

Aus dem Department für Nutztiere und öffentliches Gesundheitswesen
der Veterinärmedizinischen Universität Wien

Universitätsklinik für Wiederkäuer
(Leitung: Univ.-Prof. Dr. med. vet. Thomas Wittek, Dipl. ECBHM)

**Concentration of Vitamins of Alpaca (*Vicugna pacos*) Colostrum and Milk at
different stages of lactation**

Diplomarbeit

Veterinärmedizinische Universität Wien

vorgelegt von
Maria Magdalena Hirtl, MSc.

Wien, im Juli 2022

Department for Farm Animals and Veterinary Public Health

University of Veterinary Medicine Vienna

University Clinic for Ruminants

(Head: Univ.-Prof. Dr. med. vet. Thomas Wittek, Dipl. ECBHM)

**Concentration of Vitamines of Alpaca (*Vicugna pacos*) Colostrum and Milk at
different stages of lactation**

Diploma thesis submitted for the fulfilment of the requirements for the degree of

MAGISTRA MEDICINAE VETERINARIAE

University of Veterinary Medicine Vienna

submitted by

Maria Magdalena Hirtl, MSc.

Vienna, July 2022

Supervisor and first reviewer: Univ.-Prof. Dr. med. vet. Thomas Wittek, Dipl. ECBHM

Head of University Clinic for Ruminants

Department for Farm Animals and Veterinary Public Health

University of Veterinary Medicine Vienna

Second reviewer: Ao. Univ.-Prof. Dr. med. vet. Christine Iben, Dipl. ECVCN

Institute of Animal Nutrition and Functional Plant Compounds

Department for Farm Animals and Veterinary Public Health

University of Veterinary Medicine Vienna

Contents

1. Introduction	6
2. Overview of scientific literature	8
<i>Vitamin A</i>	8
<i>Vitamin E</i>	8
<i>Vitamin requirements of alpaca</i>	9
<i>Reference species</i>	9
Camel (<i>Camelus dromedarius</i> and <i>Camelus bactrianus</i>)	10
Cow (<i>Bos taurus</i>)	11
Buffalo (<i>Bubalus bubalis</i>).....	12
Goat (<i>Capra aegagrus hircus</i>)	12
Sheep (<i>Ovis gmelini aries</i>)	13
Pig (<i>Sus scrofa domesticus</i>).....	13
Horse (<i>Equus caballus</i>)	14
Donkey (<i>Equus asinus</i>).....	14
Human (<i>Homo sapiens</i>)	15
3. Materials and Methods	17
<i>Animals and Sample Collection</i>	17
Colostrum Sampling	17
Milk Sampling	17
Sample Management	17
<i>Laboratory Analyses</i>	18
Determination of retinol, retinyl esters, α -tocopherol and β -carotene in milk samples ...	18
<i>Statistical Analyses</i>	19
4. Results	20
<i>Composition of Colostrum</i>	20
<i>Differences in Colostrum Composition over time</i>	23
<i>Correlation of vitamin and fat concentration in alpaca colostrum</i>	27
<i>Correlation of vitamin and protein concentration in alpaca colostrum</i>	27
<i>Correlation of vitamin and lactose concentration in alpaca colostrum</i>	28
<i>Composition of Milk</i>	32
<i>Differences in Milk Composition over time</i>	35
<i>Correlation of vitamin and fat concentration in alpaca milk</i>	40
<i>Correlation of vitamin and protein concentration in alpaca milk</i>	40

	<i>Correlation of vitamin and lactose concentration in alpaca milk</i>	41
	<i>Comparison of alpaca colostrum and milk</i>	43
5.	Discussion.....	45
	<i>Temporal variations in alpaca colostrum and milk</i>	45
	<i>Differences in alpaca colostrum and milk</i>	47
	<i>Differences in colostrum and milk of alpaca and other species</i>	48
6.	Summary.....	51
7.	Zusammenfassung	52
8.	Acknowledgements	53
9.	References	54
10.	Index of figures	61
11.	Index of tables.....	63
12.	Appendix.....	65

1. Introduction

Vitamins are essential in several physiological processes in most living creatures, regardless of biological domain. Not only animals (CHEW, 1996; DEBIER et al., 2005; WINTERGERST et al., 2007; TANUMIHARDJO et al., 2016; MAQBOOL et al., 2017) but also plants (SMITH et al., 2007) and bacteria (ELÍAS-ARNANZ, 2020) depend on various vitamins. In mammals, a wide variety of vitamins are needed for functioning of different life-sustaining processes like a working immune system (CHEW, 1996; WINTERGERST et al., 2007) and other physiological functions (MAQBOOL et al., 2017; FAYE et al., 2019). Among others, fat-soluble vitamins such as Vitamin A and E are essential in new-born, juvenile as well as adult animals (CHEW, 1996; DEBIER et al., 2005; GÓMEZ et al., 2006; AYUSO et al., 2015) and humans (e.g. JENKINS and YUDKIN, 1943; WEST, 2003; ELMADFA and MEYER, 2012; TRABER, 2014; DOWLING, 2020.). These facts have been known for many decades. For example, VOGT (1928) already emphasised the importance of vitamins during the first period of life and NATVIG (1941) stated their importance in resistance against bacterial infection. Vitamin A and E are summarized in the category of fat-soluble vitamins next to Vitamin D and Vitamin K. While Vitamin A is found as many different forms of retinoids and carotenoids, Vitamin E is a wide designation for four different tocopherols and tocotrienols, respectively (DEBIER and LARONDELLE, 2005; MAQBOOL et al., 2017). Both are transmitted to neonates via colostrum and milk of mothers and support development and resistance of the young (e.g. DEBIER et al., 2005; DEBIER and LARONDELLE, 2005). Therefore, knowledge about vitamin composition of mammary secretions along with concentration of fat, protein, lactose and minerals are important for nutrient evaluation and the formulation of milk replacers in order to enable optimal growth and healthy development of new-borns (e.g. AUESTAD et al., 1989; MÖSSLER et al., 2021). Also, lifetime performance is influenced by early life development (SOBERON et al., 2012). Milk replacers are well established in species abundantly used for dairy production (KERTZ et al., 1979; HEINRICHS et al., 1995; SOBERON et al., 2012), meat production (WOLTER et al., 2002; DE GREEFF et al., 2016) and scientific research (AUESTAD et al., 1989).

The alpaca, *Vicugna pacos*, in comparison, is an animal used for its fibre and meat at a relatively small scale. As a member of the South American Camelids (SAC), alpacas are one of the two domesticated SAC species. They are closely related to llamas (*Lama guanaco* and *Lama glama*) and vicuñas (*Vicugna vicugna*) (KADWELL et al., 2001; PARRAGUEZ et

al., 2003). Initially being kept for meat production, nowadays these animals gain more and more attention in wool production and as pets and are being exported and bred all over the world (GAULY et al., 2019a). While using remastication, new world camelids are not considered true ruminants and nutrient requirements – although only modelled - vary from those species (VAN SAUN, 2006). Their milk is usually almost exclusively used for rearing their offspring (crias) (CHAD et al., 2014; MARTINI et al., 2015) in a long lactation period (PARRAGUEZ et al., 2003). Orphaned crias are fed predominantly with various other milk substitutes due to the lack of a species-specific colostrum and milk replacer (SCROGGINS, 2012, MÖSSLER et al., 2021.) Investigation of colostrum and milk composition of alpaca colostrum and milk therefore is indispensable. Recent attempts were made by CHAD et al. (2014), MARTINI et al. (2015) and MÖSSLER et al. (2021), who investigated fat, protein, lactose and mineral composition at different geographical positions. However, to our knowledge there are no publications on vitamin content of alpaca colostrum and milk. This study serves this purpose and is supposed to address the following hypotheses:

1. Variations of vitamin concentration in alpaca milk and colostrum can be shown over time.
2. There are differences regarding vitamin content of alpaca colostrum and milk, respectively.
3. Differences of alpaca colostrum and milk from colostrum and milk of other species are found on the basis of comparison with scientific publications.

2. Overview of scientific literature

Vitamin A

A variety of different molecules are implied in the general term of vitamin A. Included therein are retinol, retinal, retinoic acid, retinyl esters as well as provitamin A carotenoids like β -carotene. Dietary vitamin A usually is found as retinyl esters or β -carotene, is later partly transformed to retinol, distributed in the body, and stored in various tissues. Mostly, Vitamin A is re-esterified after absorption and is stored in the stellate cells of the liver as retinyl esters.

As an essential nutrient, it is crucial in many aspects of physiological functioning of the mammal organism. Vitamin A does not only play an important role in immunity and vision, but also in cell differentiation, reproduction, growth, and development and as an antioxidant. During gestation, the foetus is supplied with Vitamin A through the placenta because the development of various organs as well as skeleton is highly dependent on the vitamin. However, although transplacental transfer is strongly correlated to the homeostasis of the mother it is generally limited compared to other nutrients. Foetuses therefore have limited Vitamin A liver storage after parturition and need colostrum to prevent deficiency. As a result, colostrum normally contains higher levels of the vitamin than mature milk to ensure sufficient supply in the first hours of life (DEBIER et al., 2005; DEBIER and LARONDELLE, 2005).

Vitamin E

Eight compounds of Vitamin E occur naturally, the most biologically active being RRR- α -tocopherol. It makes around 90 % of the Vitamin E found in animal tissue. Next to tocopherol, tocopherol esters are present as dietary Vitamin E. Absorption of dietary Vitamin E is highly dependent on the fat content of the diet. This relationship is maintained in the blood transport, as plasma α -tocopherol levels are correlated with plasma lipid levels. As Vitamin A, Vitamin E too contributes to the well-functioning of the living organism. It is responsible for high antioxidant activity by protecting cellular membranes in mammal tissues from oxidation. Furthermore, Vitamin E is believed to be essential for physiological function of the immune system and the development of the immature immune system in neonates. It also appears to be of importance in gene expression and the inhibition of cell proliferation.

Like Vitamin A, transplacental transfer is limited for Vitamin E but is not affected by dietary intake of the mother. Nevertheless, the level of α -tocopherol increases steadily throughout the foetal life, probably correlating with the enhancement of foetal lipid mass. Due to the limited

transplacental transfer, new-borns are highly dependent on rapid intake of colostrum to stimulate the development of their immune system and to provide a crucial defence against oxidation. Colostrum usually contains higher Vitamin E levels than mature milk and timing at which colostrum intake occurs is important. Intestinal absorption of Vitamin E appears to be reduced after the first 24 hours of life. Similar to Vitamin A, Vitamin E also does not follow the milk lipid profile with usually increasing lipid values during progressing lactation. Instead Vitamin E decreases in milk after the first days of lactation (DEBIER et al., 2005; DEBIER and LARONDELLE, 2005).

Vitamin requirements of alpaca

VAN SAUN (2006) used a factorial approach to estimate nutrient requirements of South American camelids and stated vitamin requirements in these animals to be poorly understood. He concluded that the fat-soluble vitamins A, D and E are the most important for camelids. Vitamin A may be sufficient if animals feed on fresh pasture due to the abundant carotenoids contained. When fed with stored sun-dried hay Vitamin A and E intake might be insufficient. Since Vitamin E metabolism is also inadequately defined in cattle and sheep and Selenium intake is an important modifier for Vitamin E intake, numbers might vary further. In different models based on data for beef cattle and sheep, they calculated the average requirement for llamas and alpacas to be between 45 and 88 IU/kg bodyweight for Vitamin A and 0.28 to 1.1 IU/kg bodyweight for Vitamin E. These numbers, however, are based on adult animals and should be consulted with care in the context of crias as requirement in neonatal and young animals might differ.

Vitamin D – which is not addressed in this study – seems to be slightly better understood due to some cases of rickets syndrome in crias and adult alpacas (HILL et al., 1994; STIEGER-VANEGAS et al., 2013; KOFLER and WITTEK, 2015; VAN SAUN, 2015).

Reference species

Although some references can be found for composition of alpaca colostrum and milk, none of the publications address the concentrations of vitamins. Therefore, colostrum and milk vitamin concentrations of other species will be displayed in this chapter. Units of results are given as in the original publications. A detailed overview of the results, animals and analytical methods are given in Table 22. A visualisation of the conducted comparison of the literature results can be found in Figure 1 and 2.

Camel (Camelus dromedarius and Camelus bactrianus)

Camel species are frequently used as dairy animals in arid and semi-arid areas of the world. Their milk is high in Vitamin C, supplying humans in regions with scarce vegetation and little to no growing of vegetables and fruits (e.g. SAWAYA et al., 1984; FARAH et al., 1992). Therefore, the composition of camel milk is rather well known and has been compared to various other species (CLAEYS et al., 2014).

ZHANG et al. (2005) investigated the chemical composition of colostrum and milk during the first 90 days of lactation in ten primiparous two-humped Bactrian camels of the Alxa breed. They found Vitamin A levels to be higher and Vitamin E levels to be lower in milk compared to colostrum, respectively. On day 90 of lactation, content of Vitamin A was 0.97 mg/L and Vitamin E was 1.45 mg/L.

FARAH (1993) summarized based on SAWAYA et al. (1984) and KNOESS (1977), that levels of Vitamin A and E, thiamine, riboflavin, folic acid and pantothenic acid in camel milk seem to be lower compared to cows' milk. However, values relevant for this study are only given for Vitamin A from SAWAYA et al. (1984) and are stated as 0.15 mg/kg.

FAYE et al. (2019) composed a well-prepared review of the vitamins of camel milk: They stated to have found during their inquiry a mean Vitamin A value of 390 µg/L in Egyptian dromedary camel milk based on AHMED et al. (1977), which was supported by IBRAHIM and KHALIFA (2015) with a value of 380 µg/L. HADDADIN et al. (2008) reported a mean value of 267 µg/L. WERNERY (2003; *fide* FAYE et al., 2019) have shown 100 to 150 µg/L while WANG et al. (2011) found a range of 100 to 380 µg/L Vitamin A in Bactrian camel milk. CLAEYS et al. (2014) reviewed a Vitamin A concentration of 50 to 970 µg/L while SINGH et al. (2017) showed values around 201 µg/L.

BENMEZIANE – DERRADJI (2021) compared various studies regarding composition of camel milk and pointed out different vitamin contents in different regions of the world. In Kenya, 20 animals of the indigenous breed of *Camelus dromedarius* were used and samples contained an average Vitamin A content of 0.10 mg/L and Vitamin E content of 0.56 mg/L (FARAH et al., 1992). In the United Arab Emirates (Dubai and Abu Dhabi region) STAHL et al. (2006) investigated dromedary milk and colostrum resulting in 20.1 µg/dL of Vitamin A and 32.7 µg/dL of Vitamin E. In China Vitamin E levels were 126.9 mg/L (given after WANG et al., 2011) and in Saudi Arabia KONUSPAYEVA et al. (2014) detected concentrations of Vitamin A of 419.9 IU/L and Vitamin E of 20.6 µg/dL.

Seasonal changes of content of fat-soluble vitamins in camel milk were observed, correlating to the seasonal variations of fat-concentration (FAYE et al., 2019).

Cow (Bos taurus)

Bovine milk is the most abundantly used type of milk around the world (CLAEYS et al., 2014; GEROSA and SKOET, 2012), covering 83 % of milk produced globally (FOROUTAN et al., 2019). Compared to milk of other animal species, cow milk is mostly standardized due to its industrial production (CLAEYS et al., 2014). Nutrient and immunoglobulin composition of colostrum is of importance to the industry (MORRILL et al., 2012). Vitamin content of colostrum and milk has been addressed in various studies and different attempts of iatrogenic influence have been conceived (e.g., QUIGLEY and BERNARD, 1995). Various authors have contributed to the knowledge of bovine milk composition and vitamin content over the last decades. HIDIROGLOU (1989) reported 190 µg/dL α-tocopherol in colostrum and 30 µg/dL in mature milk, results gained from six dairy cows by analysis with HPLC. FARAH et al. (1992) used 0.27 mg/L (Vit. A) and 0.60 mg/L (Vit. E) for comparison with camel milk. DEBIER et al. (2005) represented values of 2.33 – 3.69 µg/mL and 0.33 – 0.57 µg/mL Vitamin A in colostrum and mature milk, respectively. They furthermore found 1.91 – 5.3 µg/mL Vitamin E in colostrum while mature milk contained 0.28 – 0.92 µg/mL. Concentrations of 4.90 µg/g retinol, 2.92 µg/g tocopherol, 0.68 µg/g β-carotene and 77.17 µg/g of fat of Vitamin E were given in KEHOE et al. (2007). HAUG et al. (2007) reported 280 µg/L of Vitamin A and 0.6 mg/L of Vitamin E. MEDHAMMAR et al. (2012) stated Vitamin A concentrations of 46 µg/100g and Vitamin E concentration of 0.07 mg/100g cow milk. ABD EL-SALAM and EL-SHIBINY (2011) reported variable vitamin content in different lactations (based on LAL and NARAYANAN (1984)). Vitamin A levels were found to be increasing from 27.8 µg/dL during the first 3 lactations to 30.7 µg/dL in lactations exceeding lactation 7. Carotene values were 6.8 µg/dL (lactation 1 – 3) to 7.2 µg/dL (> 7 lactations). Vitamin E levels ranged from 30.2 µg/dL (lactation 1-3) to 34.6 µg/dL (> 7 lactations). ABD EL-FATTAH et al. (2012) investigated colostrum composition over the first 14 days after parturition and stated 250.00 IU/dL (at calving), 187.50 IU/dL (24 h), 166.66 IU/dL (48 h), 208.33 IU/dL (72 h), 138.20 IU/dL (120 h) and 136.70 IU/100 mL (336 h) of vitamin A. As for Vitamin E, 234.00 IU/dL (at calving), 201.00 IU/dL (24 h), 174.00 IU/dL (48 h), 186.00 IU/100 mL (72 h), 220.67 IU/dL (120 h) and 318.00 IU/dL (336 h) were observed.

CLAEYS et al., 2014 used values ranging from 17 to 50 µg/dL (Vitamin A) and 20 to 184 µg/dL (α-tocopherol) for interspecific comparison of milk composition.

An attempt to display a complete overview of chemical composition of cow's milk was given by FOROUTAN et al. (2019). They reported different results for milk of various fat contents.

Vitamin A ranged from 0.55 μM in 3.25 % milk to 0.265 μM in skim milk. Vitamin E varied between 0.9 μM in 3.25 % to 0.5 μM in skim milk. Except the aforementioned, most authors reported using a given protocol of HPLC for analysis of fat-soluble vitamins and different sample sizes were used.

Buffalo (Bubalus bubalis)

After cow, buffalo is the second most used species in dairy production around the world and its milk takes an essential place in human nutrition especially in the developing world. Although originally being kept as multi-purpose animal, many milking breeds have been introduced and are used globally (ABD EL-SALAM and EL-SHIBINY, 2011). The same authors stated in their extensive review Vitamin A contents of 28.7 $\mu\text{g/dL}$ (lactation 1 to 3) to 30.7 $\mu\text{g/dL}$ (> 7 lactations) and Vitamin E contents of 23.8 $\mu\text{g/dL}$ (lactation 1 to 3) to 26.6 $\mu\text{g/dL}$ (> 7 lactations). Carotene was not detected in buffalo milk in the reviewed publications. A publication composed by ABD EL-FATTAH et al. (2012) compared the composition of cow's and buffalo's colostrum and its transition to normal milk. They investigated the first 14 days after parturition. Vitamin A levels were varying and started at 166.67 IU/dL at calving, 188.03 IU/dL at 24 hours, 171.90 IU/dL at 48 hours, 156.12 IU/dL at 72 hours, 138.20 IU/dL at 120 hours (5 days) and 139.7 IU/dL at 336 hours (14 days). Vitamin E values were similarly varying and started at 342.00 IU/dL at calving, 396.00 IU/dL at 24 hours, 387.00 IU/dL at 48 hours, 378.00 IU/dL at 72 hours, 341.67 IU/dL at 120 hours (5 days) and 330.00 IU/dL at 336 hours (14 days). A detailed overview of the temporal variations can be found in the original publication.

Goat (Capra aegagrus hircus)

Goat milk is used as food and in food production in many parts of the world (LIU et al., 2020) of increasing intensity due to their ability to live in desert and mountainous regions (PANDYA and GHODKE, 2007). Also, its specific taste and texture are often appreciated (RAYNAL-LJUTOVAC et al., 2008), especially in the Mediterranean area (PANDYA and GHODKE, 2007). Most authors agree on the strong influence of various factors such as breed, diet and season on composition and vitamin content of mammary secretions (PARK et al., 2007; RAYNAL-LJUTOVAC et al., 2008; REVILLA et al., 2017). CHANDA and OWEN (1952) found β -carotene levels ranging from 12 to 46 $\mu\text{g/dL}$ in goat colostrum. Content of fat-soluble vitamins in goat milk were reported in RAYNAL-LJUTOVAC et al. (2008) with 0.04 mg/100g retinol, 0.00 mg/100g β -carotene and 0.04 mg/100g tocopherol.

PANDYA and GHODKE (2007) stated Vitamin A levels of 2074 mg/L in goat milk. Values for Vitamin A were given as 185 IU/100g goat milk in PARK et al. (2007). CLAEYS et al. (2014) summarized 50 to 68 µg/dL (Vitamin A) in mature goat milk.

Sheep (Ovis gmelini aries)

Data on vitamin concentration of sheep milk used to be sparse, although dairy sheep farming is vital in many countries (PARK et al., 2007). As of today, more publications on the topic exist since ewe's milk is of increasing value in arid and mountainous, often rural economies (PANDYA and GHODKE, 2007). However, many recent publications concentrate on enhancing the biological availability of tocopherol and selenium in colostrum and milk (e.g., PULIDO et al., 2019) or the establishment of new, more efficient methods of measurement (e.g., REVILLA et al., 2017). As in goat's milk, many authors approve the influence of breed, environment and other factors. Vitamin content of ewe's milk was investigated or summarized by various authors. Generally, in sheep milk no β -carotene is found because it is completely modified into retinol (RAYNAL-LJUTOVAC et al., 2008; REVILLA et al., 2017).

Vitamin A levels of 146 IU/100g in ewe's milk are given in PARK et al. (2007). DEBIER et al. (2005) used Vitamin A levels of 6.54 µg/mL (colostrum) and 0.32 µg/mL (mature milk) and Vitamin E levels of 3.3 – 4.5 µg/mL (colostrum) and 1.0 – 1.65 µg/mL (mature milk) for comparison with other species. RAYNAL-LJUTOVAC et al. (2008) summarized retinol content to be 0.08 mg/100g and tocopherol content to be 0.11 mg/100g in sheep milk. REVILLA et al. (2017) used average retinol and α -tocopherol values of 57.86 µg/100g and 99.58 µg/100g, respectively, as their reference data.

Pig (Sus scrofa domesticus)

Contrary to many other species discussed, pigs are exclusively used in meat production and knowledge of the composition of colostrum and milk is used for formulation of milk replacers (CSAPÓ et al., 1996). Colostrum intake is essential for piglets since prenatal transfer of fat-soluble vitamins (next to many more nutrients and immunoglobulins) seems to be low (MAHAN and VALLET, 1997; DEBIER et al., 2005; MATTE and AUDET, 2020). Valuable publications on progress of vitamin content over the lactation period can be found. For example, CSAPÓ et al. (1996) reported mean Vitamin A levels of 1.61 mg/kg at 0 hours after parturition, 1.83 mg/kg at 68 to 76 hours after parturition and 0.92 mg/kg at 45 to 60 days after parturition, covering colostrum as well as mature milk. For the same time intervals

Vitamin E levels of 3.69 mg/kg, 3.74 mg/kg and 2.53 mg/kg were stated. MATTE and AUDET (2020) reviewed Vitamin A and E content of 2.5 mg/L and 17.9 mg/L, respectively, in porcine colostrum (in HÅKANSSON et al., 2001).

Horse (Equus caballus)

Viewed as alternative milk, equine milk is regaining attention in human nutrition, especially in continental Europe. It is usually produced on a local scale, being subject to variations due to various external factors (CLAEYS et al., 2014, MARKIEWICZ-KĘSZYCKA et al., 2014). In some areas of central Asia, mare's milk is widely used for the production of traditional beverages (MUSAEV et al., 2021).

