

Linoleic and alfa linolenic acids in pastures as a source of n-3 PUFAs and CLA

P T Garcia^{1*} and J J Casal²

¹Institute of Food Technology, Agroindustry Research Center. National Institute of Agricultural Technology INTA, CC 77 (B1708 WA) Moron, Buenos Aires, Argentina

²Forum of Food, Nutrition and Health (FANUS) Bolsa de Cereales de Buenos Aires, Argentina

***Corresponding Author:** P T Garcia, Institute of Food Technology, Agroindustry Research Center. National Institute of Agricultural Technology INTA, CC 77 (B1708 WA) Moron, Buenos Aires, Argentina.

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Abstract

Forage plants found in pastures are high in polyunsaturated fatty acids (PUFA), especially C18:2 n-6 and C18:3 n-3 fatty acid types. Fresh forage contains a high proportion (50-75%) of its total fatty acid (FA) content in the form of C18:3 n-3. Sources of variation of forage lipid concentration are plant species, growth stage, and temperature and light intensity dependant. Levels of C18:3 n-3 FA vary with plant species and factors such as stage of maturity and with light treatment. When forages represent a high proportion of ruminant diet they provide a significant quantity of FAs. Considering the importance of these FAs as substrate for conjugated linoleic acid (CLA) and n-3 PUFA concentration in beef and milk, the FA composition of forages needs to be delineated in grass, beef meat, milk and production systems. It is important to know the degree of variation in both total lipids and individual FAs in forages with a view to establishing the potential to cultivate grasses for a higher content of n-3 PUFA and hence the opportunity to deliver more n-3 PUFAs and CLA into beef and milk.

Keywords: Forages; Fatty acid; Alfalfa; Winter forages; Cutting; Season

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Introduction

Fat is an important component of the human diet, but present levels of intake are considered to be high and the overall FA composition found to be imbalanced. There is an excessive intake of saturated fatty acids (SFAs) relative to PUFAs, expressed usually as the P/S ratio, and the consumption of n-6 PUFAs is high relative to n-3 PUFAs. The ratio of n-6/n-3 PUFAs is a risk factor in cancers and coronary heart disease, especially the formation of blood clots leading to heart attack. More recently, nutritionists have focused on the type of PUFA and the balance in the diet between n-3 PUFAs formed from Alpha linolenic acid (ALA; C18:3 n-3) and the n-6 PUFAs from linoleic acid (LA; C18:2 n-6) (Russo, 2009). LA and ALA are the precursor molecules from which the rest of fatty acids belonging to the n-3 and n-6 fatty acid family can be synthesized through a series of elongation and desaturation reactions. These two fatty acid families not only share these enzymes, but they also compete for the same enzymes (Brenner, 1999). The conversion of ALA to long chain fatty acids EPA (C20:5 n-3), DPA (C22:5 n-3) and DHA (C22:6 n-3) depends on the dietary total PUFA, the ratio LA/ALA and the availability of ALA (Barcelo-Coblijn & Murphy, 2009).

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Forage plants found in pastures are high in PUFAs, specifically C18:2 n-6 and C18:3 n-3 fatty acids. FA profiles are distinctive to particular species, which confirm that FA composition of forages is under considerable genetic control (Dewhurst, *et al.* 2001). This offers the potential to select forages with higher concentrations or altered FA composition. When harvested at the same stage of development Boufaied, *et al.* (2003) found significant differences both within species of the same family (grass or legumes) and between the two families. Legumes had higher concentrations of C14:0, C16:0, C18:0, C18:1 and C18:2 n-6 and total FAs and lowers concentrations of C18:3 n-3 but large variations among species within each family were also observed. Dewhurst, *et al.* (2001) also found distinct between-species differences in FAs profiles of forage grasses cut at the same date and a significant interaction effect between species and cutting date.

