



Faculty of Bioscience Engineering

Academic year 2010 – 2011

**Determination of milk odd and branched  
fatty acids in tropical countries  
Case study: Cuba**

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Master's dissertation submitted in partial fulfillment of the requirements  
for the degree of Master of Nutrition and Rural Development,  
main subject: Tropical agriculture, major Animal Production

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**Ghent University, June, 2011**

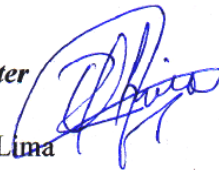


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## ABSTRACT

In the tropics, most ruminants are fed with low quality roughages, agricultural crop-residues and industrial by – products which basically contain high levels of lignocellulosic materials, low levels of fermentable carbohydrates and low levels of good-quality protein. Hence, optimal rumen function is essential under tropical conditions. In this context, odd and branched-chain fatty acid (OBCFA) in ruminant products could be interesting because of their potential as a diagnostic tool of rumen function. However, milk OBCFA as biomarkers have been studied in detail for dairy cattle of temperate regions and the information related to these specific fatty acids in tropical conditions is hardly available.

The main objective of this master’s dissertation is to determine the fatty acid pattern in milk of cows from practical farms in Central Cuba under different feeding regimes, where dairy systems are based mainly on grazing without concentrate supplementation. Emphasis is put on milk OBCFA as they were proposed as biomarkers for rumen function. These milk fatty acids will be compared with the milk OBCFA from cows of temperate regions. Finally, a further aim is to assess whether levels in milk of OBCFA can be linked with milk production levels and mineral supplementation.

As an attempt to answer the above problem, a research study was performed in “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010). Milk and feed samples from 6 dairy farms were taken over a period of 5 weeks and kept in sterilized glass bottles. Samples were stored at Universidad Central “Marta Abreu” de Las Villas in Santa Clara until analysis. Determination of chemical composition and milk fatty acids profile was carried at Lanupro – Ghent University. Analyses of fat, protein and lactose content were obtained in collaboration with the Walloon Agricultural Research Centre, Quality Department - Gembloux.

The results of the study showed that feed resources in “Desembarco del Granma” Cooperative is based on *Dichantium spp.* and *Pennisetum purpureum var. CT-115*. OBCFA proportions showed higher levels in Cuban milk from native Cuban cows or crossbreeds as compared to milk from temperate region and Holstein Friesian cows. Increased proportions of iso-fatty acids were observed in relation to the sum of microbial OBCFA, especially for iso C14:0 and iso C15:0, which is an indicative of high levels of lignocellulosic materials in central Cuban cows’ diet. Nevertheless, as all milk OBCFA (except anteiso C17:0), were influenced positively, we suggested that the origin of this increase could be either an indication of general increase in microbial activity or an indication of limited amounts of dietary fat. However, variation within OBCFA concentration was shown to evolve quite independently from external fatty acids or fatty acids which are de novo synthesized in the mammary gland. Further, a positive relation between crude protein and iso C17:0 was observed. In addition, OBCFA were not influenced by different levels of mineral supplementation but were linked with differences between farms. Finally, more research is still necessary to determine OBCFA concentrations in milk of dairy cows: monitoring should be performed during the whole year and in different tropical environments as well as under more controlled experimental conditions e.g. using fistulated dairy cows.

## **ACKNOWLEDGEMENTS**

The preparation of the present master's dissertation would not have been possible without the contribution and support of many people. There is no better place for me to thank them all than at the start of this final document.

In the first place I would like to thank my promotor Prof. dr. Veerle Fievez. She has contributed enormously to the genesis of this master's dissertation both in terms of intellectual input and of support. Not a word of mine has appeared in print or in this document which she has not relentlessly read and reflected and commented on. This master's dissertation would simply not have reached its current level without you Prof. Fievez, thank you very much.

I am especially grateful to Ir. Anne-Marie Remaut-De Winter, Coordinator, MSc. Nutrition and Rural development for giving me the opportunity to study this master and for her continuous help and encouragement during the period of study.

I like to acknowledge for the cooperation and support to conduct this research to Dr. Raciél Lima and M.Sc. Ernesto Noval, Centro de Investigaciones Agropecuarias, Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas (CIAP, Santa Clara, Cuba). I also wish to thank the Belgian Technical Cooperation Agency for its financial support and for provide me with a constant guidance during my stay in Belgium and the VLIR-UOS (Master Credit Allowances – 2010) for their cooperation by sponsoring the present study research.

I wish to express my cordial thanks for their cooperation in academic and non-academic stuff to the laboratory personnel of Lanupro, Gent University and to the researchers of the Walloon Agricultural Research Centre, Quality Department Analyses in Gembloux. I also wish to extend my cordial gratitude to the teaching staff of Gent University, especially to Prof. dr. Luc D'Haese, Director, MSc. Nutrition and Rural development.

I also would like to thank all my friends who have always been there for me and made me feel like home during this time. Finally, I dedicate this work to my family for their love and support. I especially want to thank my mother for her beloved concern and my father for never stopping to believe in my possibilities and for showing me what is important in life.

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## LIST OF ABBREVIATION

ADF	=	Acid detergent fiber
AOAC	=	Association of Official Analytical Chemists
CLA	=	Conjugated linoleic acids
CP	=	Crude protein
CT-115	=	<i>Pennisetum purpureum var. CT-115</i>
DAPA	=	Diaminopimelic acid
DM	=	Dry matter
FA	=	Fatty acids
GC	=	Gas chromatograph
GDP	=	Gross domestic product
IVD	=	In vitro digestibility
NDF	=	Neutral detergent fiber
OBCFA	=	Odd and branched-chain fatty acids
OM	=	Organic matter
PB	=	Purine bases
PC	=	Principal component
PCA	=	Principal component analysis
VFA	=	Volatile fatty acids
WSC	=	Water soluble carbohydrates

# CHAPTER 1

## Introduction

The Caribbean is characterized by a limited rural zone, familiar and mixed small farm units (Alexandre *et al.*, 1997) and grazing is the main feeding resource for cattle. Cattle farmers need appropriate rules of management valid in grazing situations and taking into account the whole system of production. They need, therefore, both agronomic advice for sward and nutritional recommendations for the animals, thus ensuring a continuous supply of feed (Boval *et al.*, 2002).

In the case of Cuba, The Cuban Revolution of 1959, favored production systems using a high proportion of foreign inputs and top-down knowledge transfer, until the collapse of the Eastern European socialist block in 1989, which induced a shift towards an agricultural production which relies on only few external inputs (Leitgeb and Vogl, 2010). As elsewhere in the tropics, this resulted in feeding most ruminants with low quality roughages, agricultural crop-residues, and industrial by – products which basically contain high levels of lignocellulosic materials, low levels of fermentable carbohydrates and low levels of good-quality protein (Wanapat, 2000).

Hence, optimal rumen function is essential, but often might be impaired under tropical conditions. In this context, odd and branched-chain fatty acids (OBCFA) in ruminant products could be interesting because of their potential as a diagnostic tool of rumen function (Vlaeminck *et al.*, 2006a). However, milk OBCFA as biomarkers have been studied in detail for dairy cattle of temperate regions and the information related to these specific fatty acids in milk produced in tropical conditions is hardly available. Furthermore, most of the temperate studies dealt with ruminants raised on good quality roughages and with high levels of concentrate supplementation.

For these reasons, the aim of the present study is to determine the fatty acid pattern in milk of cows from practical farms in Central Cuba under different feeding regimes (e.g. cows

feeding *Pennisetum purpureum* var. Cuba CT-115 vs. cows feeding natural grasses), where dairy systems are based mainly on grazing without concentrate supplementation. Emphasis is put on milk odd branched chain fatty acids as they were proposed as biomarkers for rumen function. Moreover, these milk fatty acids will be compared with the milk OBCFA from cows of temperate regions. Finally, a further aim of the survey is to assess whether levels in milk of OBCFA can be linked with milk production levels and mineral supplementation (mainly Zn, Cu and Mn).

## CHAPTER 2

### Literature review

#### 2.1. Cuban agricultural context

##### 2.1.1. History

Before 1959, Cuba practiced industrial farming to meet its domestic food and export needs (Hiranandani, 2010). Even more, following the overthrow of the Batista government in 1958, Cuba adopted the Soviet model with state ownership of land and large collective farms producing sugar for export on preferential terms in exchange for subsidized machinery, fuel, chemical inputs and knowledge (Carter, 2008). However, the collapse of the socialist bloc in 1989 marked the beginning of a new era in Cuban history. Without the support of the international socialist economy, Cuba suddenly plunged into a severe economic slump. The socialist bloc had accounted for 85% of Cuba's trade, and with its collapse, Cuban imports dropped by 75% and the deficit reached 33% of GDP. Cuban agriculture, which was highly dependent on chemical inputs from the Soviet Union, suddenly confronted a 50% reduction in fertilizer and pesticide imports. Food imports, which previously accounted for up to 57% of the caloric intake of the Cuban population, also dropped off due to the shrinking import quota bill (Altieri *et al.*, 1999).

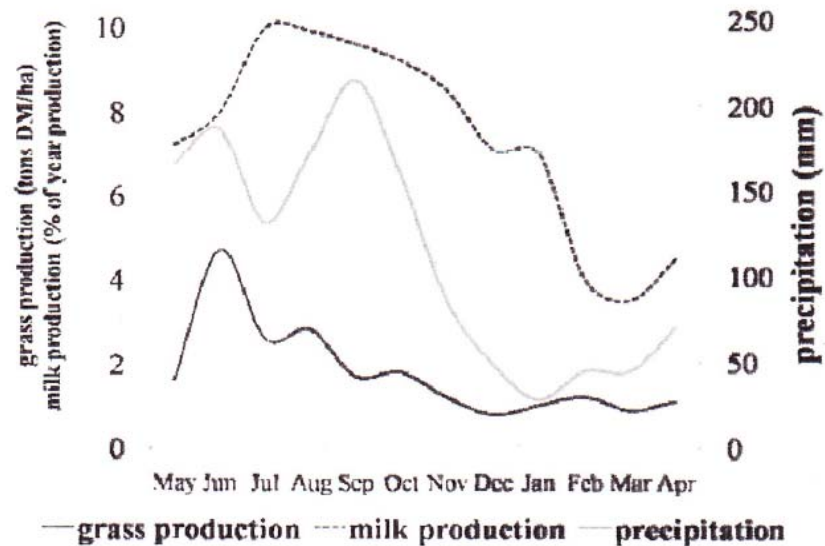
Cut off from imported foods such as meats, grains and processed foods, Cuba was forced to produce almost all its food domestically. In this desperate situation, Cuba revived traditional cultural practices like crop rotation, mixed planting and livestock manuring, which were used before the advent of modern chemicals (Hiranandani, 2010). Moreover, Cuba diversified the use of land once used to grow monocropped sugar into greater vegetable and livestock production. In fact, Cuba transformed its agricultural policy and practice from an industrialized, high-input and highly subsidized system to one that is more autonomous and self sufficient. High input feedlot livestock farms shut down, replaced by low external input systems with local rather than exotic breeds, and using crop waste,

browse and pasture legumes, for example with fodder hedges (Carter, 2008). However, body condition of dairy herds which had been created in the past 30 years was lost and milk production decreased to almost 50 percent: from 879 million liters of milk produced in 1990 (6liters/cow/day), to 425 million liters (3,1liters/cow/day) in 1992 (Perez, 1999). Nowadays, Cuban farmers and scientists are responding to this challenge of food security with a whole array of alternative agricultural technologies to sustain agricultural productivity in the farm sector (Altieri *et al.*, 1999).

