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Milk composition, fatty acids profile and fat globule size of Bottlenose Dolphin (Tursiops truncatus, Montagu 1821) milk at early lactation

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Abstract:	Research data on milk composition in cetaceans are scattered and fragmentary. This paper analyses the gross and mineral composition, the fatty acids profile and the fat globule size of bottlenose dolphin (Tursiops truncatus) colostrum and milk at early lactation. The milk samplings were carried out on three lactating female of bottlenose dolphins at the 1st, 4-5 and 20- 42 day post partum. High percentages of dry matter (51.88%), fat (26.08%) and protein (13.83%) were found in the colostrum on the first day, while there was a tendency for these components to decrease in the milk. The average diameter the milk fat globule (7.07 um) of this species was assessed for the first time. The milk was rich in unsaturated fatty acids, which were more than twice compared to the saturated fatty acids (unsaturated / saturated ratio = 2.6). The main fatty acids of dolphin milk were C16: 0, C18: 1 n-9, C20: 1 n-11, C20: 5, C22: 6 n6. Furthermore C12: 0 showed double contents in milk compared to colostrum, while C20: 1 n9 and C20: 1 n7 were respectively five and three times higher in milk than colostrum. Finally, C18: 3 n3 was approximately 4 times lower in milk than in colostrum while C24: 0 and C24: 1 tended to halve. The changes found in the fatty acid profile could be linked to the animals' energy metabolism and lipomobilization during lactation. This study contributes to improve scientific knowledge on dolphin milk composition and nutrient supply in dolphin calves.

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Iolanda Altomonte

Milk composition, fatty acids profile and fat globule size of Bottlenose Dolphin (*Tursiops truncatus*, Montagu 1821) milk at early lactation

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ABSTRACT

Research data on milk composition in cetaceans are scattered and fragmentary. This paper analyses the gross and mineral composition, the fatty acids profile and the fat globule size of bottlenose dolphin (*Tursiops truncatus*) colostrum and milk at early lactation. The milk samplings were carried out on three lactating female of bottlenose dolphins at the 1st, 4-5 and 20- 42 day post partum. High percentages of dry matter (51.88%), fat (26.08%) and protein (13.83%) were found in the colostrum on the first day, while there was a tendency for these components to decrease in the milk. The average diameter the milk fat globule (7.07 um) of this species was assessed for the first time. The milk was rich in unsaturated fatty acids, which were more than twice compared to the saturated fatty acids (unsaturated / saturated ratio = 2.6). The main fatty acids of dolphin milk were C16: 0, C18: 1 n-9, C20: 1 n-11, C20: 5, C22: 6 n6. Furthermore C12: 0 showed double contents in milk compared to colostrum, while C20: 1 n9 and C20: 1 n7 were respectively five and three times higher in milk than colostrum. Finally, C18: 3 n3 was approximately 4 times lower in milk than in colostrum while C24: 0 and C24: 1 tended to halve. The changes found in the fatty acid profile could be linked to the animals' energy metabolism and lipomobilization during lactation. This study contributes to improve scientific knowledge on dolphin milk composition and nutrient supply in dolphin calves.

Keywords: *Tursiops truncatus*, bottlenose dolphin milk, colostrum, early lactation, fatty acids, milk fat globule

Introduction

The bottlenose dolphin (*Tursiops truncatus*; order Cetacea, suborder Odontocetes) is a cosmopolitan species of Delphinidae family and lives in temperate and tropical seas areas, while is absent in polar waters (Connor et al., 2000). Wild bottlenose dolphins are found in diverse habitats such as bays, sounds, shallow estuarine systems as well as deep oceanic waters (Wells et al., 2004; Vollmer and Rosel, 2013); they live in groups and are piscivorous species, top-level predators of the marine food web (Vollmer and Rosel, 2013).

This mammalian species is commonly maintained in sea-life parks under human care. Adult bottlenose dolphin is grey in colour, with an average length of 2.2-3.8 m and a medium weight ranging from 250 to 500 kg. New-born bottlenose dolphin has a length of 0.8-1.4 m and a mean weight of 14-20 kg (Jenkins, 2009).

Bottlenose dolphins can live as long as 40-50 years in the wild, the sexual maturity is reached at 5-10 years in females and 8-13 years in males. Gestation lasts about 12 months and usually only a single offspring is calved (Robeck et al., 2018) lactation in the free living animals is prolonged. Wild bottlenose dolphin calves are nutritionally dependent on their mother for a minimum of 1–2 years but weaning time varies between populations and individuals (Senigaglia et al., 2019)

The close association mother-calves continues for an average of about 4 years and some mothers continuing to suckle calves for up to 8 years (Karniski et al., 2018).

For the first two months of life, the nutritional requirements of calves are completely dependent upon milk suckling. In captivity, calves generally start voluntary mouthing fish at 4-8 month and usually begin to hand feeding at 7 to 12 month (Townsend, 1999). The long lactation of dolphins is presumably related to the need of the offspring to achieve social and cognitive skills, foraging techniques and predator-avoidance strategies (West et al., 2007). Although many studies have been done on bottlenose dolphin ecology and biology, research data on milk composition and on variation of milk composition during lactation is scattered and fragmentary. The reasons for the lack of data have been reviewed by Oftedal (1997) and are related to the fact that:

a) collection of milk samples from wild mammals requires that animals be captured and restrained or chemically immobilized
b) In the first studies on dolphins milk samples have often been collected from carcasses of animals killed during whaling operations or obtained from stranded animals. For such samples, post-mortem or pathological changes may result in abnormal results.
c) During the samplings the lactation in dolphins has been often indirectly estimated based on the oldest calves found with lactating females in captured groups (cow-calf method), or behavioral observations, and or by mean of the milk present in calf stomachs; thus the estimation are subject to methodological error eg accompanying calf not be captured and aged, or is frequent adoptive suckling or milk stealing.

For the above mentioned reasons according to Oftedal (1997) it was impossible to reliably assign lactation stage to most milk samples from Odontocetes described in the literature. More recently, captive animals, such as bottlenose dolphins, have been trained to present for milk collection (Oftendal, 2011).

Successful rearing requires milk formulas to meet the nutritional requirements during calves' growth, to improve their survival and to support natural physiological development. In this regard, scientific knowledge on milk composition can improve neonatal care (eg artificial feeding and formulations) with appropriate nutrient supply (West et al., 2007).

To the best of our knowledge there are no published studies on the nutritional value of milk from bottlenose dolphins during the first week of lactation. Bottlenose dolphin milk fatty acids profile and mineral composition is almost unexplored, and to the best of our knowledge, the milk fat globule size of this specie has never been analysed.

This paper aimed to analyse the gross and mineral composition, the fatty acids profile and the fat globule size of bottlenose dolphin in colostrum and in milk at early lactation.

Materials and Methods

Animals and samples collection

The milk samplings were carried out on three lactating female of bottlenose dolphins. Dolphin A and B were housed at

Acquario di Genova and dolphin C at Riccione Dolphinarium, both facilities are located in Italy.

The three females dolphins were maintained with their suckling calves and were healthy throughout the research period. The three animals fed similar diet with an average energy content of 204541±5911 kcal during the period studied. The mean food intake was 8.62 ±2.33 kg/day, the diet consisted of 80.31% of herrings (*Clupea harengus*), 18.67% of capelin *(Mallotus villosus*), and 0.09% of squids (*Loligo vulgaris*).

Bottlenose dolphins were trained to present for voluntary milk collection by the aquarium staff. Animals were placed on a side to expose the mammary slit out of the water and modified syringes without needle were used reverted to create vacuum to collect the milk samples. Milk collection was in fact possible due to the negative pressure that was creating by pulling back the syringe plunger.

Samplings were collected from each animal at of 1st day post partum, 4-5 days of lactation and from 20 to 42 days, immediately refrigerated at 4° C and then transported to laboratory for the analysis, within 24 hours of milk collection. In total 6 individual raw milk samples were analysed in duplicate or triplicate each.

Gross composition and mineral analysis

Dry matter, crude protein and ash content were determined by AOAC Method (1990). Crude protein was calculated as 6.38 times the total nitrogen. Fat content was determined through gravimetric determination after extraction by Rose-Gottlieb method (IDF, 1996). Ca, Mg, Na, K, Mn and Zn through atomic absorption spectrophotometer and P through UV spectrophotometer according to the AOAC methods (1990).

Morphometric analysis of milk fat globules

The diameter and the number of fat globules per mL of milk (μm) in each sample were measured by florescence microscopy following our direct method (Salari et al., 2016). Diameter (μm) and the number of fat globules per mL of milk were determined directly in each sample of fresh milk as described by Salari et al. (2016). In brief, each sample of fresh milk was diluted 1:100 in distilled water, and was stained by adding a 0.1% solution of Acridine Orange (Sigma-Aldrich, Milan, Italy) in a 0.1 M Phosphate buffer (pH 6.8); the ratio of milk and staining solution was 10% (v/v). The analysis was performed by a fluorescence microscope Leica Ortomat Microsystem (Leika SPA, Milan, Italy) equipped with a camera (TiEsseLab, Milan, Italy) and an Image software TS view 2.0 (C & A Scientific, Manassas, VA).

