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EFFECT OF SPECIES AND HARVEST MATURITY ON THE FATTY ACIDS PROFILE OF TROPICAL FORAGES

N. A. Khan[1,2,*], M. W. Farooq[1], M. Ali[3], M. Suleman[4], N. Ahmad[1], S. M. Sulaiman[1], J. W. Cone[2] and W. H. Hendriks[2]

[1]Department of Animal Nutrition, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, 25130, Pakistan
[2]Animal Nutrition Group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, The Netherlands
[3]Institute of Animal Sciences, University of Agriculture, Faisalabad, 38040, Pakistan
[4]Department of Agriculture Chemistry, The University of Agriculture Peshawar, Khyber Pakhtunkhwa, 25130, Pakistan
[*] Corresponding Author E mail: nak126@gmail.com

ABSTRACT

The aim of this study was to quantify the fatty acid (FA) content and composition of forages commonly fed to dairy animals in the tropics. Twelve forage species, namely, *Trifolium alexandrinum, Cichorium intybus, Hordeum vulgare L., Medicago sativa, Avena sativa, Pennisetum purpureum, Setaria anceps, Sorghum almum, Panicum maximum, Rumex nepalensis, Panicum coloratum and Panicum antidotale* were evaluated. Each forage species was grown in four replicate plots under standard agronomic conditions, and sampled at early, normal and late stages of maturity. The result of the present study showed that the chemical composition, dry matter digestibility and FA contents varied ($P < 0.001$) among forage species and harvest maturity. Linolenic acid (C18:3n-3), palmitic acid (C16:0) and linoleic acid (C18:2n-6) were the predominant FAs with an average content of 8.65, 3.61 and 2.38 g/kg dry matter (DM), contributing on average to 53%, 22% and 14% of the total measured FAs, respectively. Among the individual FAs, C18:3n-3 had the largest variation ranging from 4.26 to 17.43 g/kg DM at first harvest. The content of C16:0, C18:2n-6 and C18:3n-3 decreased ($P < 0.001$) with maturity, with the largest decrease being observed in C18:3n-3. This study highlights that harvest management is an important tool to manipulate the FA contents and composition within a forage species.

Key words*:* Fatty acids, Grasses, Harvest maturity, Polyunsaturated fatty acids, Tropical forages

INTRODUCTION

Increasing the dietary supply of polyunsaturated fatty acids (PUFAs) in dairy ration can relatively quickly and favourably modulate the fatty acid (FA) composition of milk fat, and potentially benefit long term human health (Khan *et al*., 2012a). Forages are often the major source of PUFA, particularly of linolenic acid (C18:3n-3), in dairy rations. Forages are relatively low in FA content (< 35 g/kg dry matter (DM)), but due to their higher intake and C18:3n-3 content (11.17 g/kg DM), forages strongly influence the intake of PUFA in dairy animals (Khan *et al*., 2012b).

Extensive research has shown that there is considerable variation in the FA content and composition of forages due to species, maturity and environmental conditions (Clapham *et al*., 2005). Temperate grasses had a higher content of C18:3n-3 and lower contents of palmitic acid (C16:0) and linoleic acid (C18:2n-6) compared to legumes (Dierking *et al*., 2010). There was, however, a large variation in the FA content and composition within grass and legume species. The FA content declines with plant maturity, due to a decrease in leaf/stem ratio, and initiation of flowering and leaf senescence (Khan *et al*., 2011b). In the tropics, forages generally grow and mature faster, and reach the age of senescence quicker as compared to temperate conditions (Bezabih *et al*., 2013). Moreover, ambient temperature can affect the FA content of forages, by changing the amount of unsaturated FAs in membrane lipids (Allakhverdiev, 2009). A decrease in temperature during growth induces the enzymatic desaturation of FAs to regulate membrane fluidity to provide an optimal environment for photosynthesis and maintain trans-membrane transport (Allakhverdiev, 2009). On the other hand a high growth temperature (36 *vs.* 17 °C) decreased the content of C18:3n-3 and increased the contents of C16:0 and C18:2n-6 in the total FAs of *Arabidopsis* (Falcone *et al*. 2004).

