0.06 ± 0.05^{a}	0.09 ± 0.02^{a}	0.12 ± 0.06^{ab}	0.16 ± 0.07^{b}	0.16 ± 0.05^{b}	0.14 ± 0.05^{ab}	0.09 ± 0.03^{a}	0.16 ± 0.06
C20:0 (g/100 g fat)	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
C20:1 (g/100 g fat)	0.33 ± 0.04^{ABab}	0.32 ± 0.05^{ABab}	0.34 ± 0.04^{Aa}	0.33 ± 0.03^{Aa}	0.30 ± 0.04^{ABab}	0.28 ± 0.05^{ABab}	0.26 ± 0.04^{Bb}	0.31 ± 0.04
C20:2n6 (g/100 g fat)	0.65 ± 0.07^{ABCabc}	0.64 ± 0.07^{ABab}	0.69 ± 0.07^{Aa}	0.66 ± 0.07^{ABab}	0.59 ± 0.08^{ABCabc}	0.55 ± 0.06^{BCbc}	0.47 ± 0.07^{Cc}	0.60 ± 0.08
C20:3n3 (g/100 g fat)	0.13 ± 0.06	0.12 ± 0.02	0.10 ± 0.03	0.09 ± 0.01	0.09 ± 0.01	0.10 ± 0.03	0.11 ± 0.04	0.09 ± 0.02
C20:3n6 (g/100 g fat)	0.26 ± 0.02^{ABab}	0.24 ± 0.03^{ABab}	0.26 ± 0.07^{ABab}	0.29 ± 0.07^{Aa}	0.28 ± 0.07^{Aa}	0.25 ± 0.06^{ABab}	0.18 ± 0.04^{Bb}	0.27 ± 0.06
C20:4n6 (g/100 g fat)	1.37 ± 0.17^{Aa}	1.26 ± 0.22^{Aa}	1.05 ± 0.22^{ABab}	0.87 ± 0.08^{BCbc}	0.84 ± 0.12^{BCbc}	0.83 ± 0.11^{BCbc}	0.72 ± 0.11^{Cc}	0.85 ± 0.11
C20:5n3 (g/100 g fat)	0.15 ± 0.02^{Aa}	0.12 ± 0.02^{ABab}	0.11 ± 0.03^{ABb}	0.11 ± 0.01^{ABab}	0.10 ± 0.03^{ABab}	0.10 ± 0.01^{Bb}	0.10 ± 0.01^{Bb}	0.10 ± 0.02
C21:0 (g/100 g fat)	ND	ND	ND	ND	ND	ND	ND	ND
C22:0 (g/100 g fat)	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C22:1n9 (g/100 g fat)	0.21 ± 0.12	0.13 ± 0.09	0.21 ± 0.18	0.10 ± 0.04	0.12 ± 0.06	0.11 ± 0.05	0.15 ± 0.11	0.11 ± 0.05
C22:2n6 (g/100 g fat)	0.03 ± 0.03	0.04 ± 0.02	0.04 ± 0.03	0.03 ± 0.02	0.05 ± 0.03	0.03 ± 0.01	0.02 ± 0.02	0.04 ± 0.02
C22:5n3c (g/100 g fat)	0.64 ± 0.21^{ABa}	0.55 ± 0.09^{Aa}	0.43 ± 0.12^{ACab}	0.37 ± 0.10^{ACab}	0.36 ± 0.04^{BCb}	0.37 ± 0.04^{BCb}	0.32 ± 0.05^{Cb}	0.37 ± 0.07
C22:6n3 (g/100 g fat)	0.86 ± 0.10^{ABa}	0.78 ± 0.13^{Aa}	0.60 ± 0.11^{ACab}	0.53 ± 0.07^{Cb}	0.52 ± 0.06^{Cb}	0.53 ± 0.07^{BCb}	0.50 ± 0.10^{Cb}	0.53 ± 0.07
C23:0 (g/100 g fat)	0.44 ± 0.10^{Aa}	0.41 ± 0.09^{Aa}	0.55 ± 0.46^{Aa}	0.30 ± 0.03^{ABab}	0.30 ± 0.05^{ABab}	0.28 ± 0.03^{ABb}	0.24 ± 0.04^{Bb}	0.29 ± 0.04
C24:0 (g/100 g fat)	ND	ND	ND	ND	ND	ND	ND	ND

(*continued*)

Table 2. (Continued)

