Technological and seasonal variations of vitamin D and other nutritional components in donkey milk

Journal:	*Journal of Dairy Science*
Manuscript ID	JDS-18-14776.R1
Article Type:	Short Communications
Date Submitted by the Author:	02-May-2018
Complete List of Authors:	Martini, Mina; University of Pisa, Scienze veterinarie; University of Pisa, Interdepartmental Research Center"Nutraceuticals and Food for Health", Altomonte, Iolanda; Università di Pisa, Dipartimento di Scienze Veterinarie Licitra, Rosario; Università di Pisa, Dipartimento di Scienze Veterinarie Salari, Federica; Università di Pisa, Dipartimento di Scienze Veterinarie
Key Words:	donkey milk, vitamin D, seasonal variability, pasteurization

SHORT COMMUNICATION: VITAMIN D IN DONKEY MILK

Technological and seasonal variations of vitamin D and other nutritional components in donkey milk

Mina Martini[*†], Iolanda Altomonte[*], Rosario Licitra[*], Federica Salari*[1]

**Dipartimento di Scienze Veterinarie, Università di Pisa, Italy*

[†] Centro Interdipartimentale di Ricerca Nutraceutica e Alimentazione per la Salute, Università di Pisa, Italy

[1]Corresponding author: federica.salari@unipi.it

ABSTRACT

Vitamin D is an essential nutrient that plays a crucial role in calcium homeostasis and bone metabolism and also acts as a hormone. Although there have been several studies on the content of vitamin D in bovine milk, there is little information available regarding donkey milk. In the context of the nutritional assessment of donkey milk, the aim of this study was to assess the vitamin D content in donkey milk and its chemical profile with particular reference to seasonal and technological modifications after pasteurization. The study was conducted on a dairy farm that produces donkey milk for human consumption located in central Italy. At the sampling time, an aliquot of total bulk milk production was sampled before and after pasteurization (63°C for 30 min without homogenization) with a total of 20 raw and 20 pasteurized milk samples. The samples were collected for 10 months, every 15 days, from May to February 2017. All the samples were analysed for the chemical composition and vitamin D_2 and D_3 content by HPLC after saponification. The donkey milk analysed showed a higher average vitamin D content (raw milk: D_2=1.68 D_3=0.60 µg/100 mL; pasteurized milk: D_2=1.38; D_3=0.30 µg/100 mL) than reported for bovine and human milk. The results of the effect of pasteurization on milk did not highlight significant differences in the total content of vitamin D. However, vitamin D_3 has a poor thermal stability which led to a significant reduction in content in pasteurized milk compared to raw milk. Also during the seasons, the total vitamin D content of donkey milk did not show significant variations, however a higher concentration of vitamin D_3 was found in spring/summer. In conclusion, raw and pasteurized donkey milk showed a high content of vitamin D, which could be useful in meeting the deficiencies of this vitamin in humans. Further investigations are needed to improve the vitamin D content in donkey milk by increasing its endogenous synthesis and/or its transfer in milk and to clarify other variability factors.

Key words: donkey milk, vitamin D, seasonal variability, pasteurization

Short Communication

Vitamin D plays a key role in calcium homeostasis and bone metabolism and also acts as a hormone (Muller et al., 2011). Vitamin D_2 (ergocalciferol) is derived from the ultraviolet radiation of ergosterol (in particular UVB radiation), which is a vitamin D precursor naturally found in plants, fungi, and invertebrates. Vitamin D_3 (cholecalciferol) is derived by sunlight exposure from 7-Dehydrocholesterol which is a precursor of cholesterol and also acts as a provitamin D_3 (Schmid and Walther, 2013).

The major source of vitamin D for children and adults is exposure to natural sunlight that is required for ultraviolet-B (UVB)-induced vitamin D production in the skin. Vitamin D that comes from the skin or diet is biologically inert and requires its first hydroxylation in the liver to $_{25}(OH)D_3$. The latter requires a further hydroxylation in the kidneys to form the biologically active form of vitamin D $_{1,25}(OH)_2D$.