Little information is available about the effect of the nature of pasture lipids on beef FA acid composition but several studies have compared concentrate to forage-based diets to determine how fat in finished beef is altered. Dietary C18:3 n-3 is the best source of C18:3 n-3 in meat and milk, while the conjugated isomers of C18:2 (CLA) derives from C18:2 n-6 and C18:3 n-3. Increasing amounts of PUFAs n-3, CLA and a lower n-6/n-3 ratio were found in beef on forage-based diet compared to concentrate diets (French, *et al.* 2000-2003; Garcia, *et al.* 2008, 2017). Milk and ruminant meats are the only significant source of CLA in the human diet and this appears to be related to the consumption by ruminant of fresh pastures. Elgersma, *et al.* (2003) utilizing different perennial ryegrass cultivars as source of pasture in dairy cattle increased milk CLA content while grazing the cultivar containing the greatest PUFA concentrations compared to cattle consuming the cultivar lowest in PUFA.

The forage-based diets increases ALA in lamb Longissimus dorsi muscle as compared with feeding concentrates, agreeing with previous studies with beef cattle comparing alfalfa silage (Mandell, *et al.* 1998) or pasture (French, *et al.* 2000). The increased concentrations of EPA, DPA and DHA in muscle of animals fed on grass suggests that the high availability of ALA in the diet has resulted in an enhanced synthesis of these n-3 long-chain PUFAs (Nuernberg, *et al.* 2005). The FA proportion of polar lipids of fat from *L. dorsi* presented similar proportions of ALA and EPA but less DPA and DHA compared with beef heifers offered pasture only (Noci, *et al.* 2007). The feeding regime, herbage or concentrate affected the total n-3 PUFA in *L. dorsi muscle*. The herbage regime presented more ALA and EPA but no significant changes in DHA (Vasta, *et al.* 2009). The grazing on *Trifolium subterraneum* as monoculture and associated with *Lolium multiflorum* in the proportion T/L =66/33 has increased the linolenic acid of lamb meat (Chiofalo, *et al.* 2010). The types of forage, crop variety, cutting, season, year, of forage crops for grazing forage beef production (Garcia, *et al.* 2015).

Meat, fish, fish oils and eggs are the only significant sources of long-chain n-3 PUFAs for humans. Although meat has lower concentrations of these FAs compared to oily fish, it is a very significant source for many people under low dietary fish intake. The low PUFAs concentration and the high concentrations of saturated FA in ruminant tissues result from the bio hydrogenation of dietary PUFAs in the rumen. The potential use of livestock products as vehicles to deliver n-3 fatty acids has been the subject of intensive research (Moghadasian, 2008). Dewhurst, *et al.* (2009) describe a higher content of C18:2 n-6 and C18:3 n-3 in milk and meats of ruminants grazing red or white clover legumes, when compared to milk and meat of animals fed with grasses and other legumes. It is believed that in red clover rich diets the fermentation and bio hydrogenation in the rumen are different from those obtained with perennial ryegrass (Lourenco *et al.* 2008) due to the inhibition of proteolysis and lipolysis by the plant enzyme polyphenol oxidase, which is present in clover. The polyphenol oxidase converts phenols in quinones, which bind with proteins and reduce the proteolysis and lipolysis in the rumen (Lee, *et al.* 2007; Van Ranst, *et al.* 2010).

The most important factors influencing the FA concentrations of fresh forages appears to be cutting date and interval, which reflect maturity differences. Garcia, *et al.* (2015) found that cutting date affected significantly ($p < 0.001$), in alfalfa cultivars, all FAs, with the exception of the oleic acid [Table 1]. Dewhurst, *et al.* (2001) found that a cutting interval of 20 days compared 38 days increased the concentrations of all FA in perennial ryegrass and Italian ryegrass (*L. multiflorum*). Several studies have shown decline FAs concentrations with increasing maturity. Barta (1975) noticed reductions in total FAs of more than 30% in six grass species as the plants increased in maturity. Boufaied, *et al.* (2003) found reductions in C16:0, C16:1, C18:2 and C18:3 and TFA with advancing maturity of