Item	Postnatal day 0 (n = 9)	Postnatal day 2 (*n* = 18)	Postnatal day 8 (*n* = 18)	Postnatal day 14 (n = 18)	Postnatal day 20 (*n* = 18)	Postnatal day 26 (*n* = 16)	Postnatal day 32 (*n* = 13)	Mid-lactation (n = 52)
C24:1n9 (g/100 g fat)	ND	ND	ND	ND	ND	ND	ND	ND
SFAs[a] (g/100 g fat)	53.26 ± 3.53^{Aa}	56.89 ± 3.69^{ABa}	63.31 ± 4.10^{BCb}	65.47 ± 1.96^{Cb}	67.42 ± 2.35^{Cb}	68.44 ± 2.52^{Cb}	68.31 ± 1.93^{Cb}	67.06 ± 2.56
UFAs[b] (g/100 g fat)	46.74 ± 3.53^{Aa}	43.11 ± 3.69^{ABa}	36.69 ± 4.10^{BCb}	34.53 ± 1.96^{Cb}	32.58 ± 2.35^{Cb}	31.56 ± 2.52^{Cb}	31.69 ± 1.93^{Cb}	32.94 ± 2.56
MUFAs[c] (g/100 g fat)	21.64 ± 1.66^{Aa}	19.78 ± 1.59^{ABa}	16.98 ± 1.81^{BCb}	16.27 ± 1.20^{Cb}	15.01 ± 1.48^{Cb}	14.49 ± 1.87^{Cb}	15.11 ± 1.36^{Cb}	15.29 ± 1.68
PUFAs[d] (g/100 g fat)	25.10 ± 2.48^{Aa}	23.32 ± 2.51^{Aab}	19.71 ± 2.71^{ABbc}	18.26 ± 1.31^{Bc}	17.57 ± 1.18^{Bc}	17.07 ± 1.00^{Bc}	16.59 ± 1.28^{Bc}	17.66 ± 1.25
SFAs/PUFAs	2.15 ± 0.35^{Aa}	2.48 ± 0.45^{ABab}	3.30 ± 0.68^{BCbc}	3.61 ± 0.34^{Cc}	3.86 ± 0.37^{Cc}	4.03 ± 0.37^{Cc}	4.15 ± 0.41^{Cc}	3.82 ± 0.39
n3-FAs[e] (g/100 g fat)	3.23 ± 0.38^{Aa}	2.92 ± 0.34^{Aab}	2.40 ± 0.40^{ABbc}	2.22 ± 0.25^{Bc}	2.12 ± 0.17^{Bc}	2.12 ± 0.16^{Bc}	2.04 ± 0.24^{Bc}	2.16 ± 0.20
n6-FAs[f] (g/100 g fat)	21.84 ± 2.14^{Aa}	20.36 ± 2.18^{Aab}	17.27 ± 2.32^{ABbc}	16.00 ± 1.16^{Bc}	15.40 ± 1.08^{Bc}	14.92 ± 0.89^{Bc}	14.52 ± 1.05^{Bc}	15.46 ± 1.12
n6/n3	6.79 ± 0.45	6.98 ± 0.27	7.25 ± 0.55	7.25 ± 0.63	7.27 ± 0.53	7.06 ± 0.40	7.15 ± 0.42	7.20 ± 0.53
MCFAs[g] (g/100 g fat)	19.01 ± 1.55^{ABabc}	18.98 ± 1.91^{ABac}	16.23 ± 2.11^{Aa}	18.54 ± 2.60^{ABac}	20.75 ± 2.18^{BCbc}	23.09 ± 3.02^{BCbd}	25.98 ± 3.02^{Cd}	20.71 ± 3.15
LCFAs[h] (g/100 g fat)	78.80 ± 1.46^{ABabc}	79.02 ± 1.75^{ABabc}	81.92 ± 1.96^{Aa}	80.06 ± 2.63^{ABab}	77.74 ± 2.51^{BCbc}	75.57 ± 3.00^{BCcd}	72.78 ± 2.88^{Cd}	72.88 ± 3.23
VLCFAs[i] (g/100 g fat)	2.20 ± 0.36^{ADa}	1.93 ± 0.31^{Aa}	1.84 ± 0.48^{ABab}	1.35 ± 0.15^{BCbc}	1.36 ± 0.16^{BCDbc}	1.34 ± 0.09^{BCbc}	1.25 ± 0.18^{Cc}	1.35 ± 0.14
OCFAs[j] (g/100 g fat)	0.90 ± 0.08^{Aa}	0.80 ± 0.09^{ABab}	0.91 ± 0.48^{ABCabc}	0.64 ± 0.06^{ABCbcd}	0.62 ± 0.08^{BCcd}	0.58 ± 0.06^{Cd}	0.58 ± 0.06^{Cd}	0.62 ± 0.07

Values with no lowercase or uppercase letters in common in the same row are significantly different at α = 5% or α = 1%. The values at mid-lactation (at postnatal days 14 to 26) were not included in the comparison. ND, not determined.