Much of the recent work on the quantification of FA content and composition of forages has been carried out on temperate species under temperate environmental conditions. However, to the author's knowledge, the FA profile of tropical fodder species and grasses under tropical environment has not been investigated. The present study was, therefore, designed to quantify the FA content and composition of forage species commonly fed to dairy animals in tropical countries. A second aim was to investigate the dynamics of FA content and composition during a range of maturity at which these forages are usually harvested and fed to dairy animals.

MATERIALS AND METHODS

Experimental site and forage management: Twelve forage species, comprising of seven grasses and five fodder species, were evaluated in the present study (Table 1). Each grass species was sown in four replicate plots (10 m × 15 m) at the range research station of the Pakistan Forest Institute Peshawar, while each fodder species was sown in 4 replicate plots (15 m × 25 m) at the dairy research fields of The University of Agriculture, Peshawar (34°00′ N latitude, 71°30′ E longitude, and 350 m altitude). The grasses were sown on 15 March 2011, while fodder species were sown on 13th October, 2011. The plots were fertilized with 130 kg P_2O_5 /ha, 36 kg K_2O/ha and 30 kg N/ha before at sowing. The fodder plants (50%) emerged on November 3, 2011 and the range grasses emerged (50%) on April 9, 2011. Two weeks after emergence, the plots of ranges grasses were fertilised with 120 kg N/ha, while the fodder plots were fertilized with 90 kg N/ha. For the fodder plots a similar dose of fertilization was repeated after each cut.

Sampling: For the present study, samples were collected from the 1st re-growth (15th March, 2012) of range grasses and the 3rd cut (15th March; 3 weeks after the last cut) of fodder crops. The sampling was repeated at two weeks interval for normal and late harvest dates. The date of harvests and weather data during the harvest periods are shown in Table 2. The harvest dates were estimated from the established knowledge of the maturity of these forages at these stations. At each harvest maturity, four samples were randomly collected from a 1 m^2 area of each replicate plot of each species. Forages were manually cut at a height of 5 cm above the ground. Samples were kept in pre-labelled polythene zip-bags, vacuume closed, and immediately transported to the laboratory in oxygen-free (flushed with CH_4) cooling boxes. At each harvest the samples were pooled by the replicate plot of each forage type, mixed, and representative subsamples of approximately 2 kg were collected for further processing and chemical analysis. Each subsample was divided into two equal parts; one half was oven dried (60ºC for 72 h) for chemical analyses while the other half was stored at -20ºC. The plants of *Hordeum vulgare L.* were completely senescent at the third harvest and did not process for chemical analysis.

Chemical analysis: The contents of DM (method 930.15), ash (method 942.05), crude protein (CP; method 984.13; using a KjeltecTM 2400 autoanalyzer; Foss Analytical A/S, Hillerød, Denmark) and acid detergent fibre (ADF; method 973.18) were analyzed using the standard methods of AOAC (1990). The neutral detergent fibre (NDF) content was analysed according to Van Soest *et al*. (1991), with sodium sulphite and heat-stable α-amylase. The two stage *in vitro* procedure was adopted for determining the *in vitro* DM digestibility (DMD) as reported by Khan *et al* (2015).

Fatty acid analysis: The FAs analyses were carried out at the Animal Nutrition Laboratory of Wageningen University, The Netherlands. For fatty acid analysis, the lipids from forage samples were extracted with chloroform-methanol (2:1 v/v) using tridecanoic acid (C13:0, 2.25 mg/15 ml chloroform–methanol solution) as an internal standard (Khan *et al*. 2009). Fatty acids in the residual lipids were (trans) esterified, using both acid and base catalyzed methods as described by Khan *et al*. (2011a). The FA methyl esters (FAMES) were then analyzed by gas chromatography (TRACE GC Ultra™, Thermo Electron Corporation, Waltham, MA, USA). The GC was equipped with an auto-sampler, fused silica capillary column (100m × 0.25mm and 0.2µm film thickness, SP-2560; Supelco, Bellefonte PA, USA) and a flame-ionization detector. Hydrogen gas was used as a carrier at a constant flow of 1.5 ml/min. One µl of sample was injected into the GC with a split ratio of 1:50. The temperature program of the GC is described by Khan *et al*. (2011a). The FAMES were identified using external standards (S37, Supelco, Poole, Dorset, United Kingdom), and the FA contents were calculated from the peak area of the corresponding FA, using the peak area of internal standard, as described by Khan *et al*. (2009).