### **2.1.2. Cuban ruminant production system in dry and wet season**

Cuba's livestock sector has emphasized a sustainable self-sufficiency through the utilization of pasture, protein-rich fodder trees, sugar cane and other local resources (Perez, 1999). As Cuban dairy systems are based on grazing without concentrate supplementation (Ponce, 2009), there is a direct dependency of the production in relation to the weather (Figure 2.1). Cuba has two defined-weather periods: dry and rainy season with both presenting some difficulties. During the rainy season, quantity and quality of pastures are improved. However, reproductive performance of the animals could be affected due to more humid and warmer conditions. On the other hand, during the dry season there is a severe feed shortage and low feed quality (Perez, 1999). For this reason, farmers of tropical livestock production systems should be encouraged to preserve feeds for a posterior use in dry season when there is a surplus during wet season (Brown and Chavalimu, 1985). However, often tropical grasses and legumes are not ideal materials for ensiling, mainly because of their low level of water soluble carbohydrates (WSC) which is essential for successful ensiling (Titterton and Bareeba, 2000). In addition, legumes have high buffering capacity, which increases silage pH and susceptibility of legume proteins to proteolysis (McDonald *et al.*, 1991).





**Figure 2.1: Schematic representation of coincidence of annual precipitation, grass yield and milk production (average of latest five years) in Cuba**

**Source:** Lima R., (2011a). Evaluation of sorghum – soybean (*Sorghum bicolor* (L.) Moench – *Glycine max* (L.) Merr.) silage as ruminant feed. Ph.D. dissertation, Ghent University, Ghent, Belgium, pp. 173

## 2.2. Botanical and chemical composition of forage in Cuban dairy farms

### 2.2.1. Botanical composition

Since the early years of the colony in Cuba, livestock was based on pasture, mainly grasses, but always with a prevalence of native legumes like *Desmodium*, *Stylosanthes*, *Centrocema*, *Terannus* and *Macroptilium*, which made large contributions to the sustainability of livestock ecosystems. During that period, the most widespread legume was Alfalfa (*Medicago sativa*), but production results were highly variable. As this legume seemed not well adapted to climatological conditions, other species introduced like *Leucaena leucocephala* and *Neonotonia wigthii*, gave more constant results, particularly in the region closest to the Havana province (Del Pozo, 2001). In early 1960's, it became necessary to carry out a program which accelerated transformation to a more technology based agriculture. Parallel to this, Cuban farmers started to plant grasses across the country, mainly Pangola (*Digitaria decumbens*) and Bermuda cross (*Cynodon dactylon* Coastcross-1) (Del Pozo, 2001).

Unfortunately, after 1990, as a consequence of a lack of fertilizers and herbicides, pastures quickly lost their quality and were massively replaced by some natural pastures, mainly *Andropogon annulatus* L. and *Dichanthium charicosum* and by species like *Acacia fornesiana* and *Dichrostachys cinerea* which covered more than 1 million hectares or 47 percent of the area of pastures in 1994 (Paretas *et al.* 1994). Valdés and Delgado (1990) also suggested that Cuban grasslands are dominated by Jamaican star grass (*Cynodon nlemfleuensis*) and *Panicum maximum*, high-power invasive grasses, which have been genetically improved in the Cuban experimental stations according to geographic conditions, soil and management practice.

At present, various approaches were attempted to improve these pastures and develop more sustainable and self-sufficient cattle production systems, such as grass–legume associations, legume protein banks, silvo-pastoral systems, biofertilisers and production and selection of pasture species according to the needs of different regions e.g. grass CT-115 (*Pennisetum purpureum*). Still, the main constraint for success is their isolated application of one of the former approaches and, in most cases, the lack of an integrative system perspective in technology development (Funes *et al.*, 2009). However, Del Pozo (2001), reported that local pasture species under a grazing system of 2.5-3.0 cows/ha with a limited supplementation of concentrate depending on the grass availability could support milk production rates between 9000 and 9500kg/ha/year when rainfall is higher than 1300mm and between 5500 and 6000kg of milk per hectare per year when soils are poor and rainfall is 1000mm. (Table 2.1).

Accordingly Stobbs and Thompson (1978) showed that there is considerable variation between tropical pastures in their ability to supply nutrients for milk production when excess quantities of feed at the same stage of growth are provided. For example, three-week regrowth of Pangola (*Digitaria decumbens*) was shown to produce approximately 10 percent more milk than Rhodes grass (*Chloris gayana*), with Kazungula (*Setaria sphacelata* cv Kazungula) giving an intermediate level of production.

**Table 2.1: Milk production (kg/hectare/season) in two Cuban experimental stations when feeding cows different pasture species**

“La Habana” – West province of Cuba			“Las Tunas” – East province of Cuba		
Species	Dry	Rainy	Species	Dry	Rainy
<i>Digitaria decumbens - Cynodon sp.</i>	7049	7524	<i>Digitaria decumbens - Cynodon sp.</i>	3125	4277
<i>Digitaria decumbens - Cynodon sp.</i>	6178	7436	<i>Pennisetum purpureum - Pueraria sp.</i>	3326	4347
<i>Panicum maximum</i>	6534	7106	<i>Panicum maximum</i>	3645	5756
<i>Brachiaria sp.</i>	5400	5616	<i>Brachiaria sp.</i>	3780	3686
<i>Digitaria decumbens</i>	5011	9801	<i>Digitaria decumbens var. A-24</i>	3514	3929
<i>Chloris gayanus cv Callide</i>	4763	5967	<i>Brachiaria sp.- Centrocema sp.</i>	2974	6086
<b>Average</b>	<b>5823</b>	<b>7242</b>	<b>Average</b>	<b>3394</b>	<b>4680</b>

**Source:** Del Pozo P., (2001). La transformación de los ecosistemas de pastos en Cuba. Situación actual y perspectivas. In: Symposium - Producción y utilización de los pastos en Cuba. Universidad Agraria de la Habana, Cuba, adapted from Funes 2000

### 2.2.2. Chemical composition and nutritive value

Pasture can be a major source of feed for dairy cows but there are some limitations to its use. Energy and protein supplies are the most essential components in animal nutrition and, in many tropical countries, these components are often the critical limiting factors to animal production (Aminah and Chen, 1991). Therefore, determine chemical composition of this pasture vegetation is crucial, particularly in combination with in vitro digestibility, to evaluate the nutritive value of browse species which often are not yet known previously (Tufarelli *et al.*, 2010). Aminah and Chen (1991) reported that most of the tropical grasses (either native or improved pastures) have metabolisable energy values ranging from 7.0 to 11.0MJ/kg DM when cut between 2–8 weeks of regrowth, and energy concentrations of natural forages were found to be similar (7.1 to 10.1MJME/kg DM). Aumont *et al.* (1994) suggested that nutritive values of forages from the Caribbean presented low energy and nitrogen, even at a young stage of regrowth and they were usually poorly ingested by animals (Table 2.2). Based on their crop yields and nutritive value, some species are specially exploited by Cuban farmers and constitute the basis for the production of conserved ruminant feed (Lima, 2011a) (Table 2.3).

**Table 2.2: Range of variation in the chemical composition and in-vitro digestibility of Caribbean forages**

	OM (g kg <sup>-1</sup> DM)	CP (g kg <sup>-1</sup> DM)	ADF (g kg <sup>-1</sup> DM)	NDF (g kg <sup>-1</sup> DM)	IVD (%)
N	1313	1313	1313	838	1313
Mean	891	99	364	669	57.6
SD	29.7	30.2	49.3	58.7	7.72
Minimum	826	25	250	419	29.0
Maximum	986	214	450	823	79.0

**Legend:** OM, Organic matter; CP, Crude protein; ADF, NDF, acid or neutral detergent fiber; IVD, in vitro digestibility

**Source:** Aumont G., Caudron I., Saminadin G. and Xandé A., (1994). Sources of variation in nutritive values of tropical forages from the Caribbean. *Animal Feed Science and Technology*, vol. 51, 1-13

**Table 2.3: Yield and chemical composition of some Cuban feed resources, produced during the Cuban rainy season, which could be used for conservation**

	Yield (t DM/harvest/ha) <sup>A</sup>		Chemical composition of forage (g/kg DM)			
	Forage	Grain	DM <sup>B</sup>	CP	NDF	ADF
Jamaican star grass	1.89-9.00	-----	280-345	95-100	714-747	384-412
Pangola grass	6.30-7.65	-----	220-275	95-110	693-705	376-392
Guinea grass	2.20-4.50	-----	275-328	82-87	677-690	385-400
CT-115	17.5-20.0	-----	200-286	44-63	650-793	400-469
Corn	8.00-10.0	1.49-1.90	250-350	40-75	455-571	264-296
Sorghum	13.1-16.0	1.75-2.20	270-385	50-80	448-482	250-340
Soybean	4.18-4.62	0.80-1.00 <sup>C</sup>	260-375	130-247	390-573	300-432
Jack bean	8.16-19.0	1.00-2.00 <sup>C</sup>	220-370	125-220	450-626	315-432

**Legend:** <sup>A</sup> times of harvest during the rainy season: 4-5 times (Jamaican star grass, pangola grass and guinea grass); 2-3 times (CT-115); 2 times (sorghum and jack bean); one time (corn and soybean)

<sup>B</sup> **DM:** dry matter (g kg/FM), **CP:** crude protein, **NDF:** neutral detergent fiber, **ADF:** acid detergent fiber

<sup>C</sup> soybean and jack bean grain show a CP yield of 0.4 and 0.6 ton/ha, respectively

**Source:** Lima R., (2011a). Evaluation of sorghum – soybean (*Sorghum bicolor* (L.) Moench – *Glycine max* (L.) Merr.) silage as ruminant feed. Ph.D. dissertation, Ghent University, Ghent, Belgium, pp. 173

### **2.3. Comparison between temperate and tropical pastures**

There are many biological characteristics which distinguish tropical and temperate pasture: Tropical grasses possess an efficient enzyme system for CO<sub>2</sub> fixation and limited photorespiration (Barnes *et al.*, 2007). This C<sub>4</sub> photosynthetic pathway provides them an efficient use of water and nutrients, but also is associated with a higher content of structural elements resulting in a relatively low nutritive value compared with temperate C<sub>3</sub> grasses (Humphreys, 1991). In fact, tropical forages are not only more stemmy but their leaves contain more cell wall elements than temperate species, while loosely-packed mesophyll cells and widely spaced vascular bundles of C<sub>3</sub> grasses allow rapid microbial degradation of the leaf substrate. These differences lead to a lower digestibility, slower fermentation and slower particle size reduction of C<sub>4</sub> forages in the reticulorumen which also reduces the rate of passage from this organ (Hardy, 1997).