[a]SFAs (saturated fatty acids) include C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, and C24:0.

[b]UFAs (unsaturated fatty acids) include MUFAs and PUFAs.

[c]MUFAs (monounsaturated fatty acids) include C14:1n5, C15:1n5, C16:1n7, C17:1n7, C18:1n9t, C18:1n9c, C20:1, C22:1n9, and C24:1n9.

[d]PUFAs (polyunsaturated fatty acids) include C18:2n6t, C18:2n6c, C18:3n3, C18:3n6, C20:2, C20:3n3, C20:3n6, C20:4n6, C20:5n3, C22:2, C22:5n3c, and C22:6n3.

[e]n3-FAs (omega-3 fatty acids) include C18:3n3, C20:3n3, C20:5n3, C22:5n3c, and C22:6n3.

[f]n6-FAs (omega-6 fatty acids) include C18:2n6t, C18:2n6c, C18:3n6, C20:2n6, C20:3n6, and C20:4n6.

[g]MCFAs (medium-chain fatty acids) include 6 to 12 carbons.

[h]LCFAs (long-chain fatty acids) include 13 to 21 carbons.

[i]VLCFAs (very-long-chain fatty acids) include 22 or more carbons.

[j]OCFAs (odd-chain fatty acids) include 11, 13, 15, 17, and 23 carbons.

