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Influence of cultivar and cutting date on the fatty acid composition of forage crops for grazing beef production in Argentina

P. T. Garcia*,†, A. Pordomingo‡, C. D. Perez*,†, M. D. Rios†, A. M. Sancho*, G. Volpi Lagreca‡ and J. J. Casal†

*Food Technology Institute, National Institute of Agricultural Technologies (INTA), Morón, Buenos Aires, Argentina, †University of Morón, Buenos Aires, Argentina, ‡Anguil Agricultural Experimental Station, La Pampa, Argentina

Abstract

The fatty acid composition and total lipids of various forage crops at different cutting dates and seasons, which are used in forage grazing beef production in Argentina, were determined. Three experiments were carried out at the Agricultural Experimental Station of INTA, Anguil, La Pampa, Argentina Samples of alfalfa from three different cultivars with different dormancy groups (G4-5, G6-7 and G8-9) and samples of ryegrasses Bill, Florida and Queue, wheats Charrua and Guapo, and triticale Don Santiago cultivars were taken at different cutting dates and analysed for total lipids and fatty acid composition. Significant differences on the fatty acid composition, the ratios 18:3n-3/18:2n-6 and polyunsaturated fatty acids (PUFA) due to cultivar, cutting date and season were found. Taking into account the importance of 18:2n-6 and 18:3n-3 fatty acids as substrates for the conjugated isomers of 18:2n-6 (CLA) and n-3 PUFA concentrations in beef, the fatty acid composition of forages needs to be considered in forage grazing beef production systems. Data variation in both total lipids and individual fatty acid proportions in forages should be taken into account to establish the potential to cultivate crops for a higher content of n-3 PUFA and hence the opportunity to deliver more n-3 PUFA and CLA into beef.

Correspondence to: P. T. Garcia, Instituto Tecnologia Alimentos, Centro de Investigacion de Agroindustria. Instituto Nacional de Tecnologia Agropecuaria INTA, CC 77 (B1708 WA) Morón, Buenos Aires, Argentina.
E-mail: garcia.pilar@inta.gob.ar

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Introduction

Although fats are an important component of the human diet, current levels of intake are considered too high and the overall fatty acid composition imbalanced. There is an excessive intake of saturated fatty acids (SFA) relative to polyunsaturated fatty acids (PUFA), expressed usually as the P/S ratio, and the consumption of n-6 PUFA is too high relative to n-3 PUFA (Williams, 2000). In Western countries, the ratio of n-6/n-3 PUFA is a risk factor in cancers and coronary heart diseases, especially the formation of blood clots that eventually may lead to a heart attack, and nutritionists have focused on the type of PUFA and the balance in the diet between n-3 PUFA formed from 18:3n-3 and the n-6 PUFA from 18:2n-6 (Russo, 2009). The 18:3n-3 and 18:2n-6 acids are the precursor molecules from which other 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 18:3n-3 to long-chain fatty acids 20:5n-3 (eicosapentaenoic, EPA), 22:5n-3 (docosapentaenoic, DPA) and 22:6n-3 (docosahexaenoic, DHA) depends on the dietary total PUFA, the ratio 18:2n-6/18:3n-3 and the availability of 18:3n-3 (Barcelo-Coblijn and Murphy, 2009; Brenna *et al.*, 2009).

Forage plants found in pastures are high in PUFA, specifically 18:2n-6 and 18:3n-3 fatty acids. Fresh forage contains a high proportion (50–75%) of its total fatty acid content in the form of 18:3n-3. Sources of variation of forage lipid concentration are plant species, growth stage, temperature and light intensity.

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Fatty acid profiles are distinctive in particular species, which confirms that fatty acid composition of forages is under considerable genetic control (Dewhurst *et al.*, 2001). This offers the potential to select forages with higher concentrations or altered fatty acid composition. When harvested at the same stage of development, Boufäied *et al.* (2003) found significant differences, both in species of the same plant functional group (grass or legumes) and between the two groups. Legumes had higher concentrations of 14:0, 16:0, 18:0, 18:1 and 18:2n-6 and total fatty acids and lower concentrations of 18:3n-3, but large variations among species found in each functional group were also observed. Dewhurst *et al.* (2001) found distinct among-species differences in fatty acid profiles of forage grasses cut at the same date and a significant interaction effect between species and cutting date.