Nevertheless, these potential limits might be minimized by appropriate harvesting of herbage through efficient and well-planned grazing strategies in a favorable environment (Da Silva and Carvalho, 2005). Furthermore, the C<sub>4</sub> species often outperform C<sub>3</sub> species under conditions of water stress, low soil moisture, and high soil salinity although they also might be productive and competitive in wet environments and may even dominate northern temperate ecosystems e.g. C<sub>4</sub> – dominated tallgrass prairies reached to 50°N latitude in North America (Barnes *et al.*, 2007).

### **2.4. Importance of rumen fermentation**

The digestive anatomy and physiology of cattle and other ruminants is markedly different to that of monogastric animals such as man (Hart *et al.*, 2007). Digestion of food in the rumen occurs by a combination of microbial fermentation and physical breakdown during rumination. As a result of the location of the rumen, anterior to the abomasum, feedstuffs consumed by ruminants are exposed to microbial attack prior to gastric and intestinal digestion.

The rumen is essentially a fermentation chamber in which microbial attack helps digest the diet. The partly fermented food and the micro-organisms pass through the omasum, into the abomasum and then into the small intestine (Hart *et al.*, 2007). Microbial attack is carried out by a mixed population of bacteria and ciliate protozoa, together with a smaller, but possibly metabolically important, population of anaerobic fungi (Dehority, 2003). Microbial populations of the rumen are characterized into those organisms free in the rumen fluid, those associated with feed particles and those associated with the rumen wall. In a fully functioning rumen, there is a dynamic equilibrium, as ruminal microbes adhering to and detaching from feed particles are constantly leaving or re-entering the fluid compartment. Metabolic interactions between these different populations are critical for their collective survival (Fellner, 2002).

Rumen fermentation is recognized as an essential fermentation producing end-products, particularly volatile fatty acids (VFAs) i.e. glycolytic and lipogenic compounds like propionate (C<sub>3</sub>), acetate (C<sub>2</sub>) and butyrate (C<sub>4</sub>), and microbial protein synthesis i.e. NH<sub>3</sub>-N as an essential source of nitrogen, as major energy and protein for the ruminant host (Khampa and Wanapat, 2007). Hence, a more efficient rumen fermentation supports optimal synthesis of end-products (Khampa and Wanapat, 2007). Indeed, VFA formed in the rumen can supply the majority of the animal's energy requirement (approximately 80%; France and Siddons, 1993), while microbial protein leaving the rumen can account for much (typically 60–85%, Orskov, 1982) if not all of the protein entering the small intestine (Hart *et al.*, 2007). Therefore, a healthy rumen is essential and should support an optimal microbial ecology (bacteria, protozoa and fungi) and pH, through a balanced supply of substrates (e.g. roughage, energy, effective fiber, etc.) (Khampa and Wanapat, 2007).

It was reported that a microbial population established in the rumen could be affected by types of feeds and roughage to concentrate ratios (Wanapat, 2000). Cellulolytic organisms or fiber digesters will be the dominant population in grazing ruminants or otherwise eating roughages. On the other hand, the starch digesting (amylolytic) organisms will be the dominant population in feedlot cattle or other ruminants on high-grain rations (Purina, 2006). Microbial species possesses specific characteristics, e.g. types of substrates, ratios of

fermentation products and growth yield. Hence, proportions of fermentation end products may change according to predominance of microbial species within the microbial population, and these changes, can affect both milk composition and the efficiency with which feeds are utilized for production (Baldwin, 1995).

## **2.5. Milk Odd- and branched-chain fatty acids (OBCFA) in dairy cows**

### **2.5.1. Definition**

The main odd- and branched-chain fatty acids (OBCFA) in milk of dairy cows are isomers of tetradecanoic acid (iso C14:0), pentadecanoic acid (C15:0, iso C15:0 and anteiso C15:0), hexadecanoic acid (iso C16:0) and heptadecanoic acid (C17:0, iso C17:0 and anteiso C17:0). OBCFA only occur at trace levels in most plants, but are distinct components of milk and adipose tissue in cattle, sheep and goats, as well as other animals with symbiotic fermentations, such as beavers as they are largely derived from bacteria leaving the rumen (Vlaeminck *et al.*, 2006a). The OBCFA profile of rumen bacteria seems largely determined by two types of fatty acid synthetases, straight-chain and branched-chain fatty acid synthetase, on the micro-organisms, and to a lesser extent by physiological and culture conditions. This suggests that variations in the profile of OBCFA leaving the rumen are mainly a reflection of changes in the relative abundance of specific bacterial populations in the rumen rather than altered bacterial fatty acid synthesis related to the availability of primers, as the latter differences are assumed to be small compared to the former (Vlaeminck *et al.*, 2006a).

### **2.5.2. OBCFA as biomarkers of rumen function**

Vlaeminck *et al.* (2006b) suggested that the rumen microbial ecosystem is reflected in the profile of milk fatty acids particularly milk OBCFA as these are originating from rumen bacteria after outflow to the duodenum and absorption. Hence, their excretion in milk may reflect the microbes and microbial activity. OBCFA have also been used as colonization markers of freshly ingested grass (Kim *et al.*, 2005). In addition, their potential as markers

to quantify bacterial matter leaving the rumen (Vlaeminck *et al.*, 2005) was studied as well as to provide a qualitative description of the proportions of different classes of microbes leaving the rumen (Vlaeminck *et al.*, 2004). Further, shifts in the rumen microbial population due to, e.g. dietary changes (e.g. Dehority and Orpin, 1997) could be expected to enhance changes in the rumen OBCFA profile. Vlaeminck *et al.*, (2006a) reported that higher dietary starch increased the proportion of C15:0 and C17:0, whereas increased neutral detergent fibre content was positively related to anteiso C15:0 concentrations, which is in agreement with the importance of these fatty acids in respectively amyolytic and cellulolytic bacteria. Consequently, OBCFA in ruminant products also are of further interest because they have shown a strong relation with the molar proportions of individual volatile fatty acids in the rumen (Vlaeminck *et al.*, 2006b).

In line with their correlation to volatile fatty acid proportion, there is also scope for the development of a non-invasive tool for monitoring methane losses. Indeed, a close relationship exists between rumen fermentation pattern and methane losses. As the former is closely related to the excretion of odd- and branched-chain fatty acids (OBCFA) in milk, a link between the latter and rumen methane could be expected. However, this relationship between OBCFA and methane losses should first be verified through direct methane measurements before their use as an indicator (Tamminga, 2007).



## CHAPTER 3

### Materials and Methods

#### 3.1. Experimental design

In this research 6 dairy farms of the “Desembarco del Granma” Cooperative, located in Santa Clara, Cuba, were selected. Dairy farms were monitored during the Cuban rainy season (July and August 2010). The attempt was to select according to production level (high, medium and low) in order to reflect the diversity of milk production in the area. However, milk production registration and available information in this respect was limited (Table 3.1).

**Table 3.1: Grazing area (ha), number of animals in production and milk yield (kg/d/cow) of 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010), according to the report of the Cuban project coordinator**

Dairy farm	CT – 115 (ha)	Natural grasses (ha)	Total cows and heifers	Cows on A. I.	Cows on milking	Milk yield (kg/d/cow)
Alemán	16.0	62.3	76	63	40-55	6-10
La Carmita	16.4	55.4	74	63	40-55	5-9
El Compás	17.4	54.5	63	57	40-50	4-8
Tres Caminos	23.8	66.2	93	69	50-60	6-10
San Quintín	17.5	53.8	70	59	40-50	4-8
Los Sánchez	20.4	75.3	80	69	50-60	4-8

#### 3.2. Sampling and storing

##### 3.2.1. Botanical composition

Botanical composition of the pastures was assessed according to the ranking method (De Vries, 1933). Two fields per farm (one for natural pasture areas and one for CT-115 areas,

respectively) were evaluated once during the first week of August 2010. Three random set of 100 observations were selected for each field. The three species in each observation that were judged to contribute most were ranked and listed to determine frequency. Proportions were counted for each field independently and divided by the number of random set observed for each field (3).

### **3.2.2. Chemical composition**

One sample of mixture of natural grasses, CT-115 and concentrate used to feed dairy cows was taken per farm at the end of August 2010. They were transferred to the Universidad Central “Marta Abreu” de Las Villas in Santa Clara for conservation. Samples of natural grasses and CT-115 were weighted and dried at 65°C for 72 h as a pretreatment of storage. The dried samples were ground in a hammer mill (1-mm sieve). Samples from the 6 farms were pooled, according to the type of feed, which resulted in 3 samples (one for natural grasses, one for CT-115 and one for concentrate) stored in glass bottles at room temperature until utilization.

### **3.2.3. Milk samples**

Milk samples were obtained from native Cuban cows (Criollo) and crossbreeds. In order to collect milk, animals were carried from grazing areas into an outdoor milking station and manually milking twice daily.

#### **3.2.3.1. Bulk milk samples**

Bulk milk samples from 6 dairy farms of the “Desembarco del Granma” Cooperative were taken 3 times per week over a period of 5 weeks, for a total of 16 samples per farm (n = 96 samples). Bulk milk samples (50ml each one) were taken twice daily (morning and evening) from cooling tanks at the end of each milking and kept in sterilized glass bottles. Daily samples were pooled per farm and stored frozen (max -5°C) at Universidad Central “Marta Abreu” de Las Villas in Santa Clara until analysis.

### **3.2.3.2. Individual milk samples**

For the analysis of effect on mineral supplementation, 2 dairy farms of the “Desembarco del Granma” Cooperative were monitored (“Alemán” and “La Carmita”). From an average herd of 40-55 cows on milking, 20 Cows per farm were selected and split randomly into four categories based on different level of mineral supplementation (control = 0cc; treatment 1 = 2.5cc; treatment 2 = 5.0cc and treatment 3 = 7.5cc). Animals were injected with Zn, Cu and Mn, according to their category to ensure a good assimilation of the minerals, two weeks before the experiment started. The sampling process was done taking once milk sample individually per cow. Milk samples (50ml) were collected twice per day (morning and evening) at the middle of the individual milking, kept in sterilized glass bottles and stored frozen (max -5°C) at Universidad Central “Marta Abreu” de Las Villas in Santa Clara until analysis which resulted in a collection of 40 samples.