Statistical analysis: The effect of forage species and harvest maturity on the nutrient composition, DMD and FA content was determined using PROC MIXED Procedure of the Statistical Analysis System (SAS, 2009). The model used was,

$$Y_{ijk} = \mu + F_i + H_j + FH_{ij} + e_{ijk}$$

where, $Y_{ijk,}$ is the depended variable; µ, is the overall mean; F_i, is the fixed effect of forage species; H_j, is the fixed effect harvest maturity; FH_{ij}, is the fixed effect of the interaction of forage species and harvest maturity; e_{ijk} is the random error. Harvest maturity was considered as a repeated factor. Replication was considered as a random effect. When significant ($P < 0.05$) differences were detected, post-hoc analyses were carried out using Tukey-Kramer test to compute pair-wise differences in the means.

RESULTS

Chemical composition and in vitro digestibility: There was large variation ($P < 0.001$) in the chemical composition and *in vitro* DMD due to forage species and harvest maturity Table 3. The content of CP decreased ($P < 0.001$), and those of ADF and NDF increased ($P < 0.001$) with maturity in all forages. The decrease in CP content ranged from 12% in *S. anceps* to 47% in *M. sativa*, whereas, the increase in NDF content ranged from 6% in *P. maximum* to 23% in *P. coloratum*. The *in vitro*

DMD decreased with maturity in all forages. However, there was a large variation in the decrease in the *in vitro* DMD among forage species ranging from 5% in *P. purpureum* to 40% in *M. Sativa.*

Fatty acid content and composition: Results on the variation in the major individual and total FA content due to forage type and harvest maturity are presented in Table 4. In all forages, C18:3n-3 was the predominant FA with an average content of 9.21 g/kg DM, contributing on average 51% to the total measured FAs. C16:0 was the next abundant fatty acid with an average content of 3.64 g/kg DM, contributing 22% to the total FAs. Whereas, the content of C18:2n-6 averaged 2.72 g/kg DM, contributing 16% to the total FAs. Combined, these three major FAs contributed to ~ 90% of the total FAs. Of the remaining minor FAs, palmitoleic acid (C16:1), stearic acid (C18:0) and oleic acid (C18:1n-9) had an average content of 0.34 to 0.42 g/kg DM, contributing on average to 2 to 3% of the total FAs.

The content of all individual and total FAs showed a large variation ($P < 0.001$) due to forage species. Among the individual FAs, C18:3n-3 showed the largest variation, and ranged from 4.26 (*H. vulgare L.*) to 17.43 (*A. sativa*) g/kg DM at first harvest. Whereas, the content (g/kg DM) of C16:0 ranged from 2.97 (*H. vulgare L.*) to 4.72 (*C. intybus*), and that of C18:2n-6 ranged from 1.93 (*H. vulgare L.*) to 4.47 (*T. alexandrinum*) at first harvest. The contents of C16:0, C18:2n-6, C18:3n-3 and total FA decreased ($P < 0.001$) with harvest maturity in all forages. Among the individual FAs, C18:3n-3 had the largest decrease with maturity. The average content of C18:3n-3 was 10.95, 9.18 and 5.81 g/kg DM in the first, second and third harvest, respectively (data not shown). Although the content of C18:3n-3 declined in all forages, the magnitude of the decrease varied due to forage-type. The lowest decrease (20%) in C18:3n-3 content was observed in *R. nepalensis* and the highest decrease (68%) in *S. anceps.* Due to the larger decrease in the content of C18:3n-3 compared to the other FAs, the average proportion of C18:3n-3 in the total FA decreased from 50% at first harvest to 40% at third harvest. As a consequent, the average proportion of C16:0 in the total FAs increased from 20 to 24%, and that of C18:2n-6 increased from 14 to 17%.

Table 1. Description of the tropical forage species used in this study.

Forage species	Common name	Forage type
Fodder species		
Trifolium alexandrinum	Barseem	Legume
Cichorium intybus	Kasni	Forb
Medicago sativa	Lucerne	Legume
Avena sativa	Oats	Cereal
Hordeum vulgare L.	Barley	Cereal
Range grasses		
Pennisetum purpureum	Mott grass	Grass
Panicum antidotale	Bansi/Perenial Sodan grass	Grass
Panicum coloratum	Buffalo grass	Grass
Panicum maximum	Guinea grass	Grass
Rumex nepalensis	Jangali palak	Grass
Setaria anceps	Golden bristle grass	Grass
Sorghum almum	Columbus grass	Grass

Table 2. Sampling dates and weather conditions during the growing interval preceding the harvest dates.