Limited data are available considering the effect of the nature of pasture lipids on beef fatty acid composition, but several studies have shown that concentrations in forage-based diets determine how fat in finished beef is altered. Dietary 18:3n-3 is the best source for the long-chain PUFA n-3 in meat and milk, while CLA derives from 18:2n-6 and 18:3n-3 (Van Elswyk and McNeill, 2014). In some beef systems, grass conserved as silage is used and should lead to similar benefits to grazed grass, compared with concentrate diets (French *et al.*, 2000; Garcia *et al.*, 2008). 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 cattle of fresh pastures. On the one hand, Elgersma *et al.* (2003) utilizing different perennial ryegrass cultivars as source of pasture in dairy cattle increased milk CLA content, but on the other hand, grazing the cultivar containing the greatest PUFA concentrations, compared to cattle consuming the cultivar with lowest PUFA, reached lowest levels in PUFA. The forage-based diets increased 18:3n-3 in *Longissimus dorsi* muscle compared with feeding concentrates, consistent with previous studies with beef cattle comparing alfalfa silage (Mandell *et al.*, 1998) or pasture (French *et al.*, 2003). The increased concentrations of 20:5n-3, 22:5n-3 and 22:6n-3 in beef muscle fed on grass suggest that the high availability of 18:3n-3 in the diet has resulted in an enhanced synthesis of these n-3 long-chain PUFA (Nuernberg *et al.*, 2005). The fatty acid proportion of polar lipids of fat from *Longissimus dorsi* muscle showed similar proportions of 18:3n-3 and 20:5n-3 but lower proportions of 22:5n-3 and 22:6n-3 compared to beef heifers that were offered pasture only (Noci *et al.*, 2007). The feeding regime as herbage or concentrate affected the total n-3 PUFA in *Longissimus dorsi* muscle of lambs. The herbage regime presented more 18:3n-3 and 20:5n-3, but no significant changes in 22:6n-3 (Vasta *et al.*, 2009). The grazing on *Trifolium subterraneum* as monoculture and associated with *Lolium multiflorum* has increased the linolenic acid of lamb meat (Chiofalo *et al.*, 2010).

Meat, fish, fish oils and eggs are the only significant sources of long-chain n-3 PUFA in human diets. Although meat has lower concentrations of these fatty acids compared to oily fish, it is a very significant source for many people under low dietary fish intakes. The low PUFA concentration and the high concentrations of SFA in ruminant tissues result from the biohydrogenation of dietary PUFA in the rumen. The potential use of livestock products to deliver n-3 fatty acids has been the subject of intensive research (Moghadasian, 2008). Dewhurst *et al.* (2009) and Diaz *et al.* (2005) describe a higher content of 18:2n-6 and 18:3n-3 in milk and meats of ruminants grazing red or white clovers, 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 biohydrogenation in the rumen are different from those obtained with perennial ryegrass (Lourenço *et al.*, 2008) due to the inhibition of proteolysis and lipolysis by the plant enzyme polyphenol oxidase, which is found 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.*, 2011).

The aims of this study were to evaluate, at different cutting dates and seasons, the variations in fatty acid proportions of plant lipids used as forage in grazing beef production in Argentina.

Material and methods

The three experiments described in this study were carried out in the Agricultural Experimental Station of INTA, Anguil, La Pampa, Argentina (36°31′S/64°01′W), during different cutting dates and seasons between 2006 and 2008. The average annual rainfall in the Anguil area is distributed with peaks in spring and autumn, with dry summer and some precipitation in the form of snow in winter. The annual average mean, minimum and maximum annual temperatures are 15·6, 7·8 and 22·6°C, respectively, and the average mean precipitation is 759·5 mm (Casagrande *et al.*, 2012). No soil fertilization was used.

Experiment 1

Forty-five representative samples of three different alfalfa (*Medicago sativa*) cultivars from 3 different dormancy groups (G4-5, G6-7 and G8-9) were obtained during spring, summer and autumn (five subsamples for each cultivar and dormancy group). The experimen-

tal period covered 23 weeks between 15 October (spring) and 24 April (autumn) divided in to 5 periods. Each period lasted approximately 1 month except for period 5 which lasted 3 months. The average mean, minimum and maximum temperatures were as follows: in January 23, 15·6 and 30·4°C; in April 16·1, 8·5 and 23·7°C; in October 17·2, 10 and 24·3°C; in November 19, 10·5 and 27·4°C; and in December 23·1, 14·8 and 31·3°C respectively (Casagrande *et al.*, 2012).

Experiment 2

Sixty samples of 6 cultivars of annual forage crops: ryegrasses *(Lolium multiflorum*) cvs. Bill (RGB), Florida (RGF) and Quehue (RGQ); wheat (*Triticum aestivum*) cvs. Charrua (WC) and Guapo (WG); and triticale (*X Triticosecale* Wittmack) cv. Don Santiago (TDS), were harvested. Five replicate samples from each of these forage crops were cut at the end of the winter (first growth) and 1 month later (first regrowth). The average mean, minimum and maximum temperatures were as follows: in August 9·4, 0·4 and 16·0°C and in September 11·8, 0·4 and 20·0°C respectively (Casagrande *et al.*, 2012).

Experiment 3

Sixty samples of tricepiro, obtained from crossing triticale (*Tritium* × *Secale* L.) and Trigopiros (*Tritium* L. × *Thinopyrum A. Löve*), oats (*Avena sativa* L.); ryegrass *(Lolium multiflorum*); and triticale (*X Triticosecale* Wittmack), harvested during June, July and August months (winter). The average mean, minimum and maximum temperatures were as follows: in June 6·5, −2·6 and 15·5°C; in July 5·5, −2·3 and 13·3°C; and in August 6·5, −1·5 and 14·5°C respectively (Casagrande *et al.*, 2012).