### **3.3. Experimental determination**

Determination of chemical composition and milk fatty acids profile was carried at Lanupro – Ghent University. Analyses of main compounds of Cuban dairy milk (fat, protein and lactose) were obtained in collaboration with researchers of the Walloon Agricultural Research Centre, Quality Department Analyses in Gembloux.

#### **3.3.1. Chemical analysis**

Samples were dried at 60°C for 96h to constant weight. Following drying, samples were weighted to determine the dry matter (DM). Content of crude protein, crude fat, NDF and ADF and crude ash, were determined in grams per kg DM. Determination of crude protein (Kjeldhal method, Lynch and Barbano, 1999), crude fat (Soxhlet method) and ash components of composited samples of feeds, were analyzed using method described by AOAC (1990); while content of NDF and ADF was determined according to Van Soest and Wine (1968).

### 3.3.2. Milk fatty acid profile and OBCFA determination

### 3.3.3. Fatty Acids analysis

Milk fat from thawed milk samples were extracted based on the mini Roese-Göttlieb procedure (adapted from Chouinard *et al.*, 1997). For each sample, a homogenized sub-sample (2.0mL) was weighed into a Mojonnier-type fat-extraction flask. Ammonium solution 25% (0.3mL) was added and the mixture was shaken vigorously. Further, in a first extraction step, 2.0mL of ethanol 95%, 5.0mL of diethyl ether, and 5.0mL of petroleum ether were added and mixed gently. The upper layer was transferred into a methylation tube after phase separation (approx. 15 – 20min.). Fatty acids in extracted lipids were methylated as described by Stefanov *et al.* (2010). The solvents in the lipid solution were evaporated under N<sub>2</sub> at room temperature. Then, the extracted lipids were mixed with hexane, methyl acetate, methanol and sodium methylate. The extracted lipids were methylated for 10 minutes at room temperature. The reaction was terminated by addition of a termination reagent consisting of oxalic acid and diethyl ether (saturated solution). Finally, an aliquot of 0.5ml was taken and added to 0.5ml hexane for gas chromatograph (GC) analysis. The methylated fatty acids were analyzed on a Hewlett-Packard 6890 gas chromatograph (Hewlett-Packard Co, Brussels, Belgium) with a CP-Sil88 column for fatty acid methyl esters (100m × 0.25mm × 0.2m; Chrompack Inc., Middelburg, the Netherlands) according to Stefanov *et al.* (2010). Two different temperature programs were used to analyse methylated fatty acids. According to Vlaeminck *et al.* (2005), the first temperature program was used: 70°C for 4 min, followed by an increase at 10°C/min. to 150°C, then increased at 1°C/min. to 165°C, held at 165°C for 20 min., increased at 2°C/min. to 170°C, held at 170°C for 10 min., increased at 4°C/min. to 215°C, and held at 215°C for 20 minutes.

Agilent Chemstation software (Rev. B.01.01) was used for data analysis. Peaks were identified by comparison of retention times with a GC reference fatty acids methyl ester standard.

### **3.3.4. Protein, fat and lactose analysis**

Milk samples were thawed at room temperature (25°C) on water bath. Then, raw milk samples were heated at 39°C in a water bath, pre-homogenized with an IKA Ultra-Turrax instrument (IKA ® WERKE GmbH, Staufen, Germany) for 60 seconds at 6000 rpm. Finally, milk protein, protein and lactose were determined using FT-IR spectroscopy (LactoScope Automatic Fourier transform infrared instrument, Delta Instruments, Drachten, Netherlands) based on corresponding calibration equations.

## **3.4. Calculations and statistical analysis**

### **3.4.1. Simple descriptive statistics**

Simple statistical analysis was carried out using the SAS 9.1.3 package (SAS Institute Inc., Cary, NC, USA). Milk fatty acids including Odd- and branched-chain fatty acids were represented as proportion of total FA (g/100g of milk fatty acids) and as proportion of total OBCFA (g/100g of OBCFA). Mean, median, standard deviation, minimum and maximum values were obtained and compared with ranges reported by Vlaeminck *et al.* (2006a) for milk OBCFA in temperate regions. Means values of OBCFA (g/kg of milk fatty acids) were compared between farms by applying Tukey's test ( $P < 0.05$ ).

### **3.4.2. Principal component analysis**

All fatty acids including OBCFA were represented using Principal component analysis in SPSS (SPSS software for Windows, release 16.0, SPSS Inc., Chicago, IL). First, PCA based on the correlation matrix was conducted to illustrate relationships between milk fatty acids. Secondly, PCA was used to determine components which account for most of the total variation within OBCFA i.e. iso C14:0, iso C15:0, iso C16:0, iso C17:0, anteiso C15:0, anteiso C17:0, C15:0, C17:0 and. C9C17:1. Third, PCA was performed to show relations between OBCFA and main compounds of Cuban dairy milk (fat, protein and lactose). The principal component scores or loadings were calculated and represented to

evaluate their relationship with other possible variables e.g. distribution of OBCFA concentrations between farms, sampling time or the type of dietary feed used in order to group the results.

### **3.4.3. Linear regression model**

Linear regression model was performed using the SAS 9.1.3 package (SAS Institute Inc., Cary, NC, USA) to determine significance levels of the dependent variables. Least squares means of milk FA (g/100g of milk fatty acids) per mineral supplementation (treatment) and per dairy farm were estimated according to the following linear regression model:

$$Y_{ij} = \mu + M_i + F_j + MF_{ij} + \epsilon_{ij}$$

where  $Y_{ij}$  = dependent variable, individual milk FA (g/100g of milk FA),  $\mu$  = overall mean,  $M_i$  = effect of mineral supplementation (treatments) ( $i = 1, 2, 3, 4$ ) (treatment 1 (control diet) vs. test treatment 2 (2.5cc.) vs. test treatment 3 (5.0cc.) vs. test treatment 4 (7.5cc.)),  $F_j$  = effect of dairy farm ( $j = 1, 2$ ) (“La Carmita” vs. “Alemán”),  $MF_{ij}$  = interaction between mineral supplementation and dairy farm ( $ij = 1, 2, \dots, 21$ ) and  $\epsilon_{ijk}$  = residual error. Effects with the former structure were used for all OBCFA  $P < 0.05$  were considered statistically significant. Means were compared by applying Tukey’s test in cases where were necessary ( $P < 0.05$ ).

## CHAPTER 4

### Results

#### 4.1. Botanical and chemical composition of feed resources in Cuban dairy farms

Table 4.1 shows the average botanical composition of the areas indicated as natural grasslands. In general, these areas are mainly dominated by *Dichantium spp.*, *Paspalum notatun* and *Sporobolus indicus* which cover more less 85% of the total surface. Other species like *Mimosa pudica*, *Digitaria decumbens* and *Alysicarpus vaginalis* were also observed but in lower percentages. In fact, *Dichantium spp.* was consistently predominant for all the 6 farms evaluated during the experiment, yet it varying from 32 to 57%.

**Table 4.1: Botanical composition (%) of natural grassland areas of “Desembarco del Granma” Cooperative (Santa Clara - Cuba), per farm and during the Cuban rainy season (July and August 2010)**

Species	Common name	Tres Caminos	San Quintín	Los Sánchez	Alemán	El Compás	La Carmita	Average
<i>Dichantium spp.</i>	Angleton grass	32	57	47	53	42	38	45
<i>Paspalum notatun</i>	Bahia grass	28	19	26	23	29	34	27
<i>Sporobolus indicus</i>	Smut grass	21	6	15	12	10	18	13
<i>Mimosa pudica</i>	Sleeping grass	14	8	5	7	9	7	8
<i>Alysicarpus vaginalis</i>	Alyce clover	3	5	2	2	7	2	4
<i>Digitaria decumbens</i>	Pangola grass	2	5	5	3	3	1	3

Similarly, the average botanical composition for CT-115 areas is shown in Table 4.2. Although it was expected to find 100% of *Pennisetum purpureum var. CT-115*, some others natural pasture species like *Dichantium spp.*, *Cynodon nlemfuensis*, *Digitaria decumbens*, *Sporobolus indicus* and *Mimosa pudica* were found. San Quintín showed the most deviated

area from the original monoculture as compared with the others five farms, with only 45% of *Pennisetum purpureum* var. CT-115.

**Table 4.2: Botanical composition (%) of CT-115 areas of “Desembarco del Granma” Cooperative (Santa Clara - Cuba), per farm and during the Cuban rainy season (July and August 2010)**

Specie	Common name	Tres Caminos	San Quintín	Los Sánchez	Alemán	El Compás	La Carmita	Average
<i>P. purpureum</i> var. CT-115	Cuba CT-115	93	45	92	95	87	74	81
<i>Dichantium spp.</i>	Angleton grass	2	30	4	1	4	9	8
<i>Cynodon nlemfuensis</i>	Jamaican star grass	1	6	1	-	7	6	4
<i>Mimosa pudica</i>	Sleeping grass	1	10	2	2	-	5	3
<i>Sporobolus indicus</i>	Smut grass	-	5	-	1	-	3	2
<i>Digitaria decumbens</i>	Pangola grass	2	2	1	1	-	1	1
Denuded patches		1	2	-	-	2	2	1

In table 4.3, the chemical composition is given of a mixture of natural grasses, CT-115 and concentrate used to feed dairy cows in the “Desembarco del Granma” Cooperative. The CP content for concentrate samples was highest whereas for natural grass and CT-115, it was similar (80.3 and 93.4g/kg DM). Natural grass samples had the highest NDF, ADF and ash content whereas NDF, ADF and ash content for concentrate samples was lowest.

