

Milk fatty acid profile of Peruvian Criollo and Brown Swiss cows in response to different diet qualities fed at low and high altitude

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(Received 13 June 2008; accepted 1 September 2008)

Two identical experimental protocols were followed at 200 and 3,600 m above sea level (a.s.l.) determining the changes of the milk fatty acid (FA) profile of Brown Swiss (BS) and indigenous Peruvian Criollo cows (CR) as a response to diets which were designed to cover the variation in feed quality caused by season. At each site (altitude), six BS and six CR cows, adapted to >3,500 m a.s.l., were fed three dietary treatments (DS, dryseason forage; RS rainy-season forage; OC, diet optimised to meet the cow's requirements) in a 2 × 2 × 3-factorial arrangement. Intakes of FA and milk yield increased from diet DS (low quality diet) to RS and OC (high quality diet) for both cow types. Milk fat proportions of conjugated linoleic acid (CLA), C18:3 c9,c12,c15, total n-3 and polyunsaturated FA (PUFA) were highest (p < 0.05) with diet OC and higher in the lowlands than in the highlands. Low intakes of diet DS obviously resulted in a ruminal energy deficiency and body lipid mobilisation. The ruminal energy deficiency with diet DS was especially pronounced in BS, apparently reducing biohydrogenation rate and leading to lower proportions of C18:0 and higher proportions of C18:3 c9,c12,c15 in milk fat (p < 0.05). Especially C18:3 c9,c12,c15 intake did not concur with its proportion in milk fat, suggesting a strong dependence on energy status. Milk yield and FA excretion with milk were higher for BS than for CR (p < 0.05) with all three diets although milk fat content was lower (p < 0.05) for BS than CR. Milk fat of BS was richer in CLA and PUFA than milk fat of CR (p < 0.05). The desaturase indices for 18 FA were also higher for BS than CR (p < 0.05), suggesting a slightly higher Δ 9desaturase activity for BS, especially with diet DS. Milk fat content was generally higher at the high altitude than at the lowland site (p < 0.05), whereas the FA profile was unexpectedly similar across sites. Various interactions were found among diet type, cow type and altitude (site) indicating that a combination of these factors contributes to the characteristic FA profile of the respective milk.

Keywords: dairy cows; milk fat; altitude; n-3 fatty acids; conjugated linoleic acid; desaturation

1. Introduction

Options to improve the quality of milk for human nutrition, especially with respect to the fatty acid (FA) profile of milk fat have been investigated extensively (De Henauw et al. 2007) and special attention has been paid to conjugated linoleic acids (CLA) (Collomb et al. 2006) and n-3 FA (Palmquist and Griinari 2006), among them C18:3 c9,c12,c15

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(α-linolenic acid; Dewhurst et al. 2006). The influence of the cow's diet on milk fat composition has been shown in various studies (e.g., Boufaïed et al. 2003; Chilliard and Ferlay 2004). Other factors such as breed and management also seem to determine milk FA profile but less information is available concerning these interrelationships. Some authors compared different breeds with respect to their milk fat composition and found different proportions of short-chain, medium-chain and long-chain FA between breeds (Carroll et al. 2006) and breed-characteristic CLA levels (White et al. 2001, Kelsey et al. 2003). Breed-specific responses in FA profile to diet changes have also been reported (Ferlay et al. 2006). Further studies showed that high-genetic merit cows, as opposed to low-genetic merit cows, have a down-regulated $\Delta 9$ -desaturase system and a tendency for a lower proportion of monounsaturated FA (MUFA) in their milk fat (Kay et al. 2005). Another factor influencing the milk FA profile seems to be high altitude grazing. Milk products from Alpine regions were shown to be especially rich in n-3 FA and CLA (Leiber et al. 2005), and its characteristic FA profile has even been suggested as a biomarker for authentication of its alpine origin (Engel et al. 2007). Results of detailed investigations on cows moved to altitudes of up to 2,000 m above sea level (a.s.l.) (Leiber et al. 2005) indicated that the repeatedly described high CLA proportion found in alpine milk (Kraft et al. 2003; Collomb et al. 2006) can be attributed to grazing alpine pastures rather than to altitude as such. Leiber et al. (2005) also noted a clear increase of C18:3 c9,c12,c15 proportion in milk fat with altitude. They hypothesised that this might be explained by mobilisation of this FA from body tissue due to energy shortage at high altitude, ruminal ecosystem changes or specific plant secondary compounds prevalent in the diverse vegetation at that altitude, while they ruled out higher C18:3 c9,c12,c15 concentrations in alpine herbage as an explanation.

In Peru, cows kept on pastures at up to 4,000 m a.s.l. are exposed to all factors outlined above. The productivity of the cows is generally low (FAO 2003). The dominant cow type is the local "Peruvian Criollo" (*Bos taurus*) which is still scarcely studied and little is known about its productive traits. After their introduction from Europe in the 16th century, the South American Criollo cattle adapted by natural selection to different environments; in the Andean region these were especially altitude and harsh climate (Scotto Espinoza and Rosemberg Barrón 2001). Currently, upgrading is practiced especially with Brown Swiss, imported originally from the US. A further limitation to dairy husbandry in the Peruvian highlands is the scarcity of good quality feeds, especially during the dry season.

The objective of the present study was to determine the influence of the factors diet quality (as typical for the highlands compared to that typical for the lowlands) and altitude (low oxygen partial pressure) on the milk fat composition of Peruvian Criollo and Brown Swiss cows. The hypotheses tested were: (i) That the extreme altitude differences between lowlands and highlands in Peru will lead to even more pronounced alterations in FA, especially n-3 FA and CLA, than those found in the Alps, and (ii) that this response depends on diet type and cow type. For this purpose two identical protocols were run at two altitudes with three diet types (poor, medium and high quality) and two cow types (indigenous and improved) representing a $2 \times 2 \times 3$ -factorial arrangement.