Harvesting Date	Weather during preceding 14-day period			
	Mean T[1] (°C)	Max T (°C)	Min T (°C)	Rain (mm)
15-3-2012	29	32	21	39
30-3-2012	33	35	22	0
15-4-2012	37	41	23	24

[1], T = temperature

Table 3. Dry matter (g/kg), chemical composition (g/kg DM) and *in vitro* dry matter digestibility (DMD; g/kg) of several tropical forage species as affected by stage of maturity.

Forage species	Maturity	DM	CP	ADF	NDF	Ash	*In vitro* DMD
Fodder species							
Trifolium alexandrinum	Early	148[b]	201[a]	289[c]	499[b]	102[b]	367[a]
	Medium	151[b]	168[b]	329[b]	515[b]	110[a]	314[b]
	Late	176[a]	140[c]	349[a]	609[a]	115[a]	317[c]
Cichorium intybus	Early	178[c]	193[a]	269[c]	499[c]	134[a]	338[a]
	Medium	193[b]	157[b]	299[b]	535[b]	125[a]	293[b]
	Late	237[a]	147[c]	369[a]	589[a]	106[b]	285[b]
Medicago sativa	Early	101[b]	242[a]	270[c]	543[c]	169[a]	409[a]
	Medium	107[b]	220[b]	300[b]	559[b]	171[a]	347[b]
	Late	243[a]	128[c]	339[a]	620[a]	123[b]	245[c]
Avena sativa	Early	170[c]	177[a]	239[c]	529[c]	126	391[a]
	Medium	193[b]	161[b]	270[b]	550[b]	128	339[b]
	Late	200[a]	152[c]	290[a]	580[a]	120	320[c]
Hordeum vulgare L.	Early	130[b]	164[a]	290[b]	439[b]	109	260[a]
	Medium	144[a]	143[b]	320[a]	464[a]	114	216[b]
Range grasses							
Panicum antidotale	Early	179[c]	209[a]	249[c]	525[c]	132[a]	387[a]
	Medium	211[b]	169[b]	319[b]	555[b]	125[a]	356[b]
	Late	248[a]	147[c]	339[a]	590[a]	101[b]	302[c]
Panicum coloratum	Early	148[c]	166[a]	260[c]	565[c]	105[b]	354[a]
	Medium	169[b]	139[b]	290[b]	580[b]	105[b]	324[b]
	Late	246[a]	140[b]	359[a]	694[a]	129[a]	293[c]
Panicum maximum	Early	256[c]	168[a]	330[c]	604[b]	123[b]	348[a]
	Medium	361[b]	167[a]	359[b]	609[b]	141[a]	342[a]
	Late	379[a]	136[b]	419[a]	634[a]	130[a]	308[b]
Rumex nepalensis	Early	163[c]	200[a]	160[c]	499[c]	114[b]	450[a]
	Medium	190[b]	173[b]	180[b]	549[b]	125[a]	413[b]
	Late	205[a]	156[c]	190[a]	590[a]	108[b]	390[c]
Setaria anceps	Early	204[c]	230[a]	200[c]	514[c]	101[b]	459[a]
	Medium	231[b]	212[b]	210[b]	554[b]	111[a]	409[b]
	Late	251[a]	203[c]	220[a]	605[a]	99[b]	371[c]
Sorghum almum	Early	137[c]	243[a]	210[c]	519[c]	157[b]	426[a]
	Medium	164[b]	228[b]	249[b]	550[b]	150[b]	386[b]
	Late	183[a]	213[c]	270[a]	589[a]	171[a]	330[c]
Standard Error		7.6	10.2	18.2	20.1	9.1	10.1
Significance							
Forage		***	***	***	***	***	***
Maturity		***	***	***	***	NS	***
Forage × Maturity		***	***	***	***	NS	***

[abc] Means with different superscripts within column and within forage species are significantly (P<0.05) different.
DM, dry matter; CP, crude protein; ADF, acid detergent fiber; NDF, neutral detergent fiber; DMD, dry matter digestibility
***,P<0.001; NS, non-significant

Table 4. Effect of forage species and harvest maturity on the content (g/kg DM) of major individual and total fatty acids (FAs).