**Table 4.3: Chemical composition of Cuban natural grass, CT-115 and concentrate, which are used to feed dairy cows in “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010)**

	DM	CP	FAT	NDF	ADF	ASH
		g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM
Natural grass	303	80	30	797	487	146
CT-115	231	93	36	718	456	165
Concentrate	908	246	153	698	190	45

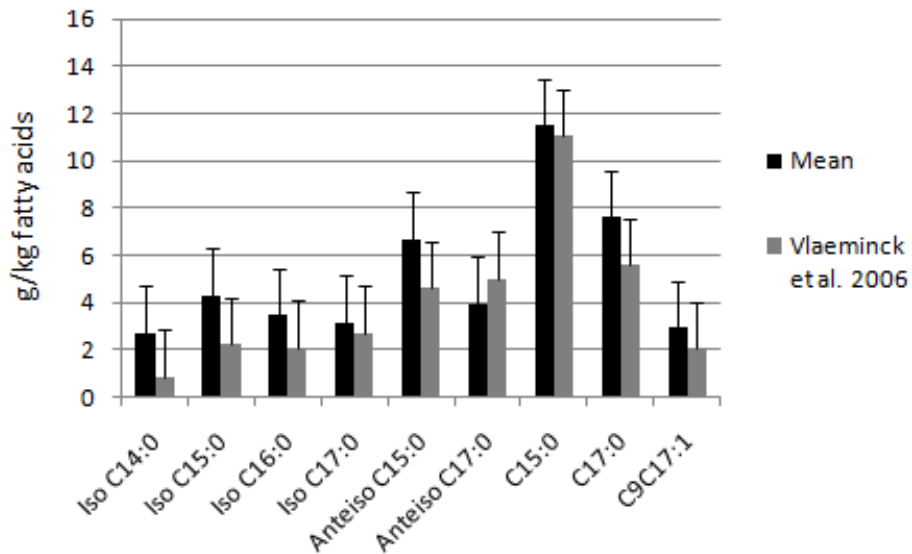


## 4.2. Average odd and branched-chain fatty acid concentrations and comparison with averages from milk of cows from temperate regions

Table 4.4 shows the results of average odd and branched-chain fatty acid concentrations in bulk milk samples of the Cuban farms studied in this experiment during the rainy season. Highest values were observed for C15:0, C17:0 and anteiso C15:0 whereas lowest concentrations were found for iso-fatty acids, anteiso C17:0 and C9C17:1. OBCFA in milk obtained under central Cuban conditions were then compared with average values of OBCFA reported by Vlaeminck *et al.* (2006a) from milk of cows from temperate regions. Almost all OBCFA, including odd chains, showed higher levels in Cuban milk from native Cuban cows or crossbreeds as compared to milk from temperate region and Holstein Friesian cows (Figure 4.1). Indeed, increased proportions of milk OBCFA (g/100g of milk fatty acids), especially iso-fatty acids, were found e.g. for iso C14:0 (2.69 vs. 0.89g/kg milk fatty acids), iso C15:0 (4.33 vs. 2.24g/kg milk fatty acids) and iso C16:0 (3.48 vs. 2.09g/kg milk fatty acids). Similar results were obtained for C15:0, C17:0, C9C17:1 and anteiso C15:0 (11.48 vs. 11.08g/kg milk fatty acids, 7.60 vs. 5.57g/kg milk fatty acids, 2.95 vs. 2.07g/kg milk fatty acids and 6.71 vs. 4.62g/kg milk fatty acids, respectively), it was lower in the case of anteiso C17:0 (3.96 vs. 5.01g/kg milk fatty acids).

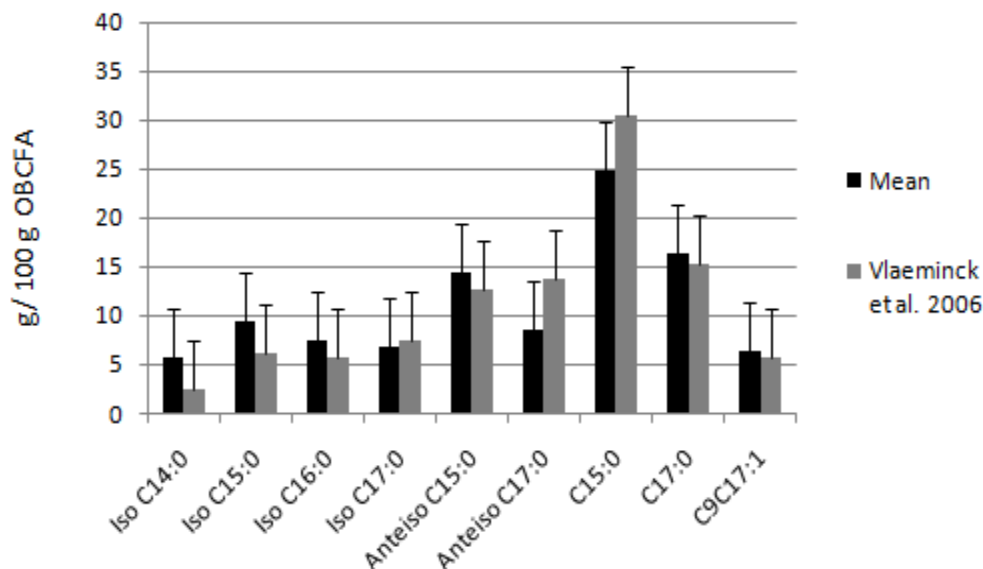
**Table 4.4: Total milk odd and branched fatty acid concentrations in milk fat obtained from 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010) (n=96)**

OBCFA	g/kg milk fatty acids			
	Mean	SD	Min	Max
Iso C14:0	2.69	0.260	1.70	3.19
Iso C15:0	4.33	0.650	2.55	5.88
Iso C16:0	3.48	0.460	2.12	4.40
Iso C17:0	3.16	0.910	1.03	5.13
Anteiso C15:0	6.71	0.790	4.15	8.85
Anteiso C17:0	3.96	0.690	2.34	5.71
C15:0	11.48	1.410	8.16	14.64
C17:0	7.60	0.970	5.17	10.34
C9C17:1	2.95	0.480	1.99	4.31
∑OBCFA	46.36	6.620	29.21	62.45



**Figure 4.1: Comparison of average milk odd and branched fatty acid concentrations in milk fat obtained from 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010) with averages as reported by Vlaeminck *et al.* (2006a) from milk of Holstein Friesian cows in temperate regions (g/kg milk fatty acids)**

Further milk OBCFA proportions were expressed relative to the sum of these microbial fatty acids (Figure 4.2). This could give a better idea of shifts in rumen microbial groups. Comparing these results in g/100g OBCFA, we can notice increased proportions of milk OBCFA for iso C14:0 (5.80 vs. 2.46g/100g OBCFA), iso C15:0 (9.33 vs. 6.18g/100g OBCFA), iso C16:0 (7.50 vs. 5.77g/100g OBCFA) and anteiso C15:0 (14.50 vs. 12.75g/100g OBCFA); and to a smaller extent for iso C17:0, C17:0 and C9C17:1 (6.82 vs. 7.50g/100g OBCFA, 16.40 vs. 15.37g/100g OBCFA and 6.37 vs. 5.71g/100g OBCFA, respectively) whereas anteiso C17:0 (8.54 vs. 13.82g/100g OBCFA) and C15:0 (24.80 vs. 30.46g/100g OBCFA) are less represented in Cuban milk samples as compared with milk from temperate regions.

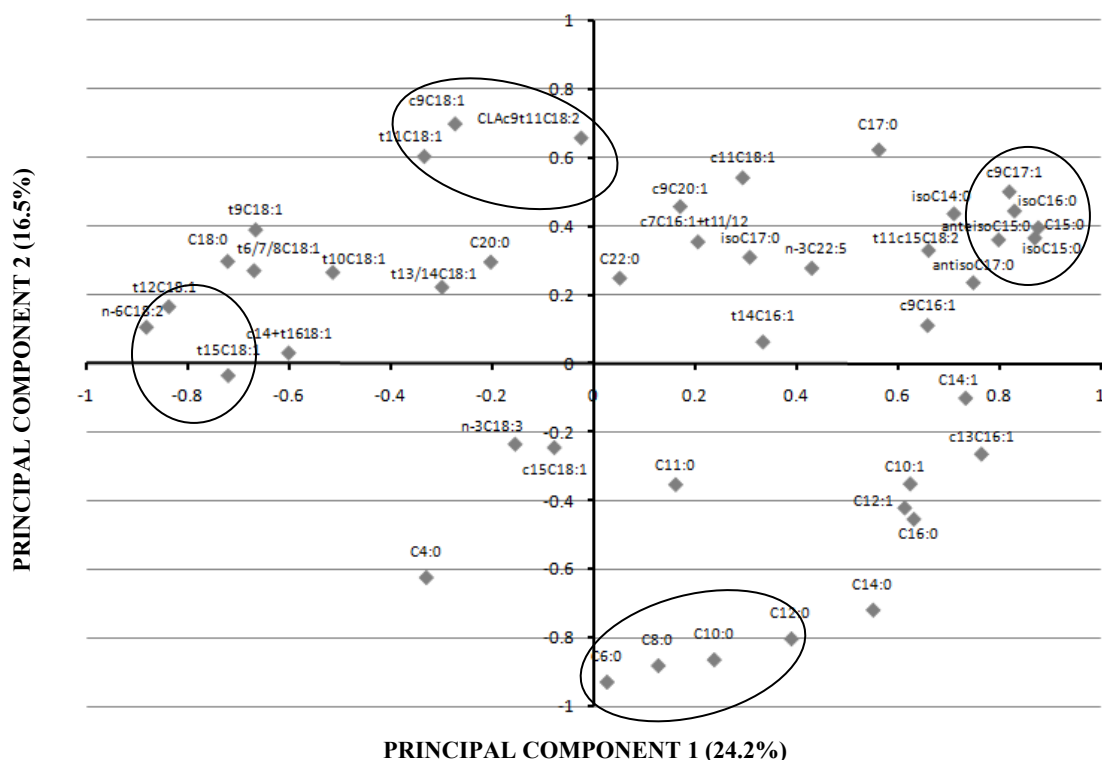


**Figure 4.2: Comparison of average milk odd and branched fatty acid concentrations in milk fat obtained from 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010) with averages as reported by Vlaeminck *et al.* (2006a) from milk of Holstein Friesian cows in temperate regions (g/100g OBCFA)**

#### **4.3. Relation between odd- and branched-chain fatty acids and other milk fatty acids**

As correlations among variables can be identified in loading plots of principal component analysis (PCA) the latter was used to find relations between milk fatty acids. From the data, two principal components (PC) could be extracted describing 40.7% of the total variation in the fatty acid profile (Figure 4.3). In the present analysis, the first principal component (PC1) explained 24.2% of the variation and was mainly due to negative relation between milk odd- and branched-chain fatty acids (mainly C15:0, iso C15:0, anteiso C15:0, iso C16:0 and C9C17:1) with t-12C18:1, t-15C18:1 and n-6C18:2. The second component (PC2), accounting for a further 16.5% of the variation and was mainly determined by a negative relation between medium chain fatty acids (C6:0, C8:0, C10:0 and C12:0) and the major hydrogenation intermediates products i.e. t-11C18:1 as well as C9C18:1 and C9t-11 C18:2. The most common conjugated linoleic acid (C9t-11 C18:2) is produced during

biohydrogenation of linoleic acid although its production through  $\Delta^9$ -desaturase activity with t-11C18:1 as precursor, is the major formation route.