The globules were grouped into three size categories: small globules (SG) with a diameter <2 μm, medium-sized globules (MG) with a diameter of 2–5 μm, and large globules (LG) with a diameter >5 μm.

Fatty acid analysis

Each sample of previously extracted fat (2.2) was used for preparation of methyl esters of fatty acids (FAME) by transesterification with sodium methoxide according to Christie (1982). The fatty acids composition was determined by gas chromatography by PerkinElmer Clarus 480 (PerkinElmer, Norwalk, CT, USA) equipped with a flame ionisation detector and a capillary column (ThermoScientific TR-FAME 60 m × 0.25 mm ID; film thickness 0.25 µm, Fisher Scientific, Loughborough, Leicestershire, UK). The peak of individual fatty acid methyl esters were identified by using standard injection (Food Industry FAME Mix – Restek Corporation, Bellefonte, PA, USA). C9:0 was used as internal standard for the quantification of the peak areas. Fatty acids are expressed as a g of individual fatty acid methyl ester (FAME) per 100 g total FAME

Mean and standard deviations for colostrum (1st day in milk), milk (4-42 days in milk) were calculated. For minerals general mean and standard deviations of two individuals are reported.

Results

The gross composition and the average diameter of the milk fat globules of colostrum (1st day in milk) and milk (4-42 days in milk) of the bottlenose dolphin milk are presented in Table 1.

Table 1 – here

The analysed samples showed on the average percentages of dry matter, protein and fat of 41.64 ± 8.06 %, 11.54 ± 1.99 % and 20.57 ± 6.12 % respectively.

Regarding fat, it showed a decreasing tendency from colostrum to milk ranging from a peak of 26.08% at the first day colostrum to 19.7% in mature milk. Similarly, protein showed maximum values in colostrum (12.90%) and minimum 10.08% in milk.

The mean diameter of the milk fat globules was 7.07 µm ± 1.276; an increasing trend in globule diameter (from 6.84 to 8.40 µm), and a decrease in number of globules per mL (from 4.02 to 1.17 $*10^7$) was found from colostrum to milk alongside with the decreases in fat.

Regarding ash, it was on the average 1.04 ± 0.55%; the main milk minerals (Table 2) were potassium and phosphorus (2.145 and 1.45 g/kg respectively) followed by calcium, sodium and magnesium (0.59, 0.29, 0.04 g/kg respectively); the analysed milk also contained about 2.72±0.202mg/kg of zinc.

Table 2 –here

In the dolphin milk samples (Table 3) short-chain fatty acids (SCFA-shorter than 12C) were absent. On the contrary, the long chains (LCFA: greater than or equal to 18C) predominated and were on the average 67.02 % of the total FAMEs. Compared to saturated (SAT), unsaturated fatty acids (UNS) were more than double quantity (on the average UNS / SAT ratio was 2.64 ± 0.384). Monounsaturated fatty acids (MUFA) account on average for 54.46% ± 6.325 of the total FAMEs and PUFAs were 17.82% ± 3.238. The main SFA in milk were C14: 0 and C16: 0 (on the average 4.92; 11.50 g of individual FAME per 100 g total FAMEs).

C16: 1 n7, C18: 1 n9; C20: 1 n9; C22: 1 n11 were the most represented among the MUFA (on the average 11.50, 10.71; 8.44, 3.87, g of individual FAME per 100 g total FAME respectively).

As for the polyunsaturated fatty acids (PUFA), the main ones were C20: 5 n3 and C22: 6 (on the average 8.44 and 5.33 g of individual FAME per 100 g total FAME).

Table 3 –here

Some variations of the FA profile between colostrum and milk were observed, in particular C12: 0 recorded double contents in milk compared to colostrum. In addition, increases in the isomers of C20: 1 passing from colostrum to milk were found, in particular of C20: 1 n9 and C20: 1 n7 quintupled and tripled respectively. Finally, decreases in C18: 3 n3, C24: 0 and C24: 1 were observed. In particular, C18: 3 n3 was approximately 4 times lower in milk than in colostrum, while in C24: 0 and C24: 1 tended to halve in milk compared to colostrum.

Discussion

The value of dry matter found in this study fell within the range described in the same species (24.5-64.7%) (Eichelberger et al., 1940; Jennes and Sloan, 1970; Yablokov et al., 1974). Fat percentage was in agreement with the most frequently values reported in the literature for milk in this species (10 - 30%) (Jenness & Sloan 1970; Pervaiz & Brew, 1986). Furthermore, the range of milk fat reported from different authors in *Tursiops truncatus* is exceptionally wide from 6.8 to 51% (Ridgway et al., 1995; West et al., 2007; Yablokov et al., 1974). Fat variability reported by the literature may mostly be related to sampling activity: for example incomplete mammary evacuation could give a potential underestimation of milk fat as normally happens in terrestrial species (Oftendal et al., 2011). In fact, it is well known that, among many farm animals, fat concentration of sampled milk is affected by the degree of filling of the mammary gland and the residual milk is usually the richest in lipid. For the same reason also samples collected before and after a suckling event might differ.

In general, milks of aquatic mammals are high in fat, likely because new-born mammals typically has thin subcutaneous body fat and must acquire considerable quantities of body fat to thermoregulate in the aquatic environment (Oftendal, 1997; Matzushiro et al., 2020). In support of this hypothesis is the fact that blubber represents a substantial proportion of the total body mass of a marine mammal and ranges from 20 to 80% in the different species of dolphins (Koopman, 2007). Blubber is the lipid-rich hypodermis of marine mammals, is a modified form of adipose tissue consisting of adipocytes contained in a supportive matrix of collagen and elastin; fat stores are contained in blubber as triacylglycerols and wax esters (Koopman, 2007).

To our knowledge this is the first investigation that reports the composition of dolphin colostrum; as regards the percentage of fat, a recent paper on another Odontocete (Harbor porpoise, *Phocoena phocoena*) found similar values in the colostrum (from 1-14 days) (30% fat).

Furthermore, the decreasing tendency for fat from the first-day colostrum to mature milk we found can has been also reported in the dairy animals as cow, sheep, goat, donkey (Pecka-Kiełb et al., 2018; Martini et al., 2012; Martini et al., 2020).

A single paper in the literature concerns the variation of gross composition during lactation in this species by West et al. (2007). The authors analysed the milk of three captive bottlenose dolphins from 1–30 months postpartum and found a significant increase in milk fat content during the course of lactation starting from the first month after delivery. Unfortunately, the results of our study are not directly comparable with that by West et al. (2007) in which sample collection started from the1^{st} month after parturition.

In our study, the protein (table 1) were within the range found in Cetaceans (about 8-12%) (Oftedal, 2011). The higher protein content in colostrum compared to milk is linked to the presence of immunoglobulins, which contribute to passive immunity of neonatal Cetacean calves. However, higher protein percentage in day one colostrum would have been expected as observed in mammals with epitheliochorial placenta (Martini et al., 2020). In fact, the epitheliochorial placenta do not allow prenatal immunoglobulin transfer, thus the transfer of immunoglobulins in the offspring through the colostrum is essential for the acquisition of passive immunity. An explanation for the lower than expected protein in colostrum could be due to the fact that changes in protein content occur rather rapidly in the first hours post-partum as found in the dairy animals (Martini et al., 2020).

Some authors reported the protein content in Cetacean milks may rise over lactation, but the degree and direction of change vary with species and also among individuals and with maternal condition (Oftendal 2011; West et al., 2007).

In dolphins, as in the other mammals, the milk lipids are packed into a membrane as milk fat globules. The size of the fat globules can affect the nutritional intake and influence the digestive kinetics of the nursing offspring (Martini et al., 2016). To our knowledge, this is the first study that analyses the average size and number of fat globules in the dolphin. The mean diameter was higher than average diameter between in the main livestock species (7.07 vs 2 -5 μm) (Martini et al., 2016). Similarly to the present results, Oftendal (2011) reports that in the Weddell seal (Phocidae), another deep diving marine mammal, the milk fat globules are relatively large.

Regarding ash content, the results were slightly higher compared to the values of 0.70 and 0.77% reported by Peddemors et al. (1989) and Jennes and Sloan (1970), respectively, but similar to those observed in the milk of Otaridae and Focidae (Oftendal, 2011).

In the data literature on the individual mineral content in dolphins are scarce and outdated.

The detected individual mineral content (Table 2) were lower than the previous reports for calcium (0.60 vs 0.74 and 1.30 g/kg) (Eichelberger et al., 1940; Peddemors et al., 1989), magnesium (0.03 vs 0.07 g/kg) and sodium (0.25 vs 1.56 g/kg) (Eichelberger et al., 1940). However, the high sodium values reported in the literature for some Cetacean are suspect due to the possibility of seawater contamination (Oftendal, 2011). We also found an exceptionally high potassium content

(2.145 vs 0.8 g/kg) compared to the only value report in the literature (Eichelberger et al., 1940).