An oral intake of vitamin D may be an important source in winter, when the UV-B-related synthesis is limited and for people who are not exposed to sunlight (Gill et al., 2016). Vitamin D deficiency is well known, and the concentration in blood serum of the hydroxylated form of calciferol [$_{25}(OH)D_3$] is recognized as a sensitive accurate indicator of the functional status of vitamin D (Heaney, 2004). The Institute of Medicine (IOM, 2011) defined a vitamin D deficiency as a content of $_{25}(OH)D_3$ less than 20 ng/mL in serum. In addition, the widespread risk of deficiency and insufficiency worldwide has been reported (Holick et al., 2011) due to the current mostly indoor life style.

In Italy, vitamin D deficiency and insufficiency were detected in Italy 49.9% and 32.3% of adolescents, respectively and 8.9% were severely deficient (Vierrucci et al., 2014).

Vitamin D dietary intake is also of great importance, and animal foodstuffs (e.g., fish, meat, offal, eggs, dairy) are the main sources for naturally-occurring cholecalciferol (vitamin D_3). However, it is difficult to cover the requirements of vitamin D solely by foodstuffs (Schmid and Walther, 2013). Milk mainly contains two forms of vitamin D (D_3 and D_2).

Research has highlighted the variability factors of vitamin D in milk by analysing endogenous, exogenous, and technological factors such as season, age of the animal, treatment and conservation (Jakobsen and Saxholt, 2009; Weir et al., 2016). However, most of the studies regard bovine milk, whereas there is little information regarding donkey milk (Gentili et al., 2012; Bulgari et al., 2015). The properties of donkey milk have been known since ancient times however in the last few decades there has been renewed interest from the scientific community due to its use as a therapeutic product for children with bovine milk protein allergy (CMPA) (Martini et al., 2017).

Despite having a low bacterial count, the thermal treatment of donkey raw milk is recommended in order to avoid the risk of food born diseases (Martini et al., 2016). Pasteurization is known to eliminate any pathogenic microorganisms that could be present in milk and guarantees its preservation. Furthermore, the literature on the effects of pasteurization on nutritional characteristics of donkey milk is not yet exhaustive.

In addition, differently from cow milk, that is mostly standardized, donkey milk has a certain variability in terms of its components

Nutritional characteristics of donkey milk are especially interesting since this milk is targeted for consumption by children for its similarity of human milk. In addition, the consumption in the elderly has been proposed given the donkey milk good calcium content, its low fat and the easy digestibility. These categories of people are at particularly risk for developing nutritional deficiencies. Therefore, they require a careful management for nutrition, from a quantitative and qualitative point of view in order to avoid conditions of undernourishment and malnutrition.

In the context of the nutritional assessment of donkey milk, the aim of this paper was to carry out an evaluation of the vitamin D content in donkey milk and its chemical profile, with particular reference to seasonal and technological modifications after pasteurization.

The study was conducted on dairy farm that produces donkey milk for human consumption. The farm, located in central Italy, has raised about 160 Amiata donkeys, reared outdoors, in a semi-intensive system. The animals are fed about 2.5 kg per day per head of concentrate for dairy donkeys (PROGEO S.C.A., Italy) and polyphite hay ad libitum. The jennies are routinely machine milked twice a day.

At sampling time, an aliquot of total daily bulk milk production was sampled before and after pasteurization (63°C for 30 min without homogenization). The samples were collected for 10 months, every 15 days, from May to February 2017 (20 raw and 20 pasteurized milk samples) with a total of 40 samples.

All the samples were analysed for dry matter, fat and lactose content by infrared analysis (Milkoscan, Italian Foss Electric, Padova, Italy); proteins, caseins and ashes (AOAC, 2004). Individual mineral content (Ca, P, Mg, K, Na, Zn)(mg/L) was determined by atomic absorption spectroscopy and ultraviolet–visible spectroscopy according to Horwitz (2000), and Murthy and Rhea (1967) Milk fat extraction was performed following Rose-Gottlieb's method (AOAC 933.05, 1995), and methyl esters of fatty acids were prepared using methanolic sodium methoxide according to Christie (1982). A Perkin Elmer Clarus 480 (Perkin Elmer, Norwolk, CT, USA) equipped with a flame ionization detector and a capillary column (60 m× 0.25 mm; film thickness 0.25 mm (ThermoScientific TR-FAME 60 m x 0.25 mm ID; film thickness 0.25 μm, Fisher Scientific UK) were used.