2. Materials and methods

2.1. Experimental diets

Two of the three experimental diets were designed to represent the nutritional values of typical highland feeds from either the dry or the rainy season. The use of these diets should

simulate the effects of the nutrition-related seasonal fluctuations as typical for the central Peruvian Andes. The diet representing the typical highland dry season feed (diet DS) consisted of oat straw and maize stover mixed at a ratio of 0.54:0.46. Accordingly, in diet DS the content of nitrogen (N, Table 1) as the first limiting nutrient in many tropical diets (Hess et al. 2003), was adjusted to the N content of 14 samples of hays and straws (6.5 g/kg DM on average) known to represent typical local dry season feeds (own collection and analysis). The diet typical for the rainy season in the highlands (diet RS) was composed of a 0.54:0.32:0.14 mixture of oat hay, alfalfa hay and maize stover. The N content of diet RS was in the range of the N contents of six samples of fresh local oat varieties (13.9 g/kg DM on average), a typical rainy season feed in the highlands. A third diet (diet OC) consisted of higher quality forages (alfalfa hay and maize stover mixed at a ratio of 0.81:0.19) and was supplemented with concentrate. Diet OC represented a diet typical for the more intensive milk production systems in the coastal region of Peru and was designed to be without any nutritional constraints to the cow genotypes characteristic for the highlands. Contents of N and net energy lactation of diet OC were adjusted to cover requirements of cows of 454 kg body weight (BW), 15 kg milk/d and 40 g fat/kg milk (NRC 2001). The concentrate contained, at a ratio of 0.57:0.41:0.01:0.01, maize, wheat middlings (ground wheat mixed with bran), NaCl and a vitaminised mineral mix (Premix Leche Prime® 100, Battilana Internacional S.A.C, Lima, Peru, Table 1). Diet OC was allocated proportionately to metabolic BW (BW^{0.75}; 17 and 26 g/kg BW^{0.75} for CR and BS cows, respectively). Thus, both cow types consumed approx. 130 g concentrate per kg total DM intake. In all groups cows had ad libitum access to forages, fresh water and NaCl. For cows on diets DS and RS, NaCl was mixed with the same mineral supplement which was included in the OC concentrate.

The forage components of the diets had been either grown specifically for the experiment (oat hay, oat straw) or purchased (alfalfa hay, maize stover). Oat hay and

Table 1. Contents of total fatty acids and selected fatty acids, nitrogen and fibre [g/kg DM] of the complete diets as offered.

		Diets*	
	Dry season (DS)	Rainy season (RS)	Optimised diet (OC) [†]
Total fatty acids	9.96	8.94	12.51
Fatty acids			
C16:0	2.11	1.87	2.59
C16:1	0.04	0.19	0.28
C18:0	0.46	0.34	0.50
C18:1 <i>c</i> 9	3.26	1.54	1.69
C18:2 c9,c12	2.20	1.75	3.34
C18:3 c9,c12,c15	0.16	1.51	1.86
SFA [§]	4.01	3.80	4.86
$MUFA^{\S}$	3.53	1.91	2.43
PUFA [§]	2.40	3.30	5.26
Nitrogen	6.61	12.88	20.94
$NDF^{\widetilde{H}}$	734	580	512

^{*}The mineral mix offered with all diets contained per kg, as stated by the producer: vitamin A, 6,000,000 IU; vitamin D₃, 625,000 IU; vitamin E, 11,000 mg; Fe, 5 g; Cu, 10 g; Mn, 10 g; Zn, 50 g; I, 0.5 g; Se, 0.2 g; Co, 0.1 g; fill material: CaCO₃; [†]Reflecting average intake across all cows getting different amounts of concentrate; [§]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; [#]Ash-free neutral detergent fibre.

straw had been harvested from the same, non-fertilised, field at an altitude of about 3,700 m a.s.l. at different stages of maturity (hay, milk grain stage; straw, over-mature stage with loss of most oat grains). Oat hay and straw were cut into pieces of 2–9 cm size shortly before the start of each sub-experiment, and alfalfa hay and maize stover (dry leaves and stems of the maize plants) were purchased readily chopped to particle sizes of 0.5–3 cm. All forages were thoroughly mixed with the other components in the proportions required for the experimental diets before feeding. Separate batches of alfalfa hay and maize stover were needed for each sub-experiment due to limited storage capacities, but differences in the forage FA profile between the batches were small (less than 0.7 g/kg DM for individual FA and FA groups). The experimental forages differed in total FA content (highest in OC, lowest in DS) and in contents of individual FA (Table 1). Diet DS was elevated in C18:1 c9 and, consequently in monounsaturated FA (MUFA), while diet RS contained more PUFA (especially C18:2 c9,c12) but still less than OC, which had highest concentrations of total FA, SFA and PUFA (including both C18:2 c9,c12 and C18:3 c9,c12,c15).