Forage species	Maturity[1] stage	C14:0	C16:0	C16:1	C18:0	C18:1n-9	C18:2n-6	C18:3n-3	C20:0	C24:0	Total FA
Fodder species											
Trifolium alexandrinum	Early	0.08	4.29^{a}	0.09	0.60	0.51	4.47^{a}	13.65^{a}	0.07	0.13	24.23^{a}
	Medium	0.07	3.93^{b}	0.08	0.48	0.42	3.86^{b}	10.53^{b}	0.05	0.11	19.59^{b}
	Late	0.09	3.90^{b}	0.09	0.48	0.49	3.69^{b}	8.60^{c}	0.06	0.10	17.79^{c}
Cichorium intybus	Early	0.13	4.72^{a}	0.53	0.31	0.44	3.57^{a}	13.39^{a}	0.07	0.14	23.43^{a}
	Medium	0.13	4.42^{b}	0.52	0.27	0.53	3.43^{b}	12.11^{b}	0.06	0.11	21.71^{b}
	Late	0.07	4.36^{c}	0.53	0.32	0.43	3.22^{b}	11.70^{b}	0.06	0.11	20.96^{c}
Medicago sativa	Early	0.13	3.87	0.41	0.51	0.44	2.95	8.91^{a}	0.07	0.12	17.50^{a}
	Medium	0.12	3.84	0.56	0.56	0.56	2.98	8.05^{b}	0.07	0.10	16.96^{b}
	Late	0.13	3.77	0.59	0.58	0.59	2.88	7.09^{c}	0.06	0.11	15.93^{c}
Avena sativa	Early	0.10	4.47^{a}	0.40	0.27	0.56	2.62^{a}	17.43^{a}	0.05	0.11	26.11^{a}
	Medium	0.05	3.91^{b}	0.43	0.22	0.49	2.41^{b}	13.18^{b}	0.04	0.09	20.87^{b}
	Late	0.04	3.82^{b}	0.40	0.18	0.44	2.31^{c}	12.10^{c}	0.04	0.10	19.47^{c}
	Medium	0.11	2.04^{b}	0.09	0.15	0.76	1.39^{b}	2.36^{b}	0.08	0.05	7.14^{b}
Range grasses											
Pennisetum purpureum	Early	0.11	4.47^{a}	0.57	0.43	0.29	2.90^{a}	13.81^{a}	0.10	0.14	22.93^{a}
	Medium	0.10	3.81^{b}	0.49	0.32	0.27	2.57^{b}	11.33^{b}	0.09	0.12	19.19^{b}
	Late	0.05	3.23^{c}	0.48	0.29	0.30	2.36^{b}	7.29^{c}	0.08	0.12	14.25^{c}
Panicum antidotale	Early	0.07	3.83^{a}	0.34	0.32	0.53	2.94^{a}	9.98^{a}	0.12	0.12	18.32^{a}
	Medium	0.06	3.66^{a}	0.37	0.28	0.53	2.91^{a}	7.94^{b}	0.10	0.09	15.56^{b}
	Late	0.05	3.02^{b}	0.34	0.28	0.47	2.40^{b}	5.12^{c}	0.09	0.08	11.90^{c}
Panicum coloratum	Early	0.12	3.51^{a}	0.28	0.22	0.41	2.16^{a}	8.04^{a}	0.84	0.11	15.81^{a}
	Medium	0.10	3.23^{a}	0.23	0.26	0.45	1.99^{ab}	6.92^{b}	0.91	0.09	14.28^{b}
	Late	0.09	2.39^{b}	0.24	0.24	0.38	1.31^{b}	4.12^{c}	0.82	0.09	9.77^{c}
Panicum maximum	Early	0.07	3.64^{a}	0.41	0.34	0.29	2.53^{a}	5.86^{a}	0.06	0.15	13.42^{a}
	Medium	0.07	3.49^{b}	0.31	0.29	0.25	2.23^{ab}	4.73^{b}	0.06	0.14	11.64^{b}
	Late	0.06	2.77^{c}	0.38	0.26	0.21	1.51^{b}	2.26^{c}	0.06	0.13	7.70^{c}
Rumex nepalensis	Early	0.17	4.42	0.70	0.32	0.51	3.76	15.64^{a}	0.10	0.18	26.15^{a}
	Medium	0.13	4.51	0.69	0.30	0.44	3.81	13.99^{b}	0.11	0.17	24.47^{b}
	Late	0.12	4.32	0.66	0.28	0.42	3.76	12.58^{c}	0.09	0.15	22.5^{c}
Setaria anceps	Early	0.06	3.46^{a}	0.52	0.43	0.30	3.63^{a}	11.05^{a}	0.19	0.17	19.77^{a}
	Medium	0.05	3.49^{a}	0.49	0.51	0.30	3.14^{b}	10.56^{b}	0.18	0.15	18.77^{b}
	Late	0.06	2.16^{b}	0.45	0.48	0.22	2.09^{c}	3.50^{c}	0.22	0.14	8.93^{c}
Sorghum almum	Early	0.12	3.58^{a}	0.44	0.39	0.24	2.39^{a}	9.39^{a}	0.08	0.12	16.82^{a}
	Medium	0.06	3.53^{a}	0.42	0.30	0.30	2.34^{a}	8.42^{b}	0.09	0.09	15.81^{b}
	Late	0.05	2.43^{b}	0.49	0.34	0.26	1.64^{b}	6.66^{c}	0.10	0.11	11.84^{c}
Standard Error		0.028	0.072	0.017	0.056	0.018	0.056	0.198	0.007	0.002	0.354
Significance[2]											
Forage		***	***	***	***	***	***	***	***	***	***
Maturity		NS	***	NS	NS	NS	***	***	NS	NS	***
Forage × Maturity		NS	***	NS	NS	NS	***	***	NS	NS	***