Forty samples of ryegrass *(Lolium multiflorum*), oats (*Avena sativa*) and alfalfa (*Medicago sativa*) harvested from March (autumn) to September (spring) months were analysed to determine fatty acid composition changes. Five replicate samples from each forage and cutting date were harvested for fatty acid composition analyses. The average mean, minimum and maximum temperatures were as follows: in March 19·1, 11·7 and 26·4°C; in April 14·0, 5·0 and 23·0°C; in June 8·5, 3·5 and 13·4°C; in July 8·3, 1·6 and 15·1°C; in August 9·4, 2·9 and 16·0°C; in September 11·8, 4·1 and 20·0°C; and in October 15·0, 7·3 and 28·4°C respectively (Casagrande *et al.*, 2012).

Chemical analyses

All forage samples, 5 cm above the soil, were harvested, placed in a box, cut into pieces, dried immediately at 60°C for 24 h in a forced-air oven and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm screen prior to analysis. Rapid freezing with dry ice or liquid N_2 followed by storage at −20°C in an inert (N_2) atmosphere has been considered to be the best method to preserve plant tissues (Christie, 1993). However, comparison of various techniques showed limited effects on fatty acid proportions, and drying of samples has been shown to be an adequate method for preparing samples for fatty acid analysis in samples collected in diverse experiments, both field and feeding experiments, which were not specially designed to investigate fatty acid concentrations (Arvidsson *et al.*, 2009). Five grams of the dried sample were used for total fat extraction and fatty acid analysis. Total lipid was determined in an aliquot dry sample of 2 g with boiling hexane in a Tecator equipment (SOXTEC SYSTEM HT 1043 Extraction Unit) according to official methods (AOAC, 1992). Lipids from an other aliquot sample were extracted using chloroform–methanol mixture (2:1) according to the Folch *et al.* (1957) method. Fatty acid methyl esters (FAMEs) were transmethylated with 4% HCl acid in anhydrous methanol according to the method of Pariza *et al.* (2001). FAMEs were measured using a GC Chrompack CP 900 equipment, fitted with a flame ionization detector. The injector and detector temperatures were set at 240°C. FAMEs were separated using a fused silica capillary column Chrompack CP-Sil 88 (100 m × 0·25 mm i.d.) Varian, Walnut Creek, CA, USA, using nitrogen as carrier gas. The temperature ramp for the GC oven was as follows: 70°C for 4 min; increase to 170°C at 13°C min^{-1}; increase to 200°C at 1°C min^{-1} for a total run time of 90 min. Individual fatty acids were identified on the basis of reference materials (Supelco 37 Component FAME mix, Supelco Bellefonte, PA, USA). Analytical results were expressed as percentages of total fatty acids. The content of fatty acids was estimated on the basis of the lipid content and the fatty acid profile.

Statistical analyses

Experiment 1

Statistical analyses were performed using SAS software version 8.0, Institute, Inc., Cary, NC. USA. The alfalfa cultivars were sown in plots of 1·6 × 1·6 m as a randomized block design with three replications. The principal blocks were 3 dormancy groups, with 3 cultivar per group and three seasons (spring, summer and autumn). The means were separated by LSD when the differences were statistically significant.

Experiment 2

Treatments were compared using the software SPSS-Advanced Statistics 12 (SPSS Inc., Chicago, IL, USA). The statistical model used was as follows:

$$Y_{ijk} = \mu + C_i + B_j + (CB)_{ij} + e_{ijk},$$

where Y represent the response, C is the cultivar, B is the cutting date, CB is the interaction between cultivar and date of cutting, and e is the error term. Mean differences were estimated using the Bonferroni test.

Experiment 3

Treatments were compared by analysis of variance using the GLM procedure (SAS 8.0 SAS Institute, Inc., Cary, NC, USA). The data were subjected to analysis of variance, with sampling month as an independent variate. Significant differences between pasture months were calculated from the Tukey's significant difference test. The cultivar differences were excluded from analysis because the samples were taken from a different pasture site.

Results

Experiment 1

The fatty acid composition of alfalfa lipids from three different cultivars with different dormancy groups (G4-5, G6-7 and G8-9) is shown in Table 1. Significant differences ($P < 0{\cdot}05$) were identified in all fatty acids except 18:2n-6. The fatty acid composition of alfalfa cultivars from 5 cutting dates is shown in Table 2. Cutting date affected significantly ($P < 0{\cdot}001$) all fatty acids except 18:1n-9. The effect of season on the fatty acid composition is shown in Table 3. No interactions were detected between dormancy group and season ($P < 0{\cdot}344$). During the autumn, 18:2n-6 increased and 16:0, 16:1, 18:0 decreased. Differences between spring and summer were observed for 16:1, 18:0, 18:2n-6 and 18:3n-3. The lipid percentage and contribution of 18:2n-6 and 18:3n-3 were higher in autumn compared with the other seasons. The total 18:3n-3 + 18:2n-6 percentages were also higher in autumn except the ratios 18:3n-3/18:2n-6 that were higher in spring compared to summer and autumn (Table 4).

Experiment 2

The fatty acid composition, total lipid (g/100 g DM) and the 18:3n-3/18:2n-6 ratios of six annual forage crops are shown in Table 5. Cutting date and variety affected significantly the concentrations of all fatty acids except 18:1n-9, which was only affected by cultivar, while 18:0 was only affected by cutting date. The 18:3/18:2 ratios were significantly affected by both, cutting date and cultivar. The total lipid (g/100 g DM) was significantly lower in cut 2 compared to cut 1 in all cultivars.