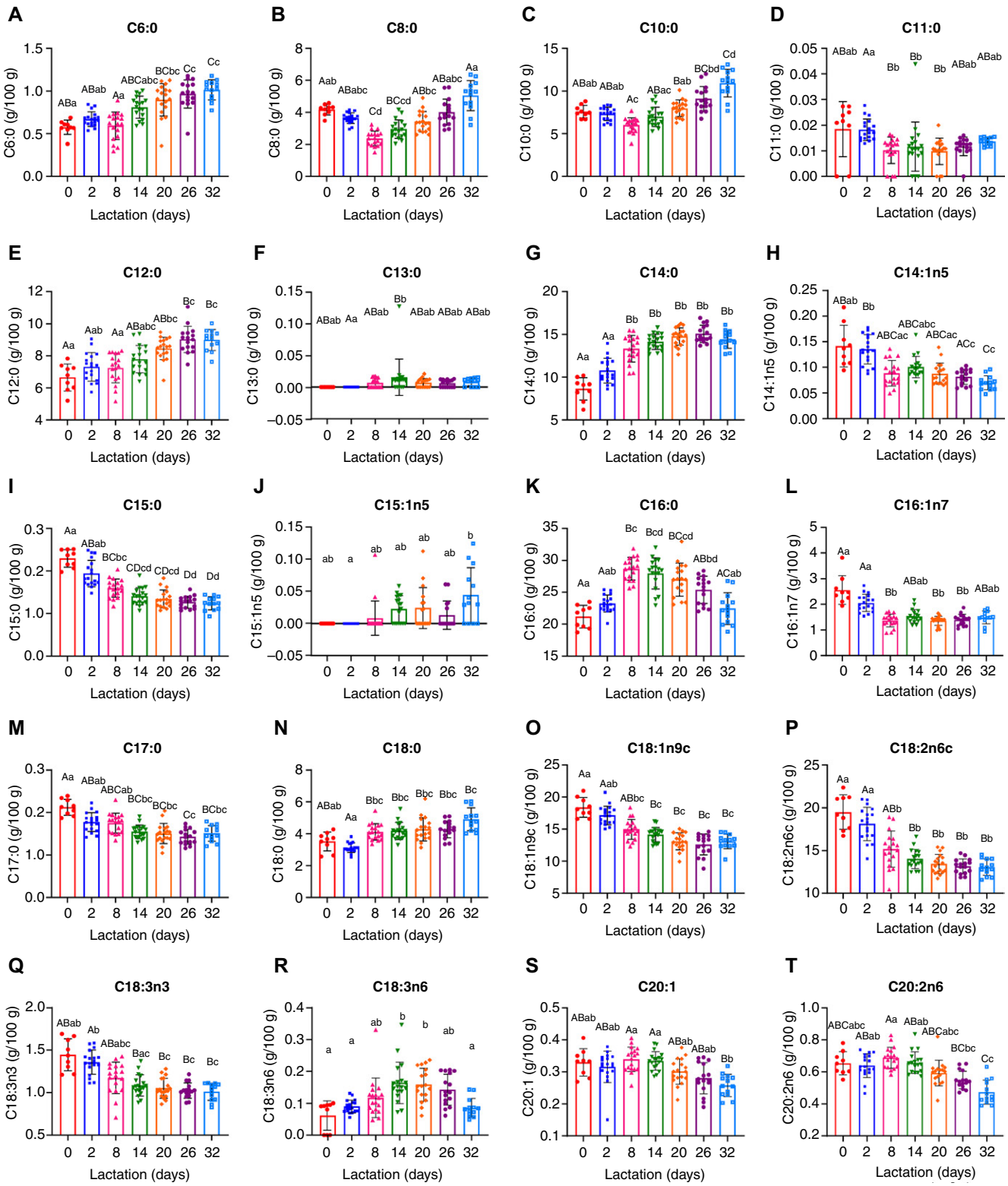

Figure 3. Changes in the relative proportions of (A) C6:0, (B) C8:0, (C) C10:0, (D) C11:0, (E) C12:0, (F) C13:0, (G) C14:0, (H) C14:1n5, (I) C15:0, (J) C15:1n5, (K) C16:0, (L) C16:1n7, (M) C17:0, (N) C18:0, (O) C18:1n9c, (P) C18:2n6c, (Q) C18:3n3, (R) C18:3n6, (S) C20:1, and (T) C20:2n6 during lactation. Each point represented a single sample. Bars represent the mean ± SD. Means that do not share any lowercase letters are significantly different at the 5% level, while those that do not share any uppercase letters are significantly different at the 1% level. A Welch ANOVA with a Tamhane T2 test (for normally distributed data with heterogeneous variances) or a nonparametric Kruskal–Wallis test with a Dunn test (for nonnormally distributed data) was used.

tree shrews commonly roost separately from their pups and nurse their young every other day during the initial weeks.[4,9] This strategy further necessitates the production of highly concentrated milk, as mammary glands can hold only a limited amount of milk. Conversely, only small amounts of lactose or other sugars were present in tree shrew milk. This observation is in line with the pattern whereby sugar content is inverse to fat content in milk due to the diluting effect of lactose.[23]