In addition, the phosphorus content was higher than previous studies (1.45 vs 0.18 and 1.10 g/kg). (Eichelberger et al., 1940; Peddemors et al., 1989). Unfortunately, for zinc there are not available data on this species for a direct comparison, however our result is comparable to that of marine mammal milks that contain about 1–8 mg/kg of zinc (Oftendal, 2011).

The only study on fatty acid composition of dolphin milk dates back to 1971 and concerns a single wild bottlenose dolphin on which milk was collected post mortem (Ackman and Eaton, 1971).

The absence of short-chain fatty acids (SCFA-shorter than 12C) in our samples was in agreement with the literature on dolphins (Ackman and Eaton, 1971), and on other marine mammals such as the beluga whale (*Delphinapterus leucas*) and Fin whale (*Balaenoptera physalus*) (Lauer and Baker, 1968) and on northern elephant seals *Mirounga angustirostris* (Fowler et al., 2014).

The preponderance of LCFA represent a similarity with human milk in which LCFA are about 63% of the total fatty acids (calculated by Xiang et al. 2000; Altomonte et al., 2017; 2019).

The average UNS/SAT ratio (2.64) was in agreement to what has been reported by Ackman and Eaton (1971) (calculated UNS / SAT ratio= 2.3).

Among the most represented individual MUFA, C16: 1 n-7 is a common product of biosynthesis in marine mammal blubber (Budge et al. 2004; Koopman et al., 2007); C20: 1 n-9 and C22: 1 n11 are also found in the blubber and are commonly linked to carnivorous feeding (Herman et al. 2005).

The high presence of C 16: 0, C18: 1 n9, in dolphin milk has similarities with the milk of other marine mammals such as northern elephant seals (Fowler et al., 2014) and with the blubber (Montie et al., 2008; Fowler et al., 2014).

Compared to human milk, dolphin milk has an excess of this two particularly important long chain PUFA (C 22: 6n-3 and C 20: 5n-3). In fact, milk FA also reflect the dietary intake and C 22: 6 n-3 and C 20: 5 n-3 mainly derive from marine source diets (Iverson, 2009). PUFA longer than C20 have been also shown to be very important in neonatal development for humans (Altomonte et al., 2019).

The changes of the FA profile between colostrum and milk, in particular of C12: 0 suggests a derivation of this FA from *ex novo* synthesis. In fact, even if the mechanisms and extent of *de novo* synthesis are not known in dophin mammary gland, in ruminants and humans it is known that FA with 6–14 C constitutes the major product of de novo FA synthesis in the mammary gland (Mahmoud et al., 2014).

The trend we found towards the increase of C20: 1 isomers passing from early and late lactation was also observed by Fowler et al. (2014) in northern elephant seals.

In small Odontocetes, milk fatty acids also reflect mobilization from fat depots (blubber) (Budge et al., 2006; Koopman et al., 2007). Lactating dolphins may preferentially mobilize longer-chain fatty acids that are found to be more prevalent in adipocytes in the deep blubber; the latter is preferentially mobilized during lactation (Montie et al., 2008; Samuel and

Worthy, 2004).

Some authors (Fowler et al., 2014) found unclear results with respect to the pattern of mobilization and changes in the FA profile of milk in Cetacean; they hypothesized that when mobilized, C20: 1 isomers are preferentially directed to milk synthesis, while mothers may preferentially use PUFA and SFA for her own metabolism.

In conclusion, this study reports for what we believe the first time the nutritional value of milk in the first weeks of lactation from bottlenose dolphins held under human care. The high concentration of fat in milk seems to have the adaptive meaning of favouring the thermoregulation of the offspring in the aquatic environment. This is the first investigation on the fatty acid composition of dolphin milk with *in vivo* sampling. Dolphin milk was rich in long chains and monounsaturated acids; the main fatty acids were C16: 0, C18: 1n-9, C20: 1n-11 and C20: 5. Increases found in some individual fatty acids from colostrum to milk could be linked to the animals' energy metabolism and lipomobilization. How the fatty acid composition and the diameter of the milk globules contribute to the health and development of the calves and what potential adaptive significance is yet little known. This study improves scientific knowledge on dolphin milk composition and could support intervention for neonatal care (eg artificial feeding and formulations) and nutrient supply in dolphin calves.

Declarations

Funding No funding was received for conducting this study.

Conflicts of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

Material and Code availability Software: JMP, RRID:SCR_014242;

Authors' contributions Conceptualization: Mina MARTINI, Matteo, Federico SOMMER, Claudia GILI; Methodology: Matteo, Federico SOMMER, Iolanda ALTOMONTE, Federica SALARI; Formal analysis and investigation: Iolanda ALTOMONTE, Federica SALARI, Rosario LICITRA; Writing - original draft preparation: Iolanda ALTOMONTE, Rosario LICITRA; Writing - review and editing: Federica SALARI, Federico SOMMER, Claudia GILI; Resources: Mina MARTINI; Supervision: Mina MARTINI

Ethics approval This research study was conducted from data obtained for clinical purposes. All animals reported in this study are humanely managed according to the national and international regulations (Italy: DM 469/2001, D.lgs 73/2005 and by following specific protocols and guidelines disseminated through the marine mammal and zoo animal management associations (EAAM Standards and Guidelines, 2019). Milk sampling was carried out by expert veterinarians for animal health management and under a strict Veterinary Protocol specifically prepared for animal care purposes, thus no sample was collected or manoeuvre performed for this study only.

Consent to participate Not applicable

Consent for publication Not applicable

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References

1. Ackman RG, Eaton CA, Mitchell ED (1971) The bottle-nosed dolphin *Tursiops truncates*: fatty acid composition of milk triglycerides. Can J Biochem 49:1172–1174. https://doi.org/10.1139/o71-168
2. Altomonte I, Salari F, Licitra R, Martini M (2019) Donkey and human milk: Insights into their compositional similarities. Int Dairy J 89:111-118. https://doi.org/10.1016/j.idairyj.2018.09.005.
3. Altomonte I, Salari F, Martini M (2017) Milk fat components and milk quality. In: Poltronieri P (ed). Microbiology in Dairy Processing, John Wiley & Sons, Ltd, pp1-10 https://doi.org/10.1002/9781119115007.ch1
4. AOAC (1990). Official methods of analysis. 15th ed. Association of Official Analytical Chemist, Arlington, VA, USA.
5. Budge SM, Iverson SJ (2003) Quantitative analysis of fatty acidprecursors in marine samples: direct conversion of wax ester alcohols and dimethylacetals to FAMEs. J Lipid Res 44:1802–1807. https://doi.org/10.1194/jlr.D300009-JLR200
6. Budge SM; Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar Mamm Sci 22:759–801. https://doi.org/10.1111/j.1748-7692.2006.00079.x
7. Christie WW (1982) A simple procedure of rapid transmethylation of glycerolipids and cholesteryl esters. J Lipid Res 23:1072–1075. https://doi.org/10.1016/S0022-2275(20)38081-0
8. Connor RC, Wells RS, Mann J, Read AJ (2000) The bottlenose dolphin: social relationships in a fission–fusion society. In: Mann J, Connor RC, Tyack PL, Whitehead H (Eds) Cetacean societies: field studies of dolphins and whales. The University of Chicago Press, Chicago, pp 91–126.
9. Cornell LH, Asper ED, Antrim JE, Searles SS, Young WG, Goff T (1987) Progress report: Results of a long-range captive breeding program for the bottlenose dolphin, Tursiops truncatus and Tursiops truncatus gilli. Zoo Biol 6:41-53. https://doi.org/10.1002/zoo.1430060106
10. Eichelberger L, Fetcher ES, Geiling EMK, Vos BJ (1940) The composition of dolphin milk. J Biol Chem 134:171–176.
11. Fowler MA, Debier C, Mignolet E., Linard C, Crocker DE, Costa DP (2014) Fatty acid mobilization and comparison to milk fatty acid content in northern elephant seals. J Comp Physiol B 184:125–135 https://doi.org/10.1007/s00360-013-0787-7
12. Herman DP, Burrows DG, Wade PR, Durban JW, Matkin CO, LeDuc RG, BarrettLennard LG, Krahn MM

(2005) Feeding ecology of eastern North Pacific killerwhales Orcinus orca from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. Mar Ecol Prog Ser 302:275–291. https://doi.org/10.3354/meps302275