CSAPÓ et al. (1995) examined colostrum and milk samples of 29 mares from different breeds in Hungary. Vitamin A and Vitamin E content in the colostrum (days 0-5) was 0.88 mg/kg and 1.342 mg/kg, respectively. In milk (days 8 - 45), concentrations of 0.34 mg/kg (Vit. A) and 1.128 mg/kg (Vit. E) were observed. Compared to bovine milk, the authors stated, equine milk contains less fat but similar levels of vitamins. Therefore, concentration of fat-soluble vitamins is relatively higher in mare's milk.

MARCONI and PANFILI (1998) compared raw (literature data) to powdered mare's milk regarding its composition and vitamin content. Various mare milk powder products were purchased and analysed. Vitamin A and E concentration in raw and powdered milk were 8.9, 26.1, 49 and 91 µg/100g fresh weight, respectively.

MARKIEWICZ-KĘSZYCKA et al. (2014) used nine Polish Cold-blooded multiparous mares in a late stage of lactation (141 – 210 d). They found Vitamin A and E to be the most abundant fat-soluble vitamins in equine milk. Vitamin E values ranged from 0.61 to 1.61 mg/L. Concentration of Vitamin A varied from 0.21 to 0.51 mg/L. In their study, β-carotene fluctuated between 0.166 and 0.599 mg/L.

Donkey (Equus asinus)

Donkeys too were used as dairy animals in ancient times and still are relevant for their milk and as packing animals in semiarid and mountainous areas (SALIMEI and FANTUZ, 2012).

SORRENTINO et al. (2005) sampled milk of 16 female Martina Franca donkeys and analysed, next to the gross composition, concentrations of Vitamin A and E. In raw milk, 1.67 ± 0.15 µg/100g trans-retinol and 5.14 ± 0.35 µg/100g α-tocopherol was detected. SALIMEI and FANTUZ (2012) reviewed 0.017 mg/L of Vitamin A and 0.051 mg/L of

Vitamin E based on results of other authors. VINCENZETTI et al. (2021) reported Vitamin A and Vitamin E levels of 58 µg/dL and 5.2 µg/dL, respectively.

Human (Homo sapiens)

In humans, a species spread all over the planet, as well as other species, new-borns, and infants in their first six months are nourished best with mothers' milk. The milk provided contains all essential nutrients as well as vitamins (LEVÊQUES et al., 2019) and new-borns fully depend on it (FUJITA et al., 2011). Concentrations of retinol in milk are higher when offspring is carried to term, nevertheless can be insufficient to meet the daily requirements of babies (SOUZA et al., 2015) especially in resource-poor countries (ERICK, 2018). Human medicine describes three stages in lactation: Colostrum (1-5 days), transitional milk (7-14 days) and mature milk (> 30 days) (LAWRENCE and LAWRENCE, 2005 *fide* ERICK 2018, ERICK 2018, DE VRIES et al., 2018). In colostrum, Vitamin A is 151 µg/dL and Vitamin E is 1.5 mg/dL. While in transitional milk Vitamin A and E levels are 88 µg/dL and 0.9 mg/dL, respectively, mature milk shows levels of 75 µg/dL (Vitamin A) and 0.25 mg/dL (Vitamin E) (ERICK 2018). DE VRIES et al. (2018) reviewed various publications on serum vitamin status of mothers and colostrum vitamin composition. Results of vitamin composition in colostrum were variable and can be found in Table 22. LIMA et al. (2017) stated retinol levels of 81.4 µg/dL in colostrum, 99.4 µg/dL in transitional milk and 58.1 µg/dL in mature milk.

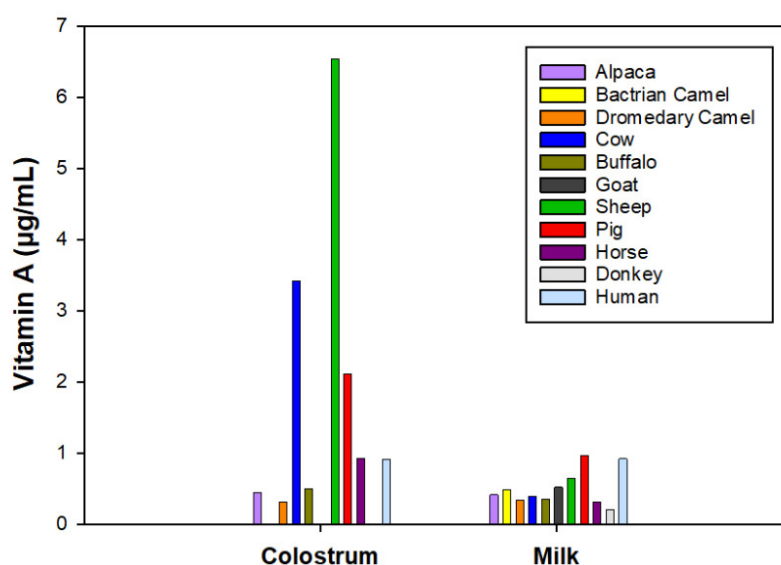


Figure 1 Comparison of average Vitamin A levels in colostrum and milk of alpaca, camel, cow, buffalo, goat, sheep, pig, horse, donkey, and human. Data has been recalculated to $\mu\text{g/mL}$ to allow comparison.

Based on: Bactrian camel: ZHANG et al., 2005; WANG et al., 2011. Dromedary: SAWAYA et al., 1984; FARAH et al., 1992; STAHL et al., 2006; HADDADIN et al., 2008; IBRAHIM and KHALIFA 2015; KONUSPAYEVA et al., 2014. Cow: RAYNAL-LJUTOVAC et al., 2008; PARK et al., 2007; KEHOE et al., 2007; ABD EL-FATTAH et al., 2012; HAUG et al., 2007; DEBIER and LARONDELLE 2005. Buffalo: ABD EL-SALAM and EL-SHIBINY 2011; ABD EL-FATTAH et al., 2012; GARAU et al., 2021. Goat: RAYNAL-LJUTOVAC et al., 2008; PARK et al., 2007; PANDYA and GHODKE 2007. Sheep: DEBIER and LARONDELLE 2005; REVILLA et al., 2017; PARK et al., 2007; RAYNAL-LJUTOVAC et al., 2008. Pig: CSAPÓ et al., 1996. Horse: CSAPÓ et al., 1995, MARCONI and PANFILI, 1998; MARKIEWICZ-KĘSZYCKA et al., 2014; MUSAEV et al., 2021. Donkey: SORRENTINO et al., 2005; SALIMEI and FANTUZ 2012; VINCENZETTI et al., 2021. Human: PARK et al., 2007; RAYNAL-LJUTOVAC et al., 2008; GARCIA 2010; GRILO 2016; GURGEL 2017; ERICK 2018; LIMA et al., 2017; CLAEYS et al., 2014.

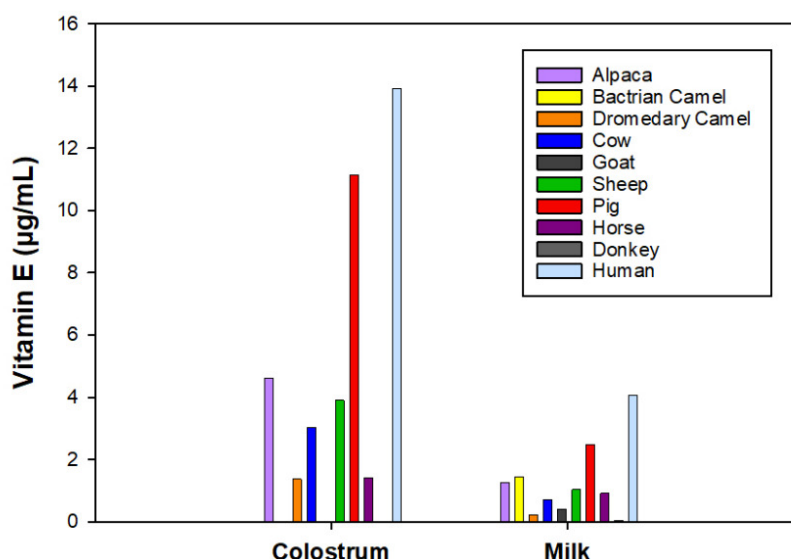


Figure 2 Comparison of average Vitamin E levels in colostrum and milk of alpaca, camel, cow, buffalo, goat, sheep, pig, horse, donkey, and human. Data has been recalculated to $\mu\text{g/mL}$ to allow comparison.

Based on: Bactrian camel: ZHANG et al., 2005; WANG et al., 2011. Dromedary: SAWAYA et al., 1984; FARAH et al., 1992; STAHL et al., 2006; HADDADIN et al., 2008; IBRAHIM and KHALIFA 2015; KONUSPAYEVA et al., 2014. Cow: HIDIROGLOU 1989; RAYNAL-LJUTOVAC et al., 2008; KEHOE et al., 2007; HAUG et al., 2007; DEBIER and LARONDELLE 2005. Buffalo: ABD EL-SALAM and EL-SHIBINY 2011; GARAU et al., 2021. Goat: RAYNAL-LJUTOVAC et al., 2008; PANDYA and GHODKE 2007. Sheep: DEBIER and LARONDELLE 2005; REVILLA et al., 2017; PARK et al., 2007; RAYNAL-LJUTOVAC et al., 2008. Pig: CSAPÓ et al., 1996; DEBIER and LARONDELLE 2005. Horse: CSAPÓ et al., 1995, MARCONI and PANFILI, 1998; MARKIEWICZ-KĘSZYCKA et al., 2014; MUSAEV et al., 2021. Donkey: SORRENTINO et al., 2005; SALIMEI and FANTUZ 2012; VINCENZETTI et al., 2021. Human: RAYNAL-LJUTOVAC et al., 2008; GARCIA 2010; CLEMENTE 2015; GRILO 2016; ERICK 2018; CLAEYS et al., 2014.

3. Materials and Methods

Animals and Sample Collection

Colostrum Sampling

Twenty multiparous alpaca mares kept in three smaller alpaca farms in the district Mürzzuschlag (Styria, Austria) were sampled for colostrum. Due to their close geographical proximity the farms were characterized with almost identical weather, husbandry and feeding conditions. All available multiparous mares of the foaling season (June - September 2018) were included in the collection of samples. The animals' age ranged from four to nine years and all sampled alpacas were of the Huacaya breed. The animals were kept on pastures on a calcareous rock in the Austrian Alps at 700 to 1100 m above sea level. In addition to the pastures, the animals were offered hay ad libitum. Sampling started on the day of birth, two to four hours after parturition (day 1) and samples were taken on four consecutive days. Mothers and crias were not separated for sampling, instead teats were closed with tape about two hours before the entire amount of colostrum was milked by hand.

Milk Sampling

Seventeen lactating alpaca mares were sampled for milk four times at monthly intervals (every 28-29 days). All mares were kept at a farm in the South Tyrolean Alps (Italy). Animals (15 Huacaya and 2 Suri mares) were between three and ten years old. Four of the mares were primiparous and 13 were multiparous. The animals were kept on alpine meadows on a calcareous source rock at 1320 to 1550 m above sea level. Mares were between lactation day 2 and 80 on the first sampling date, lactation day 2 and 109 on the second sampling date, lactation day 30 and 137 on the third sampling date and lactation day 59 and 137 on the last sampling date. Mothers and crias were separated for two hours prior to sampling and reunited immediately after. As much milk as possible was milked by hand.

Sample Management

The mares were caught and restrained by their owners using a halter and milked by hand. Prior to sampling, the udders were examined for swelling, redness, and induration in order to exclude animals suffering from clinical mastitis. Obtained samples were investigated for visible changes indicative for mastitis. Samples were collected in uncoated sample containers and transferred to a cooled polystyrene box (4°C) (Henry Schein Medical Austria GmbH, Vienna, Austria) right away. Samples were divided into subsamples within an hour after

sampling, filled in 2.0 mL Eppendorf Safe-Lock microcentrifuge tubes (Eppendorf Austria GmbH, Vienna, Austria) and 15.0 mL Greiner tubes (Greiner Bio-One GmbH, Kremsmünster, Austria), stored at -18°C and sent to the laboratory.

Laboratory Analyses

Determination of retinol, retinyl esters, α -tocopherol and β -carotene in milk samples

Vitamin A (retinol and retinyl esters), α -tocopherol and β -carotene and in milk samples were determined after organic extraction using a modified gradient reverse-phase HPLC system (Waters, Eschborn, Germany). 1000 μ l of milk samples were extracted with 2000 μ l of a mixture of n-hexane and isopropanol (3:2; v:v; stabilised with 0.05 % butylated hydroxytoluene, BHT) on a shaker for 15 min. After centrifugation (1500 x g; 10 min), the upper organic layer was removed and transferred to a fresh tube. The remaining aqueous phase was extracted again with 2000 μ l n-hexane (0.05 % BHT) and the procedure was repeated once. Then, the organic layers were combined, evaporated under dried nitrogen (30°C), resuspended in 200 μ l of isopropanol and injected onto the HPLC-system.

Separation of retinol, retinyl esters, α -tocopherol and β -carotene was performed on a C30 column (5 μ m, 250 by 3.0 mm; YMC, Wilmington, USA). The solvent system consisted of a 60-min linear gradient from solvent A with methanol/water (90/10, vol/vol; 0.4 g/liter ammonium acetate) to solvent B with methanol/methyl-*tert*-butyl-ether/water (8/90/2, vol/vol/vol, with 0.2 g/liter ammonium acetate) as the eluent at a flow rate of 0.2 ml/min. The column temperature was set to 20°C.

Retinol, retinyl esters, α -tocopherol and β -carotene were identified by comparison of retention time with an external standard (Sigma-Aldrich; Munich, Germany) using a photodiode array detector (Model 996; Waters). The quantification was done by measuring the absorption at 325 nm for retinol and retinyl esters, at 290 nm for α -tocopherol and at 450 nm for β -carotene. Accuracy and precision of the analyses were verified using standard reference material (SMR 968b; fat-soluble vitamins in human serum; National Institute of Standards and Technology, Gaithersburg, MD). The limit of detection, which was defined as analyte amount that produces a signal with a S/N ratio of 3 were 0.001 μ g/ml for retinol, 0.029 μ g/ml for α -tocopherol and 0.004 μ g/ml for β -carotene. Coefficient of variability was <4 % (Raila, *pers. comm.*).

Statistical Analyses

Microsoft Excel 2016 was used for data preparation and basic calculations. Statistical analysis was performed in RStudio (R version 4.1.1 (2021-08-10)). Since not all data were normally distributed, all indicators are shown as median and first/third quartile. Data could furthermore not be transformed to satisfyingly fulfil requirement of normal distribution by log and square root transformation. Therefore, the nonparametric Mann-Whitney U test was used for comparison of composition of milk and colostrum and the nonparametric Kruskal-Wallis H test was used to compare colostrum and milk at different times, respectively. Pairwise comparisons using Wilcoxon rank sum test with continuity correction and p value adjustment method “holm” (R default) was used as post-hoc test. The significance level in all examinations was set to 5 %. Illustrations and simple regression analyses were created using SigmaPlot 14.5 (Systat Software GmbH 2021).

4. Results

Results obtained concerning sample volumes and missing values can be taken from MÖSSLER et al. (2021). The same is true for detailed concentrations of fat, protein, and lactose in colostrum as well as milk. Those were part of the data set used in this study and are stated here for completeness but have been discussed in detail in the abovementioned publication.

A total of 77 colostrum and 60 milk samples were incorporated in this study. Missing values occurred due to the death of one animal on day 3 and development of clinical mastitis of another animal on day 4 (see MÖSSLER et al., 2021). Of the milk samples, 14 were taken in the first month of lactation, 16 in the second, 16 in the third and 13 in the fourth. Regarding sampling date irrespective of day in lactation, 12 animals were sampled on the first visit, 17 on the second, 16 on the third and 15 on the fourth.

Composition of Colostrum

The colostrum concentrations of fat, protein, lactose and vitamins on the first four days after parturition are given in Table 1 to 4.

Table 1 Minimum, maximum, median, first and third quartile of colostrum composition (n = 20) two to four hours after parturition

Colostrum Day 1					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (g/dL)	0.147	0.286	0.524	0.769	2.387
Protein (g/dL)	8.330	16.643	20.445	23.333	27.700
Lactose (g/dL)	2.860	3.783	3.950	4.493	5.400
alpha-Tocopherol (µg/mL)	1.800	2.510	4.955	6.913	8.530
Retinol (µg/mL)	0.105	0.213	0.383	0.449	0.716
Retinyl-Oleat (µg/mL)	0.001	0.003	0.006	0.012	0.021
Retinyl-Palmitat (µg/mL)	0.007	0.014	0.024	0.080	0.175
Retinyl-Stearat (µg/mL)	0.000	0.002	0.004	0.009	0.038
Sum of Retinyl Esters (µg/mL)	0.010	0.021	0.032	0.098	0.198
Total Vit A (µg/mL)	0.128	0.328	0.432	0.480	0.737
β-Carotin (µg/mL)	0.000	0.000	0.000	0.003	0.005
α-Tocopherol / Fat (mg/g)	0.00000	0.02342	0.03929	0.05878	0.48200
Retinol / Fat (mg/g)	0.00000	0.01489	0.03655	0.04723	0.27250
Retinyl-Oleat / Fat (mg/g)	0.00000	0.00019	0.00042	0.00064	0.00880
Retinyl-Palmitat / Fat (mg/g)	0.00000	0.00010	0.00020	0.00029	0.00610
Retinyl-Stearat / Fat (mg/g)	0.00000	0.00170	0.00256	0.00357	0.05510
Sum of Retinyl Esters / Fat (mg/g)	0.00000	0.00239	0.00319	0.00431	0.06520
Total Vit A / Fat (mg/g)	0.00000	0.01783	0.04046	0.05613	0.29920
β-Carotin / Fat (mg/g)	0.00000	0.00269	0.00352	0.00465	0.07560

Table 2 Minimum, maximum, median, first and third quartile of colostrum composition (n = 20) on the second day after parturition

Colostrum Day 2					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (g/dL)	0.295	0.950	2.009	2.535	6.764
Protein (g/dL)	4.860	8.495	10.370	13.450	18.120
Lactose (g/dL)	2.220	4.920	5.235	5.463	5.900
alpha-Tocopherol (µg/mL)	1.720	3.695	4.995	6.005	7.930
Retinol (µg/mL)	0.023	0.102	0.121	0.170	0.358
Retinyl-Oleat (µg/mL)	0.001	0.007	0.014	0.026	0.107
Retinyl-Palmitat (µg/mL)	0.018	0.058	0.123	0.341	0.544
Retinyl-Stearat (µg/mL)	0.002	0.007	0.011	0.028	0.104
Sum of Retinyl Esters (µg/mL)	0.025	0.068	0.145	0.389	0.755
Total Vit A (µg/mL)	0.133	0.239	0.365	0.488	0.862
β-Carotin (µg/mL)	0.000	0.002	0.003	0.004	0.010
α-Tocopherol / Fat (mg/g)	0.33808	0.52208	0.95501	1.31964	2.23625
Retinol / Fat (mg/g)	0.00607	0.04953	0.07775	0.13211	0.23172
Retinyl-Oleat / Fat (mg/g)	0.00019	0.00072	0.00132	0.00189	0.00905
Retinyl-Palmitat /Fat (mg/g)	0.00083	0.00410	0.00669	0.01094	0.05678
Retinyl-Stearat /Fat (mg/g)	0.00000	0.00039	0.00068	0.00155	0.01256
Sum of Retinyl Esters / Fat (mg/g)	0.00118	0.00582	0.00827	0.01362	0.07839
Total Vit A / Fat (mg/g)	0.01416	0.06094	0.08888	0.14447	0.23851
β-Carotin / Fat (mg/g)	0.00000	0.00000	0.00000	0.00015	0.00127

Table 3 Minimum, maximum, median, first and third quartile of colostrum composition (n = 20) on day 3 after parturition

Colostrum Day 3					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (g/dL)	1.175	2.031	2.762	4.515	9.304
Protein (g/dL)	5.740	8.235	9.480	13.610	19.040
Lactose (g/dL)	1.570	4.170	4.840	5.315	6.050
alpha-Tocopherol (µg/mL)	0.440	3.060	3.880	5.350	8.440
Retinol (µg/mL)	0.019	0.062	0.084	0.128	0.203
Retinyl-Oleat (µg/mL)	0.004	0.011	0.018	0.025	0.053
Retinyl-Palmitat (µg/mL)	0.077	0.155	0.234	0.368	1.028
Retinyl-Stearat (µg/mL)	0.004	0.010	0.029	0.049	0.347
Sum of Retinyl Esters (µg/mL)	0.103	0.173	0.268	0.440	1.397
Total Vit A (µg/mL)	0.185	0.295	0.394	0.550	1.448
β-Carotin (µg/mL)	0.000	0.003	0.003	0.004	0.006
α-Tocopherol / Fat (mg/g)	0.08323	0.18545	0.24418	0.40443	1.48184
Retinol / Fat (mg/g)	0.00075	0.00442	0.00783	0.01416	0.08668
Retinyl-Oleat / Fat (mg/g)	0.00007	0.00052	0.00075	0.00129	0.00339
Retinyl-Palmitat /Fat (mg/g)	0.00278	0.00546	0.00816	0.01022	0.02271
Retinyl-Stearat /Fat (mg/g)	0.00020	0.00031	0.00068	0.00157	0.00542
Sum of Retinyl Esters / Fat (mg/g)	0.00327	0.00697	0.01066	0.01446	0.03153
Total Vit A / Fat (mg/g)	0.00980	0.01533	0.01842	0.02167	0.10145
β-Carotin / Fat (mg/g)	0.00000	0.00004	0.00015	0.00020	0.00102

Table 4 Minimum, maximum, median, first and third quartile of colostrum composition (n = 20) on day 4 after parturition

Colostrum Day 4					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (g/dL)	3.965	4.568	5.305	5.954	8.676
Protein (g/dL)	6.060	7.213	8.300	13.508	28.370
Lactose (g/dL)	1.540	4.133	5.015	5.518	5.820
alpha-Tocopherol (µg/mL)	0.040	1.898	3.775	7.975	10.430
Retinol (µg/mL)	0.000	0.028	0.047	0.101	0.182
Retinyl-Oleat (µg/mL)	0.001	0.019	0.024	0.041	0.061
Retinyl-Palmitat (µg/mL)	0.008	0.291	0.369	0.446	0.574
Retinyl-Stearat (µg/mL)	0.001	0.021	0.028	0.063	0.115
Sum of Retinyl Esters (µg/mL)	0.010	0.359	0.458	0.518	0.745
Total Vit A (µg/mL)	0.010	0.417	0.543	0.602	0.792
β-Carotin (µg/mL)	0.000	0.003	0.004	0.006	0.008
α-Tocopherol / Fat (mg/g)	0.00473	0.06994	0.16039	0.26081	0.43404
Retinol / Fat (mg/g)	0.00037	0.00135	0.00460	0.00676	0.00834
Retinyl-Oleat / Fat (mg/g)	0.00019	0.00031	0.00057	0.00074	0.00247
Retinyl-Palmitat / Fat (mg/g)	0.00513	0.00598	0.00741	0.00965	0.01898
Retinyl-Stearat / Fat (mg/g)	0.00012	0.00052	0.00098	0.00136	0.00454
Sum of Retinyl Esters / Fat (mg/g)	0.00596	0.00701	0.00864	0.01114	0.02238
Total Vit A / Fat (mg/g)	0.00694	0.01133	0.01387	0.01586	0.03072
β-Carotin / Fat (mg/g)	0.00000	0.00005	0.00010	0.00016	0.00034

Differences in Colostrum Composition over time

The colostrum concentrations of α -tocopherol, retinol, retinyl-oleate, retinyl-palmitate, retinyl-stearate, total Vitamin A and β -carotene are shown in Figures 3 to 10. Significant differences are marked using different indices (*a-d*).