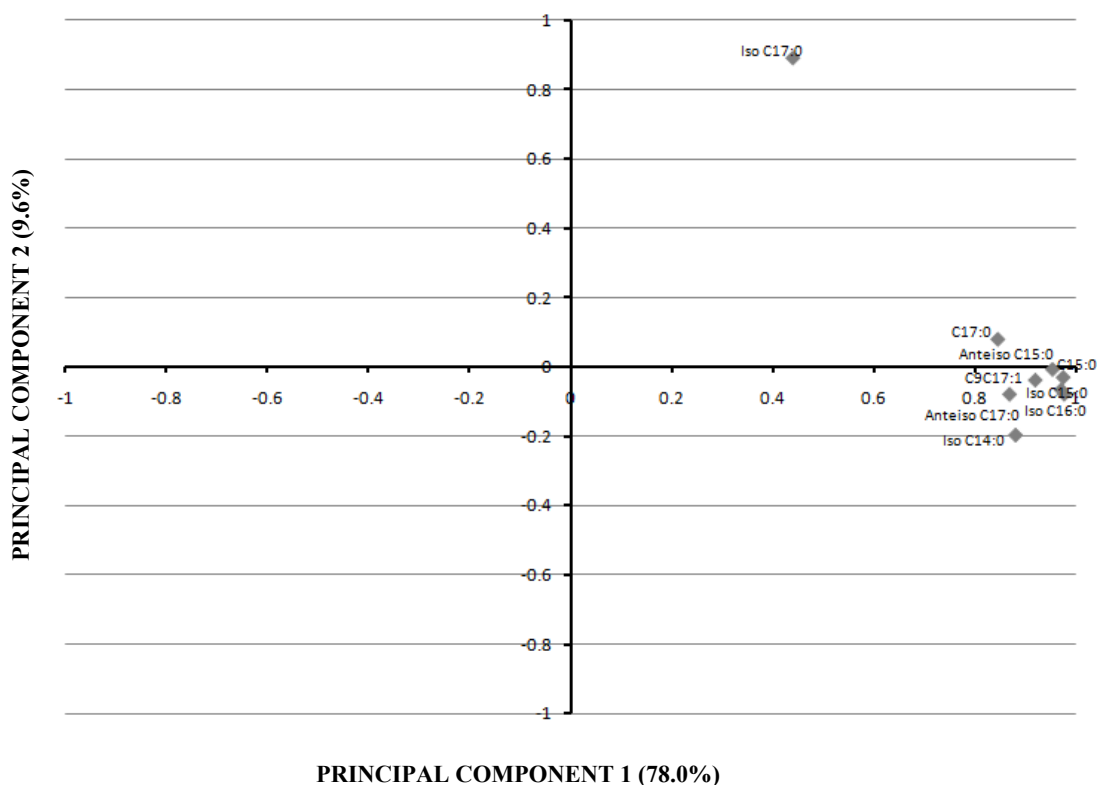
**Figure 4.3: Loading plot of a principal component (PCA), describing the relationship among odd- and branched-chain fatty acids and other milk fatty acids (g/100g milk fatty acids) in bulk milk samples from 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010)**

**Legend:** All milk fatty acids were included in the PCA, however only milk fatty acids showing a correlation greater than |0.2| were presented for clarity of the figure

#### 4.4. Factors determining changes in odd- and branched-chain fatty acids of bulk milk

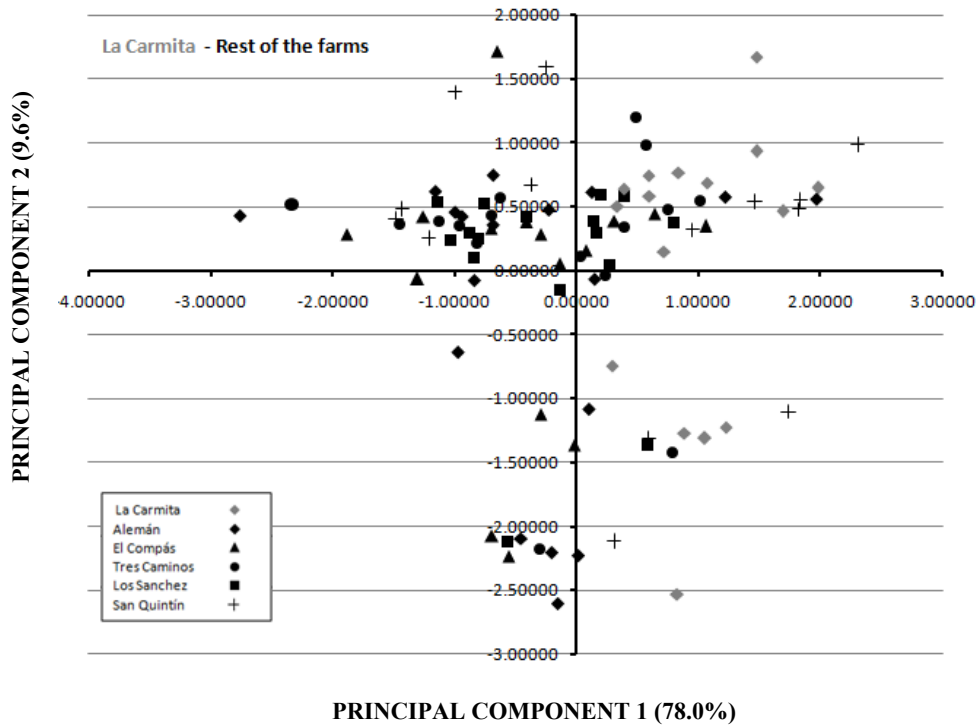
PCA was also used to find relations between odd- and branched-chain fatty acids (g/kg milk fatty acids). From the data, two PC could be extracted describing 87.5% of the total variation in the fatty acid profile (Figure 4.4). In the present analysis, PC1 explained 78.0% of the variation and was mainly determined by common variation in most of the odd- and branched-chain fatty acids (iso C14:0, C15:0, iso C15:0, anteiso C15:0, iso C16:0, C17:0

and anteiso C17:0). Iso C17:0 behaved differently from the others. PC2 accounting for a further 9.6%.



**Figure 4.4: Loading plot of a principal component (PCA), describing the relationship among odd- and branched-chain fatty acids (g/100g milk fatty acids) in bulk milk samples from 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010)**

A score plot of PCA further can indicate whether some grouped changes/variation in milk OBCFA pattern is observed within the bulk samples of the current experiment. Grouping within a score plot could give an indication of major factors determining variation in milk OBCFA concentrations. A first tendency can be seen identifying farm origin of the milk samples (Figure 4.5). “La Carmita” was the only farm of which milk OBCFA consistently showed positive PC1 values (from 0 to +3.0) compared with the other five farms (El Compás, Alemán, Los Sánchez, San Quintín and Tres Caminos) which showed positive as well as negative PC1 value. This indicates milk fat from “La Carmita” contained relatively more OBCFA as compared with milk fat from other farms (Table 4.5).



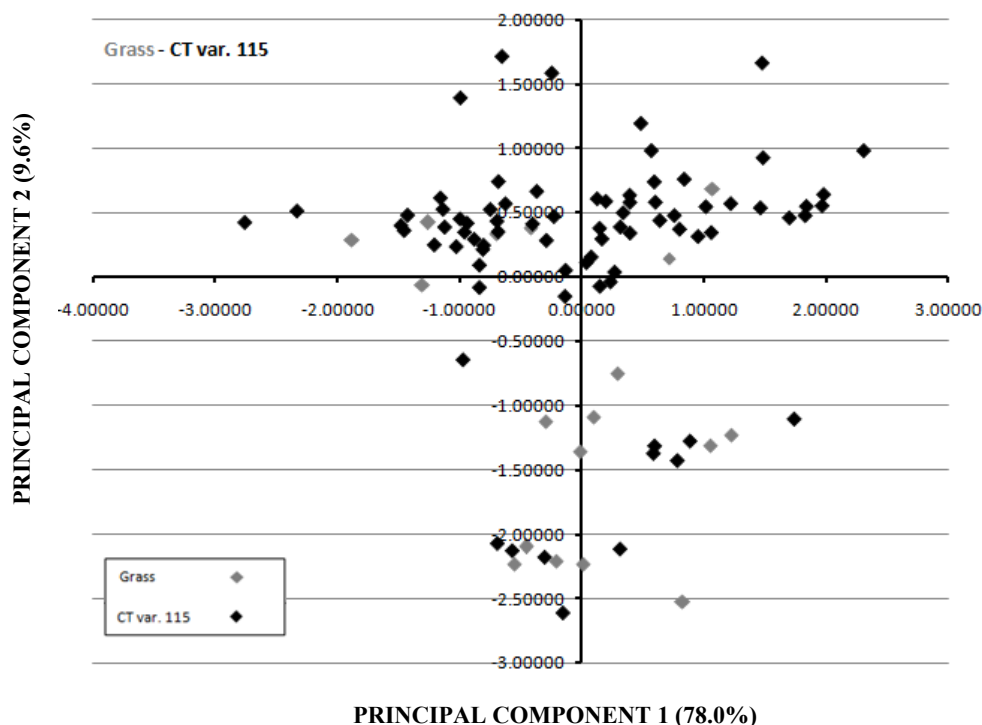
**Figure 4.5:** Score plot of a principal component analysis based on milk odd- and branched-chain fatty acids concentrations (g/100g milk fatty acids) in milk fat, showing distribution and clustering within and between 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) (“La Carmita” vs. rest of the 5 farms) during the Cuban rainy season (July and August 2010)

**Table 4.5:** Average milk odd and branched fatty acid concentrations (g/kg milk fatty acids) in milk fat obtained from 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010) (n=16)

OBCFA	g/kg milk fatty acids						
	La Carmita	Alemán	San Quintín	El Compás	Tres Caminos	Los Sánchez	SEM
Iso C14:0	2.89a	2.70 ab	2.65ab	2.64ab	2.64ab	2.62b	0.025
Iso C15:0	4.94a	4.11b	4.63ab	4.04b	4.18b	4.11b	0.059
Iso C16:0	3.96a	3.42b	3.52b	3.33b	3.39b	3.27b	0.042
Iso C17:0	3.58	2.87	3.27	2.95	3.21	3.10	0.092
Anteiso C15:0	7.33a	6.29c	7.28ab	6.55c	6.57bc	6.29c	0.069
Anteiso C17:0	4.45ab	3.73c	4.55a	3.83c	3.72c	3.54c	0.059
C15:0	12.84a	10.96b	12.03ab	10.87b	11.01b	11.21b	0.128
C17:0	8.34a	7.86ab	7.78ab	7.10b	7.46ab	7.13b	0.090
C9C17:1	3.50a	2.90b	2.94b	2.71b	2.80b	2.89b	0.043

**Legend:** a,b,c - Different superscripts on same row show significant differences (p<0.05) between farms

Some additional assessments were made using the score plot approach (e.g. Figure 4.6). It shows a difference in the distribution of OBCFA concentrations according to the type of predominant dietary feed compound used during the experiment (grass vs CT var. 115). Most of the values of PC2 were higher for cows fed with CT var.115 as compared with milk of cows fed with natural grasses. This result reflects the higher average concentration value obtained for iso C17:0 (3.34 vs. 2.41g/kg milk fatty acids, respectively). This indicates that CT var. 115 induces higher proportions of iso C17:0. However, some positive values (from 0 to +0.7) were observed for milk of cows grazing natural grasses during the last 2 weeks of the experiment in samples taken from “El Compás”. Iso C17:0 in milk fat of these two weeks on natural grasses was higher as compared with milk fat at earlier weeks when cows grazed natural grasses (2.91 vs. 1.85g/kg milk fatty acids).