13. IDF 1996. IDF-FIL. International Standard 1D. Determination of Fat Content-Gravimetric Method (Rose-Gottlieb Reference Method) International Dairy Federation—Federation Internationale de Laiterie, Brussels, Belgium
14. Iverson SJ (2009) Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In: Kainz M, Brett M, Arts M (eds) Lipids in Aquatic Ecosystems. Springer, New York, pp 281-306. https://doi.org/10.1007/978-0-387-89366-2_12
15. Jaakkola K, Willis K (2019) How long do dolphins live? Survival rates and life expectancies for bottlenose dolphins in zoological facilities vs. wild populations. Mar Mam Sci 35:1418-1437. https://doi.org/10.1111/mms.12601
16. Jenkins J (2009) Tursiops truncatus. Animal Diversity Web. https://animaldiversity.org/accounts/Tursiops_truncatus/
17. Jenness R, Sloan RE (1970) The composition of milks of various species: A review. Dairy Sci. Abstracts 32:599-612
18. Karniski C, Krzyszczyk E, Mann J (2018) Senescence impacts reproduction and maternal investment in bottlenose dolphins. Proc. R. Soc. B 285: 20181123. http://dx.doi.org/10.1098/rspb.2018.1123
19. Kastelein RA, Vaughan N, Walton S, Wiepkema PR (2002) Food intake and body measurements of Atlantic bottlenose dolphins (Tursiops truncates) in captivity. Mar Environ Re 53:199-218. https://doi.org/10.1016/s0141-1136(01)00123-4.
20. Koopman, HN (2007). Phylogenetic, ecological, and ontogenetic factors influencing the biochemical structure of the blubber of odontocetes. Mar Biol 151:277–291 https://doi.org/10.1007/s00227-006-0489-8
21. Lauer BH, Baker BE (1969) Whale milk. I. Fin whale (Balaenoptera physalus) and beluga whale (Delphinapterus leucas) milk: gross composition and fatty acid constitution. Can J Zool 47:95-97. https://doi.org/10.1139/z69-017
22. Mahmoud MA, Sunehag AL, Haymond MW (2014) De novo synthesis of milk triglycerides in humans. Am J Physiol Endocrinol Metab 306:E838-E847. https://doi.org/10.1152/ajpendo.00605.2013.
23. Martini M, Altomonte I, Salari F (2012) The lipid component of Massese ewes' colostrum: Morphometric characteristics of milk fat globules and fatty acid profile. Int Dairy J 24: 93-96. https://doi.org/10.1016/j.idairyj.2011.07.006.
24. Martini M, Licitra R, Altomonte I, Salari F (2020). Quality of donkey mammary secretion during the first ten

days of lactation, Int Dairy J 109:104781. https://doi.org/10.1016/j.idairyj.2020.104781.

25. Martini M, Salari F, Altomonte I (2016) The Macrostructure of Milk Lipids: The Fat Globules. Crit Rev Food Sci Nutr 56:1209-1221. https://doi.org/10.1080/10408398.2012.758626
26. Matsushiro M, Tsunokawa M, Matsuishi T, Kooriyama T (2020). Composition Analysis of the Colostrum and Attempted Hand-rearing of a Neglected Harbor Porpoise (Phocoena phocoena). J Zoo Wildl Med 2:81-89. https://doi.org/10.5686/jjzwm.25.81
27. Montie EW, Garvin SR, Fair PA, Bossart GD, Mitchum GB, McFee WE, Speakman T, Starczak VR, Hahn ME (2008) Blubber morphology in wild bottlenose dolphins (Tursiops truncatus) from the Southeastern United States: influence of geographic location, age class, and reproductive state. J Morphol. 269:496-511. https://doi.org/10.1002/jmor.10602.
28. Oftedal OT (1997) Lactation in whales and dolphins: evidence of divergence between baleen- and toothed-species. J. Mammary Gland Biol. Neoplasia 2:205–230. https://doi.org/10.1023/A:1026328203526
29. Oftedal OT (2011) Milk of Marine Mammals. In: John W. Fuquay (ed) Encyclopedia of Dairy Sciences, 2nd Edition), Academic Press, pp 563-580. https://doi.org/10.1016/B978-0-12-374407-4.00321-6
30. Pecka-Kiełb E, Zachwieja A, Wojtas E, Zawadzki W (2018) Influence of nutrition on the quality of colostrum and milk of ruminants. Mljekarstvo 3:169–181. https://doi.org/10.15567/mljekarstvo.2018.0302
31. Peddemors VM, de Muelenaere HJJ, Devchand K (1989) Comparative milk composition of the bottlenosed dolphin (*Tursiops truncatus*), humpback dolphin (*Sousaplumbea*) and common dolphin (*Delphinus delphis*) from southern African waters. Comp. Biochem Physiol Part A Mol Integr Physiol 94:639–641. https://doi.org/10.1016/0300-9629(89)90608-7.
32. Pervaiz S, Brew K (1986) Composition of the milks of the bottlenose dolphin (Tursiops truncatus) and the florida manatee (Trichechus manatus latirostris). Comp Biochem Physiol Part A Mol Integr Physiol 84:357-360. https://doi.org/10.1016/10.1016/0300-9629(86)90629-8.
33. Ridgway S, Kamolnick T, Reddy M, Curry C (1995) Orphan-induced lactation in Tursiops and analysis of collected milk. Mar. Mammal. Sci. 11:172–182. https://doi.org/10.1111/j.1748-7692.1995.tb00516.x
34. Robeck TR, O'Brien JK, Atkinson S (2018) Reproduction. In: Gulland F, Dierauf L, Whitman K, (eds) CRC Handbook of Marine Mammal Medicine, 3rd ed.; CRC Press, Boca Raton, FL, pp. 169–207
35. Salari F, Altomonte I, Ribeiro N L, Ribeiro MN, Bozzi R, Martini M (2016) Effects of season on the quality of Garfagnina goat milk. Ital J Anim Sci 15:568-575. https://doi.org/ 10.1080/1828051X.2016.1247658
36. Samuel AM, Worthy GAJ (2004) Variability in fatty acid composition of bottlenose dolphin (Tursiops truncatus) blubber as a function of body site, season, and reproductive state. Can. J. Zool. 82:1933-1942. https://doi.org/10.1139/z05-001

37. Senigaglia V, Christiansen F, Sprogis KR, Symons J, Bejder L (2019). Food-provisioning negatively affects calf survival and female reproductive success in bottlenose dolphins. Sci Rep 9: 8981 https://doi.org/10.1038/s41598-019-45395-6

38. Townsend (1999) Hand-rearing techniques for Neonate Cetacean. In: Fowler ME (ed) Zoo and Wild Animal Medicine. Saunders Co, Philadelphia, pp. 493-497

39. Vollmer NL, Rosel PE (2013) A Review of Common Bottlenose Dolphins (Tursiops truncatus truncatus) in the Northern Gulf of Mexico: Population Biology, Potential Threats, and Management. Southeast Nat 12:1-43. https://doi.org/10.1656/058.012.m601

40. Wells RS, Rhinehart HL, Hansen LJ, Sweeney JC, Townsend FI, Stone R, Casper DR, Scott MD, Hohn AA, Rowles TK (2004) Bottlenose Dolphins as Marine Ecosystem Sentinels: Developing a Health Monitoring System EcoHealth 1:246–254. https://doi.org/10.1007/s10393-004-0094-6.

41. West KL, Oftedal OT, Carpenter JR, Krames BJ, Campbell M, Sweeney JC (2007) Effect of lactation stage and concurrent pregnancy on milk composition in the bottlenose dolphin. J Zool 273:148–160. https://doi.org/10.1111/j.1469-7998.2007.00309.x

42. Xiang M, Alfvén G, Blennow M, Trygg M, Zetterström R (2000) Long- chain polyunsaturated fatty acids in human milk and brain growth during early infancy. Acta Paediatr 89:142-147. https://doi.org/10.1111/j.1651-2227.2000.tb01206.x

43. Yablokov AV, Belkovich VM, Borisov VI (1974). Whales and dolphins. Part 1. Kity i Del’fini, Izd-vo Nauka, Moscow. Translation JPRS 62150–1. pp528.

Milk composition, fatty acids profile and fat globule size of Bottlenose Dolphin (*Tursiops truncatus*, Montagu 1821) milk at early lactation

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ABSTRACT

Research data on milk composition in cetaceans are scattered and fragmentary. This paper analyses the gross and mineral composition, the fatty acids profile and the fat globule size of bottlenose dolphin (*Tursiops truncatus*) colostrum and milk at early lactation. The milk samplings were carried out on three lactating female of bottlenose dolphins at the 1st, 4-5 and 20- 42 day post partum. High percentages of dry matter (51.88%), fat (26.08%) and protein (13.83%) were found in the colostrum on the first day, while there was a tendency for these components to decrease in the milk. The average diameter the milk fat globule (7.07 um) of this species was assessed for the first time. The milk was rich in unsaturated fatty acids, which were more than twice compared to the saturated fatty acids (unsaturated / saturated ratio = 2.6). The main fatty acids of dolphin milk were C16: 0, C18: 1 n-9, C20: 1 n-11, C20: 5, C22: 6 n6. Furthermore C12: 0 showed double contents in milk compared to colostrum, while C20: 1 n9 and C20: 1 n7 were respectively five and three times higher in milk than colostrum. Finally, C18: 3 n3 was approximately 4 times lower in milk than in colostrum while C24: 0 and C24: 1 tended to halve. The changes found in the fatty acid profile could be linked to the animals' energy metabolism and lipomobilization during lactation. This study contributes to improve scientific knowledge on dolphin milk composition and nutrient supply in dolphin calves.