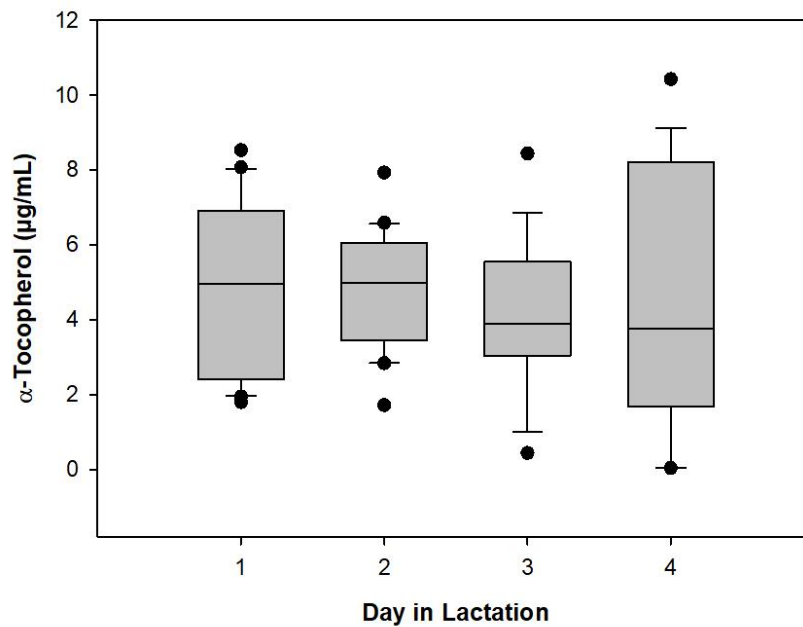


Figure 3 Boxplot of α -tocopherol concentration in alpaca colostrum samples ($n = 20$) during four days postpartum. No significant differences were observed.

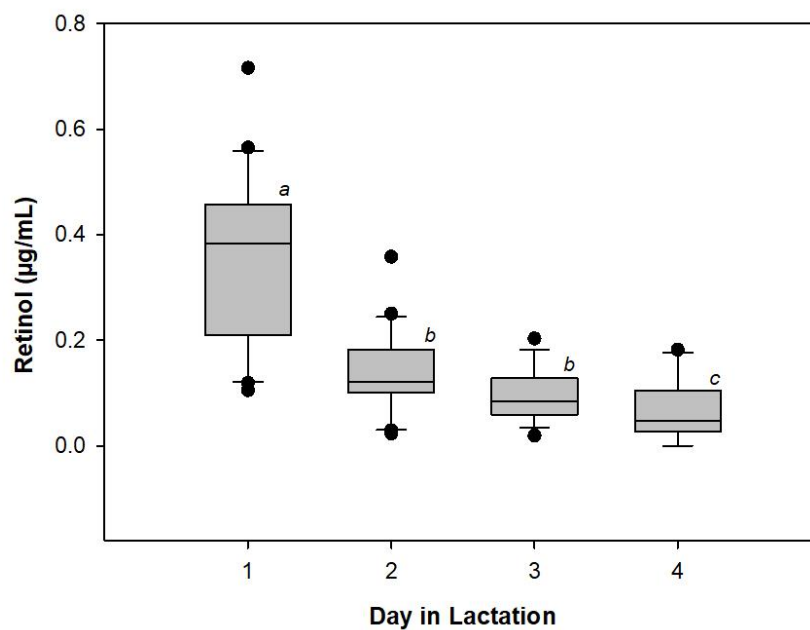


Figure 4 Boxplot of retinol concentration in alpaca colostrum samples ($n = 20$) during four days postpartum. Significant differences are marked with different indices. *a* = significantly different from all other days ($p < 0.001$), *b* = significantly different from *a* and *c* ($p < 0.01$), *c* = significantly different from day 1 and day 2 ($p < 0.01$).

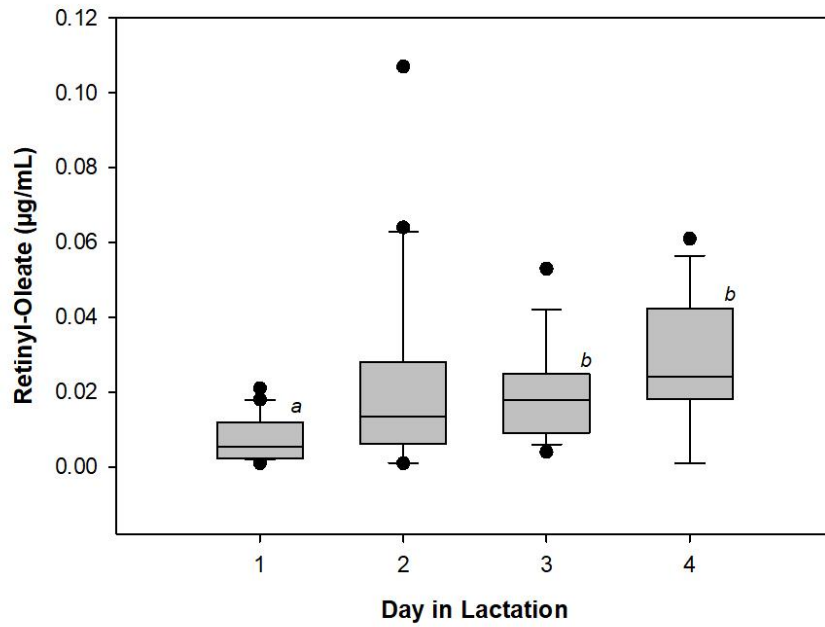


Figure 5 Boxplot of retinyl-oleate concentration in alpaca colostrum samples ($n = 20$) during four days postpartum. Significant differences are marked with different indices. $a =$ significantly different from b ($p < 0.01$)

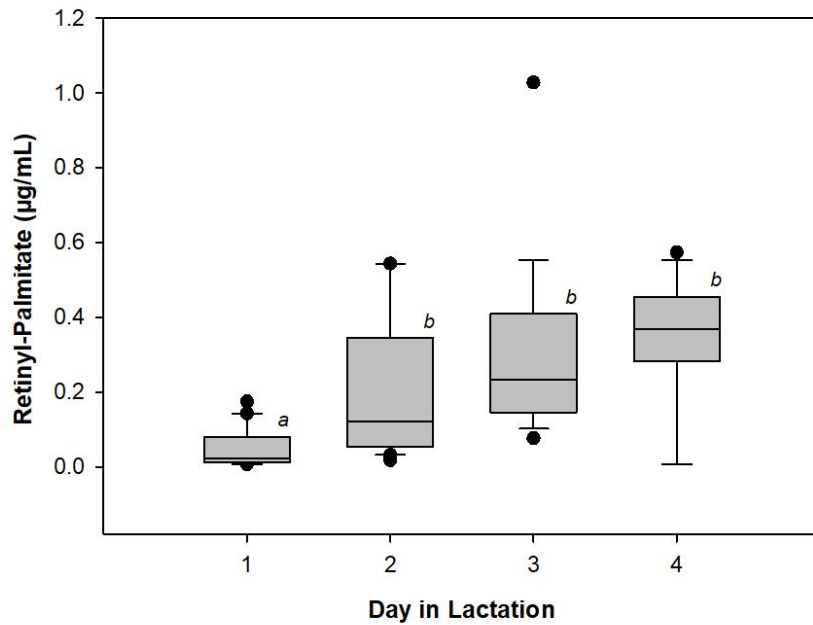


Figure 6 Boxplot of retinyl-palmitate concentration in alpaca colostrum samples ($n = 20$) during four days postpartum. Significant differences are marked with different indices. $a =$ different from b ($p < 0.01$).

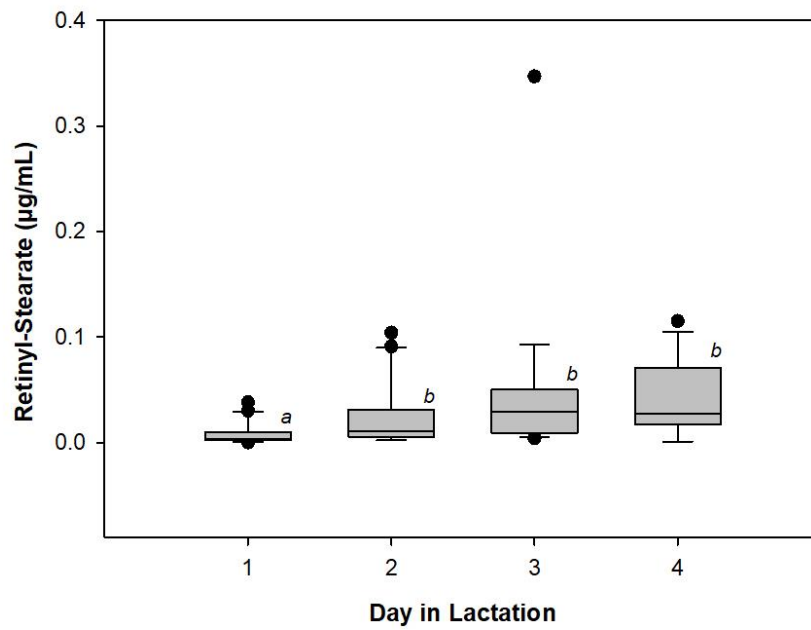


Figure 7 Boxplot of retinyl-stearate concentration in alpaca colostrum samples ($n = 20$) during four days postpartum. Significant differences are marked with different indices. $a =$ different from b ($p < 0.05$).

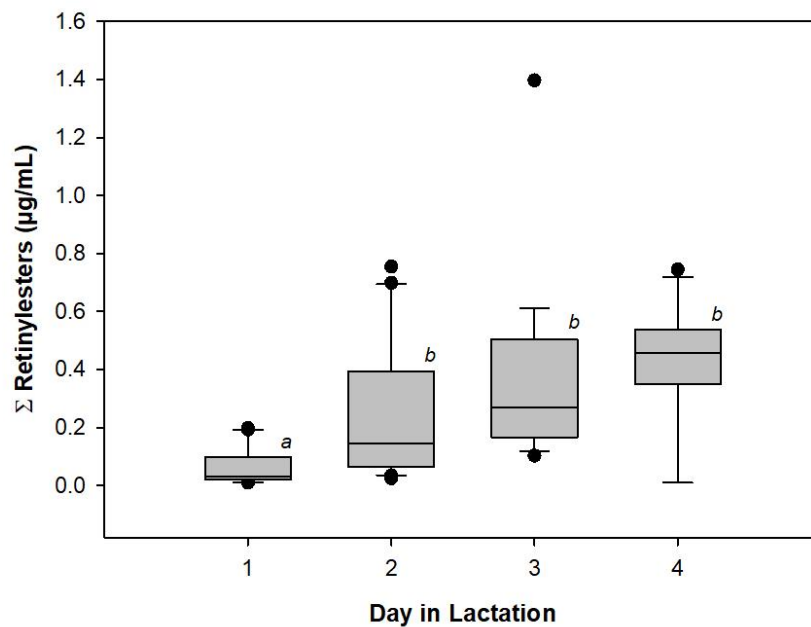


Figure 8 Boxplot of concentration of the sum of retinyl esters contained in alpaca colostrum samples ($n = 20$) during four days postpartum. Significant differences are marked with different indices. $a =$ different from b ($p < 0.01$).

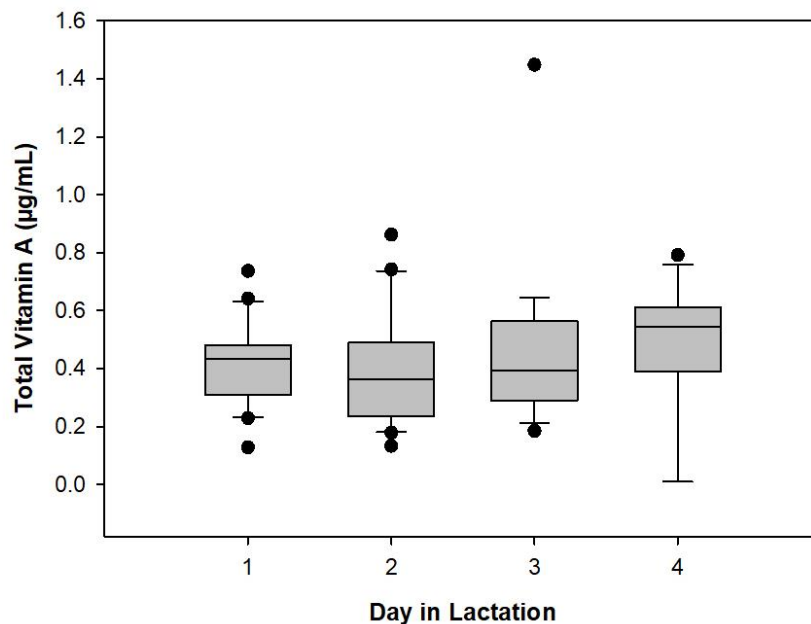


Figure 9 Boxplot of total Vitamin A concentration in alpaca colostrum samples ($n = 20$) during four days postpartum. No significant differences were observed.

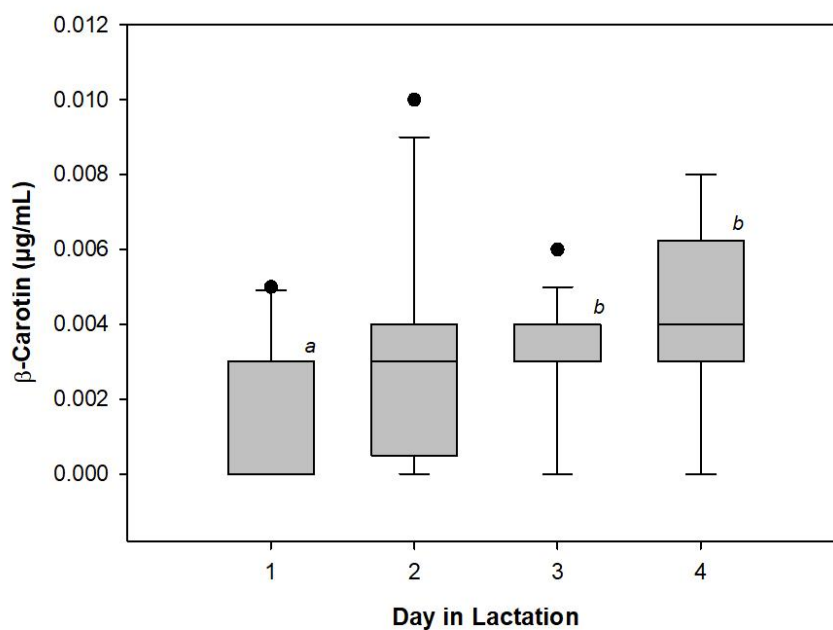


Figure 10 Boxplot of β -carotene concentration in alpaca colostrum samples ($n = 20$) during four days postpartum. Significant differences are marked with different indices. $a =$ different from b ($p < 0.05$).

Concentration of fat significantly increased over the period of the first four days postpartum ($p < 0.001$) while concentration of protein significantly decreased from day 1 to day 2 ($p < 0.001$) and stayed similar over the following two days. Content of lactose in alpaca colostrum increased significantly from day 1 to day 2 ($p < 0.001$) and was more stable over the following two days. For further details on these parameters see MÖSSLER et al. (2021). Analysis of vitamin concentration (mg) per gram fat showed significant differences for α -tocopherol ($p < 0.001$), retinol ($p < 0.001$), retinyl-oleate ($p < 0.01$) and total vitamin A ($p < 0.001$). Detailed overview of statistical results in this examination is given in Table 16 and 17 (see appendix).

Correlation of vitamin and fat concentration in alpaca colostrum

Simple linear regressions with fat as the independent and vitamins as the dependent variable, respectively, were applied in order to show possible relations between vitamins and fat. While α -tocopherol showed a widely distributed scatter plot (not pictured) and any correlation with fat ($R^2 = 0.0005$, $p = 0.85$), β -carotene could not be correlated at all. Results of retinol, retinyl-oleate, retinyl-palmitate, retinyl-stearate, sum of retinyl esters and total vitamin A are shown in Figure 11 and 12. Linear regression line, 95 % confidence intervals as well as calculation parameters are given. Model fit ranged from 20 to 55 %. Linear regression models proved to be suboptimal for these data but provided better explanation of relation than other models. Statistical details are shown in Table 13 (appendix).

Correlation of vitamin and protein concentration in alpaca colostrum

The same was performed for possible correlations between vitamins and protein content of alpaca colostrum. α -tocopherol showed no correlation with protein ($R^2 = 0.0178$, $p = 0.2471$, not shown), as well as total vitamin A ($R^2 = 0.0111$, $p = 0.3623$) and β -carotene again could not be correlated. Results of retinol, retinyl-oleate, retinyl-palmitate, retinyl-stearate, sum of retinyl esters and total vitamin A are given in Figures 12 and 13. Linear regression line, 95 % confidence intervals as well as calculation parameters are given. Model fit ranged from 15 to 66 %. Again, linear models don't provide perfect fit for these data, however performed better than other models available. Details are given in Table 14 (appendix).

Correlation of vitamin and lactose concentration in alpaca colostrum

Simple linear regressions of the investigated vitamins with lactose resulted in poor model fit for α -tocopherol ($R^2 = 0.019$, $p = 0.238$) and total vitamin A ($R^2 = 0.006$, $p = 0.5$), and only little better results for retinyl-oleate ($R^2 = 0.108$, $p < 0.01$), retinyl-palmitate ($R^2 = 0.126$, $p < 0.01$), retinyl-stearate ($R^2 = 0.069$, $p < 0.05$), sum of retinyl esters ($R^2 = 0.126$, $p < 0.01$), and retinol ($R^2 = 0.238$, $p < 0.001$). For detailed results of analysis see Table 15 (appendix).

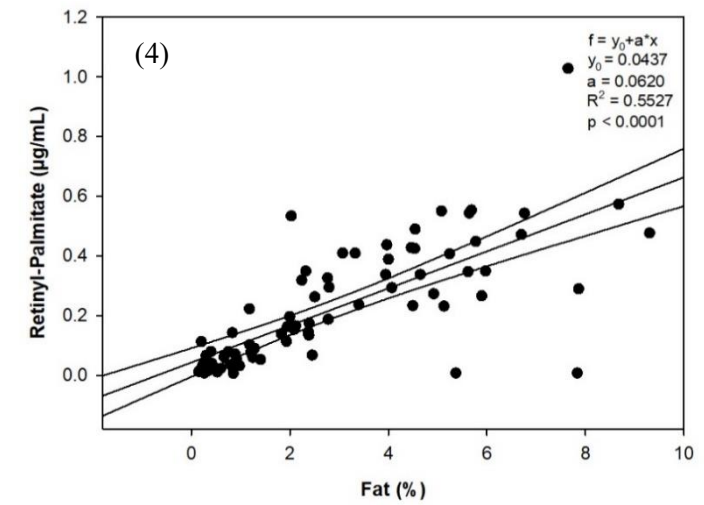
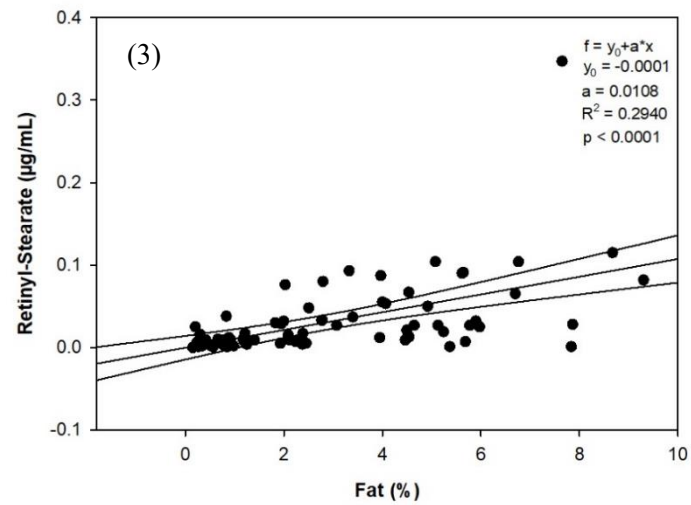
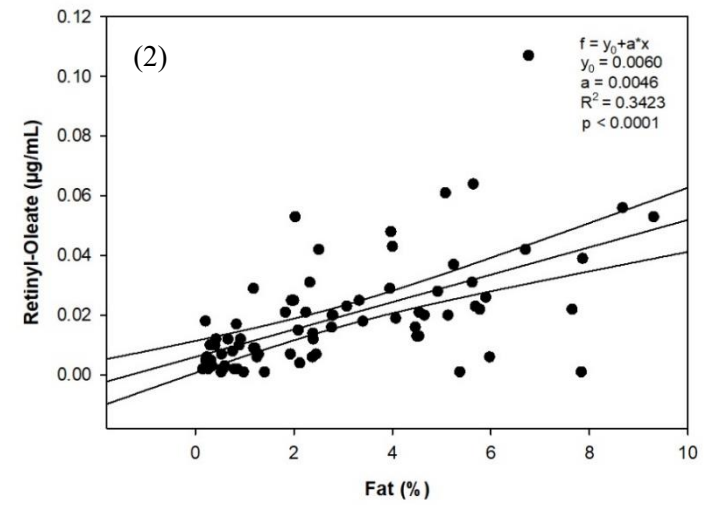
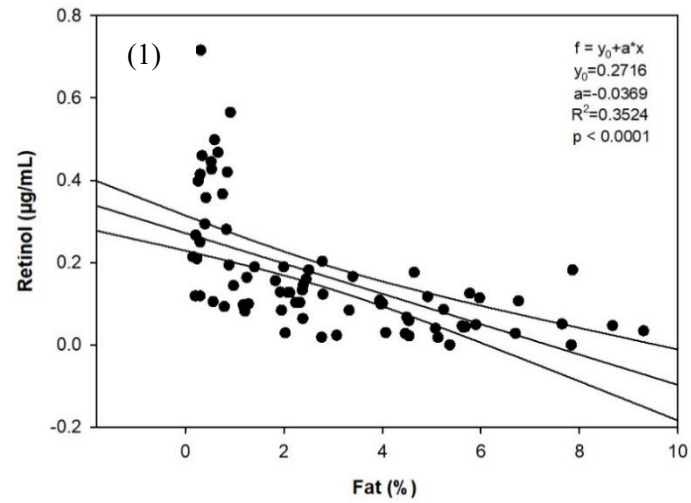


Figure 11 Correlation of colostrum fat ($n = 20$) with retinol (1), retinyl-oleate (2), retinyl-stearate (3) and retinyl-palmitate (4). Linear regression lines and 95 % confidence intervals are shown. Descriptive parameters are given in the graphs, $n = 20$. Note differences in axis scaling.