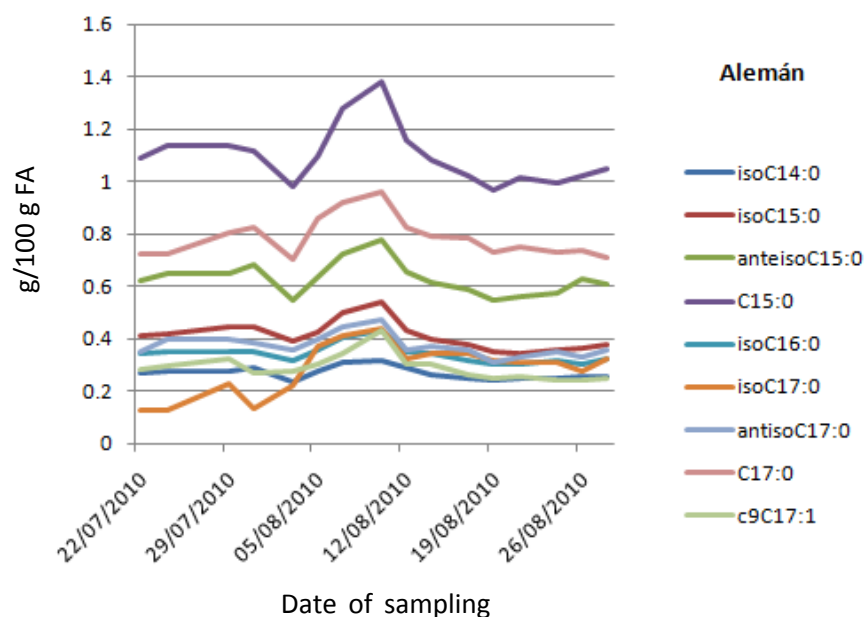


**Figure 4.6:** Score plot of a principal component analysis based on milk odd- and branched-chain fatty acids concentrations (g/100g milk fatty acids) in milk fat obtained from 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010)

**Legend:** Score plot shows distribution and clustering according to the type of predominant dietary feed compound used between farms (Grass vs. CT var. 115)

#### 4.5. Temporary changes in odd- and branched-chain fatty acids of bulk milk

Changes during the rainy season in the concentration of milk odd and branched fatty acid (g/100g milk fatty acids) are shown in Figure 4.7. The farm “Alemán” was used as an example here. Similar figures were produced for the other farms and are included as Appendix 1. OBCFA remained stable at the beginning of the evaluation (22/07/2010) with a slight increase during the last week of July (29/07/2010). Then the amount declined slightly, reached a peak before the middle of August (12/08/2010) and dropped until almost the same level as at the beginning of the evaluation.

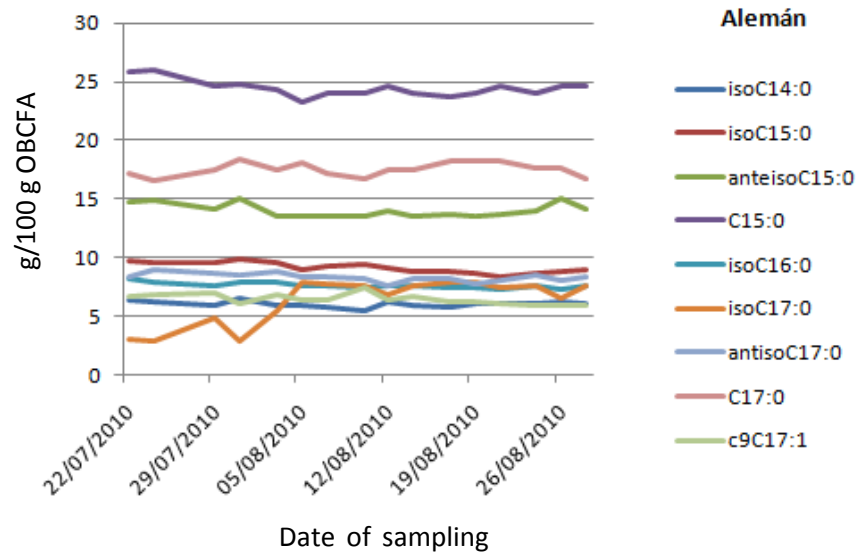


**Figure 4.7: Temporal changes in the concentration of milk odd and branched fatty acid (g/100g milk fatty acids) in milk fat, sampled at the farm “Alemán” (Santa Clara - Cuba) during the rainy season (July and August 2010)**

All OBCFA more or less behave in the same way. Hence these changes do not seem to suggest a shift within the microbial population as both fatty acids linked to cellulolytic as well as amylolytic change to a similar extent and follow the same pattern. To confirm the latter (no shifts within the microbial population) the temporary pattern of OBCFA as a proportion of these microbial fatty acids was explored (Figure 4.8 for “Alemán”, Appendix 2 for others). The hypothesis was confirmed by the constant temporary pattern in the



concentration of milk odd and branched fatty acid (g/100g OBCFA) during the rainy season (Figure 4.8).



**Figure 4.8: Temporal changes in the concentration of milk odd and branched fatty acid (g/100g OBCFA) in milk fat, sampled at the farm “Aleman” (Santa Clara - Cuba) during the rainy season (July and August 2010)**

#### 4.6. Effect of mineral supplementation in OBCFA concentrations

Results are presented in Table 4.6. Neither the sum of the milk OBCFA, nor the concentration of individual milk OBCFA changed through mineral supplementation ( $p > 0.1$ ). Total OBCFA, isoC15:0 and C15:0 tended to differ between the farms ( $0.05 < p\text{-value} < 0.1$ ). Anteiso C17:0 and C9C17:1 concentrations significantly differed between farms. These changes were consistent with those observed for bulk milk samples (Table 4.5) and with the principal component analysis which identified “La Carmita” as the only farm with consistently positive PC1-values (Figure 4.5.). There was no interaction observed ( $p = 0.700$ ) between treatments (levels) and farms evaluated (“La Carmita” and “Aleman”) for the regression model suggested.

**Table 4.6: Regression analysis of the effect of mineral supplementation in 2 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) (“La Carmita” vs. “Alemán”) during the Cuban rainy season (July and August 2010) upon the amount of OBCFA concentrations**

OBCFA	Treatment				SEM	Statistics (p – value)		
	Control	2.5 cc	5.0 cc	7.5 cc		Level	Farm	Interaction
Iso C14:0	0.249	0.278	0.265	0.298	0.008	0.143	0.533	0.884
Iso C15:0	0.392	0.436	0.418	0.451	0.013	0.416	0.074	0.875
Iso C16:0	0.324	0.354	0.343	0.377	0.011	0.296	0.241	0.984
Iso C17:0	0.345	0.374	0.348	0.325	0.013	0.439	0.216	0.702
Anteiso C15:0	0.593	0.682	0.634	0.706	0.021	0.241	0.132	0.918
Anteiso C17:0	0.361	0.398	0.372	0.395	0.013	0.403	0.040	0.608
C15:0	1.058	1.162	1.139	1.232	0.034	0.138	0.068	0.607
C17:0	0.648	0.684	0.636	0.754	0.024	0.159	0.225	0.437
C9C17:1	0.284	0.298	0.283	0.311	0.009	0.149	0.004	0.206
∑ OBCFA	4.254	4.666	4.438	4.849	0.132	0.235	0.077	0.700

## CHAPTER 5

### Discussion

First of all, the local availability of feed resources will be discussed to determine if “Desembarco del Granma” Cooperative (Santa Clara - Cuba) has sufficient local resources for feeding livestock during the Cuban rainy season. As no information is available related to OBCFA in milk produced under tropical conditions, in a second part, we will compare our results with the available literature for OBCFA from milk of Holstein Friesian cows in temperate regions. Afterwards all data collected during this study will be interpreted in an integrated way, suggesting some hypothesis to explain the observations.

#### 5.1. Feed resources in Cuban dairy farms

##### 5.1.1. Botanical composition

Botanical composition of natural grassland areas was consistently dominated by *Dichantium spp.* for all 6 farms evaluated during the experiment. *Dichantium spp.* is well adapted to most of the Cuban soils due to their tolerance to a wide range of acidity and is characterized by massive growth during the rainy season (Figueredo and Iser, 2005). Moreover, in our current study it was observed that CT-115 areas were partially replaced by *Dichantium spp.* and some other natural pasture species, especially in “San Quintín” where *Dichantium spp.* represents 30% of the surface, when it was expected to find 100% of *Pennisetum purpureum var. CT-115*. This could be interpreted as a result of poor land management practices. Production systems based on continuous grazing or overgrazing often primarily show the highest returns per hectare but have led to soil compaction, reduced fertility and the invasion of undesirable species (Donaldson and Heath, 1997).

##### 5.1.2. Chemical composition

Chemical composition for natural grasslands areas showed lower levels of crude protein compared with Aumont *et al.*, (1994) (80g/kg DM vs. 99g/kg DM) and higher levels of

ADF and NDF (487g/kg DM vs. 364g/kg DM and 797g/kg DM vs. 669g/kg DM, respectively). This indicates relatively high levels of lignocellulosic materials and low levels of fermentable carbohydrates in central Cuban cows' diets. Machado and Menéndez (1978) questioned the value of *Dichantium spp.* as a high quality pasture, although Skerman and Riveros (1992) described *Dichantium spp.* as very palatable and as producer of high quality hay. However, since animals can select a better quality diet than feed on offer when allowed to selectively graze, these issues should not be a major problem if there is enough high quality feedstuffs available.

Furthermore, results of the chemical composition for CT-115 areas in the current study were in agreement with findings of Lima (2011a) except for crude protein, where *Pennisetum purpureum var. CT-115* from the 6 dairy farms contained higher levels (93g/kg DM vs. 44 - 63g/kg DM). This situation could be attributed due to a seasonal effect. According to Machuca (2007), crude protein of *Pennisetum purpureum var. CT-115* in rainy season is around 85.6g/kg DM against 73.6g/kg DM in dry season.

