

# Production and composition of buffalo milk supplemented with agro industrial byproducts of the african palm



Producción y composición de leche de búfala bajo suplementación con residuos agroindustriales de palma africana

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### ABSTRACT

# Key words:

Buffalo milk Fatty acids Milk chemical composition Oleaginous plants The goal for this applied research was to assess the production and composition of buffalo milk when Oil Palm kernel flour and Oil Palm kernel cake are supplemented to their diet. Thirty buffaloes from the grasslands of the Andes valleys in Colombia with 1 to 3 lactations and an average weight of 575 kg were selected for the experimental. The animals were randomly assigned to one of three experimental groups: (T1 Control Group) with no diet supplements, (T2) 1000 g day<sup>1</sup> of oil palm kernel cake and 350 g day<sup>1</sup> of molasses diet supplement, and (T3) 1150 g day<sup>1</sup> of oil palm kernel flour. During the first 100 days of lactation, the milk livestock were individually weighed and milked. Milking was scheduled every 15 days, for a total of seven samples. The supplement consumption was recorded and a bromatological analysis of grasses was performed. The chemical composition of the milk was determined using an ultrasonic Ekomilk analyzer and a fatty acid full profile analysis was made using High Liquid Pressure Chromatography (HLPC). A 7 x 3 multiple variable statistical analysis was performed by sampling seven fifteen day periods and three types of diet. The average values of milk components observed were: 3.54% protein; 7.4% fat: total solids 16.9%; non-fat solids 9.5%; 2.1 fats to protein ratio. The profile of fatty acids showed 2.34% of polyunsaturated; 33.1% of monounsaturated; 64.6% of unsaturated fatty acids; and 0.96% of Omega 6 acids. In conclusion was observed partial effect to fat supplementation in the buffaloes milk production.

# RESUMEN

#### Palabras claves:

Leche de búfala Ácidos grasos Composición química de la leche Plantas oleaginosas El objetivo del presente trabajo constituyó la evaluación de la producción y composición química de la leche de búfalas sometidas a suplementación con derivados de la industria de la palma de aceite. El presente trabajo se realizó en la época de transición de lluvias en un sistema bufalino ubicado en el Magdalena Medio colombiano. Se utilizaron 30 búfalas de dos o tres partos, con pesos promedio de 575 kg y una condición corporal de 3.5 al inicio del experimento. La composición de los subproductos torta y harina de palmiste, y la de los forrajes fueron determinadas mediante análisis bromatológico. Los animales fueron dispuestos en forma aleatoria en tres grupos, control y dos suplementados con residuos agroindustriales. Los tratamientos fueron: T1 = Sin suplementación (control), T2 = torta de palmiste y, T3 = harina de palmiste. En los primeros 100 días de lactancia y cada 15 días se realizó pesaje individual, medición de la producción, perfil de ácidos grasos por HLPC y composición química de la leche por análisis ultrasónico. Se hizo análisis multivariado con arreglo factorial 7 x 3 (periodos x tratamientos). En leche la media de sólidos totales fue 16.9% y 9.5% de sólidos no-grasos; El perfil de ácidos grasos presentó porcentajes totales de 2.34 de poli-insaturados, 33.1 de mono-insaturados, 64.6 de insaturados, y 0.96 de ácidos omega 6. En conclusión se observó efecto de la suplementación sobre la producción de leche de búfala y efecto parcial sobre la composición grasa.

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igh production, protein quality and fat contents of buffalo (*Bubalus bubalis*) milk, justifies a selection of ecologically conditioned outstanding specimens from this bovine breed found in the valleys of the central mountain range of the Andes in Colombia. According to the FAO (2010) the worldwide production of buffalo milk grows at an annual rate of 248%, for various reasons apart from its high content of total solids (TS) compared to other milk cattle breeds. During the last 42 years Colombia's buffalo population has been increasing due to its capacity to adapt to different eco-systems and to the quality of its milk (Bolívar *et al.*, 2010).

Chemical composition of buffalo milk varies widely as a consequence of numerous factors such as breeds, climate and lactation period. The Murrah is the main milk production in the buffaloes breed (Malhado *et al.*, 2013).

The production of milk with buffaloes is actually an alternative for large, medium and small milk producers in Latin American and Caribbean countries. It is therefore of utmost importance for producers, technicians and the milk industry in general to know the chemical composition of this type of milk compared to other milk cattle breeds and the factors that affect it.