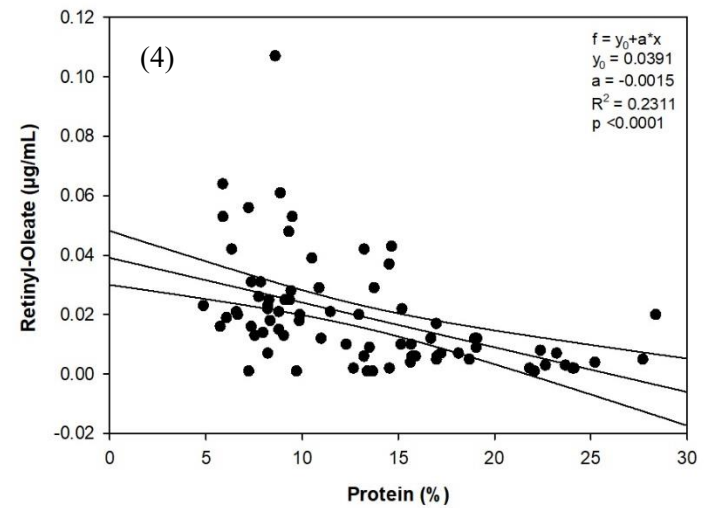
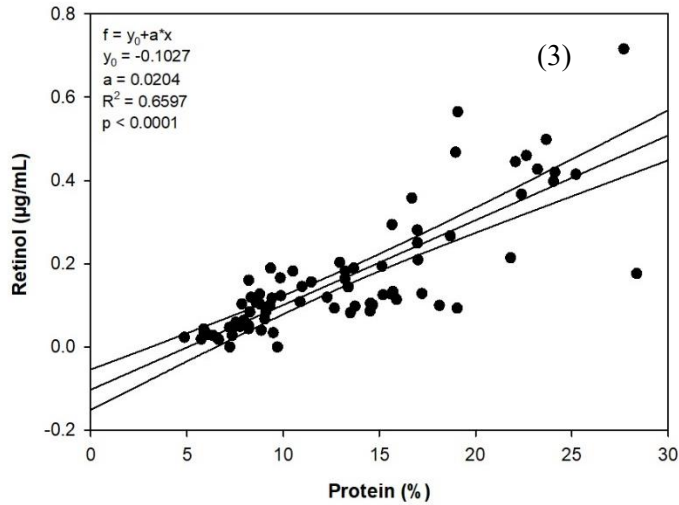
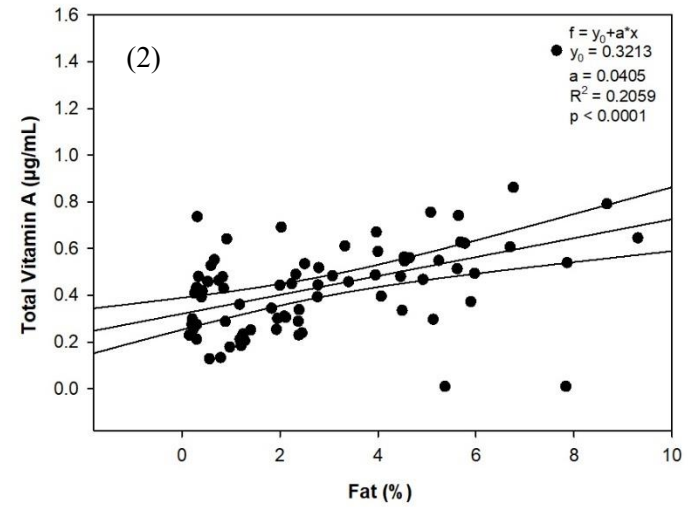
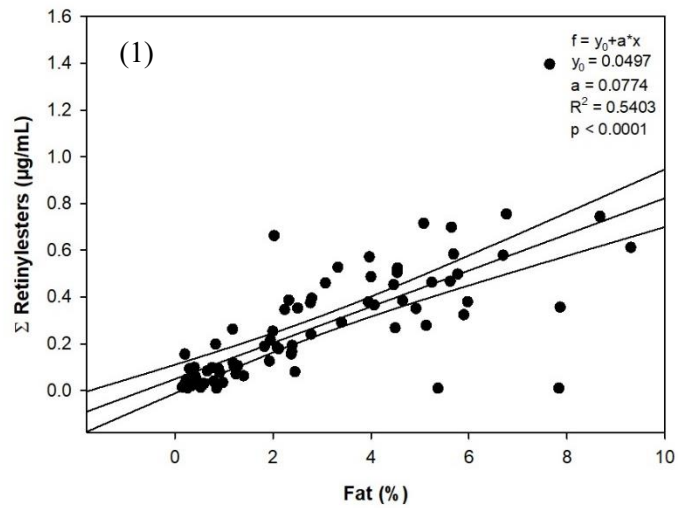


Figure 12 Correlation of colostrum concentration of fat ($n = 20$) with sum of retinyl esters (1), total vitamin A (2) and correlation of concentration of protein ($n = 20$) with retinol (3) and retinyl- oleate (4). Linear egression lines and 95 % confidence intervals are shown. Descriptive parameters are given in the graphs, $n = 20$. Note differences in axis scaling.

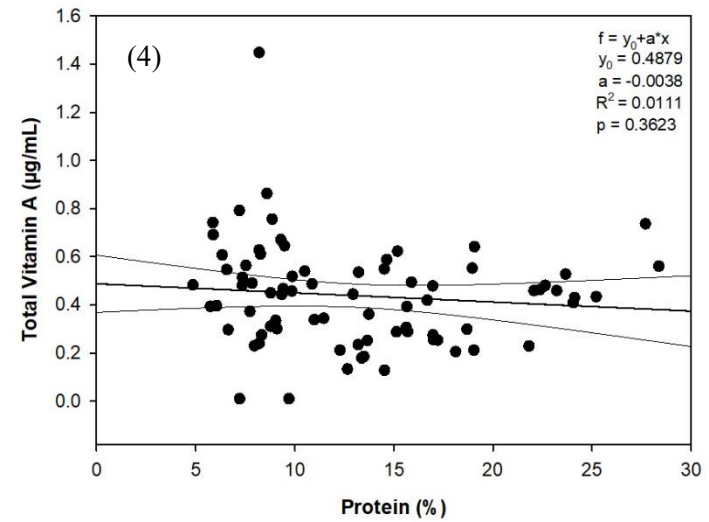
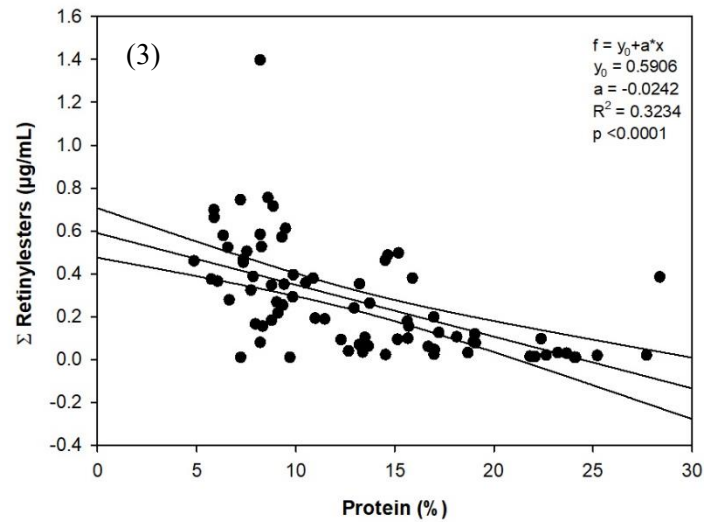
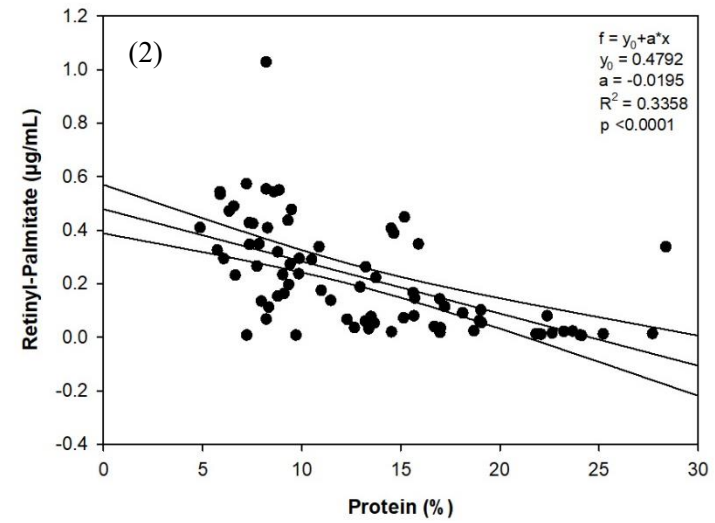
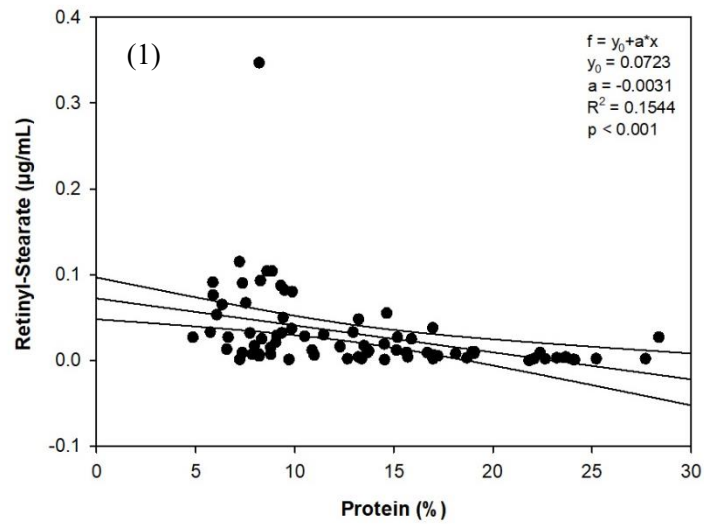


Figure 13 Correlation of colostrum concentration of protein ($n = 20$) with retinyl-stearate (1), retinyl- palmitate (2), sum of retinyl esters (3) and total vitamin A (4). Linear egression lines and 95 % confidence intervals are shown. Descriptive parameters are given in the graphs. Note differences in axis scaling

Composition of Milk

The concentrations of fat, protein, lactose and investigated vitamins contained in alpaca milk over four months of lactation are given in Table 5 to 8 with minimum and maximum values, median and first and third quartile. Samples were obtained monthly on day 1, day 29, day 57 and day 86 of the study. All samples of the whole flock available that were taken at the respective sampling date were considered, independently of individual day in lactation. Data were also grouped to monthly intervals based on day in lactation. Results can be found in Table 18 to 21 in the appendix.

Table 5 Minimum, maximum, median, first and third quartile of milk composition (n = 17) at the first sampling date. Mares were between 2 and 80 in lactation at that moment.

First Milk Sampling: Day 1, Lactation Day 2 - 80					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (g/dL)	3.000	4.185	5.420	7.795	9.930
Protein (g/dL)	4.480	4.920	7.730	9.245	16.050
Lactose (g/dL)	2.430	2.703	4.345	5.758	6.650
α-Tocopherol (μg/mL)	0.087	0.247	0.775	1.925	4.499
Retinol (μg/mL)	0.009	0.010	0.015	0.094	0.154
Retinyl-Oleat (μg/mL)	0.007	0.015	0.021	0.054	0.091
Retinyl-Palmitat (μg/mL)	0.113	0.184	0.302	0.587	0.885
Retinyl-Stearat (μg/mL)	0.014	0.040	0.068	0.098	0.114
Sum of Retinyl Esters (μg/mL)	0.146	0.232	0.416	0.751	1.076
Total Vit A (μg/mL)	0.155	0.245	0.466	0.825	1.230
β-Carotin (μg/mL)	0.000	0.003	0.005	0.008	0.013
α-Tocopherol / Fat (mg/g)	0.00290	0.00528	0.01151	0.03654	0.04531
Retinol / Fat (mg/g)	0.00014	0.00022	0.00028	0.00128	0.00251
Sum of Retinyl Esters / Fat (mg/g)	0.02900	0.05200	0.08000	0.10650	0.16600
Total Vit A / Fat (mg/g)	0.02926	0.05218	0.08033	0.10798	0.16617
β-Carotin / Fat (mg/g)	0.00000	0.00006	0.00008	0.00014	0.00019

Table 6 Minimum, maximum, median, first and third quartile of milk composition (n = 17) at the second sampling date. Mares were between 2 and 109 in lactation at that moment.

Second Milk Sampling: Day 29, Lactation Day 2 - 109					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (g/dL)	1.800	3.527	3.930	4.723	8.490
Protein (g/dL)	3.350	4.940	5.560	7.080	14.950
Lactose (g/dL)	1.870	4.300	4.760	5.730	6.240
alpha-Tocopherol (µg/mL)	0.032	0.234	0.580	1.775	4.546
Retinol (µg/mL)	0.000	0.009	0.016	0.073	0.143
Retinyl-Oleat (µg/mL)	0.001	0.014	0.017	0.022	0.041
Retinyl-Palmitat (µg/mL)	0.007	0.156	0.193	0.243	0.658
Retinyl-Stearat (µg/mL)	0.001	0.020	0.030	0.082	0.111
Sum of Retinyl Esters (µg/mL)	0.009	0.230	0.254	0.294	0.791
Total Vit A (µg/mL)	0.009	0.239	0.287	0.396	0.864
β-Carotin (µg/mL)	0.003	0.003	0.004	0.004	0.012
α-Tocopherol / Fat (mg/g)	0.00094	0.00368	0.01369	0.04331	0.12027
Retinol / Fat (mg/g)	0.00000	0.00015	0.00041	0.00150	0.00323
Sum of Retinyl Esters / Fat (mg/g)	0.00200	0.01900	0.05800	0.12675	0.19600
Total Vit A / Fat (mg/g)	0.00200	0.01915	0.05841	0.12950	0.19876
β-Carotin / Fat (mg/g)	0.00000	0.00003	0.00010	0.00013	0.00021

Table 7 Minimum, maximum, median, first and third quartile of milk composition (n = 17) at the third sampling date. Mares were between 30 and 137 in lactation at that moment.

Third Milk Sampling: Day 57, Lactation Day 30 - 137					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (g/dL)	3.620	4.032	5.130	6.027	6.830
Protein (g/dL)	3.650	5.447	6.390	7.923	12.500
Lactose (g/dL)	1.840	3.965	4.710	5.580	6.040
alpha-Tocopherol (µg/mL)	0.040	0.509	0.747	2.019	5.407
Retinol (µg/mL)	0.000	0.016	0.029	0.068	0.123
Retinyl-Oleat (µg/mL)	0.001	0.019	0.023	0.038	0.056
Retinyl-Palmitat (µg/mL)	0.008	0.201	0.236	0.391	0.788
Retinyl-Stearat (µg/mL)	0.001	0.022	0.038	0.100	0.162
Sum of Retinyl Esters (µg/mL)	0.010	0.268	0.308	0.523	0.980
Total Vit A (µg/mL)	0.010	0.296	0.395	0.629	1.073
β-Carotin (µg/mL)	0.000	0.003	0.004	0.005	0.008
α-Tocopherol / Fat (mg/g)	0.00133	0.00990	0.01223	0.04579	0.09566
Retinol / Fat (mg/g)	0.00003	0.00031	0.00040	0.00098	0.00332
Sum of Retinyl Esters / Fat (mg/g)	0.00300	0.03800	0.06350	0.08275	0.13600
Total Vit A / Fat (mg/g)	0.00000	0.02323	0.06161	0.07410	0.13931
β-Carotin / Fat (mg/g)	0.00000	0.00005	0.00008	0.00009	0.00022

Table 8 Minimum, maximum, median, first and third quartile of milk composition (n = 17) at the fourth sampling date. Mares were between 86 and 137 in lactation at that moment.

Fourth Milk Sampling: Day 86, Lactation Day 59 - 137					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (g/dL)	2.990	4.030	4.850	6.000	6.060
Protein (g/dL)	3.200	4.485	5.460	8.908	12.550
Lactose (g/dL)	2.980	3.825	4.940	5.405	6.080
alpha-Tocopherol (µg/mL)	0.028	0.249	0.440	1.483	3.381
Retinol (µg/mL)	0.000	0.007	0.016	0.035	0.108
Retinyl-Oleat (µg/mL)	0.001	0.008	0.016	0.022	0.035
Retinyl-Palmitat (µg/mL)	0.008	0.123	0.195	0.232	0.425
Retinyl-Stearat (µg/mL)	0.001	0.010	0.014	0.059	0.234
Sum of Retinyl Esters (µg/mL)	0.010	0.147	0.252	0.334	0.600
Total Vit A (µg/mL)	0.010	0.162	0.306	0.404	0.606
β-Carotin (µg/mL)	0.000	0.000	0.003	0.003	0.005
α-Tocopherol / Fat (mg/g)	0.00463	0.01993	0.02550	0.03524	0.06280
Retinol / Fat (mg/g)	0.00031	0.00032	0.00054	0.00134	0.00181
Sum of Retinyl Esters / Fat (mg/g)	0.03600	0.04600	0.06300	0.07400	0.07700
Total Vit A / Fat (mg/g)	0.00000	0.00000	0.04207	0.06689	0.07731
β-Carotin / Fat (mg/g)	0.00000	6.2*10 ⁻⁵	6.6*10 ⁻⁵	7.4*10 ⁻⁵	1.0*10 ⁻⁴

Differences in Milk Composition over time

Data were grouped to intervals of approximately 30 days in the lactation period for further analyses. Descriptive parameters including minimum, maximum, median and quartiles, of these intervals can be found in Table 18 to 21 (appendix). The illustrations of milk concentration of α -tocopherol, retinol, retinyl-oleate, retinyl-palmitate, retinyl-stearate, total Vitamin A and β -carotene in $\mu\text{g/mL}$ over four lactation months are shown in Figures 14 to 21. Furthermore, vitamin concentration (mg) per gram fat was analysed. No significant differences have been observed.

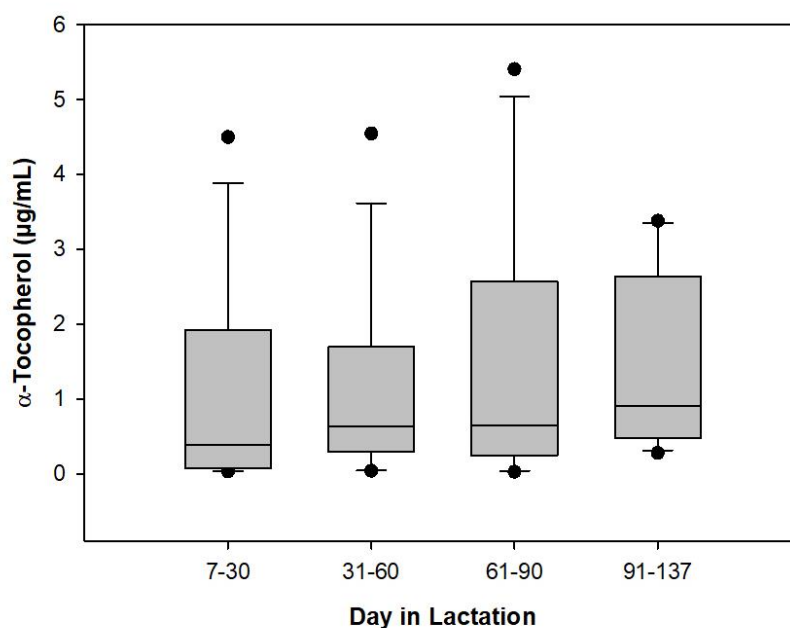


Figure 14 Boxplot of α -tocopherol concentration in alpaca milk samples ($n = 17$) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.

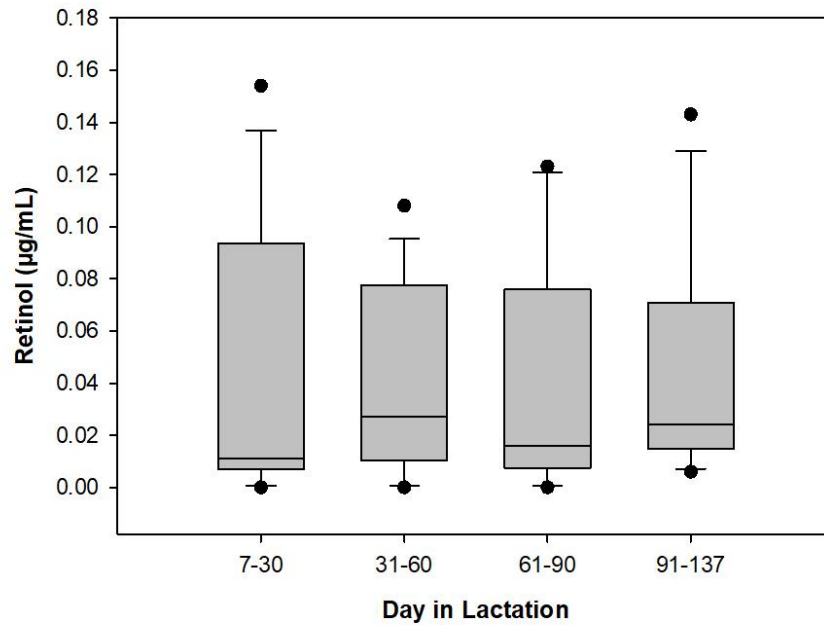


Figure 15 Boxplot of retinol concentration in alpaca milk samples ($n = 17$) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.

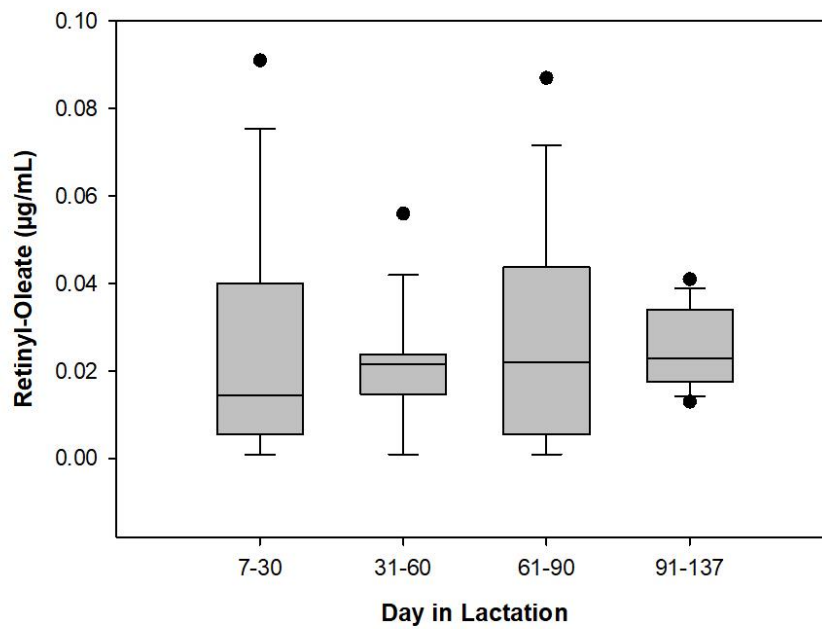


Figure 16 Boxplot of retinyl-oleate concentration in alpaca milk samples ($n = 17$) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.

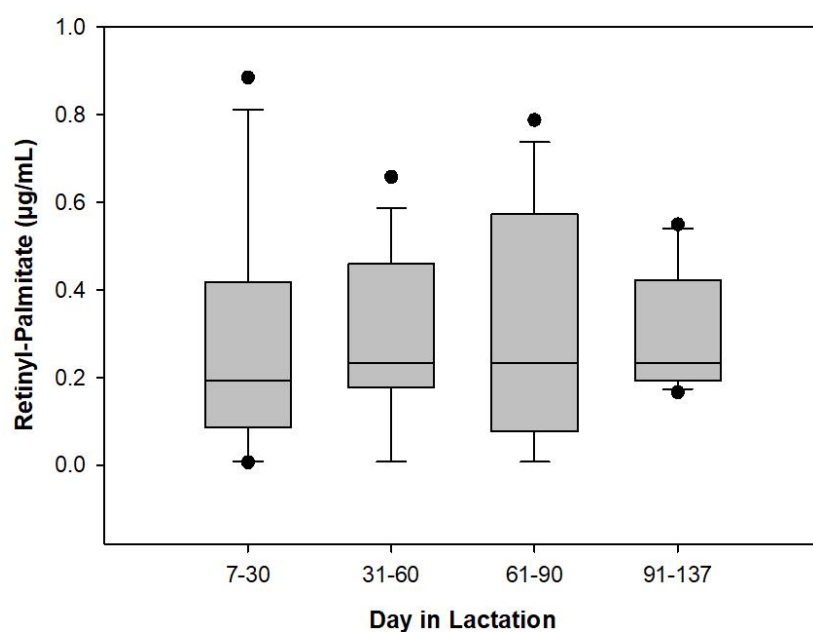


Figure 17 Boxplot of retinyl-palmitate concentration in alpaca milk samples ($n = 17$) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.

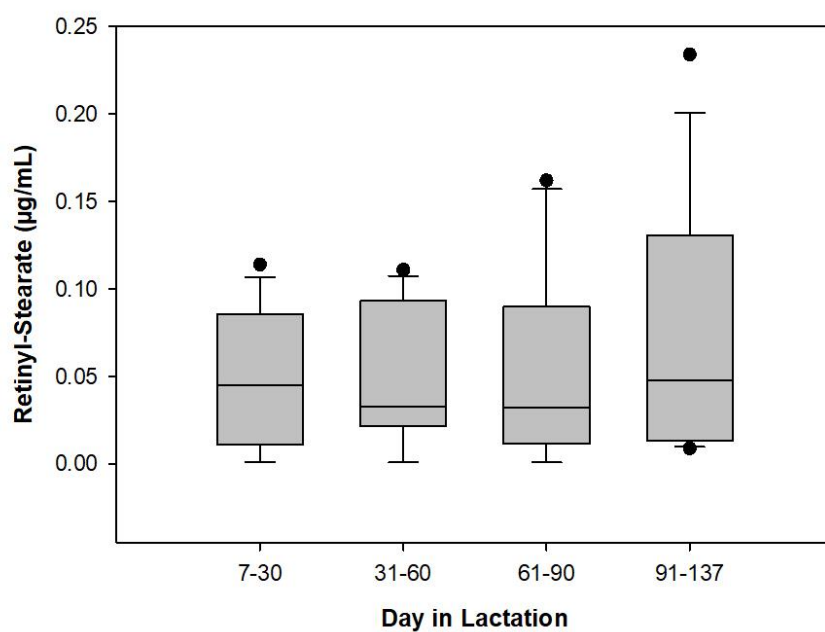


Figure 18 Boxplot of retinyl-stearate concentration in alpaca milk samples ($n = 17$) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.