The helium carrier gas flow rate was 1 mL·min^{-1}. The oven temperature program was as follows: level 1, 50°C held for 5 min, level 2, 50 to 140°C at 3°C·min^{-1} then held for 2 min, level 3, 140 to 240°C at 1°C·min^{-1} then held for 10 min. The injector and detector temperatures were set at 270 and 300°C, respectively. The peak areas of individual fatty acids (FA) were identified using an FA standard injection (Food Industry FAME Mix – Restek Corporation, 110 Benner Circle, Bellefonte, PA16823) and quantified as the percentage of total FA. In addition, nonadecanoic acid methyl ester (C19:0 Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823) was also used as an internal standard.

Milk fatty acid classes of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), short chain (SCFA), medium chain (MCFA) and long chain (LCFA) fatty acids were calculated as follows: SFA=Σ C4:0, C6:0, C8:0, C10:0, C11:0, C12:0,C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0,C21:0, C22:0, C23:0, C24:0; MUFA=Σ C14:1,C15:1, C16:1, C17:1, C18:1 t9, C18:1t11,C18:1c9, C20:1, C22:1, C24:1; PUFA=Σ C18:2 t9,12, C18:2 c9,12, C18:3 n6, C18:3 n3, C20:2, C20:3 n6, C20:4, C20:3 n3, C20:5, C22:2, C22:5, C22:6. SCFA=Σ of the fatty acids from 4 to 10 C; MCFA=Σ of the fatty acids from 11 to 17 C; LCFA=Σ of the fatty acids from 18 to 24 C.

For the determination of vitamin D, 75 mL of donkey milk were saponified by adding KOH pellets directly to the milk according to Perales et al. (2005). Since a hot temperature may lead to the isomerization of D vitamins, saponification occurred at 40°C for 32 minutes. Ethanol and double distilled water were then added to the sample in order to remove the polar compounds and to avoid foaming. Afterwards the solution was transferred to a 500 mL separatory funnel, and an initial extraction of the unsaponifiable fraction was performed using 75 mL hexane. The aqueous phase was thus drained and collected in order to repeat two extractions by adding 75 mL of hexane each time. Each time, the organic phase was collected in a rotavapor flask. Finally, the organic phase was evaporated to dryness on a rotary evaporator and the extract was resuspended in 500 μl of acetonitrile and filtered through a 0.45 μm diameter syringe filter. 100 μl of the extract were injected into an HPLC and isocratically eluted using as a mobile phase acetonitrile-methanol 97:3 at a flow of 1 mL/min, as described by Hagar et al. (1994). A Kinetex core-shell column (Phenomenex, Inc. A) was used as the stationary phase and the UV detector was set at 254 nm.

Vitamins D_2 and D_3 in the milk samples were quantified by comparison with a calibration curve obtained with the injection of the pure standards (Sigma Chemical Co., St. Louis).

All the results were analysed by ANOVA, where season (autumn-winter or spring-summer) and thermal treatment (raw or pasteurized milk) were the fixed effects. Significant differences were considered at the P level $\leq$ 0.05. The statistical analysis was carried out using JMP (2007) software.

The donkey milk analysed showed a higher average vitamin D content (raw milk: D_2=1.68, D_3=0.60 μg/100 mL; pasteurized milk: D_2=1.38; D_3=0.30 μg/100 mL)) than reported for bovine and human milk (Zhang et al., 2012; Schmid and Walther 2013). Our results shows that the main vitamin D form present in donkey milk was vitamin D_2.

The results related to the effect of heat treatment on milk (Table 1) did not highlight significant differences in the chemical composition either in relation to the total content of vitamin D or in the main constituents of milk, and fatty acid classes. Our results therefore indicate that pasteurisation treatment at 63°C does not involve substantial changes to the quality of the milk, and similar results have been previously reported by other authors (Addo and Ferragut 2015; Martini et al., 2016).

As reported by Tsai et al. (2017), vitamin D_3 has a poor thermal stability which entails a significant reduction ($P \leq 0.05$) of about 50% of D_3 in pasteurized milk compared to raw milk (Table 1).

Despite the strong decrease in the vitamin D_3 content, the total vitamin D amount was only partially affected. In fact, the total vitamin D decreased but not significantly. This can be ascribed to the large contribution of vitamin D_2, which has been described as heat-stable both after pasteurisation at 63° for 30 minutes and after sterilization (Kaushik et al., 2014).