Experiment 3

The changes in the fatty acid composition of tricepiro, oats, ryegrass and triticale cultivars sampled in June, July and August months (winter) are shown in Table 6. In all cultivars, the 16:0 increased clearly from June to August and 18:0 increased only in tricepiro and triticale cultivars. The monounsaturated 16:1 and 18:1n-9 seemed to be higher in August in all cultivars except triticale. 18:2n-6 increased only in oats and ryegrass and 18:3n-3 decreased in all culti-

Table 1 Experiment 1. Relative proportion (%) of major fatty acid of lipids from three alfalfa cultivars (1, 2 and 3) from different dormancy groups (G4-5, G6-7 and G8-9).

Cultivar	16:0	16:1	18:0	18:1n-9	18:2n-6	18:3n-3	*n*
1 (G4-5)	$28{\cdot}63 \pm 5{\cdot}58^{ab}$	$1{\cdot}97 \pm 0{\cdot}61^{ab}$	$5{\cdot}61 \pm 1{\cdot}72^{ab}$	$8{\cdot}81 \pm 1{\cdot}53^{a}$	$19{\cdot}65 \pm 4{\cdot}95$	$35{\cdot}23 \pm 4{\cdot}97^{c}$	5
2 (G4-5)	$28{\cdot}40 \pm 5{\cdot}07^{ab}$	$1{\cdot}77 \pm 0{\cdot}57^{b}$	$5{\cdot}08 \pm 1{\cdot}45^{ab}$	$5{\cdot}77 \pm 1{\cdot}37^{ab}$	$19{\cdot}97 \pm 4{\cdot}63$	$38{\cdot}77 \pm 4{\cdot}12^{abc}$	5
3 (G4-5)	$29{\cdot}02 \pm 4{\cdot}32^{a}$	$2{\cdot}02 \pm 0{\cdot}49^{ab}$	$5{\cdot}72 \pm 1{\cdot}44^{ab}$	$8{\cdot}48 \pm 2{\cdot}70^{a}$	$18{\cdot}45 \pm 2{\cdot}37$	$36{\cdot}16 \pm 6{\cdot}88^{c}$	5
1 (G6-7)	$28{\cdot}13 \pm 5{\cdot}24^{ab}$	$2{\cdot}01 \pm 0{\cdot}47^{ab}$	$6{\cdot}26 \pm 2{\cdot}19^{a}$	$6{\cdot}52 \pm 2{\cdot}56^{ab}$	$18{\cdot}15 \pm 4{\cdot}86$	$38{\cdot}61 \pm 5{\cdot}02^{abc}$	5
2 (G6-7)	$27{\cdot}99 \pm 6{\cdot}18^{ab}$	$1{\cdot}97 \pm 0{\cdot}42^{ab}$	$4{\cdot}72 \pm 1{\cdot}47^{b}$	$5{\cdot}26 \pm 0{\cdot}90^{b}$	$19{\cdot}49 \pm 5{\cdot}10$	$40{\cdot}27 \pm 4{\cdot}95^{ab}$	5
3 (G6-7)	$28{\cdot}74 \pm 6{\cdot}27^{ab}$	$1{\cdot}76 \pm 0{\cdot}57^{b}$	$5{\cdot}60 \pm 1{\cdot}73^{ab}$	$8{\cdot}14 \pm 2{\cdot}56^{ab}$	$19{\cdot}88 \pm 7{\cdot}02$	$35{\cdot}37 \pm 3{\cdot}51^{c}$	5
1 (G8-9)	$29{\cdot}43 \pm 5{\cdot}87^{a}$	$1{\cdot}89 \pm 0{\cdot}52^{b}$	$5{\cdot}51 \pm 1{\cdot}66^{ab}$	$7{\cdot}25 \pm 2{\cdot}91^{ab}$	$20{\cdot}18 \pm 5{\cdot}91$	$35{\cdot}69 \pm 7{\cdot}38^{c}$	5
2 (G8-9)	$24{\cdot}49 \pm 7{\cdot}13^{b}$	$2{\cdot}33 \pm 0{\cdot}40^{a}$	$5{\cdot}67 \pm 2{\cdot}66^{ab}$	$7{\cdot}16 \pm 3{\cdot}61^{ab}$	$19{\cdot}44 \pm 3{\cdot}82$	$40{\cdot}77 \pm 2{\cdot}19^{a}$	5
3 (G8-9)	$28{\cdot}49 \pm 4{\cdot}68^{ab}$	$1{\cdot}88 \pm 0{\cdot}27^{b}$	$5{\cdot}51 \pm 1{\cdot}56^{ab}$	7.11 ± 1.38	$20{\cdot}17 \pm 4{\cdot}82$	$36{\cdot}65 \pm 4{\cdot}46^{bc}$	5
P-value	<0·05	<0·05	<0·05	<0·05	NS	<0·05	

NS refers to the level of significance at *P*-value of >0·05. Means in the same column with different superscript letters differ at $P < 0{\cdot}05$.

vars from June to August. The total 18:2n-6 + 18:3n-3 and the ratios 18:3n-6/18:2n-3 decreased from June to August. In Table 7, the changes in the fatty acid composition of ryegrass, oats and alfalfa at different cutting dates are shown. The fatty acid composition of ryegrass from June (winter) to October (spring) showed increases of 16:0, 16:1, 18:0, 18:1n-9 and 18:2n-6 and decreases of 18:3n-3 and 18:3n-3/18:2n-6 ratios. The fatty acid composition changes of oats and alfalfa were not statistically significant.