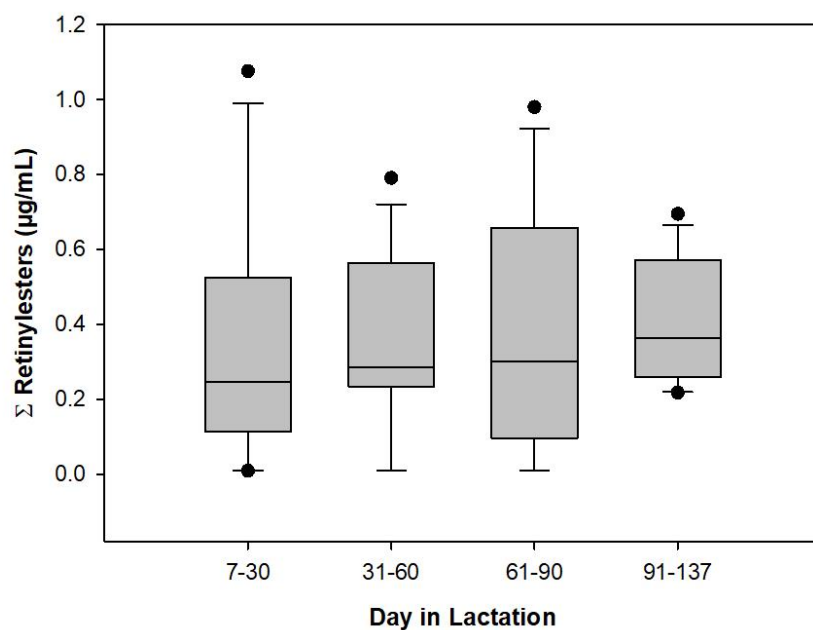


Figure 19 Boxplot of concentration of sum of retinyl esters in alpaca milk samples ($n = 17$) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.

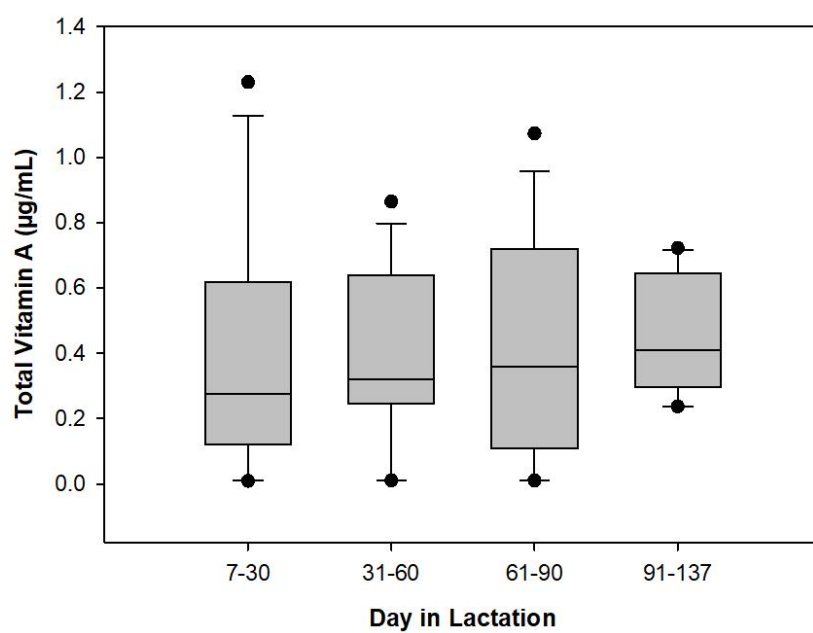


Figure 20 Boxplot of total vitamin A concentration in alpaca milk samples ($n = 17$) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.

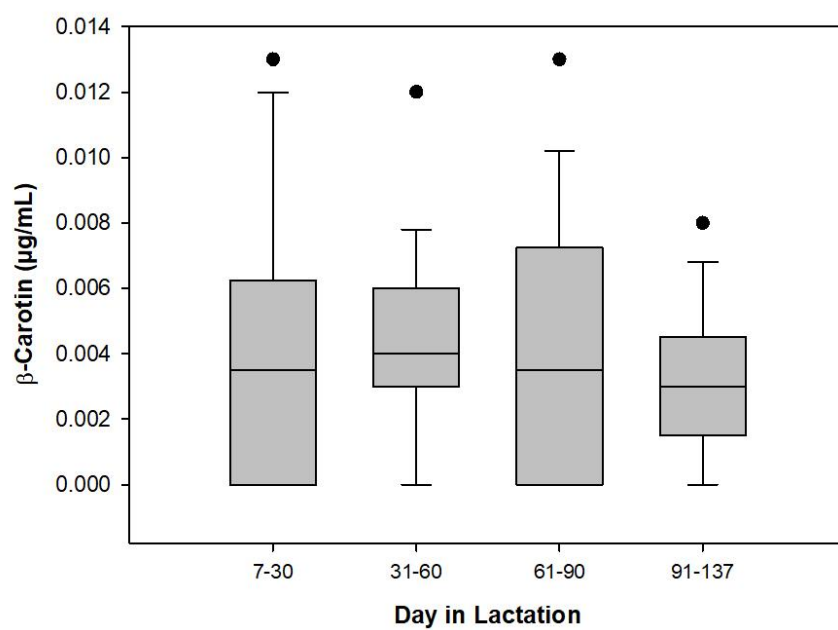


Figure 21 Boxplot of β -carotene concentration in alpaca milk samples ($n = 17$) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.

Correlation of vitamin and fat concentration in alpaca milk

As was performed for colostrum, simple linear regressions with fat as the independent and vitamins as the dependent variable, respectively, were applied for milk data. All variables scattered widely distributed. The same equation ($f = y_0 + a \cdot x$) as in the analysis of colostrum was used. Model fit ranged from 7 to 23 %, detailed information is given in Table 9. Illustrated examples are given Figure 22 (1-3). Linear regression line, 95 % confidence intervals as well as calculation parameters are given. Linear regression models proved to be suboptimal for these data but provided better explanation of relation than other models.

Table 9 Results obtained from simple linear regressions of concentration of fat and vitamins in alpaca milk (n = 17). Fat (%) was used as predictor, vitamins ($\mu\text{g/mL}$), respectively, as response variable.

Vitamin	Equation	Fat		R^2	p
		y_0	a		
α -Tocopherol	$f = y_0 + a \cdot x$	0.24	0.20	0.08	0.09
Retinol		0.01	0.01	0.07	0.12
Retinyl-Oleate		0.00	0.01	0.23	<0.01
Retinyl-Palmitate		0.01	0.00	0.00	0.84
Retinyl-Stearate		0.01	0.01	0.11	<0.05
Σ Retinylesters		0.06	0.07	0.21	<0.01
Total Vitamin A		0.07	0.07	0.20	<0.01
β -Carotin		0.00	0.01	0.11	<0.05

Correlation of vitamin and protein concentration in alpaca milk

Variables investigated for correlation of vitamin and protein concentration showed widely distributed scatter plots (not shown). The same equation ($f = y_0 + a \cdot x$) was used. Model fit ranged from no correlation at all to 13 %. Detailed results are stated in Table 10.

Table 10 Results obtained from simple linear regressions of concentration of protein and vitamins in alpaca milk (n = 17). Protein (%) was used as predictor, vitamins ($\mu\text{g/mL}$), respectively, as response variable.

Protein					
Vitamin	Equation	y_0	a	R^2	p
α -Tocopherol	$f = y_0 + a \cdot x$	0.12	0.16	0.12	<0.01
Retinol		0.00	0.01	0.20	<0.001
Retinyl-Oleate		0.01	0.00	0.12	<0.01
Retinyl-Palmitate		0.11	0.02	0.13	<0.01
Retinyl-Stearate		0.05	0.00	0.00	0.89
Σ Retinylesters		0.18	0.03	0.10	<0.05
Total Vitamin A		0.17	0.03	0.13	<0.01
β -Carotin		0.00	0.00	0.12	<0.01

Correlation of vitamin and lactose concentration in alpaca milk

Simple linear regressions of the investigated vitamins with lactose resulted in similarly poor model fit for most variables with the same equation ($f = y_0 + a \cdot x$) as mentioned above. Detailed results are found in Table 11. The model with the best fit is given as an example in Figure 22 (4). Linear regression line, 95 % confidence intervals as well as calculation parameters are given.

Table 11 Results obtained from simple linear regressions of concentration of lactose and vitamins in alpaca milk (n = 17). Lactose (%) was used as predictor, vitamins ($\mu\text{g/mL}$), respectively, as response variable.

Lactose					
Vitamin	Equation	y_0	a	R^2	p
α -Tocopherol	$f = y_0 + a \cdot x$	3.23	-0.43	0.13	<0.01
Retinol		0.14	-0.02	0.37	<0.001
Retinyl-Oleate		0.05	-0.01	0.12	<0.01
Retinyl-Palmitate		0.53	-0.05	0.09	<0.05
Retinyl-Stearate		0.05	0.00	<0.00	0.97
Σ Retinylesters		0.64	-0.06	0.07	<0.05
Total Vitamin A		0.77	-0.08	0.11	<0.05
β -Carotin		0.01	0.00	0.10	<0.05

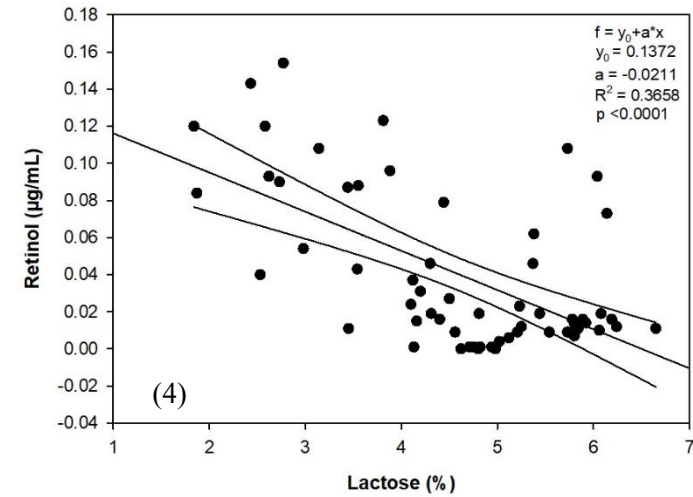
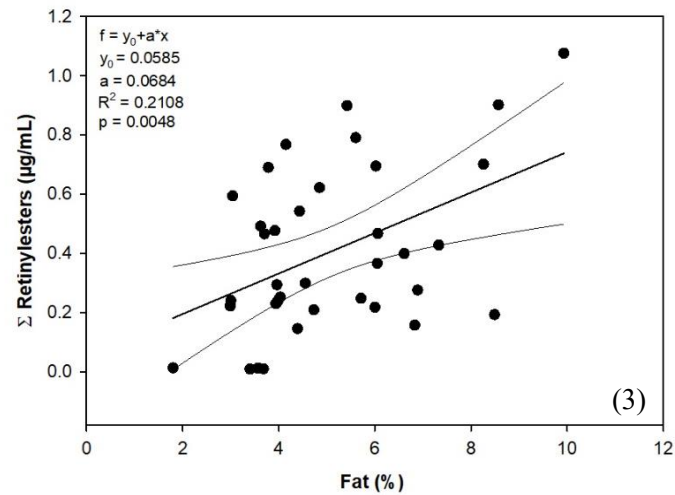
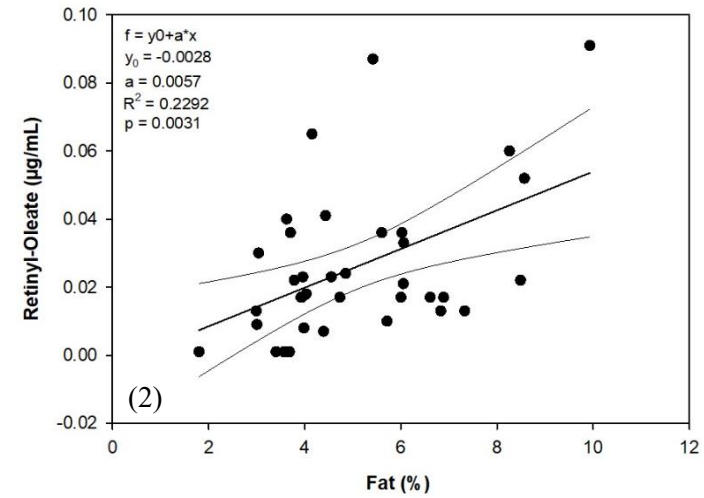
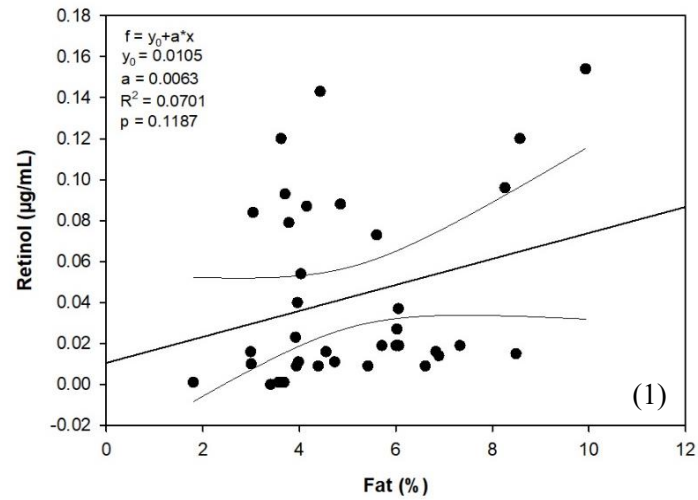


Figure 22 Correlation of milk concentration of fat ($n = 17$) with retinol (1), retinyl-oleate (2), sum of retinyl esters (3) and correlation of concentration of lactose and retinol (4). Linear egression lines and 95% confidence intervals are shown. Descriptive parameters are given in the graphs. Note differences in axis scaling

Comparison of alpaca colostrum and milk

All data obtained for colostrum and milk, respectively, were compared in order to find differences of both secret qualities. Significant differences were observed for content of fat and protein (Mann Whitney U, $p < 0.001$, respectively) but not for lactose ($p = 0.86$). Differences showed to be highly significant between alpaca colostrum and milk for α -tocopherol ($p < 0.001$, Figure 23), retinol ($p < 0.001$), retinyl-stearate ($p < 0.001$), α -tocopherol concentration per g fat ($p < 0.001$), retinol concentration per g fat ($p < 0.001$) and concentration of total vitamin A per g fat ($p < 0.001$). Further, comparison of sum of retinyl esters ($p < 0.05$), sum of retinyl esters per g fat ($p < 0.05$), β -carotene ($p < 0.05$) and retinyl-oleate per g fat ($p < 0.01$) proved to be significantly different in the two qualities. Measurements of retinyl-oleate ($p = 0.095$), retinyl-palmitate ($p = 0.068$), total vitamin A ($p = 0.27$), retinyl-palmitate per g fat ($p = 0.075$), retinyl-stearate per g fat ($p = 0.78$) and β -carotene per g fat ($p = 0.92$) did not show any significant differences. A detailed overview of results is given in Table 12.

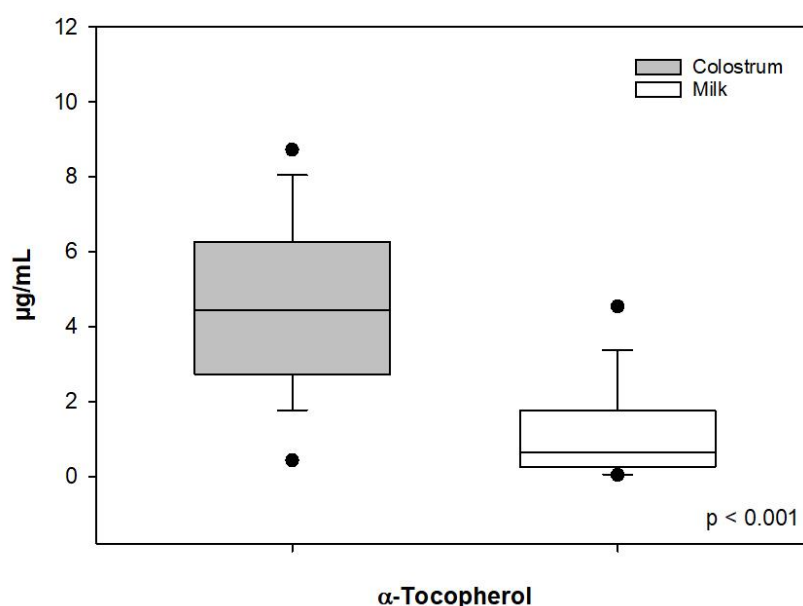


Figure 23 Boxplot of concentration of α -tocopherol in alpaca colostrum ($n = 20$) and milk ($n = 17$). Whiskers indicate first and third quartile, dots resemble 5th and 95th percentiles. Concentrations are significantly different between groups ($p < 0.001$).

Table 12 Overview of statistical analysis of comparison between alpaca colostrum (n = 20) and milk (n = 17).

Test	Variable	P	Significance
Overall comparison of milk and colostrum	Fat (%)	< 0.001	***
	Protein (%)	< 0.001	***
	Lactose (%)	0.864	n.s.
	α -Tocopherol ($\mu\text{g/mL}$)	< 0.001	***
	Retinol ($\mu\text{g/mL}$)	< 0.001	***
	Retinyl-Oleat ($\mu\text{g/mL}$)	0.095	n.s.
Mann Whitney U	Retinyl-Palmitat ($\mu\text{g/mL}$)	0.068	n.s.
	Retinyl-Stearat ($\mu\text{g/mL}$)	< 0.001	***
	Sum of Retinyl Esters ($\mu\text{g/mL}$)	< 0.05	*
	Total Vit A ($\mu\text{g/mL}$)	0.271	n.s.
	β -Carotin ($\mu\text{g/mL}$)	< 0.05	*
	α -Tocopherol / Fat (mg/g)	< 0.001	***
	Retinol / Fat (mg/g)	< 0.001	***
	Retinyl-Oleat / Fat (mg/g)	<0.01	**
	Retinyl-Palmitat /Fat (mg/g)	0.075	n.s.
	Retinyl-Stearat /Fat (mg/g)	0.784	n.s.
	Sum of Retinyl Esters / Fat (mg/g)	< 0.05	*
	Total Vit A / Fat (mg/g)	< 0.001	***
	β -Carotin / Fat (mg/g)	0.926	n.s.

5. Discussion

Temporal variations in alpaca colostrum and milk

During gestation, the embryo is unable to synthesize retinol and therefore is strongly dependent on maternal delivery of retinol or its precursors (retinyl esters and carotenes) (MARCEAU et al., 2007). However, placentation in alpacas is described as epitheliochorial and diffuse (BROWN, 2000; OLIVERA et al., 2003; MEESTERS et al., 2019) and might not allow easy transfer of fat-soluble vitamins to the foetus (GAY et al., 2004; DEBIER et al., 2005), leaving the neonatal animal fully dependent on colostrum (GAY et al., 2004; DEBIER 2007; FUJITA et al., 2011). Generally, colostrum and milk are the most valuable sources of Vitamin A and provitamin A carotenoids (e.g., TANUMIHARDJO et al., 2016) as well as Vitamin E (e.g., DEBIER et al., 2005).

In this study, clear temporal variations were found in alpaca colostrum during the first four days of lactation regarding composition of retinol and its precursors. All retinyl esters investigated showed significant differences from the first day after parturition to the following days, with a shift in the form of Vitamin A predominantly available. While retinol showed high concentrations on the first day and decreased afterwards, retinyl esters increased from day 2. Retinol can be directly absorbed in the small intestine due to the presence of CRBP (cellular retinol binding protein) in enterocytes (DEBIER and LARONDELLE, 2005), whereas retinyl esters need to be hydrolysed in the intestinal lumen before absorption is possible (TANUMIHARDJO et al., 2016). Due to the different bioavailability of different forms of Vitamin A, a possible explanation for different availability in colostrum might be: As pinocytosis and passive transfer of immunoglobulins is active in the first 24 hours postpartum but function of the intestines is not yet fully developed (BLUM and HAMMON, 2000; ONTSOUKA et al., 2016; VAN KEULEN et al., 2021), high colostrum retinol levels provide readily useable resources to stock up internal reservoirs. Since retinol bound to CRBP is protected from undesired chemical transformations (such as oxidation) (DEBIER and LARONDELLE, 2005), this way provides a secured Vitamin A supply of the new-born, lasting for the first month of life when first colostrum intake occurs at maximal 13 hours after birth (DEBIER et al., 2005).

Contrary to retinol, α -tocopherol as the most biologically active form of Vitamin E does not have a specific serum transport protein and its absorption in the small intestine is dependent on micelle formation (DEBIER et al., 2005). During the first four days after parturition, no

significant differences in α -tocopherol concentration of mammary secretion was observed, although subtle variations with slightly higher median values on the first two days of lactation were shown here. It is commonly stated that Vitamin E concentration is much higher in colostrum than it is in milk (LOUDENSLAGER et al., 1986; HIDIROGLOU 1989; DEBIER et al., 2005). However, definition of colostrum is varying. While in this study colostrum is defined as mammary secretion produced in the first four days after parturition, e.g., GAY et al. (2004) define colostrum as being produced in the first 24 hours postpartum and ABD EL-FATTAH et al. (2012) state a period of five to seven days after parturition. Colostrum interval is highly dependent on the species investigated and will be further discussed later. Also, fat content is of importance regarding the concentration of fat-soluble vitamins in colostrum and milk. Many authors state a close positive correlation of concentration of fat-soluble vitamins and fat in colostrum and milk (e.g., CSAPÓ et al., 1995; CSAPÓ et al., 1996; MARCONI and PANFILI, 1998; HADDADIN et al., 2008). However, the Vitamin E accumulation in colostrum cannot solely be explained with an increased secretion of total lipids since milk fats have shown to be similar or lower in colostrum compared to milk in various species (DEBIER et al., 2005). Based on our data and MÖSSLER et al. (2021), fat content increased significantly in the first four days of lactation and was slightly varying yet similar in the early to late lactation period. While α -tocopherol showed no clear increase with increasing concentration of fat, retinol decreased significantly with increasing fat content. The retinyl esters investigated, however, increased significantly according to the fat content observed. Since retinyl esters represent storage forms of retinol and happen to be products of retinol bound to fatty acids (specifically: esterified to fatty acyl groups) (O'BYRNE and BLANER, 2013), these results are explainable. Most publications consulted during preparation of this study did not investigate differences regarding retinol and retinyl esters but instead showed total Vitamin A or retinol. Therefore, comparison in this context is difficult. ABD EL-FATTAH et al. (2012) reported high Vitamin A levels early in the lactation period, i.e., colostrum, with a declining trend in the following days. Generally, this seems to be true for most terrestrial mammals but not for seals (DEBIER and LARONDELLE, 2005). Total Vitamin A in this study was not significantly different in the colostrum and the milk period, respectively. A subtle peak was observed in colostrum at day 4 of lactation. With a decreasing trend towards the end of lactation following, these results concur with the scientific literature (e.g., GAY et al., 2004; DEBIER and LARONDELLE, 2005; ABD EL-FATTAH et al., 2012). Some authors investigated β -carotene concentration in colostrum and milk, most often finding overall low or varying concentrations, traces or no

β -carotene at all due to transformation into retinol. Results were strongly depending on species (PARK et al., 2007; RAYNAL-LJUTOVAC et al., 2008) and other factors like breed, age, stage of lactation and nutrition (MARKIEWICZ-KĘSZYCKA et al., 2014). In this study, however, β -carotene increased significantly from the first to the fourth day of lactation, again reflecting properties of fat content in colostrum. As β -carotene originates from plant tissues (DEBIER and LARONDELLE, 2005), its content in colostrum and milk likely could be dependent on diet and environment. Vitamin E concentration of colostrum and transitional milk were variable in ABD EL-FATTAH et al., (2012), similar to the observations in this study although in our data a decreasing trend was observed from colostrum to mature milk. However, no publications concerning vitamin content of alpaca colostrum and vitamins were found, further complicating any attempts of intraspecific comparison. Interspecific comparison will be discussed later. Although pointed out before and often stated by various authors (see above), correlations of fat, protein and lactose with vitamins in this study did not show strong correlation. In this case the model used for analysis most probably was suboptimal, resulting in rather poor model fit. Nevertheless, results were significant and showed positive correlations of retinol and most retinyl esters in colostrum and of retinol, most retinyl esters and β -carotene in mature milk with fat, protein and lactose content, respectively. The positive correlation of Vitamin A and fat content is supported by results reviewed in FAYE et al. (2019). In this publication also a correlation of Vitamin E and fat content is pointed out, which is not given in our investigation.