In areas where agricultural activities produce residues with high potential use for animal nutrition, it is important to assess its effects during the Buffalo first lactation phase in which milk production coincides with nutritional unbalance of buffaloes, a problem still unresolved by research in animal production (Terramoccia *et al.*, 2005).

The African Palm produces 200 million tons of biomass per year worldwide; of which only 10% is used. An important part of this by-product is burned, which causes  $CO_2$  contamination. It is estimated that every hectare of this crop produces 25 tons of plant residue (Pauli, 1999), that could be used to produce energy as biomass and in animal nutrition; with which an environmentally friendlier palm cultivation could be achieved (Abdalla *et al.*, 2008).

In various countries the oil palm subproducts are use in ruminant nutrition (Castro *et al.*, 2016; Hassim *et al.*, 2010). This agro-industry produces two different by-products depending on the extraction processes: the oil palm kernel flour and the oil palm kernel cake. The first is obtained

by applying mechanical pressure to the seeds after they have been mashed with help of chemicals solvents, while the palm kernel cake is made by desolventizing the oils once obtained by chemical lixiviation. This last residue contains less fat, higher amount of fiber (NDF), more protein and raw energy. The chemical composition and the digestibility of the palm cake vary in content of the tegument of the seed, and the amount of residual oil. The palm flour is 20% more digestible for its low tegument residue content (FEDNA, 2003).

The greater percentage of milk fat of the buffalo is related to the presence and composition of the polyunsaturated fatty acids (FA) (Patiño *et al.*, 2008), which are important in the development of nutrients that have a preventive action on certain pathogens; this is the main reason why the present study is oriented towards obtaining nutraceuticals rich in special fatty acids (Hernández and Serna, 2003; Valdés, 2011).

The primary objective of this study was to evaluate the effects of the supply of palm kernel meal and palm cake expeller in the production and composition of the milk produced by the buffaloes during the first 100 days of lactation, grazing in the mid-grass lands of the Andes Valley in Colombia.

### MATERIALS AND METHODS

The study was conducted at a specialized buffalo milk production system located in the fertile soil of the Mid-Magdalena River Valley (6°19'N and 73°57'O). Thirty female Murrah breed buffaloes with two or three calvings; between 550 and 600 kg in weight and with at least a 3.5 body condition score on a scale of one to five (Alapati *et al.*, 2010). The selected buffaloes were pregnant with an estimated calving date due for the first week of September. The assessment period was programmed for the 1st stage of lactation (0 - 100 days). The study was approved for the committee of Animal experimental and ethics of the Facultad de Ciencias Agrarias.

The selected livestock were previously conditioned during 15 days by being fed with one kg of palm kernel flour and 1.15 kg of oil palm kernel cake. The supplement was offered once a day after milking and its consumption was total. The buffaloes were milked by hand once a day and the scientific measuring and analysis of the chemical

compositions of the milk was taken every 15 days. The livestock grazed in rotation system on "Angleton" (*Dichatium aristatum* Benth), "Climacuna" (*Dichantium annulatum*), "German" (*Echhynochloa polystachya*) and "Puntero" (*Hyparrhenia rufa*) grass, with mineralized salt (8% phosphorus) and water *ad libitum*. Openly available to groups of 10 animals each as follows: T1: Control group, no diet supplement; T2: Diet supplement (1 kg oil palm kernel cake with 350 g of molasses per animal per day) and T3: Diet supplement (1.15 kg of oil palm kernel flour without molasses). The groups were considered to be isoproteic and isonergetic. The qualities of the oil palm kernel cake and flour as well as the pastures were assessed with help of bromatological analysis (Table 1).

Table 1. Bromatological analysis\* for oil palm kernel cake and oil palm kernel flour.

Determination (%)	Oil palm kernel flour	Oil palm kernel cake		
Dry matter	91.28	94.39		
Ash	3.59	3.26		
Protein	16.20	14.03		
Fat (ether extract)	5.59	19.90		
NDF	68.57	53.77		
Carbohydrates	6.07	9.04		
Energy (Mcal·k <sup>-1</sup> )	4600.10	5376.29		

\* Weende method

The milk samples for measurement and analysis (n=210) were taken on milking days 1, 16, 31, 46, 61, 76, and 91 after calving. Protein, fat and Total solids analysis was made using the Ekomilk Fast automated machine. A profile of fatty acids (FA) was made by using a Shimadzu GC-14A gas chromatograph, equipped with flame detector (260 °C), flow gas carrier to 15 psi and reverse phase capillary column.