Discussion

The effects of season and cutting date on fatty acid composition of lipids from several alfalfa cultivars were significant, indicating the importance of the forage cutting date on the contribution of almost all fatty acids. In the present study, some slight differences in fatty acid composition of alfalfa lipids, according to the three different dormancy groups, were also found. It seems that an increase in the winter survivability, measured either by winter hardiness or autumn dormancy, has some basic impact on the fatty acids levels, even under non-freezing conditions (Alarcon Zuñiga, 2003). The proportions of 18:2n-6 and 18:3n-3 were higher in autumn compared with the other seasons, but the ratios 18:3n-3/18:2n-6 were higher in spring compared to summer and autumn. Cutting date also significantly affected in Experiment 2 the concentration of almost all fatty acids, total lipids

Table 2 Experiment 1. Effects of cutting date on the major fatty acid proportions (%) of alfalfa lipids.

Cutting/date	16:0	16:1	18:0	18:1n-9	18:2n-6	18:3n-3	*n*
1/15 October	29·13^{a}	2·26^{a}	5·47^{b}	7·68	16·74^{cd}	38·40^{ab}	9
2/25 November	29·66^{a}	2·22^{a}	5·77^{b}	6·01	15·46^{d}	40·44^{a}	9
3/26 December	32·20^{a}	1·65^{bc}	7·62^{a}	8·59	19·50^{b}	30·32^{c}	9
4/27 January	29·71^{a}	1·92^{b}	5·58^{b}	6·67	18·62^{bc}	37·52^{b}	9
5/24 April	20·03^{b}	1·51^{c}	3·15^{c}	6·89	27·41^{a}	40·83^{a}	9
s.e.	0·63	0·09	0·27	0·73	0·62	0·86	
P-value	<0·001	<0·001	<0·001	NS	<0·001	<0·001	

NS refers to the level of significance at *P*-value of >0·001. s.e., standard error. Means in the same column with different superscript letters differ at $P < 0{\cdot}001$.

Table 3 Experiment 1. Effect of season on the major fatty acid proportions (%) of alfalfa lipids.

Season	16:0	16:1	18:0	18:1n-9	18:2n-6	18:3n-3	*N*
Spring	29·40^{b}	2·35^{c}	5·62^{b}	6·84	16·10^{a}	39·42^{b}	18
Summer	30·96^{b}	1·78^{b}	6·60^{c}	7·63	18·91^{b}	33·92^{a}	18
Autumn	20·03^{a}	1·51^{a}	3·15^{a}	6·89	27·41^{c}	40·83^{b}	9
s.e.	0·80	0·097	0·22	0·61	0·66	1·05	
P-value	<0·05	<0·05	<0·05	NS	<0·05	<0·05	

NS refers to the level of significance at *P*-value of >0·05. s.e., standard error. Means in the same column with different superscript letters differ at $P < 0{\cdot}05$.

Table 4 Experiment 1. Effect of season on total lipids (g/100 g DM), the contribution of 18:2n-6 and 18:3n-3 (g/100 g DM) and on the ratio 18:3n-3/18:2n-6 in alfalfa lipids.

Season	Total lipids	18:2n-6	18:3n-3	18:2 + 18:3	18:3/18:2	*N*
Spring	2·0^{a}	0·33^{a}	0·80^{a}	1·13^{a}	2·47^{c}	18
Summer	2·2^{a}	0·41^{b}	0·75^{a}	1·16^{a}	1·80^{b}	18
Autumn	3·3^{b}	0·89^{c}	1·33^{b}	2·22^{b}	1·52^{a}	9
s.e.	0·07	0·02	0·048	0·055	0·076	
P-value	0·05	0·05	0·05	0·05	0·05	

NS refers to the level of significance at *P*-value of >0·05. s.e., standard error. Means in the same column with different superscript letters differ at $P < 0{\cdot}05$.

Table 5 Experiment 2. Effects of type of cultivar, first and second cut at the end of the winter, on the major fatty acid proportions (%) and total lipids (g/100 g DM).