Timothy (Phleum pratense) from stem elongation to early flowering. Dewhurst, *et al.* (2001) found that the highest levels of TFA concentrations in *L. perenne*, *L. multiflorum* and *L. boucheanu* were noted when the grass was in vegetative growth stages. A similar pattern of seasonal change in perennial ryegrass (*L. perenne*) was found by Guilliard, *et al.* (2002). There is some evidence indicating that the flowering process can also cause a reduction in FA concentrations (Dewhurst, *et al.* (2002). Changes in leaf: stem ratios can partly explain the declining proportions of FA as plants mature (Boufaied, *et al.* (2003); Dewhurst, *et al.* (2003). The proportion of leaves to stems decreases with maturity (Belanger & McQueen, 1996). Exposure to chilling temperatures induce changes in plants' membrane lipids, notably their degree of FA unsaturation and phospholipids contents increase during cold acclimatization. Falcone, *et al.* (2004) showed that FA concentrations in Arabidopsis are affected by changes in temperature. Following a reduction in temperature, from 36°C to 17°C, increases in concentrations of C16:3 and C18:3 were found while the concentrations of C16:1 and C18:2 decreased. Somewhat contradictory results were obtained by Kuiper, *et al.* (1970), who found that lucerne (*Medicago sativa*) grown at 15°C contained higher proportions of both C18:2 and C18:3 than plants grown at 30°C. Light intensity may affect the FA composition of forage plants by influencing the chloroplast content. Dewhurst & King (1998) found that shading grass with a black plastic sheet for 24 hr prior to cutting results in reductions of the TFA content and % 18:3 in silage obtained from it.

The effect of season on the alfalfa FA composition also was important. During the fall C18:2 increased and C16:0, C16:1, C18:0 decreased. Differences between spring and summer were observed for C16:1, C18:0, C18:2 and C18:3. The EE % and contribution of C18:2 and 18:3 were higher in fall as compared with the other seasons. The total C18:3 n-3 + C18:2 n-6 percentage was higher in fall and the ratio C18:3 n-3/C18:2 n.6 was higher in spring compared to summer and fall [Table 1] (Garcia, *et al.* 2015). Seasonal fluctuations were assessed in perennial ryegrass between April and November. It was found that during the months of June and July forages contained the least amount of total fatty acids. Elgersama, *et al.* (2003) showed values decreasing to about 2.7 and 17.4 g kg⁻¹, respectively for C18:2 and C18:3, while Dewhurst, *et al.* (2001) reported values as low as 1.4 and 4.4 g kg⁻¹. However forages collected in April and November contained the highest amounts, reaching 2.8 g kg⁻¹ for 18:2 g kg⁻¹ and 10.4 g kg⁻¹ for C18:3 respectively (Dewhurst, *et al.* 2001).

Season	EE%	C18:2	C18:3	CC18:2+18:3	C18:3/C18:2
Spring	2.0a	0.33a	0.80a	1.13a	2.47c
Summer	2.2a	0.41b	0.75a	1.16a	1.80b
Autumn	3.3b	0.89c	1.33b	2.22b	1.52a
SE ¹	0.07	0.02	0.048	0.055	0.076
p-value<	**	**	**	**	**

SE¹ Standard error; abc within a column denote different letters significantly different P < 0.05.

Table 1: Effect of season on the ether extract (EE% dry matter) and in the contribution of C18:2 n-6 and C18:3 n-3 in alfalfa lipids (Garcia, *et al.* 2015).

Winter hardiness also impact the FA acid found in forages. Somola, *et al.* (1998) found increases PUFAs concentrations in the lipid membranes of winter hardy varieties of Bermuda grass after the plants were subjected to a cold treatment. Mel'uchova, *et al.* (2008) found that ALA significantly decreased from 62% to 39% of total FAs from May to August and subsequently slightly increased from August to September, compared with the beginning of pasture season. The pasture seasonal variations in C18:3 n-3/C18:2 n-6 ratio were directly proportional to the corresponding content of CLA and indirectly proportional to the ratio in ewes' milk fat. The results suggests that the seasonal variations in CLA content in ewes' milk fat are related primarily to the seasonal variations in 18:3 n-3 content in grass lipids. Fatty acid concentration decreases as plant mature, making forage management a central determinant in FA concentration by livestock (Loyola, *et al.* 2002, Clapham, *et al.* 2005, Pestana, *et al.* 2012).