^{abc}, Means with different superscripts within column and within forage species are significantly (P<0.05) different.
C14:0, myristic acid; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, steraic acid; C18:1n-9, oleic acid; C18:2n-6, linoleic acid; C18:3n-3, linolenic acid; C20:0, arachidic acid; C24:0, lignoceric acid.
NS, not significant; ***, P<0.001.

DISCUSSION

Research in the past decade has established that forages are often the major source of PUFA, particularly C18:3n-3, in the ration of dairy animals, and high PUFA containing forages can be used to favourably modulate milk FA profile of dairy animals (Khan *et al*., 2012a). The present study reports the first dataset on FA content

and composition of tropical forages commonly fed to dairy animals. The results showed a large variation in the content of all FAs, particularly in C18:3n-3, due to forage species and harvest-maturity. This variation in FA content can result in large differences in FAs intake, because dairy animals usually consume large quantities of forages. For example, if a dairy cattle consume 10 kg DM of forage with the highest (17.43 g/kg DM) or the lowest (2.36 g/kg DM) C18:3n-3 content observed in the present study, the overall difference in C18:3n-3 intake would be 151 g/d. The large variation in the chemical composition also affects nutrient bioavailability of forages (Ali *et al*., 2012; 2013). This highlights the need for evaluating forage quality in terms of their FA content and composition. Moreover, the large variation in FA content among forage species presents an opportunity to further improve the FA content through plant breeding. This study also showed that harvest management markedly affect the FA content and FA composition of forages, thereby harvest management present another opportunity to improve the FA content of forages.

The decrease FA content with forage maturity is consistent with earlier findings (Clapham *et al*., 2005; Khan *et al*., 2011b). In photosynthetic tissues of forages, FAs are present in membranes lipids, predominantly in thylakoid membranes of chloroplasts, which on average contain 20% lipids on DM basis. In a number of forage species, a strong positive correlation have been found between the content of chlorophyll and total FAs content (Dierking *et al*., 2010). The total amount of FA in forages, therefore, is strongly influenced by the concentration of chloroplasts. The decline in chloroplast lipids with advancing maturity of forages, due to a decrease in leaf/stem ratio (Boufaïed *et al*., 2003; Khan *et al.*, 2012a), maturation of the leaves (Khan *et al*., 2011b), as well as initiation of flowering (Dewhurst *et al*., 2006) and senescence (Mishra and Sangwan, 2008; Yang and Ohlrogge, 2009) can explain the decrease in FA content with maturity in the present study. Management practices that prevent extended maturation and the decrease in leaf/stem ratio, such as increasing the number of cuttings, can minimize the decrease in FA content in forages during the growing season. Frequent cutting of perennial ryegrass prevented the normal decline in FA content during the summer with a progressive increase in total FAs content (from 20.8 to 34.6 g/kg DM) during the growing season (Dewhurst *et al*., 2002). In addition, the application of N fertilization increases the FA content in forage plants and delays the decline in FA content with advancing herbage maturity (Witkowska *et al*., 2008).