Table 1. Effects of pasteurization (63°C for 30 min) on nutritional characteristics and vitamin D content of donkey milk (N=40).

Item	Raw milk	STD error	Pasteurized milk	STD error
Fat %	0.15	0.021	0.15	0.022
Protein %	1.64	0.054	1.58	0.057
Dry matter %	9.30	0.070	9.28	0.074
Ash %	0.37	0.008	0.36	0.008
Ca mg/L	647.25	49.969	674.69	47.314
P mg/L	375.11	21.682	390.70	20.530
Mg mg/L	101.31	10.740	101.72	10.167
K mg/L	652.88	29.193	626.75	27.642
Na mg/L	202.25	34.677	176.69	32.835
Zn mg/L	2.88	0.560	3.28	0.530
SCFA %	11.79	0.472	11.96	0.499
MCFA %	42.60	0.877	41.61	0.927
LCFA %	45.61	1.049	46.43	1.109
SFA %	51.70	1.135	50.75	1.200
MUFA %	28. 56	0.773	29.29	0.818
PUFA %	19.74	0.729	19.97	0.771
UFA/SFA	0.94	0.035	0.98	0.037
n3/n6	0.69	0.082	0.73	0.087
Total Vit D μg/100mL	2.23	0.240	1.68	0.253
Vit D_2 μg/100 mL	1.64	0.211	1.38	0.223
Vit D_3 μg/100mL	0.60**a**	0.127	0.30**b**	0.134

$^{a–b}$ Means within a row with different superscripts differ ($P < 0.05$)

STD= Standard error; SCFA = short chain fatty acids, MCFA = medium chain fatty acids; LCFA = long chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, UFA = unsaturated fatty acids.

Seasonal variations in donkey milk quality were found (Table 2). In particular, dry matter content and several mineral salts such as phosphorus and zinc were significantly ($P \leq 0.05$) higher in the spring summer season. These findings are supported by a previous study by Martini et al. (2014) in which seasonal changes in milk quality were found and were suggested as being the result of the better adaptability of the Amiatina donkey to a warm and temperate climate.

In addition, significant differences in the fatty acid classes were recorded, in particular regarding unsaturated fatty acids (UFA). In fact, in the spring /summer season there was a significantly lower content of MUFA ($P \leq 0.01$) and a higher content of PUFA ($P \leq 0.05$) and of the n3/n6 ratio ($P \leq 0.01$), and therefore a greater incidence of n3 on the total PUFA.

In vitro studies have found that the vitamin D binding protein, the main carrier of vitamin D in blood and milk, competitively binds some monounsaturated fatty acids (Williams et al., 1988; Calvo and Ena 1989) such as C18:1 cis9, which is one of the main MUFAs in donkey milk (Martini et al., 2015).

Although other factors may be involved in the decrease in the synthesis of MUFA in summer milk, competition for the carrier protein due to the increase in vitamin D secretion may to some extent contribute to the decrease in this family of fatty acids in milk. However, this hypothesis needs further investigation.

In donkey milk, the total vitamin D content did not undergo significant variations, however a higher ($P \leq 0.01$) concentration of vitamin D_3 was found in spring/summer than in autumn/winter, in particular the vitamin D_3 content was four times higher than in the autumn/winter. This is probably due to the different sun exposure of the animals facilitated by the outdoor farming system. Seasonal variations in vitamin D content of milk have been well documented in cows, with higher concentrations in the summer months than in the winter (Jakobsen and Saxholt, 2009; Weir et al., 2016)

Table 2. Effects of the season on the nutritional characteristics and vitamin D content of donkey milk (N=40).