	16:0	16:1	18:0	18:1n-1	18:2n-2	18:3n-3	18:3/18:2	Total lipids	*n*
RGB									
Cut 1	18·67 ± 0·98^{c}	2·04 ± 0·12^{b}	2·45 ± 0·56^{c}	4·51 ± 1,51^{B}	9·97 ± 1·51^{bCD}	62·23 ± 3·14^{a}	6·4 ± 0·97^{a}	2·3 ± 0·01^{a}	5
Cut 2	23·84 ± 1·56^{ab}	1·85 ± 0·24^{bc}	4·39 ± 0·72^{ab}	5·57 ± 1·67^{B}	11·78 ± 0·65^{aCD}	52·41 ± 3·54^{bc}	4·5 ± 0·32^{bc}	1·5 ± 0·03^{b}	5
RGF									
Cut 1	19·59 ± 0·44^{c}	1·79 ± 0·12^{bc}	2·59 ± 0·43^{c}	4·68 ± 1·94^{B}	8·49 ± 0·41^{bD}	62·55 ± 2·40^{a}	6·4 ± 0·44^{a}	2·2 ± 0·01^{a}	5
Cut 2	24·86 ± 1·08^{ab}	1·90 ± 0·15^{bc}	4·52 ± 0·71^{ab}	5·83 ± 1·78^{B}	10·93 ± 0·69^{aD}	51·64 ± 4·24^{bcd}	4·8 ± 0·61^{b}	1·4 ± 0·04^{b}	5
WC									
Cut 1	21·33 ± 1·95^{bc}	1·99 ± 0·44^{b}	3·28 ± 0·90^{abc}	7·18 ± 2·82^{AB}	12·22 ± 0·70^{bBC}	53·77 ± 4·90^{ab}	4·4 ± 4·41^{bc}	2·2 ± 0·03^{a}	5
Cut 2	25·83 ± 1·56^{a}	1·82 ± 0·18^{bc}	4·89 ± 1·21^{a}	7·98 ± 1·78^{AB}	12·69 ± 0·53^{aBC}	46·07 ± 2·54^{bcd}	3·7 ± 1·18^{cde}	1·5 ± 0·01^{b}	5
WG									
Cut 1	24·73 ± 1·79^{ab}	2·54 ± 0·44^{a}	3·02 ± 1·84^{bc}	5·92 ± 0·98^{AB}	12·39 ± 0·79^{bAB}	51·63 ± 1·34^{bcd}	3·90 ± 0·91^{bcde}	2·3 ± 0·02^{a}	5
Cut 2	25·37 ± 1·66^{a}	1·98 ± 0·28^{b}	3·96 ± 0·60^{abc}	8·59 ± 1·52^{AB}	14·97 ± 1,55^{aAB}	45·31 ± 1·83^{bcd}	3·00 ± 0·36^{e}	1·6 ± 0·02^{b}	5
TDS									
Cut 1	22·27 ± 3·19^{abc}	1·93 ± 0·25^{b}	3·50 ± 1·20^{abc}	7·34 ± 3·13^{A}	12·07 ± 0·82^{bAB}	52·76 ± 6·77^{bc}	4·4 ± 0·61^{bcd}	2·2 ± 0·03^{a}	5
Cut 2	24·01 ± 2·81^{ab}	1·54 ± 0·16 ^{cd}	3·88 ± 1·00^{abc}	9·10 ± 2·93^{A}	15·65 ± 0·98^{aAB}	45·77 ± 7·03^{bcd}	3·1 ± 0·90de	1·6 ± 0·01^{b}	5
RGQ									
Cut 1	25·22 ± 1·05^{a}	1·72 ± 0·27^{bcd}	4·79 ± 1·35^{a}	10·20 ± 3·11^{A}	13·44 ± 3·53^{bA}	43·14 ± 5·42^{d}	3·4 ± 0·97^{cde}	2·2 ± 0·01^{a}	5
Cut 2	25·91 ± 0·86^{a}	1·38 ± 0·14^{d}	3·35 ± 0·47^{abc}	8·27 ± 1·64^{A}	16·52 ± 1,29^{aA}	44·34 ± 3·92 ^{cd}	2·7 ± 0·37^{e}	1·7 ± 0·02^{b}	5
Cultivar	0·001	0·001	NS	0·001	0·001	0·001	0·001	NS	
Cut	0·001	0·001	0·001	NS	0·001	0·001	0·001	0·001	
Interaction	0·001	0·003	0·003	NS	NS	0·01	0·01	NS	

NS refers to the level of significance at *P*-value of >0·001. Means in the same column with different superscript letters differ at $P < 0{\cdot}001$. RGB (Ryegrass Bill), RGF (Ryegrass Florida), WC (Wheat Charrua), WG (Wheat Guapo), TDS (Triticale Don Santiago) and RGQ (Ryegrass Queue).

Table 6 Experiment 3. Major fatty acid proportions (%) of lipids from tricepiro, oats, ryegrass and triticale cultivars during winter months. Average five samples each month and cultivar (Mean ± s.d.)