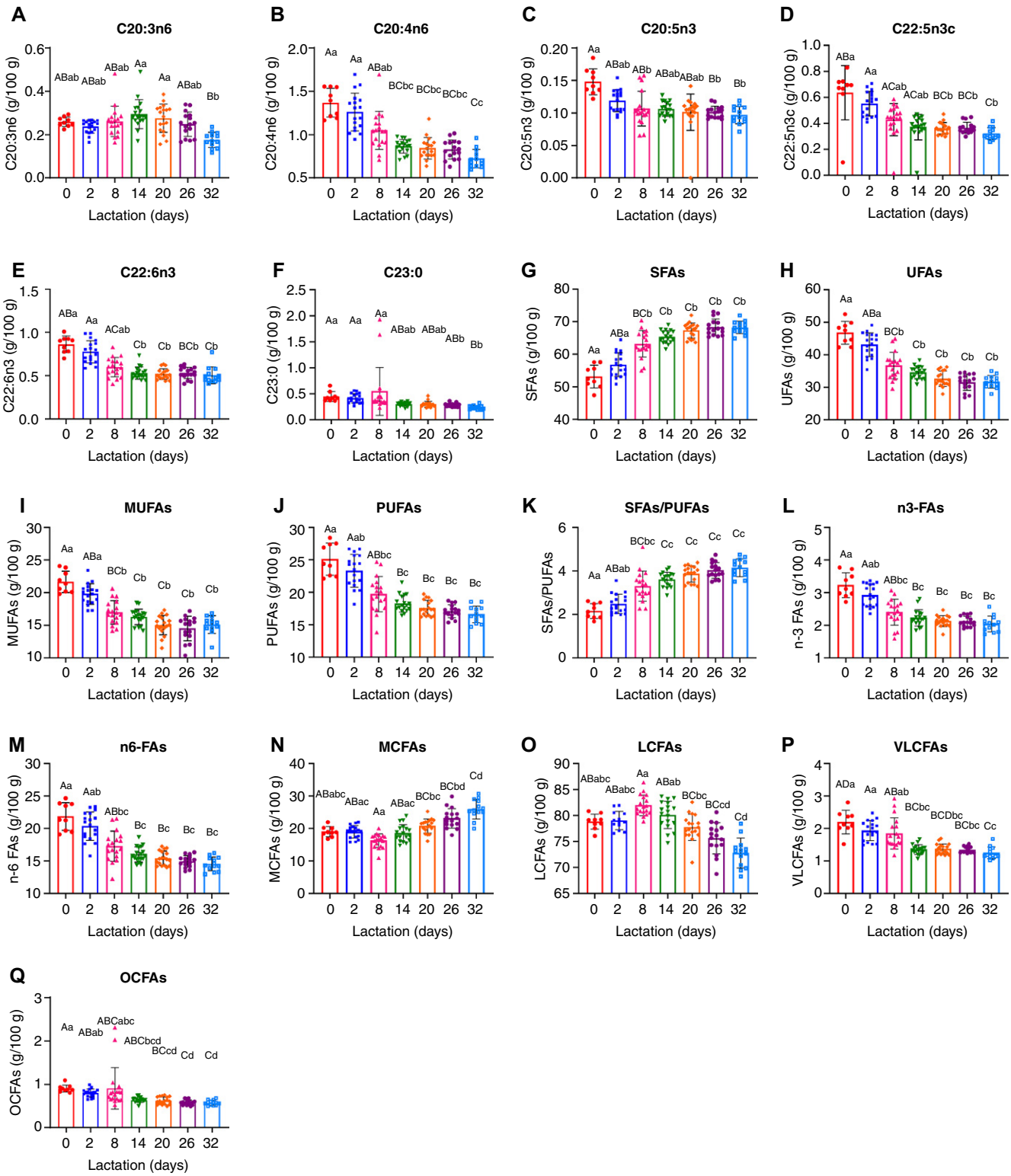

Figure 4. Changes in the relative proportions of (A) C20:3n6, (B) C20:4n6, (C) C20:5n3, (D) C22:5n3c, (E) C22:6n3, (F) C23:0, (G) SFAs, (H) UFAs, (I) MUFAs, (J) PUFAs, (K) SFAs/PUFAs, (L) n3-FAs, (M) n6-FAs, (N) MCFAs, (O) LCFAs, (P) VLCFAs, and (Q) OCFAs during lactation. Each point represented a single sample. Bars represent the mean ± SD. Means with no lowercase or uppercase letters in common are significantly different at α = 5% or α = 1%. A Welch ANOVA with a Tamhane T2 test for normally distributed data that violated the assumption of homogeneity of variance or a nonparametric Kruskal–Wallis test with a Dunn test for the nonnormally distributed data was used. SFAs (saturated fatty acids) include C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, and C24:0. UFAs (unsaturated fatty acids) include MUFAs (monounsaturated fatty acids) and PUFAs (polyunsaturated fatty acids). MUFAs include C14:1n5, C15:1n5, C16:1n7, C17:1n7, C18:1n9t, C18:1n9c, C20:1, C22:1n9, and C24:1n9. PUFAs include C18:2n6t, C18:2n6c, C18:3n3, C18:3n6, C20:2, C20:3n3, C20:3n6, C20:4n6, C20:5n3, C22:2, C22:5n3c, and C22:6n3. n3-FAs (omega-3 fatty acids) include C18:3n3, C20:3n3, C20:5n3, C22:5n3c, and C22:6n3. n6-FAs (omega-6 fatty acids) include C18:2n6t, C18:2n6c, C18:3n6, C20:2n6, C20:3n6, and C20:4n6. MCFAs (medium-chain fatty acids) include 6 to 12 carbons. LCFAs (long-chain fatty acids) include 13 to 21 carbons. VLCFAs (very-long-chain fatty acids) include 22 or more carbons. OCFAs (odd–chain fatty acids) include 11, 13, 15, 17, and 23 carbons.