In the present study, the content of C18:3n-3 declined at a preferentially faster rate than the contents of C16:0 and C18:2n-6. As a consequence, C18:3n-3 as a proportion of the total amount of FAs decreased with plant maturity. Our results are supported by earlier findings (Khan *et al*., 2011b). In contrast, Clapham *et al.* (2005) reported that the FAs contents of forages decreased with maturity, however, the proportion of individual FAs in total FAs did not change with forage maturity. Discrepancy between the two studies could be related to the differences in forage species and environment condition. In tropical conditions forages mature faster as compared to temperate environment (Bezabih *et al*., 2013). Notably, there was a strong positive relationship between the concentrations of C18:3n-3 and *in vitro* digestibility of fodder crops (R^2 = 0.46, P<0.001) and range grasses (R^2 = 0.64, P<0.001). The digestibility of forages is strongly influenced by maturity and forage family, which can explain the significant correlation between digestibility and fatty acid content within grass and fodder species. This finding also highlights the scope for developing regression equations for estimation of FA content of individual forage species or forage family from their digestibility. Although the FA contents of forages can be determined using GC, which is an accurate method, it requires time consuming extractions, derivitisation and chromatography steps, making this method unfeasible for routine analysis of silages on dairy farms. Alternatively, regression equations based on digestibility or other easily measurable parameters can be developed to estimate the fatty acid contents of forages.

In the present study, the average proportion of C18:3n-3 in the total FAs was much lower (53% *vs.* 63% and 60-76%) than those reported by Clapham *et al*. (2005) and Dierking *et al*. (2010) for temperate forages. Whereas, the proportion of C16:0 in the total FAs was much higher (22% *vs*. 14% and 11-16%) in the present study. The lower proportion of C18:3n-3 and the higher proportion of C16:0 in the total FAs in the present study could be, in part, related to the effect high ambient temperature. A higher ambient temperature decreases the proportion of C18:3n-3 in the total FAs with a concomitant increase in the proportion of C16:0 and C18:2n-6 (Falcone *et al*. 2004; Allakhverdiev, 2009).

The large differences in FA content and composition among forage species are consistent with earlier findings (Clapham *et al*., 2005; Dierking *et al*., 2010). Genetic differences in FA content among forages are related to inherent differences in plant materials and their physiological maturity (Kalač and Samková, 2012). Genetic differences in FA content are, however, more pronounced in young growing plants, whereas leaf/stem ratio, initiation of flowering and senescence become increasingly important in the more mature plants.

Conclusions: The content of all major individual and total FAs showed a large variation among forage species. Among the individual FAs, C18:3n-3 had the largest variation, ranging from 4.26 (*H. vulgare L.*) to 17.43 (*A. sativa*) g/kg DM at first harvest. The content of C16:0, C18:2n-6 and C18:3n-3 decreased (P < 0.001) with

maturity. Due to preferentially faster decrease in C18:3n-3 content, the proportion of C18:3 per unit of total FAs decreased with maturity. The large variation in FA content among forage species presents an opportunity to further improve the FA content through plant breeding. Harvest management is an important tool to manipulate the FA content and composition within a forage species, thereby presenting an opportunity to improve the FA profile of forages and ruminant products.

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REFERENCES

Ali, M., M.R. Weisbjerg, J.W. Cone, G. van Duinkerken, M.C. Blok, M. Bruinenberg and W.H. Hendriks (2012). Postruminal degradation of crude protein, neutral detergent fibre and starch of maize and grass silages in dairy cows. Anim. Feed Sci. Technol. 177: 172-179.

Ali, M., G. van Duinkerken, J.W. Cone, A. Klop, M.C. Blok, J.W. Spek, M. Bruinenberg and W.H. Hendriks (2014). Relationship between chemical composition and in situ rumen degradation characteristics of maize silages in dairy cows. Animal 8: 1832-1838.

Allakhverdiev, S.I. (2009). Regulatory roles in photosynthesis of unsaturated fatty acids in membrane lipids, In: Wada H, Murata N (Ed.), Lipids in photosynthesis: essential and regulatory functions, Springer, Dordrecht, the Netherlands. pp. 265–282.

AOAC, (1990). Association of Official Analytical Chemists; Official methods of analysis, 15th edition, AOAC, Arlington, VA, USA.