Item	Autumn/winter	STD error	Spring/summer	STD error
Fat %	0.14	0.020	0.16	0.018
Protein %	1.57	0.048	1.64	0.042
Dry matter %	9.09**b**	0.079	9.46**a**	0.070
Ash %	0.37	0.008	0.36	0.007
Ca mg/L	603.36	53.706	699.38	44.015
P mg/L	354.02**b**	23.304	402.17**a**	19.098
Mg mg/L	90.71	11.541	108.75	9.458
K mg/L	675.24	31.376	616.20	25.714
Na mg/L	164.22	37.270	208.81	30.545
Zn mg/L	2.16**b**	0.601	3.69**a**	0.493
SCFA %	12.20	0.422	11.60	0.374
MCFA %	41.63	0.805	42.52	0.712
LCFA %	46.16	0.960	45.88	0.849
SFA %	50.77	1.032	51.61	0.913
MUFA %	30.57**A**	0.734	27.60**B**	0.650
PUFA %	18.66**b**	0.680	20.78**a**	0.601
UFA/SFA	0.98	0.038	0.94	0.033
n3/n6	0.54**B**	0.083	0.84**A**	0.074
Total Vit D µg/100mL	1.65	0.250	2.21	0.240
Vit D_2 µg/100 mL	1.49	0.224	1.53	0.210
Vit D_3 µg/100 mL	0.16**B**	0.133	0.68**A**	0.127

[a–b]Means within a row with different superscripts differ (P < 0.05)

[A–B]Means within a row with different superscripts differ (P < 0.01)

STD= Standard error; SCFA = short chain fatty acids, MCFA = medium chain fatty acids; LCFA = long chain fatty acids; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, UFA = unsaturated fatty acids

In conclusion, raw and pasteurized donkey milk showed a high content of vitamin D. Although donkey's milk is a niche product, the interest of our results derives from its use in consumers at risk of nutritional deficiencies. In fact, for these categories of people, donkey milk could be helpful (together with appropriate integrations) in meeting the deficiencies of vitamin D.

Seasonal variations in vitamin D content as well as of other milk components were also highlighted, in particular the spring/summer season tends to increase the vitamin D_3 concentration in milk. Pasteurization affected the vitamin D_3 content which is more thermolabile than D_2, however it did not influence the total vitamin D uptake. Further investigations are needed to improve the vitamin D content in donkey milk through increasing its endogenous synthesis and/or its transfer in milk and to clarify other variability factors.

ACKNOWLEDGMENTS

The research was funded by the Regional Government of Tuscany: Bando pubblico per progetti di ricerca nel settore Nutraceutica. L.A.B.A.Pro.V.

REFERENCES

Addo, C.N.A. and V. Ferragut. 2015. Evaluating the Ultra-High Pressure Homogenization (UHPH) and Pasteurization effects on the quality and shelf life of donkey milk. International Journal of Food Studies 4: 104- 115. http://dx.doi.org/10.7455/ijfs/4.1.2015.a9.

AOAC (Association of Official Analytical Chemists). 1995. Official Methods of Analysis, 16th edn. Vol. II . AOAC International, Arlington

AOAC (Association of Official Analytical Chemists). 2004. Official methods of analysis. AOAC International, Gaithersburg

Bulgari, O., A. M. Caroli, S. Chessa, F. Salari, I. Altomonte, S. Savino, M. Martini and C. Gigliotti. 2015. Confronto tra il contenuto di vitamina D nel latte di differenti specie. Scienza E Tecnica Lattiero-Casearia 66: 45-49.

Calvo, M. and J.M. Ena. 1989. Relations between vitamin D and fatty acid binding properties of vitamin D-binding protein. Bioehem. Biophys. Res. Comm. 163:14-17.

Christie, W.W. 1982. A simple procedure of rapid transmethylation of glycerolipids and cholesteryl esters. J. Lipid Res. 23: 1072-1075.

Gentili, A., F.Caretti, S.Bellante, S.Ventura, S. Canepari and R. Curini 2012. Comprehensive Profiling of Carotenoids and Fat-Soluble Vitamins in Milk from Different Animal Species by LC-DAD-MS/MS Hyphenation. J. Agric. Food Chem. 61: 1628−1639. http://dx.doi.org/10.1021/jf302811a.

Gill, B.D., X. Zhu, and H.E. Indyk. 2016 The determination of vitamin D3 and 25-hydroxyvitamin D3 in early lactation and seasonal bovine milk. Int Dairy J 63:29-34.

Hagar A.F., L. Madsen, L., Wales, H. B. Bradfor. 1994. Reversed.phase liquid chromatographic determination of vitamin D in milk. J AOAC International, 77(4): 1047-1051

Holick, M.F., N.C. Binkley, H.A. Bischoff-Ferrari, C.M. Gordon, D.A. Hanley, R.P. Heaney, M. Hassan Murad and C.M. Weaver. 2011. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab, 96:1911–1930.