2.2. Experimental design, sites and cows

The experiment consisted of two identical sub-experiments carried out at two different sites (altitudes). In each sub-experiment a group of 12 cows, composed of six indigenous Criollo (CR) and six Brown Swiss cows (BS; characterised by high Brown Swiss blood levels), were fed three diets in a random sequence, with two CR and two BS cows being simultaneously on the same diet. The experimental model was a replicated 3×3 Latin square design (3 diets × 3 cows) for each cow type and at each site, respectively (total n = 72). The two sub-experiments were carried out in Peru at a site located in the lowlands (LL) at 200 m a.s.l. (Lima; Agricultural University La Molina) and at a second site in the highlands (HL) at 3,600 m a.s.l. (Pachacayo; facilities of the agricultural co-operative S.A.I.S. Tupac Amarú, situated in the district of Canchayllo, Junin). The sub-experiments were conducted sequentially, with the same staff, at similar housing conditions and with the same diet types. Differences between the two sub experiments, except altitude, were restricted to ambient temperature (ranging from 11–28°C and from -7 to 17°C at LL and HL, respectively) and experimental animals, which had to be exchanged after the first subexperiment in order to be able to perform the measurements at the same average stage of lactation. The experimental animals were reared and kept on natural (CR) or cultivated pastures (BS) near Pachacayo at altitudes of 3,500-4,000 m prior to the experiment. All animals had experienced hardly any supplementary feeding for their entire life. Animals were selected according to: (i) phenotypic characteristics for each cow type, (ii) number of days in milk at the start of the experiment (mean \pm standard deviation; CR, 69 \pm 31 and 54 ± 29 ; BS, 82 ± 17 and 61 ± 12 at LL and HL, respectively), (iii) age (CR, 4.7 ± 1.2 and 5.0 \pm 2.8 years; BS, 7.7 \pm 1.8 and 7.8 \pm 2.9 years at LL and HL, respectively), and (iv) number of lactations (CR, 2.2 \pm 1.3 and 2.5 \pm 2.1; BS, 3.2 \pm 0.8 and 4.5 \pm 2.4 at LL and HL, respectively). Data on individual milk yield and milk composition prior to the experiment were not available. Cows at the LL site suffered from transport and had to adapt to coastal temperatures which at first caused a reduction of milk yield, especially in CR. However, cows recovered quickly before the first data and sample collection started. This is confirmed by the observation that there was no significant period effect on either milk yield or milk fat content during the experiment. In the experiment, cows were housed individually in paddocks and separated from their calves (LL, 20 m² of sandy ground; HL, 12 m² of concrete floor littered with saw dust). At both sites, each paddock was equipped with a feeding trough, a water barrel and a roof covering the feeding area. The experiment started with a preparation period of 2 weeks for adaptation to housing, milking and feeding routine at each experimental site. In the LL sub-experiment, prior to road transport to the LL site, cows stayed for an additional week at S.A.I.S. Tupac Amarú in order to prepare them for transport. Thereafter the preparation period continued for two weeks at the LL site. During the preparation period, cows were treated against internal and external parasites (Biomec 120 L.A. Dorado®, containing Ivermectin, Laboratorios Biomont S.A., Lima, Peru; Fasigan Plus 20% + Minerales®, containing Triclabendazole and Albendazole, Labodec S.R.L., Lima, Peru) and were fed a mixture of maize stover and alfalfa hay (2:1 on DM basis). After the preparation period followed the first transition period in which the diet fed during the preparation period was gradually exchanged during 5-7 days by the respective first experimental diet. Cows entered this first transition period with a mean BW of 317 \pm 41 (mean \pm standard deviation) and 463 + 41 kg at LL and 331 + 55 and 437 + 50 kg at HL, for CR and BS cows, respectively. Thereafter, 17 days exclusively on the experimental diets followed, consisting of an adaptation period of 10 days and a collection period of 7 days, reserved for sampling of feed, feed refusals and milk. This time schedule was chosen following Chilliard et al. (2007), who reviewed the time-dependency of milk fatty acid response to different diets and lipid supplementation and showed that the highest response for hay-based diets can be expected about 14 days after the start of lipid supplementation. Each collection period was followed by a transition period with a gradual exchange of the diet until all cows had consumed the three experimental diets.

2.3. Sampling of feeds and milk

Feeds offered and refused were weighed daily in order to determine feed intake. During the collection weeks, samples of individual feeds and of mixed diets were taken after each preparation. Furthermore, proportionately 10% of the feed refusals were collected daily in the morning and pooled across the collection period for each cow. Cows were milked manually at 05:30 h and 17:00 h after stimulation of milk let-down by their calves. When milk flow started, the calves were immediately separated from the cow and tied near their mother. The milk yield was weighed after each milking and a 100 ml sample was taken and refrigerated for determination of fat content. Furthermore, a morning and an evening sample was taken on the first and the penultimate day of each collection period, pooled proportionately to milk amounts for each day and cow and frozen for further analysis of FA profile.

2.4. Chemical analysis of feeds and milk

Feeds and refusals were dried at 60°C and milled through a 1-mm screen. Dry matter and N contents of samples were analysed according to standard methods (Naumann and Bassler 2004) using a thermogravimetric determinator (TGA-500, Leco Corporation, St Joseph, MI, USA) for DM analysis and a C/N analyser (Leco Analysator CN-2000, Leco Corporation) for N quantification. Neutral detergent fibre (NDF) was determined on a Fibertec System M (Foss Tecator, Höganäs, Sweden) according to the method of Van Soest et al. (1991), using a heat stable amylase and expressed exclusive of residual ash. Lipids in diet components, diets and refusals were extracted, after addition of an antioxidative agent (100 mg/l of BHT), by accelerated solvent extraction (ASE 200; Dionex Corp., Sunnyvale, CA, USA) at 105°C using hexane/isopropanol (3:2 vol/vol) and transformed into FA methyl esters (FAME) using a NaOH solution for saponification and

a BF₃ methanol solution for esterification (Wettstein et al. 2001). The FAME dissolved in hexane were analysed by gas chromatography (GC; Agilent 6890N, Network GC System, Agilent Technologies, Santa Clara, CA, USA) using a fused silica capillary column (SupelcowaxTM-10, 30 m × 0.32 mm, 0.25 μm, Supelco Inc., Bellefonte, PA, USA). For analysis, detector temperature was maintained at 270°C and the injector at 60°C. The split ratio was 1:30. Samples were injected in 2 μl of hexane and hydrogen was the carrier gas used at a constant flow of 2.1 ml/min. The initial temperature was 160°C for 0.5 min, followed by an increase of 20°C/min until 190°C and thereafter of 10°C/min up to 230°C. This temperature was maintained for 8.5 min before increasing gradually (20°C/min) to the final temperature of 250°C, which was kept for further 4 min. Peaks were identified using GC ChemStation Data Software and pure methyl ester standards (Supelco 37 Component FAME mix, Supelco Inc.). Pure sunflower oil was used as a reference for the determination of response factors, and C11:0 was used as internal standard.

The fat content of the milk was determined using a portable ultrasonic milk analyser (Lactoscan 90 LCD, Milcotronic, Nova Zagora, Bulgaria). For later calculation, average daily fat contents of milk were calculated proportionately to morning and evening milk fat contents and yields. The milk analyser was calibrated using the results of 28 samples being also analysed by means of standard methods (ISO 1976).

After defrosting the milk samples for FA determination in a water bath at 38°C, these samples were transesterified and the FAME were extracted with n-heptane, both according to Suter et al. (1997). The FAME were thereafter quantified by GC (same GC system as used for feed samples), using a silica capillary column (Sil-88, 100 m \times 0.25 mm, 0.2 μ m; Varian Inc, Darmstadt, Germany). The detector temperature was maintained at 270°C and the injection occurred at 260°C. Hydrogen was used as carrier gas at a constant flow of 1.5 ml/min and 1 μ l of n-heptane containing the FAME of samples was injected into the column. The temperature programme (Collomb and Bühler 2000) started with 60°C for 5 min followed by an increase of 14°C/min up to 165°C and thereafter 2°C/min up to 225°C. After 15 min in an isothermal state, the temperature increased by 20°C/min up to 235°C and was maintained at this temperature for 5 min. The BCR 164 standard milk fat (Certified Reference Material; EC Reference Materials, Brussels, Belgium) was used as reference for the determination of response factors and the proportions of each FA were calculated based on C11:0, which was used as internal standard. The methodology by Collomb and Bühler (2000) was applied, with some modifications (Leiber et al. 2005), to identify the peaks, using GC ChemStation software (A 10.02 Revision, 1990–2003, Agilent Technologies). The FA which could not be identified with the method used, were included in the calculation of total FAME but are not separately shown in the tables (e.g., individual C18:1 FA). A further limitation of the methodology is the incomplete separation of t7,c9 from c9,t11 CLA, but according to Leiber et al. (2005), the concentration of the t7,c9 in milk is about 100-fold lower than that of c9,t11. The total daily excretion of FA was calculated as daily milk production [kg/cow and day] × content of FA in milk [g/kg]. The desaturation indices were calculated by the equation of Chilliard and Ferlay (2004) where desaturase index = (product of $\Delta 9$ -desaturase)/(product of $\Delta 9$ desaturase + substrate of $\Delta 9$ -desaturase). Products included in these calculations were C14:1 c9, C16:1 c9 and C18:1 c9 with C14:0, C16:0 and C18:0 as respective substrates.