Bezabih, M., W.F. Pellikaan, A. Tolera, N.A. Khan, W.H. Hendriks (2013). Chemical composition, *in vitro* total gas and methane production of forage species from the Mid Rift Valley grasslands of Ethiopia. Grass For. Sci. 69: 635-643.

Boufaïed, H., P.Y. Chouinard, G.F. Tremblay, H.V. Petit, R. Michaud and G. Belanger (2003). Fatty acids in forages. I. Factors affecting concentrations. Can. J. Anim. Sci. 83: 501–511.

Clapham, W.M., J.G. Foster, J.P.S. Neel and J.M. Fedders (2005). Fatty acid composition of traditional and novel forages. J. Agric. Food Chem. 53: 10068–10073.

Dewhurst, R.J., J.M. Moorby, N.D. Scollan, J.K.S. Tweed and M.O. Humphreys (2002). Effects of a stay–green trait on the concentrations and stability of fatty acids in perennial ryegrass. Grass For. Sci. 57: 360–366

Dewhurst, R.J., K.J. Shingfield, M.R.F. Lee and N.D. Scollan (2006). Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high–forage systems. Anim. Feed Sci. Technol. 131: 168–206.

Dierking, R.M., R.L. Kallenbach and C.A. Roberts (2010). Fatty acid profiles of orchardgrass, tall fescue, perennial ryegrass, and alfalfa. Crop Sci. 50: 391–402.

Falcone, D.L., J.P. Ogas and C.R. Somerville (2004). Regulation of membrane fatty acid composition by temperature in mutants of *Arabidopsis* with alterations in membrane lipid composition. BMC Plant Bio. 4: 1–45.

Kalač P.E. and E. Samkova (2010). The effects of feeding various forages on fatty acid composition of bovine milk fat: a review. Czech J. Anim. Sci. 55: 521–537.

Khan, N.A., J.W. Cone and W.H. Hendriks (2009). Stability of fatty acids in grass and maize silages after exposure to air during the feed out period. Anim. Feed Sci. Technol. 154: 183–192.

Khan, N.A., J.W. Cone, V. Fievez and W.H. Hendriks (2011a). Stability of fatty acids during wilting of perennial ryegrass (*Lolium perenne L.*): effect of bruising and environmental conditions. J. Sci. Food Agric. 91: 1659–1665.

Khan, N.A., J.W. Cone, W.F. Pellikaan, M.A. Khan, P.C. Struik and W.H. Hendriks (2011b). Changes in fatty acid content and composition in silage maize during grain filling. J. Sci. Food Agric. 91: 1041–1049.

Khan, N.A., J.W. Cone, V. Fievez and W.H. Hendriks (2012a). Causes of variation in fatty acid content and composition in grass and maize silages. Anim. Feed Sci. Technol. 174: 36-45.

Khan, N.A., S. Hussain, N. Ahmad, S. Alam, M. Bezabhi, W. H. Hendriks, P. Yu and J. W. Cone (2015). Improving the feeding value of straws with *Pleurotus ostreatus*. Animal Prod. Sci. 55: 241-245.

Khan, N.A., T.A. Tewoldebrhan, R.L.G. Zom, J.W. Cone and W.H. Hendriks (2012b). Effect of corn silage harvest maturity and concentrate type on milk fatty acid composition of dairy cows. J. Dairy Sci. 95: 1472–1483.

Mishra, S. and R. S. Sangwan (2008). Changes in fatty acid composition of polar lipids associated with growth and senescence in leaves of Catharanthus roseus. Afric. J. Plant Sci. 2: 34–37.

SAS®, 2009. Statistical Analysis System, Version 9.1.3., service pack 4. SAS Institute Inc., Cary, NC.

Van Soest P.J., J.B. Robertson and B.A. Lewis (1991). Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 73: 3583–3593.

Witkowska, I.M., C. Wever, G. Gort and A. Elgersma (2008). Effects of nitrogen rate and regrowth interval on perennial ryegrass fatty acid content during the growing season. Agron. J. 100: 1371–1379.

Yang, Z. and J. B. Ohlrogge (2009). Turnover of fatty acids during natural senescence of *Arabidopsis*, *Brachypodium*, and *Switchgrass* and in *Arabidopsis* oxidation mutants. Plant Physiol. 150: 1981–1989.