Cultivar	16:0	16:1	18:0	18:1n-9	18:2n-6	18:3n-3	18:2 + 18:3	18:3/18:2	*n*
Tricepiro									
June	17·6 ± 1·6^{c}	4·5 ± 1·8^{c}	2·2 ± 0·6^{b}	4·8 ± 1·0^{b}	10·6 ± 0·6^{a}	51·5 ± 2·7^{a}	62·1 ± 2·5^{a}	4·9 ± 0·5^{a}	5
July	20·0 ± 2·9^{b}	5·5 ± 1·7^{ba}	2·9 ± 1·0^{b}	6·3 ± 2·2^{a}	10·4 ± 0·2^{a}	47·1 ± 3·8^{b}	57·5 ± 2·2^{ab}	4·5 ± 0·3^{b}	5
August	27·0 ± 1·7^{a}	6·7 ± 2·4^{a}	8·1 ± 3·1^{a}	7·8 ± 3·0^{a}	11·0 ± 0·6^{a}	37·2 ± 2·7^{c}	48·3 ± 3·0^{b}	3·3 ± 0·5^{c}	5
Oats									
June	18·7 ± 2·4^{c}	4·1 ± 2·8^{b}	2·3 ± 0·5^{b}	4·7 ± 1·4^{a}	12·6 ± 1·4^{b}	48·7 ± 2·7^{a}	61·3 ± 3·4^{a}	3·9 ± 0·8^{a}	5
July	26·3 ± 0·8^{b}	5·4 ± 0·3^{b}	2·9 ± 0·3^{ab}	4·9 ± 0·3^{a}	15·5 ± 0·2^{a}	39·7 ± 0·2^{b}	55·3 ± 0·9^{b}	2·6 ± 0·4^{b}	5
August	33·9 ± 0·8^{a}	10·4 ± 0·6^{a}	3·4 ± 0·5^{a}	5·0 ± 0·5^{a}	17·9 ± 3·2^{a}	22·7 ± 0·7^{c}	40·7 ± 2·1^{c}	1·26 ± 0·5^{c}	5
Ryegrass									
June	21·5 ± 2·5^{c}	7·3 ± 2·0^{b}	2·1 ± 0·4^{b}	5·1 ± 0·3^{c}	13·8 ± 0·7^{b}	43·4 ± 3·4^{a}	57·3 ± 3·6^{a}	3·1 ± 0·2^{a}	5
July	29·9 ± 2·6^{b}	11·6 ± 0·8^{a}	3·5 ± 0·1^{a}	7·4 ± 1·7^{b}	15·7 ± 1·9^{b}	29·6 ± 1·3^{b}	45·1 ± 3·7^{b}	1·9 ± 0·4^{b}	5
August	38·0 ± 2·9^{a}	12·1 ± 0·9^{a}	2·8 ± 0·4^{ab}	11·7 ± 2·0^{a}	20·6 ± 2·1^{a}	22·5 ± 2·5^{c}	43·1 ± 2·6^{b}	1·1 ± 0·4^{c}	5
Triticale									
June	18·7 ± 2·1^{c}	4·9 ± 2·8^{b}	2·4 ± 1·1^{b}	6·5 ± 3·8^{b}	10·9 ± 0·9^{a}	46·9 ± 3·5^{a}	57·9 ± 5·9^{a}	4·3 ± 0·6^{a}	5
July	21·9 ± 3·7^{b}	10·4 ± 5·6^{a}	3·5 ± 1·1^{b}	8·5 ± 3·1^{a}	11·0 ± 1·2^{a}	37·4 ± 1·1^{b}	48·4 ± 2·2^{b}	3·4 ± 0·3^{b}	5
August	37·4 ± 4·2^{a}	4·1 ± 2·3^{b}	6·3 ± 2·0^{a}	8·8 ± 2·9^{a}	9·9 ± 1·3^{a}	28·9 ± 2·5^{c}	38·9 ± 2·5^{c}	2·9 ± 0·2^{c}	5
P-value	<0·05	<0·05	<0·05	<0·05	<0·05	<0·05	<0·05	<0·05	

Means in the same column and cultivar with different superscript letters differ at $P < 0·05$.

Table 7 Experiment 3. Major fatty acid proportions (%) of ryegrass, oats and alfalfa lipids sampled during March, April, June, August, September and October. Average of five samples each month and cultivar (Mean ± s.d.).

Cultivar	16:0	16:1	18:0	18:1n-9	18:2n-6	18:3n-3	18:2 + 18:3	18:3/18:2	*n*
Ryegrass									
June	23·6 ± 1·5^{b}	2·7 ± 0·5^{b}	2·3 ± 0·2a	4·5 ± 0·5^{c}	13·9 ± 1·6^{b}	53·0 ± 2·8^{a}	66·9 ± 2·0^{a}	3·9 ± 0·5^{a}	5
July	27·1 ± 1·2^{b}	2·6 ± 0·4^{b}	2·7 ± 0·5ab	5·2 ± 1·2^{ab}	15·2 ± 0·3^{b}	47·1 ± 2·6^{b}	62·3 ± 2·7^{ba}	3·1 ± 0·2^{a}	5
August	27·6 ± 3·9^{b}	2·1 ± 0·4^{b}	3·4 ± 1·7b	6·9 ± 2·2^{b}	18·0 ± 0·7^{a}	41·9 ± 7·5^{cb}	59·9 ± 7·8^{b}	2·3 ± 0·4^{ab}	5
October	34·8 ± 1·1^{a}	3·5 ± 0·2^{a}	6·6 ± 0·6a	11·7 ± 1·5^{a}	15·6 ± 0·4^{ab}	27·6 ± 2·0^{d}	43·3 ± 2·2^{c}	1·7 ± 0·1^{b}	5
Oats									
August	24·0 ± 0·5^{a}	2·8 ± 0·3^{a}	3·2 ± 0·5^{a}	4·9 ± 0·5^{a}	13·5 ± 0·6^{a}	51·4 ± 0·9^{b}	64·9 ± 1·0^{a}	3·8 ± 0·2^{a}	5
September	25·7 ± 2·0^{a}	2·6 ± 0·4^{a}	3·8 ± 0·4^{a}	5·7 ± 1·6^{a}	15·2 ± 2·0^{a}	46·8 ± 3·1^{a}	62·0 ± 3·4^{a}	3·1 ± 0·5^{a}	5
Alfalfa									
March	32·9 ± 2·9^{a}	3·8 ± 0·3^{b}	6·9 ± 1·2^{a}	9·6 ± 3·0^{a}	18·3 ± 3·0^{a}	28·4 ± 7·0^{a}	46·7 ± 6·7^{a}	1·6 ± 0·4^{a}	5
April	34·1 ± 0·8^{a}	4·2 ± 0·4^{a}	6·8 ± 0·8^{a}	9·4 ± 2·3^{a}	19·6 ± 0·1^{a}	25·9 ± 3·5^{a}	45·4 ± 3·5^{a}	1·3 ± 0·2^{a}	5
P-value	<0·05	<0·05	<0·05	<0·05	<0·05	<0·05	<0·05	<0·05	