The statistical model used for the analysis of the data was multivariate analysis with factorial arrangement  $7 \times 3$  (periods x treatments):

$$Yijk = \mu + ti + Bj + Cijk + eijk$$

Where:

- Yijk = value of the dependent variable for j that received the ijk diet supplement
- $\mu$  = overall average
- ti = fixed diet effect

Bj = j fixed time effect

- Cijk = fixed interaction effect between diet and time
- Eijk = random error of j that received the ijk diet

The level of probability was P<0.05; the variation analysis was made with the statistics program SAS 9.2 (SAS Institute, 2007).

# RESULTS AND DISCUSSION Milk Production

Significant differences for daily milk production were found among the treatments (P<0.001). The average production in the T1, T2 and T3 were 2.69, 3.82 and 3.40 L·day-1, respectively, with an average value of 3.3 L·day<sup>-1</sup> which is similar to the production of 3.21 obtained by Bolívar et al. (2010) with buffaloes in pasture grazing and slightly higher than 3.1 L·day<sup>-1</sup> found by Hurtado et al. (2005) in conditions of extensive pastures in the same region of the present study. Medina and García (2008) supplemented the diet of a commercial herd of Water Buffalo with yeast in the department of Sucre (Colombia) and found a daily average milk production of 4.49 and 3.72 L per animal in supplemented diet and non-supplemented diet, respectively; this greater production probably was due to the increased consumption of metabolic energy as is suggested by Mahyuddin and Praharani (2010). When water buffalo had a diet supplement of corn and sorghum silage (Barile et al., 2007) or with sugar cane (Franzolin and Alves, 2010), the milk production was not significantly different to that of grazing buffaloes. (Quintero et al., 2008) found that milk production is affected by the number of calvings and climate change.

During the first calving, the average maximum daily milk production per animal was of 3.81 L during rainy season

as compared to 3.76 L during the dry season. On the fifth calving, milk production during the rainy season was 4.52 L and 4.37 L during of dry season. This information shows that when the buffalo reaches its adult weight, the production of milk is higher during the rainy season.

For this experiment, the first 100 days of the milking period, correspond to a transitional climate period between the wet and dry seasons.

#### Total Solids (TS) in buffalo milk

The TS in the milk varied between the dietary treatments 16.6%, 16.7% and 17.2% for T1, T2 and T3, respectively (P<0.001). Differences were found between the results of T1 and T2 groups and T3 superior to T2, in the TS content. The percentage differences of T3 were of 2.55 versus T2 and of 3.19 in respect to T1. These differences were affected by the amount of fat in the milk, while the percentage of protein showed the least variation. The amounts of TS found in the present study are similar to those obtained by Hurtado *et al.* (2005) (16.9%) and greater to those found by Patiño et al. (2002), Patiño (2004) (15.7%) and Bastos (2005) (16.6%).

#### Milk composition

No differences were found among the dietary treatments (P>0.05) for the protein contents in buffalo milk. The average amounts found were 3.56% for the control group, 3.54% for the group with a diet supplement of oil palm kernel cake and molasses; and 3.50%, for the group with the diet supplement of oil palm kernel flour. The protein average for all the experiments was 3.53%. similar to 3.58% found by Medina and García (2008) in buffaloes supplemented with Saccharomyces cerevisiae and 3.66% by (Patiño, 2004) in a study of buffaloes fed on grass without dietary supplementation. However, the protein content found in this study was less than that reported by (Cervantes et al., 2010) in a study on the adaptability of the buffalo in Colombia, where milk proteins range between 4.07% and 4.77 % for an average of 4.40%.