2.5. Statistical analysis

Two models were applied for analysis of variance, both using Tukey's method for calculating the quartile values. Model 1 included diet type (D), cow type (C) and period of

the experiment (P) as fixed factors, the $D \times C$ interaction and animal (A) within cow type as random factor as follows:

$$Y_{ijklm} = \mu + D_i + C_j + P_k + D \times C_{ij} + A(C)_{l(i)} + \epsilon_{ijklm}$$

A second model (model 2) additionally included site (S) as fixed factor and all possible two-way interactions among D, C and S, and the three-way interaction D \times C \times S. Again, animal (A) within C and S was defined as subject for a random effect. The model reads as follows:

$$\begin{split} Y_{ijklmn} &= \mu + D_i + C_j + P_k + S_l + D \times C_{ij} + D \times S_{il} + C \times S_{jl} + D \times C \times S_{ijl} \\ &\quad + A(C \times S)_{m(il)} + \epsilon_{ijklmn} \end{split}$$

The interpretation of direct site (altitude) comparisons as performed with model 2 was limited due to the use of different cows at the two sites (altitudes). Therefore model 1 was used to calculate the least square means (LSMeans) for diets and cow types at each site and to perform multiple comparisons among these LSMeans separately for each site. Thus the effect of site (altitude) is excluded in the comparison of LSMeans. Model 2 provided the standard error (SE) and the significances of the fixed factors and interactions across both sites. The interactions including altitude (site) (D \times S, C \times S, D \times C \times S) remain widely unaffected by the uncertainty of the direct altitude effect. All statistical analyses were performed with the MIXED procedure of SAS (version 8.2; SAS, 1999–2001).

3. Results

3.1. Intake and milk yield

Daily intakes of total FA varied (p < 0.001) depending on diet quality and were lower (p < 0.05) for CR than BS (Table 2). The CR, compared to BS, consumed less FA with diets RS and OC, but similar amounts with diet DS (C × D; p < 0.05). Milk yield increased (p < 0.001) with the quality of the diet and was higher for BS than for CR (p < 0.001), showing the smallest differences between cow types with diet DS (1.9 kg) and the highest ones with diet OC (4.0 kg) (C × D; p < 0.05). The milk fat content was higher for CR than BS (p < 0.01) and higher at HL than at LL (p < 0.05) while there was no clear diet effect.

3.2. Fatty acid profile of the milk lipids

With diet DS, proportions of saturated short chain FA (SCFA; C4:0; C6:0, C8:0) and saturated medium chain FA (MCFA, C10:0; C12:0; C14:0; C16:0) showed concentrations lower (p < 0.05; diet comparisons across sites not shown in tables) than with the other diets (Table 3). This was partially compensated for by C18:0. The *cis*-C18:1 proportion of total FAME was highest (p < 0.05) with diet DS. Feeding diet OC enhanced the proportions of *trans*-C18:1, C18:2 c9,c12, C18:2 c9,c11 and CLA, resulting also in elevated (p < 0.05) proportions of PUFA, total n-3 and total n-6. The milk fat of the CR cows had higher proportions (p < 0.05) of C18:0 and lower proportions of C18:2 c9,c12, C18:2 c9,c11 and total CLA than that of BS, resulting in lower PUFA proportions. All other FA were similar in proportion for the two cow types. With diet OC, differences between CR and BS were smaller for the proportion of C18:0 and higher for total *trans*-C18:1 (D × C

Table 2. Intake of dry matter (DM), total fatty acids (FA) as well as yield and fat content of milk of Brown Swiss and Criollo cows with the experimental diets and at different sites (altitudes).

	Diet	DM intake [kg/d]	Total FA intake [g/d]	Milk yield [kg/d]	Milk fat content [†] [g/kg milk]
Highland					
Criollo	Dry season	5.03^{d}	48.6 ^d	2.59 ^d	53.3
Brown Swiss	Dry season	6.13 ^d	59.2 ^d	4.33 ^{bd}	48.0
Criollo	Rainy season	8.96°	78.5 ^{cd}	3.85^{c}	56.5
Brown Swiss	Rainy season	13.15 ^b	114.1 ^b	6.88 ^a	50.6
Criollo	Optimised diet	9.51°	110.3 ^{bc}	4.07^{c}	55.8
Brown Swiss	Optimised diet	15.18 ^a	198.5 ^a	8.13 ^a	49.2
Lowland					
Criollo	Dry season	4.57 ^v	47.7^{w}	1.88^{w}	47.3
Brown Swiss	Dry season	6.47^{vw}	66.5 ^{wx}	3.86 ^x	48.4
Criollo	Rainy season	8.87^{wx}	80.0^{x}	3.52^{x}	53.1
Brown Swiss	Rainy season	13.02 ^y	116.6 ^y	6.59 ^y	46.2
Criollo	Optimised diet	10.26^{x}	124.2 ^y	4.21 ^x	50.1
Brown Swiss	Optimised diet	15.38 ^z	207.7^{z}	8.23^{z}	44.4
SE^\S		0.552	6.41	0.458	2.14
<i>p</i> -values [§]					
Diet (D)		< 0.001	< 0.001	< 0.001	0.14
Cow type (C)		< 0.001	< 0.001	< 0.001	0.008
Site (S)		0.80	0.24	0.54	0.024
$D \times C$		< 0.001	< 0.001	< 0.001	0.15
$D \times S$		0.47	0.42	0.14	0.58
$C \times S$		0.91	0.86	0.92	0.54
$D \times C \times S$		0.52	0.73	0.93	0.28

[†]Taken from Bartl et al. (2008); [§]Calculated by model 2 (cf. *Materials and methods*); ^{a-d}LSMeans and ^{v-z}LSMeans from the highland and lowland site, respectively, within one column without common superscripts are significantly different at p < 0.05 (model 1; cf. *Materials and methods*).

interactions, p < 0.05) than with the other diets. Site (altitude) effects (p < 0.05) were mainly found for unsaturated FA (lower proportions at HL of C18:2 c9,c12, CLA, C18:3 c9,c12,c15 and various > C18 FA). Responses to diet type varied between sites (D × S; p < 0.05) in several individual PUFA as well as in *trans*-C18:1, CLA and total n-3. Although differences were small, proportions of *cis*-C18:1 and MUFA were highest for BS at LL and for CR at HL (C × S; p < 0.05), while the opposite was true for SFA.