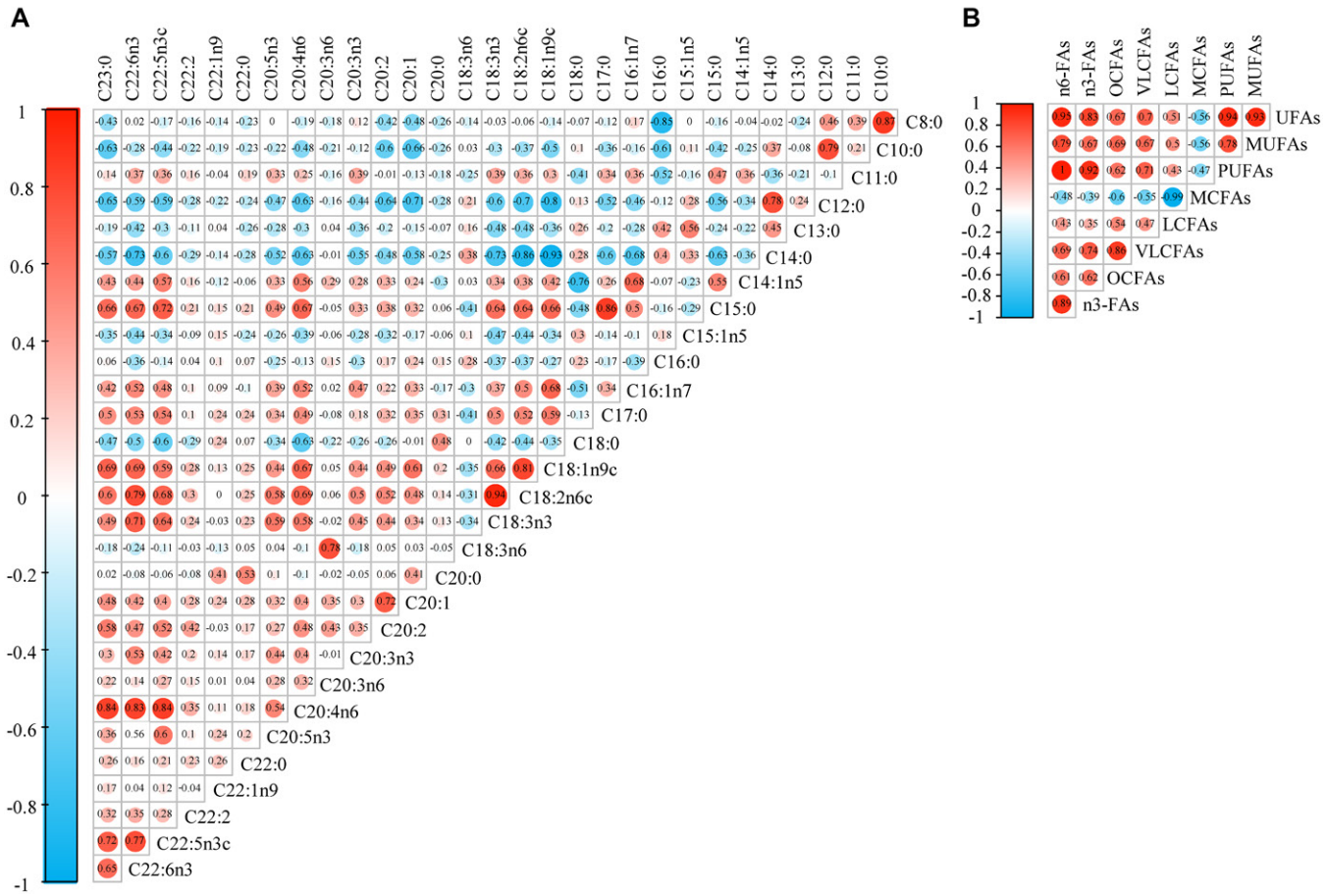

Figure 5. Correlations among fatty acids (A) and fatty acid groups (B) in milk from captive tree shrews at different lactation periods. The Spearman rank correlation test was used to evaluate the correlation between any 2 fatty acids or fatty acid groups.

In this study, lipid and protein contents varied little across lactation, similar to observations in some mammals such as naked mole-rats, bongos, and narrow-ridged finless porpoises.[17,36,53] The reasons for this constancy independent of lactation stages remain ill-defined. One possibility is that the varying nutritional needs of the young are met primarily by adjusting daily milk volume rather than altering constituent proportions. In addition, individual variation or confounding factors may obscure any changes, emphasizing the need for larger sample sizes and unbiased sampling in future research. Conversely, the DM level increased over time, primarily owing to the rising sugar and ash content. Sugars and minerals (in the form of oxides in ash) are crucial for various physiologic functions in mammalian young. For sugars, lactose plays a crucial role in energy metabolism, osmotic adjustment, and calcium absorption. Oligosaccharides, on the other hand, act as milk prebiotics, antiadhesives, and immunomodulators, helping to protect the newborn from pathogenic organisms.[38] In addition, oligosaccharides and their metabolites, such as sialic acid and 2′-fucosyllactose, contribute to support brain development and promote brain health.[22,39] Although the sugar composition of tree shrew milk was unknown, the contribution of sugars to energy was extremely low, that is, around 2% in our study. One hypothesis may be that the biologic significance of sugars in tree