It has be pointed out that for this research the female buffaloes used were in their second or third quarter of lactation, therefore, in their first 100 days of lactation; a time span during which low percentages of protein are normally registered. The average percentage of fat in buffalo milk was 7.16%, 7.25% and 7.74% for dietary treatments T1, T2 and T3, respectively (P>0.01), with an average percentage value for all the supplements of 7.38%. The absence of statistical differences between the different diet supplements was probably due to the fact that the oil palm kernel cake and the oil palm kernel flour contain low fat remnants. The fat value found coincides with the larger amount (7.36%) reported by Hurtado et al. (2005) while working with buffaloes under extensive grazing and supplemented with molasses and with the amount of 7.22% found by Patiño (2004) in a study carried out in Argentina with buffaloes fed on natural grasslands without diet supplementation. The percentage of fat in buffalo milk was 8.19% in a range between 6.89% and 9.34 similar to that found by Cervantes et al. (2010) in buffalo milk in a study for nine places of the Magdalena river valley in Colombia. Among the factors that could have caused differences in the fat readings are analytical methods, genetic factors, nutritional and physiological factors; and the environmental and sanitary conditions of the animals (Walker et al., 2004; Patiño et al., 1999).

### RESULTS

As a result, fat in this research exceeds 4.16% and 5.19% reported by Medina and García (2008) in buffaloes supplemented and not supplemented with yeast in the humid region of Colombia; as is different to 6.78% found by Patiño *et al.* (2002) and 3.56% and 4.35% in studies made by Bastos (2005). The lactose average (5.24%) did not vary (P>0.05) no statistical effect was found for diet supplement or time period.

**Determination of the fatty acids types in buffalo milk** The average percentages for short chain FA (Table 2) found were: undecilic (0.91%), and lauric (1.83%), and long chain FA were: mirístic (8.6%); miristoleic (0.62%); pentadecanoic (1.91%); palmitic (30.6%); palmitoleic (0.77%); heptadecanoic (1.04%); estearic (19.6%); vaccenic (5.47%); oleic (26.2%); linoleic (0.95%); and conjugated linoleic acid (CLA) at (1.38%). The FA profile did not show any significant differences between the dietary treatments in the FA content of monounsaturated (MUFA), nor in the FA polyunsaturated (PUFAs) and the FA saturated (SFAs). The content of saturated FA in the T1 group was 63.8%, and 36.2% in unsaturated FA (33.8% mono-unsaturated and 2.4% polyunsaturated). For T2, 63.9% of the FA content was saturated and 36.1% unsaturated, of which 33.7% were monounsaturated and 2.33% polyunsaturated. In T3 the results showed that 65.9% of the FA were saturated and 34.1% were unsaturated of which 31.8 % were monounsaturated and 2.28% polyunsaturated.

Patiño *et al.* (2008) working with Murrah buffaloes and crossbreeds between Murrah and Mediterranean breeds in natural grasslands, found the content of saturated FA in the milk was 56.9%, 6.88% less than that found in the present

study; while the content of the unsaturated FA was 43.7%, 7.47% more than the amount found in T1. The animals whose diet was supplemented with corn showed that 57.1% of FA in the milk were saturates, which represents 6.84% less of the amount than T2 and 42.9% of unsaturated FA, exceeding T2 by 6.84%.

Buffalo milk contains high amounts of unsaturated long chain FA as: palmitoleic and linoleic; while the amounts of miristoleic and oleic FA are low contributing to a better nutrition in the FA profile of milk (Verruma and Salgado, 1994).

**Table 2.** Fatty Acids in Buffalo Milk in grazing and receiving Oil Palm residues as a Diet Supplement (in two periods beginning –day 15- of lactation and at the end –day 100- of the lactation.