Keywords: *Tursiops truncatus*, bottlenose dolphin milk, colostrum, early lactation, fatty acids, milk fat globule

Introduction

The bottlenose dolphin (*Tursiops truncatus*; order Cetacea, suborder Odontocetes) is a cosmopolitan species of Delphinidae family and lives in temperate and tropical seas areas, while is absent in polar waters (Connor et al., 2000). Wild bottlenose dolphins are found in diverse habitats such as bays, sounds, shallow estuarine systems as well as deep oceanic waters (Wells et al., 2004; Vollmer and Rosel, 2013); they live in groups and are piscivorous species, top-level predators of the marine food web (Vollmer and Rosel, 2013).

This mammalian species is commonly maintained in sea-life parks under human care. Adult bottlenose dolphin is grey in colour, with an average length of 2.2-3.8 m and a medium weight ranging from 250 to 500 kg. New-born bottlenose dolphin has a length of 0.8-1.4 m and a mean weight of 14-20 kg (Jenkins, 2009).

Bottlenose dolphins can live as long as 40-50 years in the wild, the sexual maturity is reached at 5-10 years in females and 8-13 years in males. Gestation lasts about 12 months and usually only a single offspring is calved (Robeck et al., 2018) lactation in the free living animals is prolonged. Wild bottlenose dolphin calves are nutritionally dependent on their mother for a minimum of 1–2 years but weaning time varies between populations and individuals (Senigaglia et al., 2019) The close association mother-calves continues for an average of about 4 years and some mothers continuing to suckle calves for up to 8 years (Karniski et al., 2018).

For the first two months of life, the nutritional requirements of calves are completely dependent upon milk suckling. In captivity, calves generally start voluntary mouthing fish at 4-8 month and usually begin to hand feeding at 7 to 12 month (Townsend, 1999). The long lactation of dolphins is presumably related to the need of the offspring to achieve social and cognitive skills, foraging techniques and predator-avoidance strategies (West et al., 2007). Although many studies have been done on bottlenose dolphin ecology and biology, research data on milk composition and on variation of milk composition during lactation is scattered and fragmentary. The reasons for the lack of data have been reviewed by Oftedal (1997) and are related to the fact that:

a) collection of milk samples from wild mammals requires that animals be captured and restrained or chemically immobilized
b) In the first studies on dolphins milk samples have often been collected from carcasses of animals killed during whaling operations or obtained from stranded animals. For such samples, post-mortem or pathological changes may result in abnormal results.
c) During the samplings the lactation in dolphins has been often indirectly estimated based on the oldest calves found with lactating females in captured groups (cow-calf method), or behavioral observations, and or by mean of the milk present in calf stomachs; thus the estimation are subject to methodological error eg accompanying calf not be captured and aged, or is frequent adoptive suckling or milk stealing.

For the above mentioned reasons according to Oftedal (1997) it was impossible to reliably assign lactation stage to most milk samples from Odontocetes described in the literature. More recently, captive animals, such as bottlenose dolphins, have been trained to present for milk collection (Oftendal, 2011).

Successful rearing requires milk formulas to meet the nutritional requirements during calves' growth, to improve their survival and to support natural physiological development. In this regard, scientific knowledge on milk composition can improve neonatal care (eg artificial feeding and formulations) with appropriate nutrient supply (West et al., 2007).

To the best of our knowledge there are no published studies on the nutritional value of milk from bottlenose dolphins during the first week of lactation. Bottlenose dolphin milk fatty acids profile and mineral composition is almost unexplored, and to the best of our knowledge, the milk fat globule size of this specie has never been analysed.

This paper aimed to analyse the gross and mineral composition, the fatty acids profile and the fat globule size of bottlenose dolphin in colostrum and in milk at early lactation.

Materials and Methods

Animals and samples collection

The milk samplings were carried out on three lactating female of bottlenose dolphins. Dolphin A and B were housed at

Acquario di Genova and dolphin C at Oltremare park, both facilities are located in Italy.

The three females dolphins were maintained with their suckling calves and were healthy throughout the research period. The three animals fed similar diet with an average energy content of 204541±5911 kcal during the period studied. The mean food intake was 8.62 ±2.33 kg/day, the diet consisted of 80.31% of herrings (*Clupea harengus*), 18.67% of capelin *(Mallotus villosus*), and 0.09% of squids (*Loligo vulgaris*).

Bottlenose dolphins were trained to present for voluntary milk collection by the aquarium staff. Animals were placed on a side to expose the mammary slit out of the water and modified syringes without needle were used reverted to create vacuum to collect the milk samples. Milk collection was in fact possible due to the negative pressure that was creating by pulling back the syringe plunger.

Samplings were collected from each animal at of 1st day post partum, 4-5 days of lactation and from 20 to 42 days, immediately refrigerated at 4° C and then transported to laboratory for the analysis, within 24 hours of milk collection. In total 6 individual raw milk samples were analysed in duplicate or triplicate each.

Gross composition and mineral analysis

Dry matter, crude protein and ash content were determined by AOAC Method (1990). Crude protein was calculated as 6.38 times the total nitrogen. Fat content was determined through gravimetric determination after extraction by Rose-Gottlieb method (IDF, 1996). Ca, Mg, Na, K, Mn and Zn through atomic absorption spectrophotometer and P through UV spectrophotometer according to the AOAC methods (1990).

Morphometric analysis of milk fat globules

The diameter and the number of fat globules per mL of milk (μm) in each sample were measured by florescence microscopy following our direct method (Salari et al., 2016). Diameter (μm) and the number of fat globules per mL of milk were determined directly in each sample of fresh milk as described by Salari et al. (2016). In brief, each sample of fresh milk was diluted 1:100 in distilled water, and was stained by adding a 0.1% solution of Acridine Orange (Sigma-Aldrich, Milan, Italy) in a 0.1 M Phosphate buffer (pH 6.8); the ratio of milk and staining solution was 10% (v/v). The analysis was performed by a fluorescence microscope Leica Ortomat Microsystem (Leika SPA, Milan, Italy) equipped with a camera (TiEsseLab, Milan, Italy) and an Image software TS view 2.0 (C & A Scientific, Manassas, VA).

The globules were grouped into three size categories: small globules (SG) with a diameter <2 μm, medium-sized globules (MG) with a diameter of 2–5 μm, and large globules (LG) with a diameter >5 μm.

Fatty acid analysis

Each sample of previously extracted fat (2.2) was used for preparation of methyl esters of fatty acids (FAME) by transesterification with sodium methoxide according to Christie (1982). The fatty acids composition was determined by gas chromatography by PerkinElmer Clarus 480 (PerkinElmer, Norwalk, CT, USA) equipped with a flame ionisation detector and a capillary column (ThermoScientific TR-FAME 60 m × 0.25 mm ID; film thickness 0.25 μm, Fisher Scientific, Loughborough, Leicestershire, UK). The peak of individual fatty acid methyl esters were identified by using standard injection (Food Industry FAME Mix – Restek Corporation, Bellefonte, PA, USA). C9:0 was used as internal standard for the quantification of the peak areas. Fatty acids are expressed as a g of individual fatty acid methyl ester (FAME) per 100 g total FAME

Mean and standard deviations for colostrum (1st day in milk), milk (4-42 days in milk) were calculated. For minerals general mean and standard deviations of two individuals are reported.

Results

The gross composition and the average diameter of the milk fat globules of colostrum (1st day in milk) and milk (4-42 days in milk) of the bottlenose dolphin milk are presented in Table 1.

Table 1 – here

The analysed samples showed on the average percentages of dry matter, protein and fat of 41.64 ± 8.06 %, 11.54 ± 1.99 % and 20.57 ± 6.12 % respectively.

Regarding fat, it showed a decreasing tendency from colostrum to milk ranging from a peak of 26.08% at the first day colostrum to 19.7% in mature milk. Similarly, protein showed maximum values in colostrum (12.90%) and minimum 10.08% in milk.

The mean diameter of the milk fat globules was 7.07 μm ± 1.276; an increasing trend in globule diameter (from 6.84 to 8.40 μm), and a decrease in number of globules per mL (from 4.02 to 1.17 $*10^7$) was found from colostrum to milk alongside with the decreases in fat.

Regarding ash, it was on the average 1.04 ± 0.55%; the main milk minerals (Table 2) were potassium and phosphorus (2.145 and 1.45 g/kg respectively) followed by calcium, sodium and magnesium (0.59, 0.29, 0.04 g/kg respectively); the analysed milk also contained about 2.72±0.202mg/kg of zinc.

Table 2 –here

In the dolphin milk samples (Table 3) short-chain fatty acids (SCFA-shorter than 12C) were absent. On the contrary, the long chains (LCFA: greater than or equal to 18C) predominated and were on the average 67.02 % of the total FAMEs. Compared to saturated (SAT), unsaturated fatty acids (UNS) were more than double quantity (on the average UNS / SAT ratio was 2.64 ± 0.384). Monounsaturated fatty acids (MUFA) account on average for 54.46% ± 6.325 of the total FAMEs and PUFAs were 17.82% ± 3.238. The main SFA in milk were C14: 0 and C16: 0 (on the average 4.92; 11.50 g of individual FAME per 100 g total FAMEs).