Means in the same column and cultivar with different superscript letters differ at $P < 0·05$.

and the ratios
18:3n-3/18:2n-6. The effects of cultivar or cutting date seem to be different because 18:1n-9 proportions were affected for cultivar and 18:0 proportions were only affected by cutting date. The results of Experiment 3 showed also some differences in the fatty acid composition due to the cutting date.

Effects of cutting date and interval, which reflect maturity differences, on the fatty acid composition were reported in several studies. Dewhurst *et al.* (2001) found that a cutting interval of 20 days compared with 38 days increased the concentrations of all fatty acids in both perennial ryegrass and Italian ryegrass (*Lolium multiflorum* L.). Barta (1975) noticed reductions in total fatty acids of more than 30% in six grass species as plants increased in maturity. Boufäied *et al.* (2003) found reductions in 16:0, 16:1, 18:2n-6 and 18:3n-3 and total fatty acid with advancing maturity of Timothy from stem elongation to early flowering. Dewhurst *et al.* (2001) found that the highest

levels of total fatty acid concentrations in *Lolium* L. spp (*L. perenne*, *L. multiflorum and L. boucheanum*) were noticed when the grass was in vegetative growth stages. The proportion of leaves to stems decreases with maturity (Belanger and McQueen, 1996). Changes in leaf: stem ratios can partly explain the declining proportions of fatty acids as plants mature (Dewhurst *et al.*, 2002; Boufäied *et al.*, 2003). Fatty acid concentration decreases as plants mature, making forage management a central determinant in fatty acid concentration (Clapham *et al.*, 2005). There is some evidence indicating that the flowering process can also cause a reduction in fatty acid concentrations (Dewhurst *et al.*, 2002).

Winter hardiness also affects the fatty acids found in forages. Samala *et al.* (1998) found increases in PUFA concentrations in the lipid membranes of winter-hardy varieties of Bermuda grass after the plants were subjected to a cold treatment. Exposure to chilling temperatures induces changes in plant 'membrane lipids, notably their degree of fatty acid unsaturation and phospholipid contents increase during cold acclimatization. Falcone *et al.* (2004) showed that fatty acid concentrations in *Arabidopsis* are affected by changes in temperature. Following a reduction in temperature, from 36 to 17°C, increases in concentrations of 16:3 and 18:3n-3 were found, while the concentrations of 16:1 and 18:2n-6 decreased. Somewhat contradictory resulted were obtained by Kuiper (1970), who found that lucerne (*Medicago sativa* L.) grown at 15°C contained higher proportions of both 18:2n-6 and 18:3n-3 than plants grown at 30°C.

Light intensity may affect the fatty acid composition of forage plants by influencing the chloroplast content. Dewhurst and King (1998) found that shading grass with a black plastic sheet for 24 h prior to cutting resulted in reductions in the total fatty acid content, together with 18:3n-3 percentages in silage obtained from it.

Seasonal fluctuations were assessed in perennial ryegrass (*Lolium perenne* L.) between April and November. It was found that during the months of June and July, forages contained the least amount of total fatty acids. Elgersma *et al.* (2003) showed higher concentrations of total fatty acids in mid-summer than in early summer, as consistent differences in the concentrations of 18:3n-3 were found among cultivars Barlet and Magella throughout the season. A similar pattern of seasonal change in perennial ryegrass (*L. perenne*) was found by Gilliland *et al.* (2002). Mel'uchova *et al.* (2008) found that in natural pastures, 18:3n-3 acid significantly decreased from 62 to 39% of total fatty acids from May to August and subsequently it slightly increased from August to September, compared with the beginning of pasture season. The pasture seasonal variations in 18:3n-3/18:2n-6 ratios were directly proportional to the corresponding content of CLA and indirectly proportional to the ratio in ewes' milk fat. The results suggest that the seasonal variations in CLA content in ewes' milk fat are related primarily to the seasonal variations in 18:3n-3 content in grass lipids.

Conclusions

This study about the forage fatty acid composition of forages in Argentina clearly shows the significant changes in total lipids and fatty acid composition, particularly in the contribution of 18:2n-6 and 18:3n-3, the ratio 18:3n-3/18:2n-6 and the total PUFA due to cultivar, cutting date and season. Considering the importance of these fatty acids as substrates for CLA and n-3 PUFA concentrations in beef, the fatty acid composition of forages needs to be considered in forage grazing beef production systems. There is also a need for more understanding of processes that occur in the rumen. There are factors other than the fatty acids influencing the amounts of PUFA 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 fatty acids 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 beef.

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