3.3. Daily excretion of fatty acids with the milk

With diet OC, the daily excretion of total FA and all individual FA and groups, shown in Table 4, was highest (p < 0.05), but responses to RS and DS varied. Total daily excretion of all FA studied was smaller for CR than BS (p < 0.01). This was due to the effect of milk yield differences between the cow types overriding those of milk fat content. Differences between CR and BS in daily excretion of FA were smallest with diet RS, where milk fat yield of CR was lower by a factor of 0.42 than that of BS. Daily excretion of FA was not affected by site (altitude). Excretion of C18:2 c9,c12, C18:3 c9,c12,c15, CLA, PUFA was highest for diet OC at LL and comparable at the two sites with the other diets

Effect of cow type, diet type and site (altitude) on the proportions of selected fatty acids and fatty acid groups* in milk fat [% of total fatty Table 3.

acids].		JF-',					1					6				
	Diet	C16:0	C18:0	cis- C18:1	trans- C18:1	C18:2 <i>c</i> 9, <i>c</i> 12	C18:2 <i>c</i> 9, <i>t</i> 11	C18:3 c9,c12,c15	Total CLA	Total n-3	Total n-6	SCFA	MCFA	SFA	MUFA	PUFA
Highland Criollos Brown Swiss	Dry season Dry season	27.6 29.1	11.41 ^a 9.24 ^a	26.3 24.5	1.44	0.97	0.37	0.27	0.46	0.61	1.25	6.74	41.5	66.1 67.2	31.1 29.5	2.76
Criollos Brown Swiss	Rainy season Rainy season	39.3 40.4	5.44 ^b 4.91 ^b	14.9	1.06	0.71	0.26	0.41	0.35	0.71	0.93	7.43	58.1 59.4	77.6 78.2	20.0	2.36
Criollos Brown Swiss	Optimised diet Optimised diet	35.4 35.5	6.27 ^b 6.92 ^b	16.8 15.4	1.38	1.08	0.41	0.59	$0.51 \\ 0.56$	0.89	1.33	7.17	53.7 54.5	74.6 75.8	22.1 20.7	3.27
Lowland Criollos Brown Swiss	Dry season Dry season	27.9 26.4	11.56	24.4 29.2	1.09 ^x 1.08 ^x	1.04	0.34	0.27^{x} 0.42^{xy}	0.49	0.71^{y} 0.86^{y}	1.39 ^{wx} 1.66 ^{xy}	6.88	42.3	67.9 ^w 62.3 ^v	29.0^{y} 34.0^{z}	3.05 ^{xy} 3.67 ^y
Criollos Brown Swiss	Rainy season Rainy season	37.0 38.4	6.88	14.6 14.4	1.03^{y} 1.01^{x}	0.90	0.29	0.46^{y} 0.43^{xy}	0.40	0.893	1.20° 1.11°w	7.77	56.8 58.3	77.9 ^z 77.5 ^{yz}	18.9 ^x 19.5 ^x	3.14^{xy} 3.02^{x}
Criollos Brown Swiss	Optimised diet Optimised diet	32.5 32.9	7.83 6.25	16.9 16.6	1.89² 2.17 ^x	1.43	0.55	0.89^{z} 0.79^{z}	0.65	1.36^{2} 1.26^{2}	1.74^{yz} 1.85^{z}	7.63 7.46	51.4 52.7	73.6 ^{xy} 72.9 ^{wxz}	22.0 ^x 22.4 ^x	4.43^{2} 4.70^{2}
\mathbf{SE}^\S		1.13	0.474	66.0	0.143	0.088	0.047	0.053	0.050	0.070	0.100	0.216	1.36	1.22	1.10	0.238
p -values § Diet (D) Cow type (C) Site (S) D \times C D \times S C \times S D \times C		 0.001 0.52 0.023 0.64 0.45 0.58 0.40 	 < 0.001 0.003 0.19 0.008 0.31 0.15 0.15 	 < 0.001 0.947 0.231 0.164 0.693 0.027 0.082 	 < 0.001 0.16 0.75 0.034 < 0.001 0.49 0.78 	 <0.001 0.015 0.013 0.12 0.039 0.48 0.31 	 < 0.001 0.045 0.065 0.35 0.003 0.58 0.74 	<0.001 0.59 0.013 0.015 0.002 0.69 0.60	 < 0.001 0.034 0.025 0.36 0.024 0.67 0.61 	 <0.001 0.47 <0.001 0.090 0.003 0.61 0.92 	 < 0.001 0.042 0.003 0.27 0.084 0.49 0.42 	 <0.001 0.51 0.98 0.59 0.57 0.072 0.74 	 <0.001 0.26 0.057 0.90 0.82 0.34 0.15 	 < 0.001 0.44 0.11 0.23 0.51 0.045 0.21 	 < 0.001 0.70 0.37 0.27 0.65 0.019 0.22 	<0.001 0.021 <0.001 0.55 0.019 0.52 0.27

*CLA, conjugated linoleic acids; SCFA, short-chain saturated fatty acids; MCFA, medium-chain saturated fatty acids; MUFA, monounsaturated fatty acids; 8 Calculated by model 2 (cf. Materials and methods); $^{a-1}$ LSMeans and $^{x-2}$ LSMeans from the highland and lowland site, respectively, within one column without common superscripts are significantly different at p < 0.05 (model 1; cf. Materials and methods).

Table 4. Effect of cow type, diet type and site (altitude) on the daily excretion of selected fatty acids and groups of fatty acids* [g/d] with the milk.