C16: 1 n7, C18: 1 n9; C20: 1 n9; C22: 1 n11 were the most represented among the MUFA (on the average 11.50, 10.71; 8.44, 3.87, g of individual FAME per 100 g total FAME respectively).

As for the polyunsaturated fatty acids (PUFA), the main ones were C20: 5 n3 and C22: 6 (on the average 8.44 and 5.33 g of individual FAME per 100 g total FAME).

Table 3 –here

Some variations of the FA profile between colostrum and milk were observed, in particular C12: 0 recorded double contents in milk compared to colostrum. In addition, increases in the isomers of C20: 1 passing from colostrum to milk were found, in particular of C20: 1 n9 and C20: 1 n7 quintupled and tripled respectively. Finally, decreases in C18: 3 n3, C24: 0 and C24: 1 were observed. In particular, C18: 3 n3 was approximately 4 times lower in milk than in colostrum, while in C24: 0 and C24: 1 tended to halve in milk compared to colostrum.

Discussion

The value of dry matter found in this study fell within the range described in the same species (24.5-64.7%) (Eichelberger et al., 1940; Jennes and Sloan, 1970; Yablokov et al., 1974). Fat percentage was in agreement with the most frequently values reported in the literature for milk in this species (10 - 30%) (Jenness & Sloan 1970; Pervaiz & Brew, 1986). Furthermore, the range of milk fat reported from different authors in *Tursiops truncatus* is exceptionally wide from 6.8 to 51% (Ridgway et al., 1995; West et al., 2007; Yablokov et al., 1974). Fat variability reported by the literature may mostly be related to sampling activity: for example incomplete mammary evacuation could give a potential underestimation of milk fat as normally happens in terrestrial species (Oftendal et al., 2011). In fact, it is well known that, among many farm animals, fat concentration of sampled milk is affected by the degree of filling of the mammary gland and the residual milk is usually the richest in lipid. For the same reason also samples collected before and after a suckling event might differ.

In general, milks of aquatic mammals are high in fat, likely because new-born mammals typically has thin subcutaneous body fat and must acquire considerable quantities of body fat to thermoregulate in the aquatic environment (Oftendal, 1997; Matzushiro et al., 2020). In support of this hypothesis is the fact that blubber represents a substantial proportion of the total body mass of a marine mammal and ranges from 20 to 80% in the different species of dolphins (Koopman, 2007). Blubber is the lipid-rich hypodermis of marine mammals, is a modified form of adipose tissue consisting of adipocytes contained in a supportive matrix of collagen and elastin; fat stores are contained in blubber as triacylglycerols and wax esters (Koopman, 2007).

To our knowledge this is the first investigation that reports the composition of dolphin colostrum; as regards the percentage of fat, a recent paper on another Odontocete (Harbor porpoise, *Phocoena phocoena*) found similar values in the colostrum (from 1-14 days) (30% fat).

Furthermore, the decreasing tendency for fat from the first-day colostrum to mature milk we found can has been also reported in the dairy animals as cow, sheep, goat, donkey (Pecka-Kiełb et al., 2018; Martini et al., 2012; Martini et al., 2020).

A single paper in the literature concerns the variation of gross composition during lactation in this species by West et al. (2007). The authors analysed the milk of three captive bottlenose dolphins from 1–30 months postpartum and found a significant increase in milk fat content during the course of lactation starting from the first month after delivery. Unfortunately, the results of our study are not directly comparable with that by West et al. (2007) in which sample collection started from the1st month after parturition.

In our study, the protein (table 1) were within the range found in Cetaceans (about 8-12%) (Oftedal, 2011). The higher protein content in colostrum compared to milk is linked to the presence of immunoglobulins, which contribute to passive immunity of neonatal Cetacean calves. However, higher protein percentage in day one colostrum would have been expected as observed in mammals with epitheliochorial placenta (Martini et al., 2020). In fact, the epitheliochorial placenta do not allow prenatal immunoglobulin transfer, thus the transfer of immunoglobulins in the offspring through the colostrum is essential for the acquisition of passive immunity. An explanation for the lower than expected protein in colostrum could be due to the fact that changes in protein content occur rather rapidly in the first hours post-partum as found in the dairy animals (Martini et al., 2020).

Some authors reported the protein content in Cetacean milks may rise over lactation, but the degree and direction of change vary with species and also among individuals and with maternal condition (Oftendal 2011; West et al., 2007).

In dolphins, as in the other mammals, the milk lipids are packed into a membrane as milk fat globules. The size of the fat globules can affect the nutritional intake and influence the digestive kinetics of the nursing offspring (Martini et al., 2016). To our knowledge, this is the first study that analyses the average size and number of fat globules in the dolphin. The mean diameter was higher than average diameter between in the main livestock species (7.07 vs 2 -5 μm) (Martini et al., 2016). Similarly to the present results, Oftendal (2011) reports that in the Weddell seal (Phocidae), another deep diving marine mammal, the milk fat globules are relatively large.

Regarding ash content, the results were slightly higher compared to the values of 0.70 and 0.77% reported by Peddemors et al. (1989) and Jennes and Sloan (1970), respectively, but similar to those observed in the milk of Otaridae and Focidae (Oftendal, 2011).

In the data literature on the individual mineral content in dolphins are scarce and outdated.

The detected individual mineral content (Table 2) were lower than the previous reports for calcium (0.60 vs 0.74 and 1.30 g/kg) (Eichelberger et al., 1940; Peddemors et al., 1989), magnesium (0.03 vs 0.07 g/kg) and sodium (0.25 vs 1.56 g/kg) (Eichelberger et al., 1940). However, the high sodium values reported in the literature for some Cetacean are suspect due to the possibility of seawater contamination (Oftendal, 2011). We also found an exceptionally high potassium content

(2.145 vs 0.8 g/kg) compared to the only value report in the literature (Eichelberger et al., 1940).

In addition, the phosphorus content was higher than previous studies (1.45 vs 0.18 and 1.10 g/kg). (Eichelberger et al., 1940; Peddemors et al., 1989). Unfortunately, for zinc there are not available data on this species for a direct comparison, however our result is comparable to that of marine mammal milks that contain about 1–8 mg/kg of zinc (Oftendal, 2011).

The only study on fatty acid composition of dolphin milk dates back to 1971 and concerns a single wild bottlenose dolphin on which milk was collected post mortem (Ackman and Eaton, 1971).

The absence of short-chain fatty acids (SCFA-shorter than 12C) in our samples was in agreement with the literature on dolphins (Ackman and Eaton, 1971), and on other marine mammals such as the beluga whale (*Delphinapterus leucas*) and Fin whale (*Balaenoptera physalus*) (Lauer and Baker, 1968) and on northern elephant seals *Mirounga angustirostris* (Fowler et al., 2014).

The preponderance of LCFA represent a similarity with human milk in which LCFA are about 63% of the total fatty acids (calculated by Xiang et al. 2000; Altomonte et al., 2017; 2019).

The average UNS/SAT ratio (2.64) was in agreement to what has been reported by Ackman and Eaton (1971) (calculated UNS / SAT ratio= 2.3).

Among the most represented individual MUFA, C16: 1 n-7 is a common product of biosynthesis in marine mammal blubber (Budge et al. 2004; Koopman et al., 2007); C20: 1 n-9 and C22: 1 n11 are also found in the blubber and are commonly linked to carnivorous feeding (Herman et al. 2005).

The high presence of C 16: 0, C18: 1 n9, in dolphin milk has similarities with the milk of other marine mammals such as northern elephant seals (Fowler et al., 2014) and with the blubber (Montie et al., 2008; Fowler et al., 2014).

Compared to human milk, dolphin milk has an excess of this two particularly important long chain PUFA (C 22: 6n-3 and C 20: 5n-3). In fact, milk FA also reflect the dietary intake and C 22: 6 n-3 and C 20: 5 n-3 mainly derive from marine source diets (Iverson, 2009). PUFA longer than C20 have been also shown to be very important in neonatal development for humans (Altomonte et al., 2019).

The changes of the FA profile between colostrum and milk, in particular of C12: 0 suggests a derivation of this FA from *ex novo* synthesis. In fact, even if the mechanisms and extent of *de novo* synthesis are not known in dophin mammary gland, in ruminants and humans it is known that FA with 6–14 C constitutes the major product of de novo FA synthesis in the mammary gland (Mahmoud et al., 2014).

The trend we found towards the increase of C20: 1 isomers passing from early and late lactation was also observed by Fowler et al. (2014) in northern elephant seals.

In small Odontocetes, milk fatty acids also reflect mobilization from fat depots (blubber) (Budge et al., 2006; Koopman et al., 2007). Lactating dolphins may preferentially mobilize longer-chain fatty acids that are found to be more prevalent in adipocytes in the deep blubber; the latter is preferentially mobilized during lactation (Montie et al., 2008; Samuel and

Worthy, 2004).