Differences in alpaca colostrum and milk

Colostrum and milk usually are highly different regarding their composition, especially fat and protein content as well as amount of minerals, vitamins and other biologically active substances (DEBIER et al., 2005; DEBIER and LARONDELLE, 2005; GEORGIEV, 2008; ABD EL-FATTAH et al., 2012; ONTSOUKA et al., 2016). Vitamin concentrations usually are high immediately after parturition and drop rapidly in the first few days of lactation, depending on species (e.g., HIDIROGLOU, 1989; GAY et al., 2004; DEBIER and LARONDELLE, 2005). The literature data supports the results found in this study, with α -tocopherol, retinol, retinyl-stearate and β -carotene showing significant differences in colostrum and milk, respectively. Total Vitamin A did not show significant differences when measured per volume milk. However, when viewed relatively to fat content, highly significant differences were observed. Vice versa for results of β -carotene. As already

mentioned, fat content is of major importance regarding behaviour of fat-soluble vitamins. Lack of results given relative to fat content have been criticized by FAYE et al. (2019) due to the difficulty of assembling various data. Especially in comparing mammary secretions of different species with various content of fat, water and solids reference is important but prove to be relevant even in intraspecific investigations.

Differences in colostrum and milk of alpaca and other species

Comparing vitamin content of colostrum and milk of alpaca to other species comes with distinct difficulties. Mostly units used in results are not equal and sometimes are difficult to be equalized by calculation due to the need of conversion factors dependent on quality or specific gravity of the medium. In most cases, neither is stated in the publications found. An attempt to harmonize the data found was made by using average specific gravity values found in the literature for every species necessary and a conversion algorithm provided publicly. Values found for Vitamin A, E and β -carotene, respectively, were standardized to $\mu\text{g/mL}$. As a general trend in every species considered, higher colostrum vitamin levels compared to mature milk can be observed (Figures 22 and 23, high outliers found in the literature were ignored). Average total Vitamin A content in alpaca colostrum was found to be $0.44 \pm 0.21 \mu\text{g/mL}$ (mean $\pm$ SD for comparison with other species), being higher than compared to camel colostrum (STAHL et al., 2006) but lower compared to other species. Average Vitamin E level resulted in $4.62 \pm 2.39 \mu\text{g/mL}$, being considerably higher than in many other species except pig ($3.94 \pm 0.29 \mu\text{g/mL}$; CSAPÓ et al., 1996), and human (variable values from 11.89 to $16.65 \mu\text{g/mL}$). As for β -carotene, values found in alpaca colostrum ($0.0028 \pm 0.0024 \mu\text{g/mL}$) were similar as in dromedary milk ($0.0032 \mu\text{g/mL}$; STAHL et al., 2006) but clearly lower than in cow milk with ranges reported from 0.05 to $1.38 \mu\text{g/mL}$ (HIDIROGLOU, 1989; RAYNAL-LJUTOVAC et al., 2008). Harmonized results are stated in detail in Table 23. Original results are given in Table 22.

As criticized by FAYE et al. (2019) vitamin composition most often is not given in amount of vitamin per fat content. Therefore, our data can only be compared to results of GARAU et al. (2021), who reported α -tocopherol levels of $26 \mu\text{g/g}$ fat in buffalo milk and $35 \mu\text{g/g}$ fat in cow milk. Further to ABD EL-SALAM and EL-SHIBINY (2011) who reported $12.1 \mu\text{g/g}$ fat of Vitamin A and Vitamin E, respectively, in buffalo milk and KEHOE et al. (2007), who stated $77 \mu\text{g/g}$ fat in cow colostrum. In comparison, alpaca colostrum in this study contained Vitamin A values of $39 \mu\text{g/g}$ fat and α -tocopherol levels of $420 \mu\text{g/g}$ fat while mature milk is found with levels of $10 \mu\text{g/g}$ fat total Vitamin A and $30 \mu\text{g/g}$ fat α -tocopherol.

However, different methods as well as serious lack of data available or found in many species during research for this study should be considered. Furthermore, the methods used are not the same since many extraction protocols for vitamins are present in the scientific literature and are used in different laboratories of different countries. In analysis of vitamin content most often some protocol of HPLC with following spectrometry is used. In most publications viewed for this work HPLC was used except FOROUTAN et al. (2019) who tried to establish a targeted LC-MS/MS method and REVILLA et al. (2017) who investigated efficiency of NIR analysis.

Various factors mentioned by many authors potentially play a major role in alpaca milk composition as they do in other species. Dietary intake for one might influence milk composition (PARK et al., 2007; RAYNAL-LJUTOVAC et al., 2008), at least for Vitamin A (DEBIER et al., 2005). Similar as in other species, also breed and geographical location could be important factors, however, are not yet investigated in alpacas to our best knowledge. Parity and age of the mother might also be of interest in this context. While DEBIER and LARONDELLE (2005) state higher Vitamin A concentrations in colostrum and milk of primiparous female cows than multiparous ones, ABD EL-SALAM and EL-SHIBINY (2011) and ABD EL-FATTAH et al. (2012) report rising values with increasing number of lactations. Since mainly multiparous individuals were sampled in this study, a comparison of lactation number and age was not attempted. Influence of parity, along with investigation of impact of breed and geographical differences on vitamin composition of alpaca colostrum and could be a field of further research in the future.

In conclusion, all hypotheses stated in the beginning were investigated and confirmed. Vitamin content is higher in alpaca colostrum than in mature milk and differences can be shown over time, allowing to assume a transitional interval from colostrum to milk. Finally, interspecific differences could be observed, highlighting the specific needs of alpaca crias compared to offspring of other species. In respect to the rearing of orphaned crias, it can be assumed that goat and sheep milk can be used from a vitamin point of view since at least Vitamin A content is similar in the three species. However, representative values for colostrum of the two species could not be obtained and gross composition of colostrum and milk should be considered. For a strong statement on the topic, a comparative analysis of goat, sheep and alpaca colostrum and milk would be useful. Unfortunately, no information on llama vitamin composition in colostrum and milk could be developed. As the second

domesticated SAC species and being closely related to the alpaca, comparison of the two breeds would be extremely valuable.

6. Summary

As one of the two domesticated South American Camelid species, alpacas (*Vicugna pacos*) gain more and more attention around the world for their wool and as pet animals. Although using remastication, alpacas are not considered true ruminants and nutrient requirements are different. Raising orphaned alpaca offspring (crias) therefore has been a challenge for breeders and keepers. Attempts of investigation of colostrum and milk composition have been made to enable composition of a milk replacer on the long term. Gross composition of alpaca colostrum and milk considering contents of fat, protein, lactose and minerals have already been investigated by authors of different countries. Vitamin content in alpaca mammary secretion, however, have not yet been investigated. Vitamin A and Vitamin E show great importance in growth and development of neonatal and young animals. Neonates show very low serum levels of both vitamins, due to limited transplacental transfer. This makes rapid colostrum intake crucial for new-borns. The main subject of this study was to quantify Vitamin A, β -carotene and Vitamin E values in alpaca colostrum and milk. Differences over time in colostrum and milk, respectively, could be shown with higher vitamin content earlier in the lactation period. Significant differences were found between colostrum and milk. Colostrum contained 0.43 ± 0.21 $\mu\text{g/mL}$ total Vitamin A, 0.003 ± 0.002 $\mu\text{g/mL}$ β -carotene and 4.62 ± 2.39 $\mu\text{g/mL}$ Vitamin E while in mature milk 0.41 ± 0.28 $\mu\text{g/mL}$ total Vitamin A, 0.004 ± 0.003 $\mu\text{g/mL}$ β -carotene and 1.27 ± 1.41 $\mu\text{g/mL}$ Vitamin E were observed. Compared to other species, vitamin content of alpaca milk is similar to goat and sheep milk. Vitamin A level in colostrum is higher than in camel colostrum, but lower than in other species while Vitamin E level is higher than in other species except pig and human.

7. Zusammenfassung

Als eine der beiden domestizierten Arten der Neuweltkameliden gewinnt das Alpaka (*Vicugna pacos*) weltweit immer mehr an Beliebtheit und wird als Faserlieferant und Haustier gehalten. Trotz Wiederkautätigkeit und dem Vorhandensein eines Vormagensystems zählen Alpakas nicht zur Gruppe der wahren Wiederkäuer und haben einen anderen Nährstoffbedarf als solche. Das Aufziehen verwaister Alpaka-Junge (Crias) ist dementsprechend eine Herausforderung für Züchter und Halter. Untersuchungen der Zusammensetzung von Kolostrum und Milch wurden deshalb eingeleitet, mit dem Ziel der Komposition eines artspezifischen Milchaustauschers. Verschiedene Forschungsgruppen in unterschiedlichen Ländern untersuchten bereits die Zusammensetzung von Kolostrum und Milch bezüglich Fett-, Protein-, Laktose- und Mineralgehalt. Allerdings gab es bisher keine Untersuchungen des Vitamingehaltes. Vitamin A und Vitamin E sind von großer Bedeutung für Wachstum und Entwicklung neonataler und junger Tiere. Neugeborene zeigen sehr geringe Serumvitaminspiegel beider Vitamine, begründet durch limitierten transplacentären Transfer. Dadurch ist eine schnelle Versorgung mit Kolostrum essentiell. Das Hauptaugenmerk dieser Studie war die quantitative Beurteilung der Vitamin A-, β -Carotin- und Vitamin E-Gehalte in Alpaka Kolostrum und Milch. Unterschiede im zeitlichen Verlauf, mit höheren Gehalten am Beginn der Laktation, wurden beobachtet. Darüber hinaus wurden signifikante Unterschiede in Kolostrum und Milch festgestellt. Im Durchschnitt enthielt Kolostrum $0,43 \pm 0,21 \mu\text{g/mL}$ Vitamin A, $0,003 \pm 0,002 \mu\text{g/mL}$ β -Carotin und $4,62 \pm 2,39 \mu\text{g/mL}$ Vitamin E. Dagegen enthielt Milch $0,41 \pm 0,28 \mu\text{g/mL}$ Vitamin A, $0,004 \pm 0,003 \mu\text{g/mL}$ β -Carotin und $1,27 \pm 1,41 \mu\text{g/mL}$ Vitamin E. Im Vergleich zu anderen Spezies ist der Vitamingehalt in Alpakamilch ähnlich jenem von Schaf- und Ziegenmilch. Der Vitamin A-Gehalt im Kolostrum ist höher als in Kamelkolostrum, jedoch niedriger im Vergleich zu anderen Tierarten. Die Vitamin E-Konzentration ist im Verhältnis zu anderen Tierarten im Alpakakolostrum höher, in unserem Vergleich zeigen im Mittel nur Schwein und Mensch höhere Konzentrationen.

8. Acknowledgements

I would like to thank Univ.-Prof. Dr. med.vet. Thomas Wittek Dipl. ECBHM for trusting me with this challenge and the data involved and especially for his immense patience. Also I like to thank Prof. Dr. J. Raila and his Team at Potsdam University for analysing the colostrum and milk.

Thank you to Ao. Univ.-Prof. Dr. med. vet. Christine Iben Dipl. ECVCN for the incredibly fast review of this piece of work.

Many thanks to my employer Dr. Andrea Mergl, who is patient and pressing at the same time and who has taught me so much.

Thank you to Helmuth and Charlotte Mergl for keeping work away whenever school was more important.

Thanks to Fritz and Resi Höfer for introducing me into that one-of-a-kind family business.

Many thanks to Dominik Strasser, who jumps whenever I ask for anything.

And finally, the greatest “Thank you” imaginable to my mother, Veronika, without whom I would never ever have taken that step.

9. References

- ABD EL-FATTAH, A.M., ABD RABO, F.H., EL-DIEB, S.M. and EL-KASHEF, H.A. (2012): Changes in composition of colostrum of Egyptian buffaloes and Holstein cows. *BMC Vet Res* 8, 19.
- ABD EL-SALAM, M.H. and EL-SHIBINY, S. (2011): A comprehensive review on the composition and properties of buffalo milk. *Dairy Science and Technol* 91, 663–699.
- AHMED, A. A., AWAD, Y. L., and FAHMY, F. (1977). Studies on some minor constituents of camel's milk [Egypt]. *Vet. Med. J. Assiut Univ.*, 25: 51-54.
- AUESTAD, N., KORSAK, R.A., BERGSTROM, J.D. and EDMOND, J. (1989): Milk-substitutes comparable to rat's milk; their preparation, composition and impact on development and metabolism in the artificially reared rat. *Br J Nutr* 61, 495–518.
- AYUSO, M., ÓVILO, C., FERNÁNDEZ, A., NUÑEZ, Y., ISABEL, B., DAZA, A., LÓPEZ-BOTE, C.J. and REY, A.I. (2015): Effects of dietary vitamin A supplementation or restriction and its timing on retinol and α -tocopherol accumulation and gene expression in heavy pigs. *Animal Feed Science and Technology* 202, 62–74.
- BENMEZIANE – DERRADJI, F. (2021): Evaluation of camel milk: gross composition—a scientific overview. *Trop Anim Health Prod* 53, 308.
- BLUM, J.W. and HAMMON, H. (2000): Colostrum effects on the gastrointestinal tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. *Livestock Production Science* 66, 151–159.
- BROWN, B.W. (2000): A review on reproduction in South American camelids. *Animal Reproduction Science* 58, 169–195.
- CHAD, E.K., DEPETERS, E.J., PUSCHNER, B., TAYLOR, S.J. and ROBISON, J. (2014): Preliminary investigation of the composition of alpaca (*Vicugna pacos*) milk in California. *Small Ruminant Research* 117, 165–168.
- CHANDA, R., and OWEN, E. C. (1952). The effect of thyroxine and thiouracil on the composition of milk. 2. The carotene and vitamin A content. *Biochemical Journal*, 51(3), 404.
- CHEW, B.P. (1996): Importance of antioxidant vitamins in immunity and health in animals. *Animal Feed Science and Technology* 59, 103–114.
- CLAEYS, W.L., VERRAES, C., CARDOEN, S., DE BLOCK, J., HUYGHEBAERT, A., RAES, K., DEWETTINCK, K. and HERMAN, L. (2014): Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits. *Food Control* 42, 188–201.
- CSAPÓ, J., MARTIN, T.G., CSAPÓ-KISS, Z.S. and HÁZAS, Z. (1996): Protein, fats, vitamin and mineral concentrations in porcine colostrum and milk from parturition to 60 days. *International Dairy Journal* 6, 881–902.
- CSAPÓ, J., STEFLER, J., MARTIN, T.G., MAKRAY, S. and CSAPÓ-KISS, Zs. (1995): Composition of mares' colostrum and milk. Fat content, fatty acid composition and vitamin content. *International Dairy Journal* 5, 393–402.

- DEBIER, C. and LARONDELLE, Y. (2005): Vitamins A and E: metabolism, roles and transfer to offspring. *Br J Nutr* 93, 153–174.
- DEBIER, C., POTTIER, J., GOFFE, Ch. and LARONDELLE, Y. (2005): Present knowledge and unexpected behaviours of vitamins A and E in colostrum and milk. *Livestock Production Science* 98, 135–147.
- DEBIER, C. (2007). Vitamin E during pre-and postnatal periods. *Vitamins and Hormones*, 76, 357-373.
- DOWLING, J.E. (2020): Vitamin A: its many roles—from vision and synaptic plasticity to infant mortality. *J Comp Physiol A* 206, 389–399.
- ELÍAS-ARNANZ, M. (2020). Anaerobic bacteria need their vitamin B12 to digest estrogen. *Proceedings of the National Academy of Sciences*, 117(4), 1833-1835.
- ELMADFA, I. and MEYER, A.L. (2012): Vitamins for the First 1000 Days: Preparing for Life. *International Journal for Vitamin and Nutrition Research* 82, 342–347.
- ERICK, M. (2018): Breast milk is conditionally perfect. *Medical Hypotheses* 111, 82–89.
- FARAH, Z. (1993): Composition and characteristics of camel milk. *Journal of Dairy Research* 60, 603–626.
- FARAH, Z., RETTENMAIER, R. and ATKINS, D. (1992): Vitamin Content of Camel Milk. *Internat J Vit Nutr Res* 30–33.
- FAYE, B., KONUSPAYEVA, G. and BENGOUNI, M. (2019): Vitamins of camel milk: a comprehensive review. *KZB* 17–32.
- FOROUTAN, A., GUO, A.C., VAZQUEZ-FRESNO, R., LIPFERT, M., ZHANG, L., ZHENG, J., BADRAN, H., BUDINSKI, Z., MANDAL, R., AMETAJ, B.N. and WISHART, D.S. (2019): Chemical Composition of Commercial Cow's Milk. *J Agric Food Chem* 67, 4897–4914.
- FUJITA, M., SHELL-DUNCAN, B., NDEMWA, P., BRINDLE, E., LO, Y.-J., KOMBE, Y. and O'CONNOR, K. (2011): Vitamin A dynamics in breastmilk and liver stores: A life history perspective. *Am J Hum Biol* 23, 664–673.
- GAULY, M. (2011). Zoologie, Domestikation und Verbreitung von Neuweltkameliden. *Neuweltkameliden: Haltung, Zucht, Erkrankungen; Gaily, M., Vaughan, J., Cebra, C., Eds*, 13-18.
- GARAU, V., MANIS, C., SCANO, P., and CABONI, P. (2021). Compositional characteristics of mediterranean buffalo milk and whey. *Dairy*, 2(3), 469-488.
- GAY, L.S., KRONFELD, D.S., GRIMSLEY-COOK, A., DASCANIO, J.J., ORDAKOWSKI-BURK, A.O., SPLAN, R.K., DUNNINGTON, E.A. and SKLAN, D.J. (2004): Retinol, β -carotene and β -tocopherol concentrations in mare and foal plasma and in colostrum. *Journal of Equine Veterinary Science* 24, 115–120.
- GEORGIEV, I.P. (2008): DIFFERENCES IN CHEMICAL COMPOSITION BETWEEN COW COLOSTRUM AND MILK. 11.
- GEROSA, S., and SKOET, J. (2012). Milk availability: trends in production and demand and medium-term outlook. *ESA Working Paper*. No 12-01, 1-40.

- GÓMEZ, E., CAAMAÑO, J., RODRÍGUEZ, A., DE FRUTOS, C., FACAL, N. and DÍEZ, C. (2006): Bovine Early Embryonic Development and Vitamin A. *Reprod Domest Anim* 41, 63–71.
- GREEFF, A. DE, RESINK, J.W., HEES, H.M.J. VAN, RUULS, L., KLAASSEN, G.J., ROUWERS, S.M.G. and STOCKHOFE-ZURWIEDEN, N. (2016): Supplementation of piglets with nutrient-dense complex milk replacer improves intestinal development and microbial fermentation¹. *Journal of Animal Science* 94, 1012–1019.
- HADDADIN, M.S., GAMMOH, S.I. and ROBINSON, R.K. (2008): Seasonal variations in the chemical composition of camel milk in Jordan. *Journal of Dairy Research* 75, 8–12.
- HÅKANSSON, J., HAKKARAINEN, J., and LUNDEHEIM, N. (2001). Variation in vitamin E, glutathione peroxidase and retinol concentrations in blood plasma of primiparous sows and their piglets, and in vitamin E, selenium and retinol contents in sows' milk. *Acta Agriculturae Scandinavica, Section A-Animal Science*, 51(4), 224-234.
- HAUG, A., HØSTMARK, A. T., and HARSTAD, O. M. (2007). Bovine milk in human nutrition—a review. *Lipids in health and disease*, 6(1), 1-16.
- HEINRICHS, A.J., WELLS, S.J. and LOSINGER, W.C. (1995): A Study of the Use of Milk Replacers for Dairy Calves in the United States. *Journal of Dairy Science* 78, 2831–2837.
- HIDIROGLOU, M. (1989): Mammary Transfer of Vitamin E in Dairy Cows. *Journal of Dairy Science* 72, 1067–1071.
- HILL, F.I., THOMPSON, K.G. and GRACE, N.D. (1994): Rickets in alpacas (*Lama paces*) in New Zealand. *New Zealand Veterinary Journal* 42, 229–232.
- IBRAHIM, A. H., and KHALIFA, S. A. (2015). The effects of various stabilizers on physiochemical properties of camel's milk yoghurt. *Journal of American Science*, 11(1), 15-24.
- JENKINS, G.N. and YUDKIN, J. (1943): Vitamins and Physiological Function. *BMJ* 2, 265–266.
- KADWELL, M., FERNANDEZ, M., STANLEY, H.F., BALDI, R., WHEELER, J.C., ROSADIO, R. and BRUFORD, M.W. (2001): Genetic analysis reveals the wild ancestors of the llama and the alpaca. *Proc R Soc Lond B* 268, 2575–2584.
- KEHOE, S. I., JAYARAO, B. M., and HEINRICHS, A. J. (2007). A survey of bovine colostrum composition and colostrum management practices on Pennsylvania dairy farms. *Journal of dairy science*, 90(9), 4108-4116.
- KERTZ, A.F., PREWITT, L.R. and EVERETT, J.P. (1979): An Early Weaning Calf Program: Summarization and Review. *Journal of Dairy Science* 62, 1835–1843.
- KEULEN, P. VAN, MCCOARD, S.A., DIJKSTRA, J., SWANSSON, H. and KHAN, M.A. (2021): Effect of postpartum collection time and colostrum quality on passive transfer of immunity, performance, and small intestinal development in preweaning calves. *Journal of Dairy Science* 104, 11931–11944.
- KOFLER, J. and WITTEK, T. (2015): Rachitis bei einem Huacaya Alpaka mit braunem Fell - Fallbericht. *Wiener Tierärztliche Monatsschrift* 8.
- KONUSPAYEVA, G., BR, br, FAYE, B. and MUSSAAD, and. (2014): Some lipid components of the camel milk and blood in intensive farm in Saudi Arabia. *Emir J Food Agric* 26, 349.

- LAL, D., and NARAYANAN, K. M. (1984). Effect of lactation number on the poly-unsaturated fatty acids and oxidative stability of milk fats. *Asian J Dairy Res* 3:37–40.
- LAWRENCE, R. A., and LAWRENCE, R. M. (2005). Breastfeeding. A guide for the medical profession 6 th ed. *St. Louis: Mosby*.
- LEVÊQUES, A., OBERSON, J.-M., TISSOT, E.A., REDEUIL, K., THAKKAR, S.K. and CAMPOS-GIMÉNEZ, E. (2019): Quantification of Vitamins A, E, and K and Carotenoids in Submilliliter Volumes of Human Milk. *Journal of AOAC INTERNATIONAL* 102, 1059–1068.
- LIMA, M.S.R., SILVA RIBEIRO, K.D. DA, PIRES, J.F., BEZERRA, D.F., BELLOT, P.E.N.R., OLIVEIRA WEIGERT, L.P. DE and DIMENSTEIN, R. (2017): Breast milk retinol concentration in mothers of preterm newborns. *Early Human Development* 106–107, 41–45.
- LIU, H., GUO, X., ZHAO, Q., QIN, Y., and ZHANG, J. (2020). Lipidomics analysis for identifying the geographical origin and lactation stage of goat milk. *Food chemistry*, 309, 125765.
- LOUDENSLAGER, M. J., KU, P. K., WHETTER, P. A., ULLREY, D. E., WHITEHAIR, C. K., STOWE, H. D., and MILLER, E. R. (1986). Importance of diet of dam and colostrum to the biological antioxidant status and parenteral iron tolerance of the pig. *Journal of Animal Science*, 63(6), 1905-1914.
- MAHAN, D. C., and VALLET, J. L. (1997). Vitamin and mineral transfer during fetal development and the early postnatal period in pigs. *Journal of animal science*, 75(10), 2731-2738.
- MAQBOOL, M.A., ASLAM, M., AKBAR, W. and IQBAL, Z. (2017): Biological importance of vitamins for human health: A review. 02, 10.
- MARCEAU, G., GALLOT, D., LEMERY, D., and SAPIN, V. (2007). Metabolism of retinol during mammalian placental and embryonic development. *Vitamins and Hormones*, 75, 97-115.
- MARCONI, E. and PANFILI, G. (1998): Chemical Composition and Nutritional Properties of Commercial Products of Mare Milk Powder. *Journal of Food Composition and Analysis* 11, 178–187.
- MARKIEWICZ-KĘSZYCKA, M., WÓJTOWSKI, J., CZYŻAK-RUNOWSKA, G., KUCZYŃSKA, B., PUPPEL, K., KRZYŻEWSKI, J., ... and BAGNICKA, E. (2014). Concentration of selected fatty acids, fat-soluble vitamins and β -carotene in late lactation mares' milk. *International Dairy Journal*, 38(1), 31-36.
- MARTINI, M., ALTOMONTE, I., SILVA SANT'ANA, A.M. DA, DEL PLAVIGNANO, G. and SALARI, F. (2015): Gross, mineral and fatty acid composition of alpaca (*Vicugna pacos*) milk at 30 and 60 days of lactation. *Small Ruminant Research* 132, 50–54.
- MATTE, J. J., and AUDET, I. (2020). Maternal perinatal transfer of vitamins and trace elements to piglets. *Animal*, 14(1), 31-38.
- MEDHAMMAR, E., WIJESINHA-BETTONI, R., STADLMAYR, B., NILSSON, E., CHARRONDIÈRE, U.R. and BURLINGAME, B. (2012): Composition of milk from minor dairy animals and buffalo breeds: a biodiversity perspective: Composition of milk from minor dairy animals and buffalo breeds. *J Sci Food Agric* 92, 445–474.
- MEESTERS, M., OPSOMER, G. and GOVAERE, J. (2019): Macroscopic evaluation of the placenta of the alpaca (*Vicugna pacos*). *Reprod Dom Anim* 54, 996–1002.