Horwitz, W. (Ed.): Official Method of Analysis of AOAC International. 17th edition. Gaithersburg : AOAC International, 2000. ISBN: 0935584676.

Jakobsen, J. and E. Saxholt. 2009. Vitamin D metabolites in bovine milk and butter. J Food Compost Anal 22: 472–478.

JMP (2007) User's guide, version 7.0. SAS. Inst. Inc., Cary, NC, USA.

Kaushik, R., B. Sachdeva and S. Arora. 2014. Vitamin D2 stability in milk during processing, packaging and storage. Lebensm. Wiss. Technol. 56: 421.426.

Martini, M., I. Altomonte, R. Licitra and F. Salari. 2017. Nutritional and nutraceutical quality of donkey milk. J Equine Vet Sci. http://dx.doi.org/10.1016/j.jevs.2017.10.020.

Martini, M., I. Altomonte, F. Salari, and A. M. Caroli. 2014. Short communication: Monitoring nutritional quality of Amiata donkey milk: Effects of lactation and productive season. J Dairy Sci 97: 6819-6822. http://dx.doi.org/10.3168/jds.2014-8544.

Martini, M., F. Salari, I. Altomonte, G. Ragona, D. Casati and G. Brajon 2016. Conservazione del latte d’asina: aspetti nutrizionali e igienico-sanitari. Scienza E Tecnica Lattiero-Casearia 67: 63-72.

Martini, M., I. Altomonte, E. Manica, and F. Salari. 2015. Changes in donkey milk lipids in relation to season and lactation. J Food Compost Anal 41:30-34. http://dx.doi.org/10.1016/j.jfca.2014.12.019.

Müller D.N., M. Kleinewietfeld and H. Kvakan. 2011. Vitamin D review Journal of the Renin-Angiotensin-Aldosterone System 12:125-128.

Murthy G.K. and U. Rhea. 1967. Determination of major cations in milk by atomic absorption spectrophotometry. J of Dairy Sci 50: 313–317. http://dx.doi.org/10.3168/jds.S0022-0302(67)87416-2.

Nair R and A. Maseeh. 2012. Vitamin D: The “sunshine” vitamin. J Pharmacol Pharmacother. 3:118-126. doi:10.4103/0976-500X.95506.

IOM (Institute of Medicine.) 2011. Dietary reference intakes for calcium and vitamin D. Washington DC: The National Academies Press

Perales, S, A. Alegría, R. Barberá and R. Farré. 2005. Review: Determination of Vitamin D in Dairy Products by High Performance Liquid Chromatography. Food Sci Tech Int 11:451–462.

Schmid A. and B Walther. 2013. Natural Vitamin D Content in Animal Products. Adv. Nutr. 4: 453–462. http://dx.doi.org/10.3945/an.113.003780.

Tsai S.-Y., H.-Y. Lin, W.-P. Hong and C.-P. Lin. 2017. Evaluation of preliminary causes for vitamin D series degradation via DSC and HPLC analyses. J Therm Anal Calorim 130: 1357–1369. http://dx.doi.org/10.1007/s10973-017-6209-4.

Vierucci F., M. Del Pistoia, M. Fanos, P. Erba and Gi.Saggese2. 2014.Prevalence of hypovitaminosis D and predictors of vitamin D status in Italian healthy adolescents Italian Journal of Pediatrics 40:54 http://www.ijponline.net/content/40/1/54

Weir, R.R., J.J. Strain, M. Johnston, C. Lowis, A.M. Fearon, S. Stewart and L.K. Pourshahidi. 2016. Environmental and genetic factors influence the vitamin D content of cows' milk. Proceedings of the Nutrition Society 76, 76-82. http://dx.doi.org/10.1017/S0029665116000811.

Williams, M.H., E.L. Van Alstyne and R.M. Galbraith. 1988. Evidence of a novel association of unsaturated fatty acids with GC (vitamin D-binding protein). Biochem. Biophys. Res. Comm. 153: 1019-1024.

Zhang J.Y., A.J. Lucey, K. Galvin, L. Nolan, K.D. Cashman, J.R. Higgins and M. Kiely. 2012. Vitamin D content of human milk and associations with milk fat content and maternal serum 25-hydroxyvitamin D concentrations. Proceedings of the Nutrition Society 71 (OCE2), E54 http://dx.doi.org/10.1017/S0029665112001115.