Some authors (Fowler et al., 2014) found unclear results with respect to the pattern of mobilization and changes in the FA profile of milk in Cetacean; they hypothesized that when mobilized, C20: 1 isomers are preferentially directed to milk synthesis, while mothers may preferentially use PUFA and SFA for her own metabolism.

In conclusion, this study reports for what we believe the first time the nutritional value of milk in the first weeks of lactation from bottlenose dolphins held under human care. The high concentration of fat in milk seems to have the adaptive meaning of favouring the thermoregulation of the offspring in the aquatic environment. This is the first investigation on the fatty acid composition of dolphin milk with *in vivo* sampling. Dolphin milk was rich in long chains and monounsaturated acids; the main fatty acids were C16: 0, C18: 1n-9, C20: 1n-11 and C20: 5. Increases found in some individual fatty acids from colostrum to milk could be linked to the animals' energy metabolism and lipomobilization. How the fatty acid composition and the diameter of the milk globules contribute to the health and development of the calves and what potential adaptive significance is yet little known. This study improves scientific knowledge on dolphin milk composition and could support intervention for neonatal care (eg artificial feeding and formulations) and nutrient supply in dolphin calves.

Declarations

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Conflicts of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Availability of data and material The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

Material and Code availability Software: JMP, RRID:SCR_014242;

Authors' contributions Conceptualization: Mina MARTINI, Matteo, Federico SOMMER, Claudia GILI; Methodology: Matteo, Federico SOMMER, Iolanda ALTOMONTE, Federica SALARI; Formal analysis and investigation: Iolanda ALTOMONTE, Federica SALARI, Rosario LICITRA; Writing - original draft preparation: Iolanda ALTOMONTE, Rosario LICITRA; Writing - review and editing: Federica SALARI, Federico SOMMER, Claudia GILI; Resources: Mina MARTINI; Supervision: Mina MARTINI

Ethics approval This research study was conducted from data obtained for clinical purposes. All animals reported in this study are humanely managed according to the national and international regulations (Italy: DM 469/2001, D.lgs 73/2005 and by following specific protocols and guidelines disseminated through the marine mammal and zoo animal management associations (EAAM Standards and Guidelines, 2019). Milk sampling was carried out by expert veterinarians for animal health management and under a strict Veterinary Protocol specifically prepared for animal care purposes, thus no sample was collected or manoeuvre performed for this study only.

Consent to participate Not applicable

Consent for publication Not applicable

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References

1. Ackman RG, Eaton CA, Mitchell ED (1971) The bottle-nosed dolphin *Tursiops truncates*: fatty acid composition of milk triglycerides. Can J Biochem 49:1172–1174. https://doi.org/10.1139/o71-168
2. Altomonte I, Salari F, Licitra R, Martini M (2019) Donkey and human milk: Insights into their compositional similarities. Int Dairy J 89:111-118. https://doi.org/10.1016/j.idairyj.2018.09.005.
3. Altomonte I, Salari F, Martini M (2017) Milk fat components and milk quality. In: Poltronieri P (ed). Microbiology in Dairy Processing, John Wiley & Sons, Ltd, pp1-10 https://doi.org/10.1002/9781119115007.ch1
4. AOAC (1990). Official methods of analysis. 15th ed. Association of Official Analytical Chemist, Arlington, VA, USA.
5. Budge SM, Iverson SJ (2003) Quantitative analysis of fatty acidprecursors in marine samples: direct conversion of wax ester alcohols and dimethylacetals to FAMEs. J Lipid Res 44:1802–1807. https://doi.org/10.1194/jlr.D300009-JLR200
6. Budge SM; Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar Mamm Sci 22:759–801. https://doi.org/10.1111/j.1748-7692.2006.00079.x
7. Christie WW (1982) A simple procedure of rapid transmethylation of glycerolipids and cholesteryl esters. J Lipid Res 23:1072–1075. https://doi.org/10.1016/S0022-2275(20)38081-0
8. Connor RC, Wells RS, Mann J, Read AJ (2000) The bottlenose dolphin: social relationships in a fission–fusion society. In: Mann J, Connor RC, Tyack PL, Whitehead H (Eds) Cetacean societies: field studies of dolphins and whales. The University of Chicago Press, Chicago, pp 91–126.
9. Cornell LH, Asper ED, Antrim JE, Searles SS, Young WG, Goff T (1987) Progress report: Results of a long-range captive breeding program for the bottlenose dolphin, Tursiops truncatus and Tursiops truncatus gilli. Zoo Biol 6:41-53. https://doi.org/10.1002/zoo.1430060106
10. Eichelberger L, Fetcher ES, Geiling EMK, Vos BJ (1940) The composition of dolphin milk. J Biol Chem 134:171–176.
11. Fowler MA, Debier C, Mignolet E., Linard C, Crocker DE, Costa DP (2014) Fatty acid mobilization and comparison to milk fatty acid content in northern elephant seals. J Comp Physiol B 184:125–135 https://doi.org/10.1007/s00360-013-0787-7
12. Herman DP, Burrows DG, Wade PR, Durban JW, Matkin CO, LeDuc RG, BarrettLennard LG, Krahn MM

(2005) Feeding ecology of eastern North Pacific killerwhales Orcinus orca from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. Mar Ecol Prog Ser 302:275–291. https://doi.org/10.3354/meps302275

13. IDF 1996. IDF-FIL. International Standard 1D. Determination of Fat Content-Gravimetric Method (Rose-Gottlieb Reference Method) International Dairy Federation—Federation Internationale de Laiterie, Brussels, Belgium
14. Iverson SJ (2009) Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In: Kainz M, Brett M, Arts M (eds) Lipids in Aquatic Ecosystems. Springer, New York, pp 281-306. https://doi.org/10.1007/978-0-387-89366-2_12
15. Jaakkola K, Willis K (2019) How long do dolphins live? Survival rates and life expectancies for bottlenose dolphins in zoological facilities vs. wild populations. Mar Mam Sci 35:1418-1437. https://doi.org/10.1111/mms.12601
16. Jenkins J (2009) Tursiops truncatus. Animal Diversity Web. https://animaldiversity.org/accounts/Tursiops_truncatus/
17. Jenness R, Sloan RE (1970) The composition of milks of various species: A review. Dairy Sci. Abstracts 32:599-612
18. Karniski C, Krzyszczyk E, Mann J (2018) Senescence impacts reproduction and maternal investment in bottlenose dolphins. Proc. R. Soc. B 285: 20181123. http://dx.doi.org/10.1098/rspb.2018.1123
19. Kastelein RA, Vaughan N, Walton S, Wiepkema PR (2002) Food intake and body measurements of Atlantic bottlenose dolphins (Tursiops truncates) in captivity. Mar Environ Re 53:199-218. https://doi.org/10.1016/s0141-1136(01)00123-4.
20. Koopman, HN (2007). Phylogenetic, ecological, and ontogenetic factors influencing the biochemical structure of the blubber of odontocetes. Mar Biol 151:277–291 https://doi.org/10.1007/s00227-006-0489-8
21. Lauer BH, Baker BE (1969) Whale milk. I. Fin whale (Balaenoptera physalus) and beluga whale (Delphinapterus leucas) milk: gross composition and fatty acid constitution. Can J Zool 47:95-97. https://doi.org/10.1139/z69-017
22. Mahmoud MA, Sunehag AL, Haymond MW (2014) De novo synthesis of milk triglycerides in humans. Am J Physiol Endocrinol Metab 306:E838-E847. https://doi.org/10.1152/ajpendo.00605.2013.
23. Martini M, Altomonte I, Salari F (2012) The lipid component of Massese ewes' colostrum: Morphometric characteristics of milk fat globules and fatty acid profile. Int Dairy J 24: 93-96. https://doi.org/10.1016/j.idairyj.2011.07.006.
24. Martini M, Licitra R, Altomonte I, Salari F (2020). Quality of donkey mammary secretion during the first ten

days of lactation, Int Dairy J 109:104781. https://doi.org/10.1016/j.idairyj.2020.104781.