- MORRILL, K. M., CONRAD, E., LAGO, A., CAMPBELL, J., QUIGLEY, J., and TYLER, H. (2012). Nationwide evaluation of quality and composition of colostrum on dairy farms in the United States. *Journal of dairy science*, 95(7), 3997-4005.
- MÖSSLER, M., AICHNER, J., MÜLLER, A., ALBERT, T. and WITTEK, T. (2021): Concentrations of Fat, Protein, Lactose, Macro and Trace Minerals in Alpaca Colostrum and Milk at Different Lactation Stages. *Animals* 11, 1955.
- MUSAEV, A., SADYKOVA, S., ANAMBAYEVA, A., SAIZHANOVA, M., BALKANAY, G. and KOLBAEV, M. (2021): Mare's Milk: Composition, its Properties and Uses in Medicine. Archives of Razi Institute.
- NATVIG, H. (1941). The importance of vitamins for the resistance of the organism against bacterial infections. *The importance of vitamins for the resistance of the organism against bacterial infections.*, (2).
- O'BYRNE, S.M. and BLANER, W.S. (2013): Retinol and retinyl esters: biochemistry and physiology. *Journal of Lipid Research* 54, 1731–1743.
- OLIVERA, L., ZAGO, D., LEISER, R., JONES, C. and BEVILACQUA, E. (2003): Placentation in the alpaca *Lama pacos*. *Anatomy and Embryology* 207, 45–62.
- ONTSOUKA, E.C., ALBRECHT, C. and BRUCKMAIER, R.M. (2016): Invited review: Growth-promoting effects of colostrum in calves based on interaction with intestinal cell surface receptors and receptor-like transporters. *Journal of Dairy Science* 99, 4111–4123.
- PANDYA, A.J. and GHODKE, K.M. (2007): Goat and sheep milk products other than cheeses and yoghurt. *Small Ruminant Research* 68, 193–206.
- PARK, Y.W., JUÁREZ, M., RAMOS, M. and HAENLEIN, G.F.W. (2007): Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research* 68, 88–113.
- PARRAGUEZ, V. H., LATORRE, M. T. E., FERRANDO, G., and RAGGI, L. A. (2003). Milk composition in alpaca (*Lama pacos*): comparative study in two regions of Chile. *Archivos de Zootecnia*, 52(200), 431-439.
- PULIDO, E., FERNÁNDEZ, M., PRIETO, N., VI, R. B., ANDRÉS, S., LÓPEZ, S., and GIRÁLDEZ, F. J. (2019). Effect of milking frequency and α -tocopherol plus selenium supplementation on sheep milk lipid composition and oxidative stability. *Journal of dairy science*, 102(4), 3097-3109.
- QUIGLEY III, J. D., and BERNARD, J. K. (1995). Effects of addition of vitamin E to colostrum on serum α -tocopherol and immunoglobulin concentrations in neonatal calves. *Food and Agricultural Immunology*, 7:3, 295-298.
- RAYNAL-LJUTOVAC, K., LAGRIFFOUL, G., PACCARD, P., GUILLET, I. and CHILLIARD, Y. (2008): Composition of goat and sheep milk products: An update. *Small Ruminant Research* 79, 57–72.
- REVILLA, I., ESCUREDO, O., GONZÁLEZ-MARTÍN, M.I. and PALACIOS, C. (2017): Fatty acids and fat-soluble vitamins in ewe's milk predicted by near infrared reflectance spectroscopy. Determination of seasonality. *Food Chemistry* 214, 468–477.
- SALIMEI, E. and FANTUZ, F. (2012): Equid milk for human consumption. *International Dairy Journal* 24, 130–142.

- SAUN, R.J.V. Vitamin D and phosphorus interrelationships in alpacas. 5.
- SAWAYA, W.N., KHALIL, J.K., AL-SHALHAT, A. and AL-MOHAMMAD, H. (1984): Chemical Composition and Nutritional Quality of Camel Milk. *J Food Science* 49, 744–747.
- SCROGGINS, S. What Should You Put in the Cria Baby Bottle? *Alpacas Magazine* 114–117.
- SINGH, R., Mal, G., KUMAR, D., PATIL, N. V., and PATHAK, K. M. L. (2017). Camel milk: An important natural adjuvant. *Agricultural research*, 6(4), 327-340.
- SMITH, A.G., CROFT, M.T., MOULIN, M. and WEBB, M.E. (2007): Plants need their vitamins too. *Current Opinion in Plant Biology* 10, 266–275.
- SOBERON, F., RAFFRENATO, E., EVERETT, R.W. and VAN AMBURGH, M.E. (2012): Preweaning milk replacer intake and effects on long-term productivity of dairy calves. *Journal of Dairy Science* 95, 783–793.
- SORRENTINO, E., SALIMEI, E., SUCCI, M., GAMMARIELLO, D., Di CRISCIO, T., PANFILI, G., and COPPOLA, R. (2006). Heat treatment of ass's milk, a hypoallergenic food for infancy. *Technological innovation and enhancement of marginal products*, 569 – 574.
- SOUZA, G., DOLINSKY, M., MATOS, A., CHAGAS, C. and RAMALHO, A. (2015): Vitamin A concentration in human milk and its relationship with liver reserve formation and compliance with the recommended daily intake of vitamin A in pre-term and term infants in exclusive breastfeeding. *Arch Gynecol Obstet* 291, 319–325.
- STAHL, T., SALLMANN, H. P., DUEHLMEIER, R., and WERNERY, U. (2006). Selected vitamins and fatty acid patterns in dromedary milk and colostrum. *Journal of Camel Practice and Research*, 13(1), 53-57.
- STIEGER-VANEGAS, S., GARRET, R., MCKENZIE, E. and LÖHR, C. (2013): Vertebral fractures in two alpaca crias with rickets syndrome. *Aust Vet J* 91, 437–440.
- TANUMIHARDJO, S.A., RUSSELL, R.M., STEPHENSEN, C.B., GANNON, B.M., CRAFT, N.E., HASKELL, M.J., LIETZ, G., SCHULZE, K. and RAITEN, D.J. (2016): Biomarkers of Nutrition for Development (BOND)—Vitamin A Review. *J Nutr* 146, 1816S-1848S.
- TRABER, M.G. (2014): Vitamin E Inadequacy in Humans: Causes and Consequences. *Advances in Nutrition* 5, 503–514.
- VAN SAUN, R.J. (2006): Nutrient requirements of South American camelids: A factorial approach. *Small Ruminant Research* 61, 165–186.
- VINCENZETTI, S., SANTINI, G., POLZONETTI, V., PUCCIARELLI, S., KLIMANOVA, Y. and POLIDORI, P. (2021): Vitamins in Human and Donkey Milk: Functional and Nutritional Role. *Nutrients* 13, 1509.
- VOGT, E. (1928). The Importance of Vitamins during the First Period of Life. *Klinische Wochenschrift*, 7, 1941-4.
- WANG, S. Y., LIANG, J. P., SHAO, W. J., and WEN, H. (2011). Mineral, vitamin and fatty acid contents in the camel milk of dromedaries in the Anxi Gansu China. *Journal of Camel Practice and Research*, 18(2), 273-276.
- WEST, K.P. Vitamin A Deficiency Disorders in Children and Women. 13.

WINTERGERST, E.S., MAGGINI, S. and HORNIG, D.H. (2007): Contribution of Selected Vitamins and Trace Elements to Immune Function. *Ann Nutr Metab* 51, 301–323.

WOLTER, B.F., ELLIS, M., CORRIGAN, B.P. and DEDECKER, J.M. (2002): The effect of birth weight and feeding of supplemental milk replacer to piglets during lactation on preweaning and postweaning growth performance and carcass characteristics¹. *Journal of Animal Science* 80, 301–308.

Y. DE VRIES, J., PUNDIR, S., MCKENZIE, E., KEIJER, J. and KUSSMANN, M. (2018): Maternal Circulating Vitamin Status and Colostrum Vitamin Composition in Healthy Lactating Women—A Systematic Approach. *Nutrients* 10, 687.

ZHANG, H., YAO, J., ZHAO, D., LIU, H., LI, J. and GUO, M. (2005): Changes in Chemical Composition of Alxa Bactrian Camel Milk During Lactation. *Journal of Dairy Science* 88, 3402–3410.

10. Index of figures

Figure 1 Comparison of average Vitamin A levels in colostrum and milk of alpaca, camel, cow, buffalo, goat, sheep, pig, horse, donkey, and human.....	16
Figure 2 Comparison of average Vitamin E levels in colostrum and milk of alpaca, camel, cow, buffalo, goat, sheep, pig, horse, donkey, and human.....	16
Figure 3 Boxplot of α -tocopherol concentration in alpaca colostrum samples (n = 20) during four days postpartum. No significant differences were observed.....	23
Figure 4 Boxplot of retinol concentration in alpaca colostrum samples (n = 20) during four days postpartum. Significant differences are marked with different indices. a = significantly different from all other days (p < 0.001), b = significantly different from a and c (p < 0.01), c = significantly different from day 1 and day 2 (p < 0.01).....	23
Figure 5 Boxplot of retinyl-oleate concentration in alpaca colostrum samples (n = 20) during four days postpartum. Significant differences are marked with different indices. a = significantly different from b (p < 0.01).....	24
Figure 6 Boxplot of retinyl-palmitate concentration in alpaca colostrum samples (n = 20) during four days postpartum. Significant differences are marked with different indices. a = different from b (p < 0.01).....	24
Figure 7 Boxplot of retinyl-stearate concentration in alpaca colostrum samples (n = 20) during four days postpartum. Significant differences are marked with different indices. a = different from b (p < 0.05).....	25
Figure 8 Boxplot of concentration of the sum of retinyl esters contained in alpaca colostrum samples (n = 20) during four days postpartum. Significant differences are marked with different indices. a = different from b (p < 0.01).....	25
Figure 9 Boxplot of total Vitamin A concentration in alpaca colostrum samples (n = 20) during four days postpartum. No significant differences were observed.....	26
Figure 10 Boxplot of β -carotene concentration in alpaca colostrum samples (n = 20) during four days postpartum. Significant differences are marked with different indices. a = different from b (p < 0.05).....	26
Figure 11 Correlation of colostrum fat (n = 20) with retinol (1), retinyl-oleate (2), retinyl-stearate (3) and retinyl-palmitate (4). Linear regression lines and 95 % confidence intervals are shown. Descriptive parameters are given in the graphs. Note differences in axis scaling.....	29
Figure 12 Correlation of colostrum concentration of fat (n = 20) with sum of retinyl esters (1), total vitamin A (2) and correlation of concentration of protein with retinol (3) and retinyl- oleate (4). Linear egression lines and 95 % confidence intervals are shown. Descriptive parameters are given in the graphs. Note differences in axis scaling.....	30
Figure 13 Correlation of colostrum concentration of protein (n = 20) with retinyl-stearate (1), retinyl-palmitate (2), sum of retinyl esters (3) and total vitamin A (4). Linear egression lines and 95 % confidence intervals are shown. Descriptive parameters are given in the graphs. Note differences in axis scaling.....	31

Figure 14 Boxplot of α -tocopherol concentration in alpaca milk samples (n = 17) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.....	35
Figure 15 Boxplot of retinol concentration in alpaca milk samples (n = 17) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.....	36
Figure 16 Boxplot of retinyl-oleate concentration in alpaca milk samples (n = 17) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.....	36
Figure 17 Boxplot of retinyl-palmitate concentration in alpaca milk samples (n = 17) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.....	37
Figure 18 Boxplot of retinyl-stearate concentration in alpaca milk samples (n = 17) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.....	37
Figure 19 Boxplot of concentration of sum of retinyl esters in alpaca milk samples (n = 17) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.....	38
Figure 20 Boxplot of total vitamin A concentration in alpaca milk samples (n = 17) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.....	38
Figure 21 Boxplot of β -carotene concentration in alpaca milk samples (n = 17) during four months into lactation. Median, first and third quartile, and outliers are shown. No significant differences were observed.....	39
Figure 22 Correlation of milk concentration of fat (n = 17) with retinol (1), retinyl-oleate (2), sum of retinyl esters (3) and correlation of concentration of lactose (n = 17) and retinol (4). Linear egression lines and 95 % confidence intervals are shown. Descriptive parameters are given in the graphs. Note differences in axis scaling.....	42
Figure 23 Boxplot of concentration of α -tocopherol in alpaca colostrum (n = 20) and milk (n = 17). Whiskers indicate first and third quartile, dots resemble 5 th and 95 th percentiles. Concentrations are significantly different between groups (p < 0.001).....	43

11. Index of tables

Table 1 Minimum, maximum, median, first and third quartile of colostrum composition (n = 20) two to four hours after parturition.....	20
Table 2 Minimum, maximum, median, first and third quartile of colostrum composition (n = 20) on the second day after parturition.....	21
Table 3 Minimum, maximum, median, first and third quartile of colostrum composition (n = 20) on day 3 after parturition.....	21
Table 4 Minimum, maximum, median, first and third quartile of colostrum composition (n = 20) on day 4 after parturition.....	22
Table 5 Minimum, maximum, median, first and third quartile of milk composition (n = 17) at the first sampling date. Mares were between 2 and 80 in lactation at that moment.....	32
Table 6 Minimum, maximum, median, first and third quartile of milk composition (n = 17) at the second sampling date. Mares were between 2 and 109 in lactation at that moment.....	33
Table 7 Minimum, maximum, median, first and third quartile of milk composition (n = 17) at the third sampling date. Mares were between 30 and 137 in lactation at that moment.....	33
Table 8 Minimum, maximum, median, first and third quartile of milk composition (n = 17) at the fourth sampling date. Mares were between 86 and 137 in lactation at that moment.....	34
Table 9 Results obtained from simple linear regressions of concentration of fat and vitamins in alpaca milk (n = 17). Fat (%) was used as predictor, vitamins (µg/mL), respectively, as response variable.....	40
Table 10 Results obtained from simple linear regressions of concentration of protein and vitamins in alpaca milk (n = 17). Protein (%) was used as predictor, vitamins (µg/mL), respectively, as response variable.....	41
Table 11 Results obtained from simple linear regressions of concentration of lactose and vitamins in alpaca milk (n = 17). Lactose (%) was used as predictor, vitamins (µg/mL), respectively, as response variable.....	41
Table 12 Overview of statistical analysis of comparison between alpaca colostrum (n = 20) and milk (n = 17).....	44
Table 13 Results obtained from simple linear regressions of concentration of fat and vitamins in alpaca colostrum (n = 20). Fat (%) was used as predictor, vitamins (µg/mL), respectively, as response variable.....	65
Table 14 Results obtained from simple linear regressions of concentration of protein and vitamins in alpaca colostrum (n = 20). Protein (%) was used as predictor, vitamins (µg/mL), respectively, as response variable.....	65
Table 15 Results obtained from simple linear regressions of concentration of lactose and vitamins in alpaca colostrum (n = 20). Lactose (%) was used as predictor, vitamins (µg/mL), respectively, as response variable.....	65

Table 16 Detailed overview of statistical analysis of composition of alpaca colostrum (n = 20) via Kruskal-Wallis H and Mann-Whitney U multiple comparisons as post-hoc analyses.....	66
Table 17 Detailed overview of statistical analysis of composition of alpaca colostrum (n = 20) via Kruskal-Wallis H and Mann-Whitney U multiple comparisons as post-hoc analyses.....	67
Table 18 Minimum, maximum, median, first and third quartile of milk composition (n = 17) in the first month of lactation (data grouped per days in lactation). Lactation days 7 to 30 are given.....	68
Table 19 Minimum, maximum, median, first and third quartile of milk composition (n = 17) in the second month of lactation (data grouped per days in lactation). Lactation days 31 to 60 are given.....	68
Table 20 Minimum, maximum, median, first and third quartile of milk composition (n = 17) in the third month of lactation (data grouped per days in lactation). Lactation days 61 to 90 are given.....	69
Table 21 Minimum, maximum, median, first and third quartile of milk composition (n = 17) in the fourth month of lactation (data grouped per days in lactation). Lactation days 91 to 137 are given.....	69
Table 22 Overview of vitamin content and methods found in literature. Units are given as stated in the original publication.....	70, 71
Table 23 Calculated data from literature harmonized to µg/mL for easier comparison.....	72, 73

12. Appendix

Table 13 Results obtained from simple linear regressions of concentration of fat and vitamins in alpaca colostrum ($n = 20$). Fat (%) was used as predictor, vitamins ($\mu\text{g/mL}$), respectively, as response variable.

Fat					
Vitamin	Equation	y_0	a	R^2	p
α -Tocopherol	$f = y_0 + a \cdot x$	4.56	0.02	0.01	0.85
Retinol		0.27	0.04	0.35	<0.001
Retinyl-Oleate		0.01	0.00	0.34	<0.001
Retinyl-Palmitate		0.04	0.06	0.55	<0.001
Retinyl-Stearate		0.00	0.01	0.29	<0.001
Σ Retinylesters		0.05	0.08	0.54	<0.001
Total Vitamin A		0.32	0.04	0.21	<0.001
β -Carotin		0.00	0.00	0.11	0.75

Table 14 Results obtained from simple linear regressions of concentration of protein and vitamins in alpaca colostrum ($n = 20$). Protein (%) was used as predictor, vitamins ($\mu\text{g/mL}$), respectively, as response variable.

Protein					
Vitamin	Equation	y_0	a	R^2	p
α -Tocopherol	$f = y_0 + a \cdot x$	3.91	0.05	0.02	0.25
Retinol		-0.10	0.02	0.66	<0.001
Retinyl-Oleate		0.04	0.00	0.23	<0.001
Retinyl-Palmitate		0.48	-0.02	0.34	<0.001
Retinyl-Stearate		0.07	0.00	0.15	<0.001
Σ Retinylesters		0.59	-0.02	0.32	<0.001
Total Vitamin A		0.49	0.00	0.01	0.36
β -Carotin		0.00	<0.001	0.01	0.37

Table 15 Results obtained from simple linear regressions of concentration of lactose and vitamins in alpaca colostrum ($n = 20$). Lactose (%) was used as predictor, vitamins ($\mu\text{g/mL}$), respectively, as response variable.

Lactose					
Vitamin	Equation	y_0	a	R^2	p
α -Tocopherol	$f = y_0 + a \cdot x$	6.24	-0.35	0.02	0.24
Retinol		0.52	-0.08	0.24	<0.001
Retinyl-Oleate		-0.01	0.01	0.11	<0.01
Retinyl-Palmitate		-0.13	0.08	0.13	<0.01
Retinyl-Stearate		-0.03	0.01	0.07	<0.05
Σ Retinylesters		-0.17	0.09	0.13	<0.01
Total Vitamin A		0.36	0.02	0.01	0.50
β -Carotin		0.00	<0.001	0.00	0.93

Table 16 Detailed overview of statistical analysis of composition of alpaca colostrum (n = 20) via Kruskal-Wallis H and Mann-Whitney U multiple comparisons as post-hoc analyses.

	Test	df	p			
	Kruskal-Wallis H	3	0.83			
		3	see below			
α-Tocopherol	Multiple Comparisons	day 1	day 1	day 2	day 3	day 4
		day 1		0.99	0.99	0.99
		day 2	0.99		0.99	0.99
		day 3	0.99	0.99		0.99
		day 4	0.99	0.99	0.99	
	Kruskal-Wallis H	3	<0.001			
		3	see below			
Retinol	Multiple Comparisons	day 1	day 1	day 2	day 3	day 4
		day 1		<0.001	<0.001	<0.001
		day 2	<0.001		0.12	<0.01
		day 3	<0.001	0.12		0.12
		day 4	<0.001	<0.01	0.12	
	Kruskal-Wallis H	3	<0.001			
		3	see below			
Retinyl-Oleate	Multiple Comparisons	day 1	day 1	day 2	day 3	day 4
		day 1		0.09	<0.01	<0.01
		day 2	0.09		0.55	0.40
		day 3	<0.01	0.55		0.40
		day 4	<0.01	0.40	0.40	
	Kruskal-Wallis H	3	<0.001			
		3	see below			
Retinyl-Palmitate	Multiple Comparisons	day 1	day 1	day 2	day 3	day 4
		day 1		<0.01	0.13	0.13
		day 2	<0.01		0.13	0.13
		day 3	<0.001	0.13		0.13
		day 4	<0.01	0.13	0.13	
	Kruskal-Wallis H	3	<0.001			
		3	see below			
Retinyl-Stearate	Multiple Comparisons	day 1	day 1	day 2	day 3	day 4
		day 1		<0.05	<0.001	<0.01
		day 2	<0.05		0.11	0.08
		day 3	<0.001	0.11		0.61
		day 4	<0.01	0.08	0.61	
	Kruskal-Wallis H	3	<0.001			
		3	see below			
Σ Retinylester	Multiple Comparisons	day 1	day 1	day 2	day 3	day 4
		day 1		<0.01	<0.001	<0.001
		day 2	<0.01		0.13	
		day 3	<0.001	0.13		0.10
		day 4	<0.001	0.59	0.10	
	Kruskal-Wallis H	3	0.14			
		3	see below			
Total Vitamin A	Multiple Comparisons	day 1	day 1	day 2	day 3	day 4
		day 1		0.64	0.92	0.15
		day 2	0.64		0.64	0.15
		day 3	0.92	0.64		0.30
		day 4	0.15	0.15	0.30	
	Kruskal-Wallis H	3	<0.01			
		3	see below			
β-Carotin	Multiple Comparisons	day 1	day 1	day 2	day 3	day 4
		day 1		0.06	<0.05	<0.01
		day 2	0.06		0.58	0.06
		day 3	<0.05	0.58		
		day 4	<0.01	0.06	0.06	

Table 17 Detailed overview of statistical analysis of composition of alpaca colostrum (n = 20) via Kruskal-Wallis H and Mann-Whitney U multiple comparisons as post-hoc analyses.