				Diet supplement				
Fat Acid	Abbrevia- tion	Control		Oil palm kernel flour		Oil palm kernel cake + molasses		
		Beginning <sup>a</sup> *	End <sup>b</sup> **	Beginning <sup>a</sup> *	End <sup>b</sup> **	Beginning <sup>a</sup> *	End <sup>b</sup>	
Undecilic	C11:0	0.92	0.82	1.00	0.91	1.01	0.82	
Láuric	C12:0	1.49	1.20	2.03	3.01	1.53	1.73	
Miristic	C14:0	8.23	7.91	9.35	9.99	8.26	8.28	
Miristoleic	C14:1	0.23	0.41	0.36	0.52	1.82	0.43	
Pentadecanoic	C15:0	1.52	1.67	1.77	1.61	3.04	1.88	
Palmitic	C16:0	30.5	30.4	32.90	31.1	28.90	29.60	
Palmitoleic	C16:1	0.45	0.82	0.40	0.78	1.32	0.87	
Heptadecanoic	C17:0	1.42	1.15	0.31	0.92	1.31	1.17	
Estearic	C18:0	20.80	19.5	19.70	17.3	21.30	19.00	
Vaccenic	C18:1t-11	5.13	6.05	4.83	5.37	5.29	6.33	
Oleic	C18:1n-9c	26.90	27.7	25.10	26.1	24.20	27.20	
Linoleic	C18:2n-6c	1.35	0.83	1.10	0.74	0.92	0.81	
CLA	C18:2-9cis-	1.06	1.56	1.16	1.57	1.05	1.89	
	11t							
	_	100	100	100	100	100	100	
Polyunsaturated	Pufas	2.41	2.39	2.26	2.31	1.97	2.70	
Monounsaturated	Mufas	32.70	34.90	30.70	32.80	32.60	34.80	
Total Saturated	Sfas	64.90	62.70	67.00	64.90	65.40	62.50	
Total Omega 3	n - 3	0.00	0.00	0.00	0.00	0.00	0.00	
Total Omega 6	n - 6	1.35	0.83	1.10	0.74	0.92	0.81	
Total n - 6/n - 3	n - 6/n - 3	0.00	0.00	0.00	0.00	0.00	0.00	

\* a Average of 10 measurements at the beginning of the trial (day 15)

\*\* <sup>b</sup> Average of 10 observations at the end of the trial (day 100)

### CONCLUSIONS

The diet supplements of the Oil Palm kernel cake with molasses increased the production of the milk by 29.2%

when compared to the control group. The type of diet supplement did not affect protein percentage in milk. The type of diet supplement affected fat percentage in milk. It increased in group (T3) more than that of group (T2). The diet supplement of the oil palm kernel flour improved the total milk solids of the buffalo milk.

Only contents of the myristic acid and the palmitoleic were affected by diet. Medium chain acids: undecilic, lauric were unchanged. no differences were found in the long chain acids: miristoleic, pentadecanoic, palmitoleic, heptadecanoic, estearic, vaccenic, oleic and linoleic.

#### REFERENCES

Abdalla A, Silva Filho J, Godoi A, Carmo C, Eduardo J. 2008. Utilização de subprodutos da indústria de biodiesel na alimentação de ruminantes. Revista Brasileira de Zootecnia 37 Supl. 258-260. doi: 10.1590/S1516-35982008001300030

Alapati A, Kapa S, Jeepalyam S, Rangappa S, Yemireddy K. 2010. Development of the body condition score system in Murrah buffaloes: validation through ultrasonic assessment of body fat reserves. Journal of Veterinary Science 11(1): 1-8. doi: 10.4142/jvs.2010.11.1.1

Barile V, Tripaldi C, Pizzoferrato L, Pacelli C, Palocci G, Allegrini S, Maschio M, Mattera M, Manzi P and Borghese A. 2007. Effects of different diets on milk yield and quality of lactating buffaloes: maize versus sorghum silage Note II. Revista Veterinaria 21(1): 655-657.

Bastos P. 2005. Constituição físico-química, celular e microbiológica do leite de búfalas (*Bubalus bubalis*) criadas no estado de São Paulo, Tese doutorado. Universidade de São Paulo. 130 p.

Bolívar D, Cerón M, Ramírez E, Agudelo D and Angulo R. 2010. Parámetros genéticos para características reproductivas en una población de búfalos (*Bubalus bubalis, Artiodactyla, Bovidae*) en el Magdalena Medio colombiano. Revista Facultad Nacional de Agronomía 63(2): 5587-5594.

Castro T, Cabezas A, De la Fuente J, Isabel B, Manso T, and Jimeno V. 2016. Animal performance and meat characteristics in steers reared in intensive conditions fed with different vegetable oils. Animal 10(3): 520-530. doi: 10.1017/S1751731115002554

Cervantes E, Espitia A and Prieto E. 2010. Viabilidad de los sistemas bufalinos en Colombia. Revista Colombiana de Ciencias Animales 2(1): 215-224.

Food and Agriculture Organization-FAO. 2010. FAOSTAT. En: Agriculture Database, : Febrero 2014.

Blas de C, Mateos GG y Rebollar PG (eds.). 2003. Tablas FEDNA de composición y valor nutritivo de alimentos para la formulación de piensos compuestos. Segunda edición. Fundación Española para el Desarrollo de la Nutrición Animal, Madrid, España. 423 p.