	Diet	C18:0	C18:2 c9,c12	C18:3 c9,c12,c15	Total CLA	SCFA	MCFA	SFA	MUFA	PUFA
Highland Criollos Brown Swiss	Dry season Dry season	13.3 ^{ab} 17.2 ^b	0.42	0.34	0.55	7.6° 13.3 ^{bc}	46.4° 86.7°c	75° 128 ^{bc}	36.0 56.8	3.23 5.84
Criollos Brown Swiss	Rainy season Rainy season	10.6^{b} 16.3^{b}	0.37	0.77	0.67	14.6^{b} 25.3^{a}	$113.4^{\rm b}$ $193.2^{\rm a}$	151 ^b 255 ^a	38.1 59.9	4.49 10.36
Criollos Brown Swiss	Optimised diet Optimised diet	12.6 ^b 23.9 ^a	$0.51 \\ 0.52$	1.10	0.98	14.1 ^b 27.8 ^a	$105.3^{\rm b}$ $197.3^{\rm a}$	146 ^b 272 ^a	42.6 73.5	6.12 12.01
Lowland Criollos Brown Swiss	Dry season Dry season	8.5 ^x 15.8 ^y	$\frac{0.41^{2}}{0.61^{y}}$	$\frac{0.21^{\rm w}}{0.72^{\rm wx}}$	0.38^{\times} 1.01^{\times}	5.1 ^x 11.1 ^{xy}	31.7^{x} 67.2^{xy}	51 ^x 105 ^{xy}	22.1 ^w 57.5 ^y	2.33^{x} 6.20^{xy}
Criollos Brown Swiss	Rainy season Rainy season	11.4^{xy} 14.6^{xy}	0.42^{y} 0.34^{y}	0.76^{wx} 1.19^{xy}	0.65^{xy} 1.27 ^y	12.6^{y} 21.2^{z}	92.9^{y} 161.9^{z}	128^{y} 215^{z}	31.2 ^{wx} 53.4 ^y	5.18^{xy} 8.49^{y}
Criollos Brown Swiss	Optimised diet Optimised diet	13.3^{y} 21.3 ^z	0.58^{z} 0.63^{z}	1.57^{y} 2.72^{z}	$\frac{1.14^{y}}{2.69^{z}}$	13.0^{y} 25.5^{z}	86.9^{y} 181.7^{z}	125^{y} 250^{z}	38.1 ^{xy} 76.5 ^z	7.70^{y} 16.05^{z}
\mathbf{SE}^\S		2.00	0.044	0.159	0.159	1.92	14.02	18.8	5.55	1.069
$p ext{-values}^{\S}$ Diet (D) Cow type (C) Site (S) D × C D × S C × S D × C		 0.001 0.002 0.42 0.004 0.17 0.83 0.075 	 0.001 0.001 0.76 0.011 0.022 0.93 0.088 	<0.001 <0.001 0.16 0.005 0.002 0.61 0.75	<0.001 <0.001 0.31 0.001 0.019 0.27 0.39	<0.001 <0.001 0.16 0.001 0.76 0.77	 < 0.001 < 0.001 < 0.11 < 0.001 < 0.001 < 0.73 < 0.86 < 0.87 	 < 0.001 < 0.001 < 0.001 	 < 0.001 < 0.001 < 0.32 <	 < 0.001 < 0.001 0.38 0.021 0.015 0.76 0.11

*CLA, conjugated linoleic acids; SCFA, short-chain saturated fatty acids; MCFA, medium-chain saturated fatty acids; MCFA, monounsaturated fatty acids; Moleculated by model 2 (cf. Materials and methods); $^{a-c}$ LSMeans and $^{w-z}$ LSMeans from the highland and lowland site, respectively, within one column without common superscripts are significantly different at p < 0.05 (model 1; cf. Materials and methods).

(D \times S; p < 0.05) except for C18:3 c9,c12,c15 with diet DS, showing a lower excretion at LL than HL.

3.4. Desaturase indices and recovery rates

Diet type affected indices for 14 and 16 FA whereas cow type had no effect on desaturase indices (Table 5). At LL, index 18 was lower with all three diets for CR than for BS whereas at HL this index was higher for CR compared to BS with diet OC, equal with diet RS and lower with diet DS (D × C, D × S; p < 0.05). The indices 14 and 16 were highest (p < 0.05) with diets RS and DS, respectively. In index 14, a D × C interaction occurred (p < 0.05), but this included only BS where index 14 was lower by 18% with diet OC than with diet RS. Cow type differences occurred at the LL site, where index 14 was higher by 0.02 units for BS than CR (C × S, p < 0.05). The highest (p < 0.05) apparent recoveries [g excretion with milk/g intake] of ingested total C18:0, C18:2 c9,c12 and C18:3 c9,c12,c15 were found with diet DS. The recovery rate of total C18:0 was lower (p < 0.001) by 0.24 for BS than for CR. Cow type differences in recovery of C18:3 c9,c12,c15 were small with 0.01 g/g. Apparently no other experimental factors influenced FA recovery rates.

4. Discussion

The purpose of the present study was to determine the responses in milk FA profile of two cow types to three diet types at an altitude difference of 3,400 m. The diets used represented the seasonal variation in feed quality typical for the central Peruvian highlands (diets DS and RS) and also allowed the comparison with a diet not limited in nutrients and energy (diet OC). Apart from the extreme altitude difference, the design of the present study also differs from previous approaches by the application of an experimental design which allowed the separation of the influence of various factors characteristic for different altitudes and by the use of a common, but scarcely studied, cow type (Peruvian Criollo). Due to methodological reasons there were certain limitations to the altitude comparison itself allowing only careful conclusions being drawn for this factor. Although previous studies on milk FA profile were often performed with higher yielding cows, the comparison of these findings with the results of the present study remains unbiased, as only a little effect of selection for milk yield on proportions of individual FA in milk is known (Kay et al. 2005; Bobe et al. 2007).