		Test	df	p			
α-Tocopherol/Fat	Multiple Comparisons	Kruskal-Wallis H	3	< 0.001			
			3	see below			
			day 1	day 2	day 3	day 4	
			< 0.001	< 0.001	< 0.001	< 0.001	
	Multiple Comparisons		day 1	< 0.001	< 0.05	< 0.001	
			day 2	< 0.001	< 0.05	< 0.001	
			day 3	< 0.001	< 0.05	< 0.05	
			day 4	< 0.001	< 0.001	< 0.05	
Retinol/Fat	Multiple Comparisons	Kruskal-Wallis H	3	<0.001			
			3	see below			
			day 1	day 2	day 3	day 4	
			< 0.001	< 0.001	< 0.001	< 0.001	
	Multiple Comparisons		day 1	< 0.001	< 0.05	< 0.01	
			day 2	< 0.001	< 0.05	< 0.01	
			day 3	< 0.001	< 0.05	< 0.01	
			day 4	< 0.001	< 0.001	< 0.01	
Retinyl-Oleate/Fat	Multiple Comparisons	Kruskal-Wallis H	3	<0.01			
			3	see below			
			day 1	day 2	day 3	day 4	
			0.53	0.53	< 0.05	< 0.01	
	Multiple Comparisons		day 1	0.53	0.42	< 0.05	
			day 2	0.53	0.42	0.53	
			day 3	< 0.05	0.42	0.53	
			day 4	< 0.01	< 0.05	0.53	
Retinyl-Palmitate/Fat	Multiple Comparisons	Kruskal-Wallis H	3	0.43			
			3	see below			
			day 1	day 2	day 3	day 4	
			1.00	1.00	1.00	1.00	
	Multiple Comparisons		day 1	1.00	1.00	0.90	
			day 2	1.00	1.00	1.00	
			day 3	1.00	1.00	1.00	
			day 4	1.00	0.90	1.00	
Retinyl-Stearate/Fat	Multiple Comparisons	Kruskal-Wallis H	3	0.63			
			3	see below			
			day 1	day 2	day 3	day 4	
			1.00	1.00	1.00	1.00	
	Multiple Comparisons		day 1	1.00	1.00	1.00	
			day 2	1.00	1.00	1.00	
			day 3	1.00	1.00	1.00	
			day 4	1.00	1.00	1.00	
Σ Retinylester/Fat	Multiple Comparisons	Kruskal-Wallis H	3	0.39			
			3	see below			
			day 1	day 2	day 3	day 4	
			1.00	1.00	1.00	1.00	
	Multiple Comparisons		day 1	1.00	1.00	0.56	
			day 2	1.00	1.00	1.00	
			day 3	1.00	1.00	1.00	
			day 4	1.00	0.56	1.00	
Total Vitamin A/Fat	Multiple Comparisons	Kruskal-Wallis H	3	< 0.001			
			3	see below			
			day 1	day 2	day 3	day 4	
			< 0.001	< 0.001	< 0.01	< 0.001	
	Multiple Comparisons		day 1	< 0.001	< 0.01	< 0.01	
			day 2	< 0.001	< 0.01	< 0.01	
			day 3	< 0.001	< 0.01	< 0.01	
			day 4	< 0.001	< 0.001	< 0.01	
β-Carotin/Fat	Multiple Comparisons	Kruskal-Wallis H	3	0.23			
			3	see below			
			day 1	day 2	day 3	day 4	
			0.83	0.83	0.83	0.83	
	Multiple Comparisons		day 1	0.83	0.83	0.55	
			day 2	0.83	0.83	0.83	
			day 3	0.83	0.83	0.83	
			day 4	0.83	0.55	0.83	

Table 18 Minimum, maximum, median, first and third quartile of milk composition (n = 17) in the first month of lactation (data grouped per days in lactation). Lactation days 7 to 30 are given.

First Month in Lactation: Lactation Day 7 - 30					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (%)	1.800	3.570	4.390	7.330	9.930
Protein (%)	3.350	4.830	6.020	8.340	16.050
Lactose (%)	2.580	3.943	4.690	5.600	6.650
α -Tocopherol ($\mu\text{g/mL}$)	0.032	0.116	0.391	1.453	4.499
Retinol ($\mu\text{g/mL}$)	0.000	0.009	0.011	0.081	0.154
Retinyl-Oleate ($\mu\text{g/mL}$)	0.001	0.007	0.015	0.031	0.091
Retinyl-Palmitate ($\mu\text{g/mL}$)	0.007	0.129	0.194	0.373	0.885
Retinyl-Stearate ($\mu\text{g/mL}$)	0.001	0.017	0.045	0.075	0.114
Σ Retinylester ($\mu\text{g/mL}$)	0.009	0.162	0.247	0.457	1.076
Total Vitamin A ($\mu\text{g/mL}$)	0.009	0.171	0.276	0.531	1.230
β -Carotin ($\mu\text{g/mL}$)	0.000	0.001	0.004	0.006	0.013
α -Tocopherol/Fat (mg/g)	0.00094	0.00322	0.00543	0.03336	0.04531
Retinol / Fat (mg/g)	0.00000	0.00014	0.00026	0.00116	0.00251
Retinyl-Oleate / Fat (mg/g)	0.00003	0.00016	0.00026	0.00061	0.00097
Retinyl-Palmitate / Fat (mg/g)	0.00021	0.00257	0.00470	0.00659	0.01019
Retinyl-Stearate / Fat (mg/g)	0.00003	0.00030	0.00101	0.00124	0.00150
Σ Retinylester / Fat (mg/g)	0.00026	0.00333	0.00603	0.00849	0.01259
Total Vitamin A / Fat (mg/g)	0.00026	0.00353	0.00617	0.00965	0.01511
β -Carotin / Fat (mg/g)	0.00000	0.00000	0.00006	0.00011	0.00019

Table 19 Minimum, maximum, median, first and third quartile of milk composition (n = 17) in the second month of lactation (data grouped per days in lactation). Lactation days 31 to 60 are given.

Second Month in Lactation: Lactation Day 31 - 60					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (%)	3.040	3.815	3.950	5.683	8.490
Protein (%)	3.650	5.000	6.455	7.653	14.950
Lactose (%)	1.870	4.190	4.755	5.420	6.140
α -Tocopherol ($\mu\text{g/mL}$)	0.040	0.315	0.627	1.537	4.546
Retinol ($\mu\text{g/mL}$)	0.000	0.013	0.027	0.075	0.108
Retinyl-Oleate ($\mu\text{g/mL}$)	0.001	0.016	0.022	0.023	0.056
Retinyl-Palmitate ($\mu\text{g/mL}$)	0.008	0.188	0.234	0.421	0.658
Retinyl-Stearate ($\mu\text{g/mL}$)	0.001	0.025	0.033	0.086	0.111
Σ Retinylester ($\mu\text{g/mL}$)	0.010	0.244	0.286	0.506	0.791
Total Vitamin A ($\mu\text{g/mL}$)	0.010	0.260	0.320	0.562	0.864
β -Carotin ($\mu\text{g/mL}$)	0.000	0.003	0.004	0.006	0.012
α -Tocopherol/Fat (mg/g)	0.00133	0.00603	0.01491	0.03654	0.12026
Retinol / Fat (mg/g)	0.00003	0.00021	0.00046	0.00123	0.00276
Retinyl-Oleate / Fat (mg/g)	0.00003	0.00025	0.00043	0.00058	0.00099
Retinyl-Palmitate / Fat (mg/g)	0.00022	0.00343	0.00552	0.01137	0.01641
Retinyl-Stearate / Fat (mg/g)	0.00003	0.00043	0.00061	0.00168	0.00294
Σ Retinylester / Fat (mg/g)	0.00027	0.00409	0.00663	0.01364	0.01954
Total Vitamin A / Fat (mg/g)	0.00030	0.00433	0.00725	0.01476	0.02230
β -Carotin / Fat (mg/g)	0.00000	0.00005	0.00010	0.00014	0.00021

Table 20 Minimum, maximum, median, first and third quartile of milk composition (n = 17) in the third month of lactation (data grouped per days in lactation). Lactation days 61 to 90 are given.

Third Month in Lactation: Lactation Day 61 - 90					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (%)	3.620	4.250	4.985	5.893	6.830
Protein (%)	3.250	4.753	5.795	7.375	12.500
Lactose (%)	1.840	3.743	4.880	5.785	6.190
α -Tocopherol ($\mu\text{g/mL}$)	0.028	0.287	0.652	2.019	5.407
Retinol ($\mu\text{g/mL}$)	0.000	0.009	0.016	0.054	0.123
Retinyl-Oleate ($\mu\text{g/mL}$)	0.001	0.011	0.022	0.041	0.087
Retinyl-Palmitate ($\mu\text{g/mL}$)	0.008	0.113	0.233	0.432	0.788
Retinyl-Stearate ($\mu\text{g/mL}$)	0.001	0.013	0.033	0.088	0.162
Σ Retinylester ($\mu\text{g/mL}$)	0.010	0.137	0.302	0.548	0.980
Total Vitamin A ($\mu\text{g/mL}$)	0.010	0.151	0.359	0.648	1.073
β -Carotin ($\mu\text{g/mL}$)	0.000	0.000	0.004	0.006	0.013
α -Tocopherol/Fat (mg/g)	0.01104	0.01235	0.01286	0.06240	0.09566
Retinol / Fat (mg/g)	0.00017	0.00026	0.00048	0.00173	0.00331
Retinyl-Oleate / Fat (mg/g)	0.00019	0.00039	0.00081	0.00145	0.00161
Retinyl-Palmitate / Fat (mg/g)	0.00192	0.00521	0.00763	0.01240	0.01561
Retinyl-Stearate / Fat (mg/g)	0.00019	0.00053	0.00110	0.00166	0.00251
Σ Retinylester / Fat (mg/g)	0.00230	0.00618	0.01008	0.01584	0.01851
Total Vitamin A / Fat (mg/g)	0.00253	0.00673	0.01184	0.01687	0.02060
β -Carotin / Fat (mg/g)	0.00004	0.00008	0.00013	0.00021	0.00031

Table 21 Minimum, maximum, median, first and third quartile of milk composition (n = 17) in the fourth month of lactation (data grouped per days in lactation). Lactation days 91 to 137 are given.

Fourth Month in Lactation: Lactation Day 91 - 137					
	Minimum	1st Quartile	Median	3rd Quartile	Maximum
Fat (%)	2.990	4.230	4.850	6.010	6.060
Protein (%)	3.200	5.150	6.330	9.630	12.550
Lactose (%)	2.430	3.550	4.500	5.440	6.080
α -Tocopherol ($\mu\text{g/mL}$)	0.278	0.512	0.906	2.231	3.381
Retinol ($\mu\text{g/mL}$)	0.006	0.016	0.024	0.054	0.143
Retinyl-Oleate ($\mu\text{g/mL}$)	0.013	0.018	0.023	0.033	0.041
Retinyl-Palmitate ($\mu\text{g/mL}$)	0.167	0.195	0.234	0.420	0.550
Retinyl-Stearate ($\mu\text{g/mL}$)	0.009	0.014	0.048	0.128	0.234
Σ Retinylester ($\mu\text{g/mL}$)	0.218	0.265	0.364	0.543	0.695
Total Vitamin A ($\mu\text{g/mL}$)	0.237	0.306	0.410	0.606	0.722
β -Carotin ($\mu\text{g/mL}$)	0.000	0.003	0.003	0.004	0.008
α -Tocopherol/Fat (mg/g)	0.00463	0.01749	0.02550	0.04280	0.06280
Retinol / Fat (mg/g)	0.00031	0.00038	0.00054	0.00158	0.00323
Retinyl-Oleate / Fat (mg/g)	0.00028	0.00044	0.00049	0.00057	0.00093
Retinyl-Palmitate / Fat (mg/g)	0.00317	0.00557	0.00701	0.00911	0.01134
Retinyl-Stearate / Fat (mg/g)	0.00015	0.00033	0.00099	0.00152	0.00221
Σ Retinylester / Fat (mg/g)	0.00363	0.00684	0.00771	0.01190	0.01282
Total Vitamin A / Fat (mg/g)	0.00395	0.00778	0.00802	0.01332	0.01549
β -Carotin / Fat (mg/g)	0.00000	0.00007	0.00007	0.00010	0.00018

Table 22 Overview of vitamin content and methods found in literature. Units are given as stated in the original publication.

Species	Vitamin A		β-Carotene		Vitamin E		Animals	Method	Author, Year
	Colostrum	Milk	Colostrum	Milk	Colostrum	Milk			
Bactrian Camel		0.97 mg/L				1.45 mg/L	10 bactrian camels, aged 5 years	fluorescence spectrometry	ZHANG et al., 2005
		100 - 380 µg/l				126.9 ± 26.2 mg/L	unknown	unknown	WANG et al., 2011
		500 ± 6.2 IU/kg					approx. 500 camels from 11 different flocks	standard methods of AOAC (1980)	SAWAYA et al., 1984
Dromedary Camel		0.1 ± 0.27 mg/L				0.56 ± 0.60 mg/L	20 dromedary camels	HPLC, different protocols for retinol and α-tocopherol, respectively; spectrophotometry with 330 nm for retinol and 295 nm for α-tocopherol	FARAH et al., 1992
	307 ± 132 µg/L	201 ± 100 µg/L	3.2 µg/L	< 3.2 µg/L	136.9 ± 98.4 µg/dL	32.7 ± 12.8 µg/dL	different camel herds	HPLC as well as gas chromatographic methods	STAHL et al., 2006 <i>vide</i> FAYE et al., 2019 - original paper not available
		267 ± 80 µg/L				17.8 ± 5.8 µg/L	camel herd of unknown size	isocratic HPLC with UV/Visible detector; detection at 280 nm	HADDADIN et al., 2008
		0.380 0.03 mg/L				26.0 ± 1.73 µg/L	healthy uninfected dromedary camels	HPLC, photodiode-array detector; detection at 325nm for Vit. A, 295nm for Vit. E	IBRAHIM & KHALIFA 2015
		20.1 ± 10.0 µg%				32.7 ± 12.8 µg%	unknown	Review paper	SINGH et al., 2017
		12.6 ± 2.4 µg/dL				20.2 ± 1.05 µg/dL	8 dromedary camels of 4 different breeds	vitamin content analyzed at laboratory	KONUSPAYEVA et al., 2014
		0.04 mg/100g	0.02 mg/100g			0.11 mg/100g	unknown	Review paper	RAYNAL-LJUTOVAC et al., 2008
Cow		126 IU/100g					unknown	Review paper	PARK et al., 2007
					1.91 ± 0.14 µg/mL	0.28 ± 0.16 µg/mL	8 Holstein cows from immediately postpartum to 30d	HPLC	HIDIROGLOU 1989
	4.90 ± 1.82 µg/g		0.68 ± 0.63 µg/g			2.92 ± 3.65 µg/g	55 Holstein dairy herds	extraxtion according to methods from Vitamin Analysis for the Health and Food Sciences (Eitenmiller and Landen, 1999); analysis by HPLC as describes by Arnaud et al., 1991	KEHOE et al., 2007
						77.17 ± 33.51 µg/g fat			
	250.00 ± 2.65 IU/dL	128.90 ± 0.90 IU/dL			234.00 ± 3.00 IU/dL	318.00 ± 5.00 IU/dL	36 Holstein Cows, second lactation	determination after Omeh & Uzoegwe (2010) and Nasar et al., 2009	ABD EL-FATTAH et al., 2012
Buffalo		280 µg/L				0.6 mg/L	unknown	Review paper	HAUG et al., 2007
		29.8 ± 0.8 µg/dL				25.4 ± 0.5 µg/dL	unknown	Review paper, values for 4-6 lactations given	ABD EL-SALAM & EL-SHIBINY 2011
	166.67 ± 0.88 IU/dL	136.7 ± 0.26 IU/dL			342.00 ± 3.3 IU/dL	330.00 ± 2.98 IU/dL	18 Egyptian buffaloes, second lactation	determination after Omeh & Uzoegwe (2010) and Nasar et al., 2009	ABD EL-FATTAH et al., 2012
		230 IU/mL				334 µg/g	unknown	Review paper	GARAU et al., 2021
Goat		0.04 mg/100g		0.00 mg/100g		0.04 mg/100g	unknown	Review paper	RAYNAL-LJUTOVAC et al., 2008
		185 IU/100g					unknown	Review paper	PARK et al., 2007
		2074 IU/L					unknown	Review paper	PANDYA & GHODKE 2007
Sheep		63.75 ± 21.86 µg/100g				90.43 ± 58.33 µg/100g	240 milk samples of twenty commercial flocks	UPLC, photodiode-array detector scanning at 275 - 465 nm and a fluorometric detector; two separate isocratic chromatographic methods	REVILLA et al., 2017
		0.08 mg/100g				0.11 mg/100g	unknown	Review paper	RAYNAL-LJUTOVAC et al., 2008
		146 IU/100g					unknown	Review paper	PARK et al., 2007

Table 22 Cont.

Species	Vitamin A		β -Carotene		Vitamin E		Animals	Method	Author, Year
	Colostrum	Milk	Colostrum	Milk	Colostrum	Milk			
Pig	1.61 $\pm$ 0.18 mg/kg	0.92 $\pm$ 0.14 mg/kg			3.69 $\pm$ 0.28 mg/kg	2.53 $\pm$ 0.23 mg/kg	10 Danish Large White, 10 Danish Duroc and 10 Norwegian Landrace sows	extraction with alcohol-n-hexane; determination using a Pye UNICAM LC- XP HPLC; quantitative evaluation with vitamin standards from MERCK	CSAPÓ et al., 1996
	2.5 $\pm$ 0.32 mg/L				17.9 $\pm$ 3.8 mg/L		unknown	Citation	
Horse	0.88 mg/kg	0.34 mg/kg			1342 mg/kg	1128 mg/kg	29 mares of different age and 4 different breeds	extraction with alcohol-n-hexane; determination using a Pye UNICAM LC- XP HPLC; quantitative evaluation with vitamin standards from MERCK	CSAPÓ et al., 1995
		8.9 $\pm$ 0.12 μ g/100g FW		2.8 $\pm$ 0.12 μ g/100g FW		26.1 $\pm$ 0.5 μ g/100g FW	powdered and raw mare's milk, data of raw milk given	extraction with exane:ethyl acetate, determination with normal-phase HPLC (after Panfili et al., 1994)	MARCONI & PANFILI 1998
		0.403 $\pm$ 0.081 mg/L		0.388 $\pm$ 0.102 mg/L		1.13 $\pm$ 0.27 mg/L	9 Polish Cold-Blooded multiparous mares	described by Puppel et al., 2012a	MARKIEWICZ-KESZYCKA et al., 2014
		0.403 mg/L				1.13 mg/L	unknown	Review Paper	MUSAEV et al., 2021
Donkey		1.67 $\pm$ 0.15 μ g/100g				5.14 $\pm$ 0.35 μ g/100g	16 Martina Franca donkeys	extraction with exane:ethyl acetate, determination with normal-phase HPLC (after Panfili et al., 1994)	SORRENTINO et al., 2005
		0.017 mg/L				0.051 mg/L	unknown	Review Paper	SALIMEI & FANTUZ 2012
		58 μ g/dL				5.2 μ g/dL	unknown	Review Paper	VINCENZETTI et al., 2021
Human	74.49 $\pm$ 54.44 μ g/dL				1217.4 $\pm$ 959.9 μ g/dL		73 mothers, aged 14 - 36 years	HPLC-UV; spectro-photometry	GARCIA 2010
					1665.2 $\pm$ 160.2 μ g/dL		109 mothers, aged 24.1 $\pm$ 5.6 years	HPLC-UV; spectro-photometry	CLEMENTE 2015
	101.3 $\pm$ 63.3 μ g/dL				1189.4 $\pm$ 47.7 μ g/dL		88 mothers, aged 24 $\pm$ 7 years	HPLC-UV; spectro-photometry	GRILO 2016
	96.6 $\pm$ 53.5 μ g/dL						100 mothers, mean age 28.6 years	HPLC-UV; spectro-photometry	GURGEL 2017
	151 μ g/dL				1500 μ g/dL		unknown	Review Paper	ERICK 2018
		88 μ g/dL				900 μ g/dL	transitional milk, unknown	Review Paper	ERICK 2018
		75 μ g/dL				250 μ g/dL	mature milk, unknown transitional milk, 58 mothers aged 18 - 40 years	HPLC; chromatography with 325nm for retinol	LIMA et al., 2017
		99.4 $\pm$ 36.7 μ g/dL					mature milk, 58 mothers aged 18 - 40 years		
		58.1 $\pm$ 17.5 μ g/dL							
		30 - 200 μ g/dL				300 - 800 μ g/dL	unknown	Review Paper	CLAEYS et al., 2014
	0.06 mg/100g					0.23 mg/100g	unknown	Review Paper	RAYNAL-LJUTOVAC et al., 2008
	190 IU/100g						unknown	Review Paper	PARK et al., 2007

Table 23 Calculated data from literature harmonized to µg/mL for easier comparison.

Species	Vitamin A		β-carotene		Vitamin E		Author, Year
	Colostrum (µg/mL)	Milk (µg/mL)	Colostrum (µg/mL)	Milk (µg/mL)	Colostrum (µg/mL)	Milk (µg/mL)	
Alpaca	0.438 ± 0.211	0.413 ± 0.287	0.002 ± 0.002	0.004 ± 0.003	4.620 ± 2.388	1.265 ± 1.405	original data
Bactrian Camel		0.97				1.45	ZHANG et al., 2005
		0.10 - 0.38				126.9 ± 26.2	WANG et al., 2011
		1.543 ± 0.0191					SAWAYA et al., 1984
		0.1 ± 0.27				0.56 ± 0.60	FARAH et al., 1992
Dromedary Camel	0.307 ± 0.132	0.201 - 0.1	0.0032	< 0.0032	1.369 ± 0.984	0.327 ± 0.128	STAHL et al., 2006 <i>fide</i> FAYE et al., 2019
		0.267 - 0.08				0.0178 ± 0.0058	HADDADIN et al., 2008
		0.380 0.03				0.026 ± 0.0017	IBRAHIM & KHALIFA 2015
		0.126 ± .024				0.202 ± 0.0105	KONUSPAYEVA et al., 2014
Cow		0.4112 - 0.412	0.2091 - 0.2107			1.1308 - 1.133	RAYNAL-LJUTOVAC et al., 2008
		0.3886 - 0.389					PARK et al., 2007
	3.22 - 7.08		0.0523 - 1.3802		1.91 ± 0.14	0.28 ± 0.16	HIDIROGLOU 1989
	0.75 ± 0.008	0.3867 ± 0.0027			3		KEHOE et al., 2007
		0.28			1570.46 ± 20.13	2134.22 ± 33.55	ABD EL-FATTAH et al., 2012
	2.33	0.33				0.6	HAUG et al., 2007
	3.69	0.57			1.91	0.28	DEBIER & LARONDELLE 2005
					5.3	0.92	DEBIER & LARONDELLE 2005
Buffalo		0.298 ± 0.008				0.254 ± 0.005	ABD EL-SALAM & EL-SHIBINY 2011
	0.5 ± 0.0026	0.41 ± 0.0008			2295.30 ± 22.147	2214.7651 ± 20	ABD EL-FATTAH et al., 2012
		69				344.02 - 344.69	GARAU et al., 2021
Goat		0.4116 - 0.4156		0.00		0.4116 - 0.4156	RAYNAL-LJUTOVAC et al., 2008
		0.5711 - 0.5766					PARK et al., 2007
		0.6222					PANDYA & GHODKE 2007
Sheep		0.4334 - 0.889				0.4334 - 0.889	REVILLA et al., 2017
		0.8278 - 0.8307				1.1382 - 1.1422	RAYNAL-LJUTOVAC et al., 2008
		0.4532 - 0.4548					PARK et al., 2007
	6.54	0.32			3.3	1	DEBIER & LARONDELLE 2005
Pig	1.722 ± 0.192	0.966 ± 0.147			4.5	1.65	DEBIER & LARONDELLE 2005
	2.5 ± 0.32				3.9483 ± 0.2996	2.6565 ± 0.2415	CSAPÓ et al., 1996
					17.9 ± 3.8		
Horse					11.06	2.3	DEBIER & LARONDELLE 2005
	0.9046 - 0.9372	0.3495			1.38 - 1.43	1.159	CSAPÓ et al., 1995
		0.0915 ± 0.0012		0.0287 ± 0.0012		0.268 ± 0.005	MARCONI & PANFILI 1998
		0.403 ± 0.081		0.388 ± 0.102		1.13 ± 0.27	MARKIEWICZ-KĘSZYCKA et al., 2014
		0.403				1.13	MUSAEV et al., 2021

Table 23 Cont.

Donkey		0.0172 ± 0.0015		0.053 ± 0.00361	SORRENTINO et al., 2005
		0.017		0.051	SALIMEI & FANTUZ 2012
		0.58		0.052	VINCENZETTI et al., 2021
	0.7449 ± 0.5444		12.174 ± 9.599		GARCIA 2010
			16.652 ± 1.602		CLEMENTE 2015
	1.013 ± 0.633		11.894 ± 0.477		GRILO 2016
	0.966 ± 0.535				GURGEL 2017
	1.51		15		ERICK 2018
Human		0.88		9	
		0.75		2.5	ERICK 2018
		0.994 ± 0.367			LIMA et al., 2017
		0.581 ± 0.175			
		0.30 - 2		3 - 8	CLAEYS et al., 2014
	0.63			2.369 - 2.373	RAYNAL-LJUTOVAC et al., 2008
	0.5985				PARK et al., 2007