25. Martini M, Salari F, Altomonte I (2016) The Macrostructure of Milk Lipids: The Fat Globules. Crit Rev Food Sci Nutr 56:1209-1221. https://doi.org/10.1080/10408398.2012.758626
26. Matsushiro M, Tsunokawa M, Matsuishi T, Kooriyama T (2020). Composition Analysis of the Colostrum and Attempted Hand-rearing of a Neglected Harbor Porpoise (Phocoena phocoena). J Zoo Wildl Med 2:81-89. https://doi.org/10.5686/jjzwm.25.81
27. Montie EW, Garvin SR, Fair PA, Bossart GD, Mitchum GB, McFee WE, Speakman T, Starczak VR, Hahn ME (2008) Blubber morphology in wild bottlenose dolphins (Tursiops truncatus) from the Southeastern United States: influence of geographic location, age class, and reproductive state. J Morphol. 269:496-511. https://doi.org/10.1002/jmor.10602.
28. Oftedal OT (1997) Lactation in whales and dolphins: evidence of divergence between baleen- and toothed-species. J. Mammary Gland Biol. Neoplasia 2:205–230. https://doi.org/10.1023/A:1026328203526
29. Oftedal OT (2011) Milk of Marine Mammals. In: John W. Fuquay (ed) Encyclopedia of Dairy Sciences, 2nd Edition), Academic Press, pp 563-580. https://doi.org/10.1016/B978-0-12-374407-4.00321-6
30. Pecka-Kiełb E, Zachwieja A, Wojtas E, Zawadzki W (2018) Influence of nutrition on the quality of colostrum and milk of ruminants. Mljekarstvo 3:169–181. https://doi.org/10.15567/mljekarstvo.2018.0302
31. Peddemors VM, de Muelenaere HJJ, Devchand K (1989) Comparative milk composition of the bottlenosed dolphin (*Tursiops truncatus*), humpback dolphin (*Sousaplumbea*) and common dolphin (*Delphinus delphis*) from southern African waters. Comp. Biochem Physiol Part A Mol Integr Physiol 94:639–641. https://doi.org/10.1016/0300-9629(89)90608-7.
32. Pervaiz S, Brew K (1986) Composition of the milks of the bottlenose dolphin (Tursiops truncatus) and the florida manatee (Trichechus manatus latirostris). Comp Biochem Physiol Part A Mol Integr Physiol 84:357-360. https://doi.org/10.1016/10.1016/0300-9629(86)90629-8.
33. Ridgway S, Kamolnick T, Reddy M, Curry C (1995) Orphan-induced lactation in Tursiops and analysis of collected milk. Mar. Mammal. Sci. 11:172–182. https://doi.org/10.1111/j.1748-7692.1995.tb00516.x
34. Robeck TR, O'Brien JK, Atkinson S (2018) Reproduction. In: Gulland F, Dierauf L, Whitman K, (eds) CRC Handbook of Marine Mammal Medicine, 3rd ed.; CRC Press, Boca Raton, FL, pp. 169–207
35. Salari F, Altomonte I, Ribeiro N L, Ribeiro MN, Bozzi R, Martini M (2016) Effects of season on the quality of Garfagnina goat milk. Ital J Anim Sci 15:568-575. https://doi.org/ 10.1080/1828051X.2016.1247658
36. Samuel AM, Worthy GAJ (2004) Variability in fatty acid composition of bottlenose dolphin (Tursiops truncatus) blubber as a function of body site, season, and reproductive state. Can. J. Zool. 82:1933-1942. https://doi.org/10.1139/z05-001

37. Senigaglia V, Christiansen F, Sprogis KR, Symons J, Bejder L (2019). Food-provisioning negatively affects calf survival and female reproductive success in bottlenose dolphins. Sci Rep 9: 8981 https://doi.org/10.1038/s41598-019-45395-6

38. Townsend (1999) Hand-rearing techniques for Neonate Cetacean. In: Fowler ME (ed) Zoo and Wild Animal Medicine. Saunders Co, Philadelphia, pp. 493-497

39. Vollmer NL, Rosel PE (2013) A Review of Common Bottlenose Dolphins (Tursiops truncatus truncatus) in the Northern Gulf of Mexico: Population Biology, Potential Threats, and Management. Southeast Nat 12:1-43. https://doi.org/10.1656/058.012.m601

40. Wells RS, Rhinehart HL, Hansen LJ, Sweeney JC, Townsend FI, Stone R, Casper DR, Scott MD, Hohn AA, Rowles TK (2004) Bottlenose Dolphins as Marine Ecosystem Sentinels: Developing a Health Monitoring System EcoHealth 1:246–254. https://doi.org/10.1007/s10393-004-0094-6.

41. West KL, Oftedal OT, Carpenter JR, Krames BJ, Campbell M, Sweeney JC (2007) Effect of lactation stage and concurrent pregnancy on milk composition in the bottlenose dolphin. J Zool 273:148–160. https://doi.org/10.1111/j.1469-7998.2007.00309.x

42. Xiang M, Alfvén G, Blennow M, Trygg M, Zetterström R (2000) Long- chain polyunsaturated fatty acids in human milk and brain growth during early infancy. Acta Paediatr 89:142-147. https://doi.org/10.1111/j.1651-2227.2000.tb01206.x

43. Yablokov AV, Belkovich VM, Borisov VI (1974). Whales and dolphins. Part 1. Kity i Del'fini, Izd-vo Nauka, Moscow. Translation JPRS 62150–1. pp528.

Table 1 - Gross composition and average diameter of the milk fat globules of bottlenose dolphin (*Tursiops truncatus)* milk.

	Colostrum (1st DIM[a])	**Milk (4-42 DIM)**
	Mean±SD[2]	Mean±SD
Dry matter (%)	51.88±6.295	37.55±3.757
Fat (%)	26.08±0.30	19.07±4.07
Protein (%)	13.83±1.547	10.41±0.833
Ash (%)	1.34±0.791	1.08±0.463
Average diameter µm	6.84±0.387	8.40±0.231
SG[b]%	0.37±0.487	0.12±0.147
MG[c]%	. 38.05±4.815	21.09±4.102
LG[d]%	61.58±5.219	78.79±4.201
Number per mL(*10[7])	4.02±1.04	1.17±0.26

[a]days in milk; [b]standard deviation; [c]Small globules: with a diameter ≤2 µm;¶ [d]Medium globules: with a diameter between 2 and 5 µm; [5]Large globules: with a diameter ≥5 µm

Table 2 –Mineral composition of bottlenose dolphin (*Tursiops truncatus)* milk

	Mean±SD	Peddersmors et al. (1989)	Eichelberger et al. (1940)
Ca (g/kg)	0.59±0.042	1.30	0.74
P (g/kg)	1.45±0.307	1.10	0.18
Mg (g/kg)	0.04±0.005		0.07
Na (g/kg)	0.29±0.045		1.56
K (g/kg)	2.145±0.148		0.8
Zn (mg/kg)	2.72±0.202		

Table 3. Fatty acid profile of bottlenose dolphin (*Tursiops truncatus*) colostrum and milk

		Colostrum (1st DIM[b])	Milk (4-42 DIM)
C12:0	g of FAME[a] per 100 g total FAMESs	0.12±0.010	0.24±0.071
C14:0		4.11±0.255	5.49±0.352
C14:1 cis 9		0.25±0.018	0.39±0.057
C15:0		0.31±0.01	0.28±0.04
C16:0		13.51±0.33	10.39±0.87
Unknown 1		0.36±0.013	0.29±0.198
Unknown 2		0.22±0.046	0.17±0.117
C16:1		4.86±0.148	5.93±0.275
C17:0		0.25±0.035	0.26±0.045
anteiso C17:0		0.26±0.011	0.12±0.145
iso C17:0		0.37±0.015	0.36±0.078
C17:1		0.26±0.127	0.20±0.226
C18:0		2.81±0.071	2.06±0.483
C18:1 n11		1.03±0.030	1.90±0.784
C18:1 n9		12.90±0.150	9.40±0.666
C18:1 n7		2.19±0.073	2.01±0.228
C18:2 n6 (trans)		0.21±0.010	0.24±0.028
C18:2 n6 (cis)		1.10±0.055	1.09±0.092
C20:0		0.08±0.002	0.08±0.091
C18:3 n6		0.34±0.476	0.26±0.177
C18:3 n3		0.70±0.003	0.18±0.212
C20:1n11		1.41±0.074	2.51±0.595
C20:1n9		3.22±0.167	8.85±0.942
C20:1n7		0.11±0.002	0.29±0.188
C20:4 n6		0.36±0.022	0.22±0.156
C20:5		4.46±0.307	3.46±0.237
C22:1 n9		1.43±0.099	1.16±0.244
C22:1 n11		4.43±0.155	3.46±1.00
C24:0		0.21±0.002	0.13±0.090
C24:1		0.21±0.009	0.10±0.107
Unknown 3		0.18±0.044	0.15±0.101
C22:5n3		2.01±0.124	1.31±0.348
C22:6 n3		5.93±0.484	5.23±1.045
∑SFA[c]		22.02±0.741	19.44±2.047
∑MUFA[d]		32.33±0.975	44.02±3.014
∑PUFA[e]		15.16±0.561	12.00±1.848
∑MCFA[f]		24.88±1.02	24.14±1.99

∑LCFA[g]		45.40±1.27	51.91±4.85
SFA	%	31.67±0.028	25.74±0.636
MUFA		46.51±0.121	58.43±1.879
PUFA		21.81±0.09	15.83±1.245
MCFA		35.40±0.295	31.76±6.841
LCFA		64.60±1.273	68.24±4.853
UNS[h]/SAT		2.16±0.003	2.88±0.096

[a]fatty acid methyl ester, [b]days in milk; [c]Saturated Fatty Acids, [d]Monounsaturated Fatty Acids, [e]Polyunsaturated Fatty Acids, [f]Medium Chain Fatty Acids ($12 \leq C < 18$), [g]Long Chain Fatty Acids ($C \geq 18$); [h]Unsaturated Fatty Acids.