4.1. Effect of diet type

Changes in milk FA composition due to diet composition and quality have been well documented in dairy animals (reviewed by Bauman and Griinari 2003; Chilliard and Ferlay 2004). Low proportions in total FAME of SCFA and MCFA with diet DS compared to the other diets indicate a lower de-novo synthesis of FA, as FA < C16:0 in milk fat almost exclusively originate from de-novo synthesis in the mammary gland (Bauman and Griinari 2003). Deficient supply of ruminally fermentable energy, and consequently of acetate and butyrate as precursors for SCFA and MCFA in milk (Bugaud et al. 2001), might be responsible for this observation. Previous calculations showed that when opposing intake with diet DS to assumed requirements for net energy lactation (NEL) there was a clear deficiency of NEL for both cow types (Bartl et al. 2008). The simultaneously higher MUFA proportions found with diet DS were not due to an

Effect of cow type, diet type and site (altitude) on desaturase indices and recovery rates of selected fatty acids. Table 5.

		,		•			
		I	Desaturase indices*	*	Recovery	Recovery rates [g excretion with milk/g intake]	h milk/g intake]
	Diet	Index 14	Index 16	Index 18	Total C18	C18:2 c9,c12	C18:3 c9,c12,c15
Highland [§] Criollo	Dry season	0.094	0.056	0.69	1.49	0.094	0.349
Brown Swiss	Dry season	0.088	0.061	0.72	1.09	0.098	0.399
Criollo	Rainy season	0.104	0.048	0.72	0.54	0.049	0.028
Brown Swiss	Rainy season	0.094	0.048	0.72	0.35	0.042	0.018
Criollo	Optimised diet	0.099	0.052	0.72	0.43	0.039	0.038
Brown Swiss	Optimised diet	0.070	0.048	89.0	0.25	0.025	0.046
Lowland [§]							
Criollo	Dry season	0.073	0.064	29.0	1.33	0.101	0.408
Brown Swiss	Dry season	0.084	0.078	0.75	1.04	0.098	0.485
Criollo	Rainy season	0.075	0.044	29.0	0.58	990.0	0.036
Brown Swiss	Rainy season	0.091	0.054	0.72	0.37	0.041	0.023
Criollo	Optimised diet	0.070	0.051	0.67	0.40	0.048	0.041
Brown Swiss	Optimised diet	0.082	0.055	0.71	0.22	0.028	0.025
$SE^{\#}$		0.0071	0.0042	0.002	0.083	0.0084	0.0577
p-values#							
Diet (D)		0.001	< 0.001	0.19	< 0.001	< 0.001	< 0.001
Cow type (C)		0.00	980.0	0.024	<0.001	0.032	89.0
Site (S)		0.076	0.063	0.45	0.52	0.22	0.49
$D \times C$		0.041	0.23	0.017	0.31	0.28	0.58
$D \times S$		0.37	0.075	0.14	0.49	0.91	0.59
C × S		0.042	0.12	0.012	0.73	0.31	0.97
$D \times C \times S$		0.07	86.0	0.59	0.83	0.81	0.97

*Index $14 = C14:1 \cdot 9/(C14:0 + C14:1 \cdot 29)$; index $16 = C16:1 \cdot 29/(C16:0 + C16:1 \cdot 29)$; index $18 = C18:1 \cdot 29/(C18:0 + C18:1 \cdot 29)$; #Calculated by model 2 (cf. Materials and methods); *LSMeans from the highland and lowland sites have been calculated by model 1 (cf. Materials and methods).

increased intake of C18 FA (2.58, 3.96 and 6.32 g/d for diets DS, RS and OC, respectively) but seemed to have been caused by an increased availability of C18 FA due to body lipid mobilisation (Chilliard and Ferlay 2004). Indicators for mobilisation of body lipids were body weight loss, lower plasma levels of insulin, triiodothyroine and glucose and higher non-esterified FA (NEFA) levels with diet DS, compared to diets RS and OC (described in Bartl et al. 2008). Although diet DS was characterised by the lowest PUFA intake the proportions of PUFA in FAME were even slightly higher than with diet RS. This is consistent with the recovery rates of total C18, C18:2 c9,c12 and C18:3 c9,c12,c15 which were highest with diet DS and suggest an increased by-pass of PUFA (Bugaud et al. 2001), caused by a ruminal energy deficiency reducing biohydrogenation (Leiber et al. 2005). Since the diet effect was highly significant in FA recovery, it seems unlikely that major carry-over effects from the previous diets occurred. Overall the findings suggest that processes taking place in the animal in response to energy deficiency were overriding effects of the FA profile of the low-quality diet DS.

Feeding diet OC, compared to DS and RS, elevated C18:2 c9,c12, CLA and C18:3 c9,c12,c15 proportions in FAME. This was likely due to increased intakes of C18:1 c9, C18:2 c9,c12 and C18:3 c9,c12,c15 which thereafter have been either utilised directly by the mammary gland after ruminal bypass or had been subject to ruminal biohydrogenation (Collomb et al. 2006).

4.2. Effect of cow type

Differences in BW and rumen capacity led to cow-type specific intakes of DM and FA in the present study. The ratios of total daily milk fat yield [g/d] to daily FA intake were similar for both cow types (1:2.2 and 1:2.5 for CR and BS, respectively), indicating a similarly efficient utilisation of ingested FA for milk fat synthesis. There are no reports known to the authors comparing the milk FA profile of Peruvian CR and BS cows. However, various authors reported a certain effect of breed on milk fat composition but findings were often contradictory, some supporting and some disproving a breed effect on a distinct FA (e.g. concerning C18:2 c9,c12 and C18:2 c9,t11; Kelsey et al. 2003; Carroll et al. 2006; Ferlay et al. 2006). For proportions of C18:0, being higher for CR than BS in the present study, the reports quoted did not describe significant differences between different breeds. Total CLA proportion was shown to be breed-specific in studies comparing Holstein with Jersey (White et al. 2001) and with BS (Kelsey et al. 2003). In the present study CLA proportions were higher for BS than for CR. The majority of the CLA in milk fat is endogenously synthesised with the help of $\Delta 9$ desaturase from C18:1 t11 (Griinari et al. 2000). Kay et al. (2005) showed that selection for milk yield in general did not affect proportions of most FA but they also showed that dairy cows from lines selected for milk yield, versus unselected cows, express a reduced $\Delta 9$ -desaturase activity. In the present study only small differences between cow types were found in desaturase index 18 (p < 0.05) and no difference for indices 16 and 14. According to Soyeurt et al. (2006), index 14 is the best indicator for $\Delta 9$ -desaturase activity, because C14:1 c9 is exclusively generated through desaturation. This suggests that cow type differences in $\Delta 9$ -desaturase activity were not responsible for the higher CLA proportion in the BS milk compared to the CR milk. The clearly higher total daily excretion of CLA for BS compared to CR probably resulted from the correspondingly higher intake of important precursors for CLA synthesis, namely C18:2 c9,c12 (111 vs. 184 g/day for CR and BS, respectively) and C18:3 c9,c12,c15 (67 vs. 100 g/d for CR and BS). This is consistent with the positive correlation between CLA excretion in milk and the daily intake of the precursors mentioned before (r = 0.95 and 0.88 for CR and BS, respectively).