Franzolin R and Alves T. 2010. Evaluation of diets with increasing corn grain levels on rumen protozoa population and liquid outflow rate in buffalo. Revista Veterinaria 21(1): 663-665.

Hernández C and Serna S. 2003. Alimentos nutraceúticos, el futuro de nuestra alimentación. Transferencia Revista Digital de Posgrado fInvestigación y Extensión del Campus Monterrey, México. 16(61): 1-5.

Hassim HA, Lourenço M, Goel G, Vlaeminck B, Goh YM, and Fievez V. 2010. Effect of different inclusion levels of oil palm fronds on *in vitro* rumen fermentation pattern, fatty acid metabolism and apparent biohydrogenation of linoleic and linolenic acid. Animal Feed Science and Technology 162(3-4): 155–158. doi: 10.1016/j. anifeedsci.2010.09.011

Hurtado N, Cerón M, Lopera M, Bernal A and Cifuentes T. 2005. Determinación de parámetros físico-químicos de leche bufalina en un sistema de producción orgánica. Livestock Research for Rural Development 17 (1).

Malhado CHM, Malhado ACM, Ramos ADA, Carneiro PLS, Souza JCD, and Pala A. 2013. Genetic parameters for milk yield, lactation length and calving intervals of Murrah buffaloes from Brazil. Revista Brasileira de Zootecnia 42(8): doi: 10.1590/S1516-35982013000800005

Mahyuddin P and Praharani L. 2010. Effect of oil palm solid waste on milk production of Murrah buffalo. Revista Veterinaria 21(1): 648-652.

Medina S and García N. 2008. Efectos de la suplementación con levaduras (*Saccharomyces cerevisiae*) sobre la producción y calidad de la leche de búfala (*Bubalus bubalis*). En: Informativo Facultad de Ciencias Agropecuarias. Universidad Católica de Oriente.

Patiño E, Jacobo R, Méndez F, Giorgi E, Cipolini F, Stamatti G and Guanziroli M. 1999. Fatty acids, minerals and vitamins of water buffalo milk in Argentina. Italia Buffalo Newsletter 18(2): 328-330.

Patiño M, Méndez F, Cedres J and Guanziroli M. 2002. Caracterización de leche de búfalas mestizas en pastizales naturales de corrientes, Argentina. Revista Argentina Producción Animal 22(3–4): 199-203.

Patiño E. 2004. Factores que afectan las propiedades físicas y la composición química de la leche de búfalas *(Bubalus bubalis)* en Corrientes, Argentina. Revista Veterinaria 15: 21-25.

Patiño M, Judis M, Guanziroli C, Pochón D, Cedres J, Doval M, Romero A, Faisal E and Rebak G. 2008. Determinación de ácidos grasos en leche bubalina (*Bubalus bubalis*) en Corrientes, Argentina. Revista Veterinaria 19(1): 28-32.

Pauli G. 1999. Forest product for a sustainable economy. Forum for Applied Research and Public Policy 14(4): 30-39.

Quintero J, Serna J, Cerón M, Hurtado N and Agudelo D. 2008. Estimación de la curva de lactancia mediante modelos matemáticos lineales y no lineales en búfalas Colombianas. Revista Lasallista de Investigación 5(1): 34-44.

Statistical Analysis System Institute-SAS. 2007. SAS User's guide Institute Inc. Proc Mixed Statistics. Ed. Cary, NC, USA 475p.

Terramoccia S, Bartocci S and Borghese A. 2005. Nutritional requirements in buffalo cows and heifers, Chapter VII. In: Borghese, A. (ed.). Buffalo production and research. Reu Technical Series, 67, Italy. p: 145-160.

Valdés A. 2011. Contribución de la leche de búfala a la industria y a la salud humana. Revista Cubana de Ciencias Veterinarias 32(1): 43-46.

Verruma M and Salgado J. 1994. Análise química do leite de búfala em comparação do leite de vaca. Science Agriculture Piracicaba 51(1): 131–137.

Walker G, Dunshea F and Doyle P. 2004. Effects of nutrition and management on the production and composition of milk fat and protein: A review. Australian Journal Agriculture Research 55: 1009-1028. doi: 10.1071/AR03173