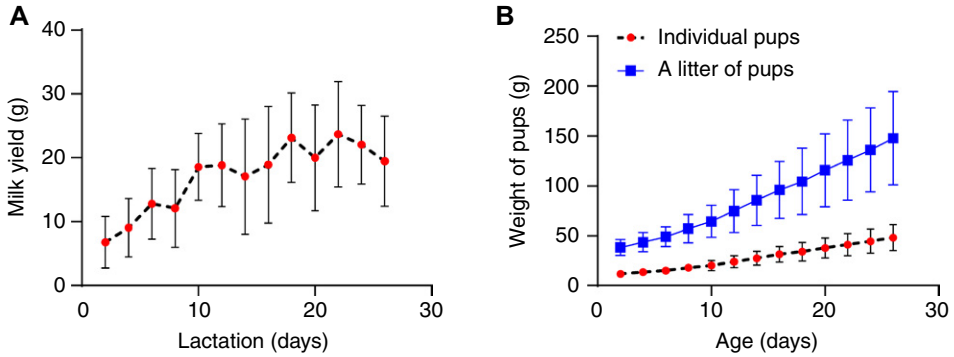

Figure 6. Changes in (A) the milk yield of lactating tree shrews from postnatal days 2 to 26 and (B) the weight of tree shrew pups before they began consuming solid food. Lactating tree shrews, $n = 18$. Tree shrew pups, $n = 58$ (27 males and 31 females) at the start of the experiment, with 3 pups dying at 9, 12, or 16 d of age. Therefore, the litter size for these 18 litters of pups was 3.22 ± 0.65 during postnatal days 2 to 8, 3.17 ± 0.71 on postnatal days 12 and 14, and 3.06 ± 0.87 from postnatal day 16 onward. Bars represent the mean ± SD.

shrew milk lies primarily in functions beyond energy supply, such as supporting brain development and promoting overall health. As a result, the ongoing increase in sugars during lactation may be driven mainly by the growth and developmental requirements. If validated, further exploration of the sugar and mineral composition in tree shrew milk, as well as their influencing factors, is warranted. Meanwhile, larger sample sizes and advanced technologies, such as HPLC and MS, should be employed for a more comprehensive understanding of the needs for these nutrients.

In tree shrew milk, the total SFAs predominated over the total UFAs, consistent with patterns observed in terrestrial mammals. However, the level of total UFAs in milk from tree shrews is comparable to that from some primates and higher than that from most ruminants whose ruminal bacteria convert UFAs into SFAs.[13,30,31] Tree shrew milk is rich in FAs from the n6 or n3 families, such as C18:2n6, C18:3n3, C20:4n6, and C22:6n3, which are essential for maintaining optimal health of the young,[14] indicating a great demand for these nutrients in tree shrew pups. The stable n6/n3 ratio of approximately 7 is higher than that from giant pandas and primates,[2,30] highlighting its significance for the growth and cognitive development of young individuals.[5] In addition, tree shrews do not produce significant quantities of SCFAs, a characteristic shared by most mammals, except for artiodactyls.[21] However, they can synthesize MCFAs, with notable levels of C8:0 (3.4%), C10:0 (8.0%), and C12 (8.4%) detected, despite low dietary amounts (Table S1).

Lactation stages significantly influence the FA composition in captive tree shrews. In the early lactation phase, some SFAs showed a significant increase (Table 2; Figure 3E, G, K, and N), consistent with findings in other mammals such as goats and camels.[10,45] The increase in these SFAs might be the consequence of an activated de novo synthesis pathway and enhanced uptake of FAs from the blood.[20] With the extension of lactation, the proportion of C16:0 (the predominant SFA) decreased, while the proportions of most de novo FAs such as MCFAs increased. Intriguingly, the proportions of C14:1 and C16:1 declined (Table 2; Figure 3H and L), which contrasts with trends observed in sows and pandas,[2,20] where these FAs followed the trend of their precursors, C14:0 and C16:0. This discrepancy may reflect time-dependent variations in the types of FAs synthesized in the mammary epithelial cells of tree shrews over time. Regarding UFAs, nearly all decreased during the early lactation period, suggesting that tree shrews experienced a negative energy balance and had to mobilize adipose tissue reserves for LCFAs, the majority of which were UFAs. This could result from the limited feed intake and suboptimal mammary function. Supporting this, our observations revealed that early in lactation, tree shrews exhibit low feed intake and MY, which gradually increase as lactation continued. During the late stage of lactation, most FA levels stabilize (Table 2), suggesting a recovery from negative energy balance. These trends are consistent with those patterns observed in some mammals such as camels and goats.[10,45]