4.3. Effect of site (altitude)

Milk and milk products of alpine origin have been found to distinctly differ in FA composition, especially concerning C18:3 c9,c12,c15 and CLA, from milk produced in the lowlands (e.g., Kraft et al. 2003; Leiber et al. 2005; Collomb et al. 2006; Engel et al. 2007). The specific botanic composition of mountainous pastures was identified as one main reason for higher CLA concentrations in alpine milk in these studies (e.g., Leiber et al. 2005). Additionally, Leiber et al. (2005) observed an increase of C18:3 c9,c12,c15 concentration in milk fat with increasing altitude, although consumption of C18:3 c9,c12,c15 decreased. The present results indicated lower CLA, C18:3 c9,c12,c15, PUFA, n-3 and n-6 and higher C16:0 concentrations in milk fat for the cows kept at 3,600 m than at 200 m a.s.l. This cannot be explained by intake differences, as intake of FA was only minimally lower in the highlands and even higher for C18:2 c9,c12 by 0.9 g/d at HL than at LL. The explanations of Leiber et al. (2005) for an increase in C18:3 c9,c12,c15 with altitude are based on a metabolic energy deficit leading to preferential mobilisation of C18:3 c9,c12,c15 from body fat or a restricted ruminal biohydrogenation, due to a ruminal energy deficiency. In the present experiment there were no indications from metabolic traits (measured plasma non-esterified FA: 0.31 ± 0.14 vs. 0.25 ± 0.10 at HL and LL, respectively; p = 0.25 for site) for a situation being energetically more restricted at one of the two sites. A certain influence of the adaptation level of animals to high altitude on energy metabolism, as also found for yellow cattle at pastures between 3,250 and 4,270 m a.s.l. (Han et al. 2003), might explain this lack of differences between the sites. Whereas in alpine systems cows are kept at high altitude sites only temporarily, cows in Peru typically stay in the highlands for their lifetime, as was true also for the experimental cows, and therefore are adapted to high altitude. The different proportions of FA at the two sites were most probably associated with the slightly elevated milk fat contents at the high altitude site, a phenomenon also reported from various other studies (e.g., Leiber et al. 2006). In most studies, this was accompanied by a reduction of milk yield due to a limited feed intake (e.g., Bianca and Puhan 1974; Leiber et al. 2006). Nevertheless, milk yield and DM intake were similar at the two sites in our experiment which indicates the presence of other than intake-induced effects of low oxygen partial pressure on milk constituents.

4.4. Interactions between diet type and other factors of influence

4.4.1. Interaction of diet type and cow type

Due to different long-term selection goals (CR, adaptation to low-quality diets; BS, high milk yield), the responses to diets might have been different between cow types. The BS fed diet DS seemed to have expressed a lower biohydrogenating activity than CR as milk fat had lower proportions of the terminal product C18:0 and higher proportions of C18:3 c9,c12,c15 (+52%). Nevertheless, higher C18:3 c9,c12,c15 intakes of BS, compared to CR, with diet DS (+22%) might have partly contributed to the effect on this FA. Alternatively, the low DM intake with diet DS might not have provided BS with sufficient precursors for de-novo synthesis of FA hence supporting MUFA and PUFA to be absorbed from the gut without being modified (Bauman and Griinari 2003). Differences between CR and BS declined with increasing diet quality as indicated by the proportions of C18:0 and C18:3

c9,c12,c15 in milk fat with diets RS and OC. With diet OC, the biohydrogenation intermediate *trans*-C18:1 accumulated in BS milk to a higher extent compared to CR milk. This could have been due to a decreased $\Delta 9$ -desaturase activity, which was higher for BS than CR with diets DS and RS but equivalent (index 18) or even slightly lower (index 14) with diet OC. This is consistent with findings by Kay et al. (2005) for diets unrestricted in energy and nutrient supply.

4.4.2. Interaction of diet type and site (altitude)

Proportions of CLA, C18:3 c9,c12,c15 and n-3, and thus also of total PUFA, were proportionately higher in milk fat at LL than at HL with all three diets, but the differences between the two sites were smallest with DS and highest with OC. This could be partly due to the higher intakes of most FA with diet OC at the LL compared to the HL site, but this 5% difference in DM intake cannot exclusively explain differences in total PUFA which were in a magnitude of 37% with diet OC (14 and 12% with RS and DS, respectively). Most likely these D \times S interactions were triggered by a combination of factors. Accordingly, the higher concentration of PUFA at LL than at HL might have resulted from a higher intake of C18:3 c9,c12,c15 at LL than HL with diet OC (+34%) and a relatively higher $de\ novo$ FA synthesis with diet DS at LL compared to HL, increasing also the proportions of long-chain PUFA relatively more at the LL than at the HL site with this diet.

5. Conclusions

Under the conditions investigated, once more an altitude effect on the milk fatty acid profile appears to have taken place. Strong interactions between the altitude and other factors such as diet type, fatty acid intake, energy status, origin and type of the cows occurred but highland milk did not have the expected more desired fatty acid profile than lowland milk. Accordingly, a direct comparison between the Alpine and the Andean milk production systems is limited, most probably due to the fact that in Europe the alpine sojourn of the cows is restricted to a few summer months, whereas in Peru cows, which are kept permanently at high altitudes, are adapted to the mountainous environment. The study shows that the most reliable way to increase the proportion of favourable FA in milk is definitely an improvement of diet quality.

Acknowledgements

We gratefully acknowledge the support by the animal husbandry staff of The University at UNA La Molina, the laboratory LENA and the S.A.I.S. Tupac Amarú and the laboratory work at ETH of Muna Mergani, Seher Ayra and Carmen Kunz. The assistance of Cristina Carrión, Raúl Cabrera and of the farmers Paulino Atanacio, Timoteo Taza and Soledad Ricarpa, by providing their cows, is very much appreciated. This study was supported by the North-South Centre of ETH Zurich (formerly 'ZIL') and the Swiss Agency for Development and Cooperation (SDC).

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