Cutting date and variety affected significantly the concentrations of all FA with the exception of C18:1 and C18:0 respectively and EE (g/100g DM) was significantly lower in cut 2 compared with cut 1 in all cultivars Garcia, *et al.* (2015). Dewhurst, *et al.* (2001) compared three ryegrass species and found C18:2 and C18:3 differing by as much as 0.34 g kg⁻¹ and 4.02 g kg⁻¹, respectively. Garcia, *et al.* (2015) studied changes in the FA composition of tricepiro, oat, ryegrass and triticale winter cultivars sampling in June, July and August months. In all cultivars the SFA C16:0 increased clearly from June to August but C18:0 increased only in tricepiro and triticale cultivars. The monounsaturated C16:1 and C18:1 seems to increase in all cultivars. The increase of C18:2 n-6 are only clear in oats and ryegrass but 18:3 n-3 increased in all from June to August. The PUFA C18:2n-6+C18:3n-3 and the ratio C18:3n-6/C18:2n-3 decreasing from June to August. The FA composition of ryegrass from June and October seems to increase in C16:0, C16:1, C18:0, C18:1 and C18:2 but decreases in C18:3 n-3 and the ratio C18:3 n-3/ C18:2 n-6 [Table 2].

	C18:2 n-6	C18:3n-3	C18:3/C18:2	C18:2+C18:3 %
Ryegrass Bill		1.45a		72.20a
Cut 1	0.23bcd	0.81cd	6.36a	64.19abcd
Cut 2	0.18aCD		4.46bc	
Ryegrass Florida				
Cut 1	0.19bD	1.42 a	6.36a	71.04ab
Cut 2	0.16aD	0.77cd	4.75b	62.57bcd
Wheat Charrua	0.27bBC	1.20b		
Cut 1	0.20aBC	0.72d	4.41bc	65.99abc
Cut 2			3.66bcde	58.76cd
Wheat Guapo	0.29bAB			
Cut 1	0.25aAB	1.20b	3.90bcde	64.03abcd
Cut 2		0.75d	3.03e	60.28cd
Triticale Don	0.26bAB			
Santiago	0.26aAB	1.15b	4.38bcd	64.83abcd
Cut 1		0.75d	3.11de	61.42cd
Cut 2				
Rye grass Queue				
Cut 1	0.29bA	0.94c	3.40cde	56.58d
Cut 2	0.28aA	0.74d	2.71e	60.86cd
Cultivar	***	***	NS	***
Cut	***	***	***	***
Interaction	NS	**	***	***

abc ABCD denotes in the same column bearing different letters different at P<0.001 level.

Table 2: Effects of type of cultivar, first (Cut 1) and second at the winter end (Cut 2) on C18:2n-6 and C18:3n-3 contribution (mg/100g dry matter), C18:3/C18:2 ratios and C18:2+C18:3 %. (Garcia *et al.* 2015).

Fatty acid concentration decreases as plant mature, making forage management a central determinant in fatty acid concentration by livestock (Clapham, *et al.* 2005)

Fatty acid variation within forage species is currently not known. Dewhurst, *et al.* (2001) compared three ryegrass species and found C18:2 and C18:3 differing by as much as 0.34 g kg⁻¹ and 4.02 g kg⁻¹, respectively. Boufaied, *et al.* (2003) found legumes contained 1.3 times more C18:2 than grass species while grasses contained 1.1 times more C18:3 than legumes on average.

Conclusions

Many factors affect the FA composition of several cultivars. Significant changes in total lipids and FA composition, particularly, in the contribution of C18:2 and C18:3, the ratio C18:3 n-3/C18:2 n-6 and the total PUFAs. Considering the importance of these FA as substrate for CLA and n-3 PUFAs concentration in grass-fed ruminants. Fatty acid composition of forages need to be considered in forage grazing beef and milk production systems. Since drying samples has been shown to be an acceptable method for preparing samples for FA analysis it would be possible to analyze a great number of samples. There is also a need for more understanding of processes that occur in the rumen. There are factors other than the FA influence the amounts of PUFAs that escape from the rumen and these factors warrant further study. It is important to know the degree of variation in both total lipids and individual FA in forages with a view to establishing the potential to cultivate grasses for a higher content of n-3 PUFA and hence the opportunity to deliver more n-3 PUFA into milk and meat from ruminant animals.

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