An intriguing observation was that, from P2 to P8, while the MY increased significantly (Figure 6A), the weight gain of both individual pups and the total litter was extremely slow (Figure 6B). In contrast, from P10 onward, MY increased only marginally, yet the weight of pups and the litter weight accelerated markedly (Figure 6). This suggests that nutrient conversion efficiency for growth is higher during the mid-lactation stage compared with the early phase. One potential explanation for this pattern is that older pups have developed thicker fur, enhanced thermoregulatory mechanisms, and a relatively smaller body surface area. Consequently, less energy is expended on maintaining body temperature and overall metabolism, which allows a greater proportion of nutrients from the milk to be allocated toward growth rather than maintenance.

The microanalysis and gas chromatography method employed in this study offers both scientific and animal welfare advantages. Collecting milk samples from small mammals poses practical and ethical challenges due to limited sample volume and high-fat content. Traditionally, researchers have pooled samples from various dams or lactation stages,[17,42,48] or collected stomach contents from suckling pups to acquire sufficient volume for analysis.[48] While pooling is cost-effective, it sacrifices individual-specific or stage-specific information. Collecting stomach contents from pups raises ethical concerns due to the invasive procedures involved and introduces potential bias from the contamination with digestive juices. In contrast, microanalysis addresses these challenges by reducing the sample volume required for analysis. Nevertheless, it is noteworthy that this approach is also associated with limitations, such as reduced precision and accuracy. Therefore, science and animal welfare should be carefully balanced when choosing between traditional analysis methods and microanalysis techniques.

In this study, the pups were found to weigh slightly less compared with those not sampled as previously reported (weight difference was less than 20%).[3] This discrepancy may be linked to the small sample size, stress induced by serial milking, or potential adverse effects of ketamine in this study. Studies in both humans and animals indicate that perinatal stress can induce variation in the MY and milk composition, including changes in micronutrients, immune components, and microbiome, ultimately impacting the development of the young.[43] To minimize the potential stress associated with serial milking, more refinement such as positive reinforcement and extending milking intervals should be considered.

This study has several flaws that may introduce potential systematic biases. First, with regard to the sampling design, tree shrews typically suckle their neonates shortly after delivery,[3] making colostrum collection nearly impossible. Moreover, obtaining milk during early lactation is difficult due to low MY and the undeveloped mother/pup relationship. Thus, milk samples at P0 and P2 were collected from dams that did not suckle their young on those days, rather than from those providing samples at P8 to P32. Second, with regard to the vacuum milking technique, we found that hand expression, an ideal milking method,[18] was ineffective for releasing tree shrew milk. We therefore had to choose vacuum pumping with a homemade machine (Figure 1B). During the collection process, we observed that some water evaporated due to airflow from the pump, particularly for individuals with low MY. This could partially explain why the macronutrient levels in this study were somewhat higher than those previously reported.[4,48] Third, with regard to incomplete milking, evacuating glands was found problematic for tree shrews, even with numerous efforts, including oxytocin injection, anesthetics, massage, and other approaches. Consequently, the collected milk may overrepresent the foremilk, which may have resulted in a lower fat content.[18]

Conclusions

In short, we characterized the milk macronutrient and FA composition in captive tree shrews and its dynamic changes over lactation. Tree shrew milk was characterized by high DM, fat, and protein content with low sugar content; the DM, sugars, and ash level gradually increased over the lactation. Thirty-one FAs were identified, and most of them underwent

complex changes throughout lactation. We speculate that the characteristics and changes in the composition of tree shrew milk may reflect the growing nutritional needs of pups and the mammary gland's biosynthetic capacity, consistent with the reproductive strategy of tree shrews. This study lays the groundwork for optimizing milk formulas to facilitate the laboratory domestication of this valuable species.

Supplementary Materials

Table S1. Chemical components of diet

Acknowledgments

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Author Contributions

J.-Q.C., L.-B.L., and H.M. conceived of the presented idea; J.-Q.C., Y.M., Q.Z., and L.-B.L. administrated and provided the animals; J.-Q.C, Q.L., and Z.H. performed the animal experiments; J.-Q.C., Q.L., M.Z., Y.L., and S.H. performed the chemical analysis of milk samples; and J.-Q.C. and H.M. wrote the manuscript. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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