## **5.2. Concentration of milk OBCFA as compared with literature data**

### **5.2.1. Differences within the rumen microbial population**

As indicated earlier, milk OBCFA proportions were expressed relative to the sum of these microbial fatty acids to give a better idea of shifts within rumen microbial groups. Increased proportions of iso-fatty acids were observed, especially for iso C14:0 and iso C15:0. Knowledge of OBCFA as biomarkers of rumen function is limited to cows on temperate diets. Here, higher levels of those iso-fatty acids were indicative of high levels of lignocellulosic materials and low levels of fermentable carbohydrates (Vlaeminck *et al.*, 2006a). This would suggest an increased proportion of cellulolytic bacteria to be associated with dairy cattle diets in Central Cuba. On the other hand, an increased proportion of amyolytic bacteria possibly would increase anteiso and particularly linear odd-chain fatty acids. Hence, differences between Cuban and temperate milk in proportions of iso-fatty acids and C15:0 might reflect differences in the rumen bacterial populations such as those

induced by variation in dietary F:C ratio (Vlaeminck *et al.*, 2006a). Moreover, Weimer *et al.* (1999), indicated that the ruminal cellulolytic bacterial population tended to increase with a higher NDF content of the diet. NDF and ADF content (571 and 342g/kg DM, respectively) in Dutch botanically diverse natural grasslands without any kind of fertilization are lower (Laurenço *et al.*, 2007) compared with our results for natural grasses (797 and 487g/kg DM, respectively) and CT-115 (718 and 456g/kg DM, respectively). Those temperate natural grasslands only represent a minor fraction of dairy cow's diets (if any) as they are considered too fibrous. Hence, it might not be surprising that higher NDF and ADF proportions resulted in higher iso C14:0 and iso C15:0 and lower C15:0, indicative for a greater ruminal cellulolytic bacterial population.

Our results related to higher levels of iso-fatty acids are also in agreement with other studies from temperate regions. Kraft *et al.* (2003) reported increased concentrations of iso-fatty acids in milk of cows that were grazing alpine pastures (1.68 and 3.40g/kg fatty acids for iso C14:0 and iso C15:0, respectively). On the other hand, low milk levels of iso C14:0 (0.4g/kg fatty acids) were reported by Jurjanz *et al.* (2004) from cows that were fed with maize silage which contains more rapidly degradable starch. Van Nespen *et al.* (2005), observed low levels of both iso C14:0 and iso C15:0 (0.38 and 1.37g/kg fatty acids, respectively) in milk of cows in early lactation receiving a highly digestible diet rich in starch.

### **5.2.2. Importance of rumen microbial activity and/or effect of dietary fat content**

Although the proportion of iso-fatty acids increased within the total OBCFA, it is striking that all milk odd- and branched-chain fatty acids (except anteiso C17:0), including both branched and odd chains, showed higher concentrations in milk fat under central Cuban conditions. The origin of this increase could be twofold: either an indication of general increase in microbial activity or an indication of limited amounts of dietary fat. Both factors are explored a bit more in detail in following paragraphs.

### 5.2.2.1. Higher microbial activity

Protein available to the ruminant for duodenal absorption is supplied by dietary bypass protein and microbial protein. The latter supplies, on average, 59% of the protein available for absorption in dairy cattle (Clark *et al.*, 1992). Hence, if there is a higher microbial activity in the rumen of Cuban dairy cattle, it could be assumed that microbial protein would contribute to a larger extent to the protein available for absorption. As we do not possess data on duodenal protein flow, the importance of microbial protein supply was assessed from the ratio duodenal flow of microbial protein to milk protein. The former was quantified based on relations of milk OBCFA secretions and duodenal microbial protein flow as established before (Vlaeminck *et al.*, 2006a). Indeed, higher microbial nitrogen flow per kg milk protein would be obtained compared with cows on temperate diets. Vlaeminck *et al.* (2005) established linear relations between the amount of OBCFA excreted in the milk and duodenal flow of purine bases (PB) and diaminopimelic acid (DAPA), which are common markers for microbial protein. Later, Vlaeminck *et al.* (2006a) combined dietary treatment means data from previous experiments to predict duodenal flow of microbial protein for an “average cow” based on milk secretion of OBCFA. Overestimates of duodenal flow of microbial protein were based on this equation and expressed relative in proportion with the total amount of protein excreted in the milk. Data of milk protein and milk yield obtained from experiments in temperate regions (Gadeyne *et al.*, 2011), indicated 258.5g/day of duodenal flow of microbial N to represent 29.8% of the total amount of protein excreted in the milk (866.4g/day). On the other hand, our results for Cuban milk samples showed lower duodenal flow of microbial N (104.5g/day) but it represented 44.3% of the total amount of protein excreted in the milk (236g/day), which indicates higher rumen microbial activity on Cuban dairy cattle compared with cows of temperate regions. However, it doesn't mean necessary that it is the most predominant reason of increased levels of OBCFA. Moreover, the application of the equation to convert OBCFA secretion in milk to duodenal flow of microbial protein should be considered with caution as not validated under tropical feed conditions and with non Holstein breeds.

### **5.2.2.2. Lower amounts of dietary fat**

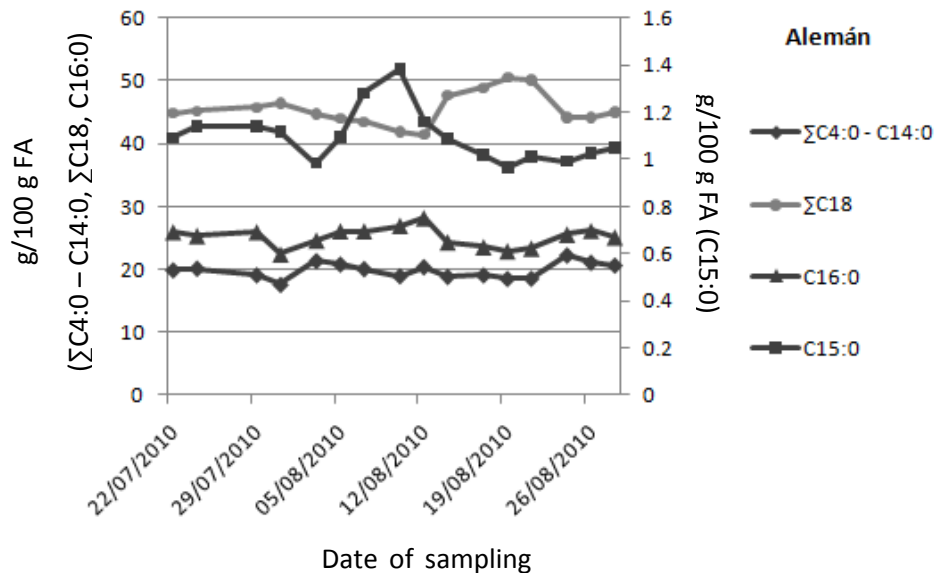
Dietary fatty acids might inhibit de novo synthesis of fatty acids by microbes (Demeyer *et al.*, 1978; Emmanuel, 1978). Further, it is energetically more beneficial for rumen microbes to incorporate dietary fat when available rather than de novo synthesize fatty acids. Hence, OBCFA increase with decreasing dietary fatty acid content. Natural grasses contain low dietary fat (30g/kg DM), whereas the fat content of CT-115 is slightly higher (36g/kg DM). Dairy cows in temperate regions usually receive a supplemental concentrate which is richer in fat. As all of the dairy farms of the “Desembarco del Granma” Cooperative based their production on grazing with a very limited concentrate supplementation, it is expected that de novo synthesis of OBCFA is higher as compared with temperate regions

### **5.2.2.3. Higher microbial activity or lower amount of dietary fat as determining factor**

In order to get idea whether microbial activity or dietary fat is the predominant reason for higher amounts of milk OBCFA, a comparison is made with other milk fatty acids. Our results showed negative negative relation between medium chain fatty acids (C6:0, C8:0, C10:0 and C12:0) which result from de novo synthesis of short and medium chain fatty acids in the mammary gland from acetate and  $\beta$ -hydroxy-butyrate and some milk C18 - fatty acids i.e. trans-11 C18:1; cis-9 C18:1 or cis-9 trans-11 C18:2 (CLA). The negative correlation between milk odd- and branched-chain fatty acids (mainly C15:0, iso C15:0, anteiso C15:0, iso C16:0 and C9C17:1) with trans-12 C18:1, trans-15 C18:1 and n-6 C18:2 suggest an inhibitory or diluting effect of long-chain fatty acids on rumen bacteria (Vlaeminck *et al.*, 2006a). Dairy cows fed supplemental fat rich in C18:2 n-6 (Collomb *et al.*, 2004; Fievez *et al.*, 2005; Rego *et al.*, 2005) and C18:3 n-3 (Collomb *et al.*, 2004; Loor *et al.*, 2005) had lower proportions of milk OBCFA.

As outlined before an increase in milk OBCFA could have a dual origin and could be an indication of an increased rumen microbial activity or decreased dietary fat supply. Higher levels in Cuban milk of both OBCFA indicative for cellulolytic and amylolytic bacteria, questioned increase of rumen microbial activity as major source for greater milk OBCFA

content. To test the second hypothesis, i.e. stimulation of OBCFA transfer from duodenum to milk in Cuban cows due to a lower supply of external fatty acids, proportions of milk fatty acids of external origin in Cuban milk was compared with 'standard fatty acid composition of average temperate milk' (Jensen, 2002). External fatty acids include all fatty acids with 18 or more carbons as well as about half of C16:0. However, the concentration of those fatty acid groups in Cuban milk is within the range (39.4 and 26.9g/100g milk fat) reported for milk of Holstein-Friesian cows in temperate regions (33-40 g C18/100g milk fat and 23.5-30 g C16:0/100g milk fat). Nevertheless, concentration ranges of fatty acids are relatively large, which might have impaired to find clear differences. Hence, another approach was also applied as we also observed variation in OBCFA concentrations within the period of sampling in Cuba. It was compared within each farm whether this temporal variation in OBCFA was associated with 1/ a similar variation in fatty acids which are de novo synthesized in the mammary gland and 2/ an opposite pattern of external fatty acids (i.e. C18 and C16:0). This pattern was more or less observed and is illustrated in Figure 5.1 for “Alemán”, Appendix 3 for others, with C15:0 as a representative for milk OBCFA.



**Figure 5.1: Temporal changes in the concentration of milk fatty acids groups originating from de novo synthesis ( $\Sigma C4:0 - C14:0$ ) in the mammary gland, dietary origin eventually after rumen metabolism ( $\Sigma C18$ ) or dual origin (C16:0) vs. milk C15:0, sampled at the farm “Alemán” (Santa Clara - Cuba) during the rainy season (July and August 2010)**



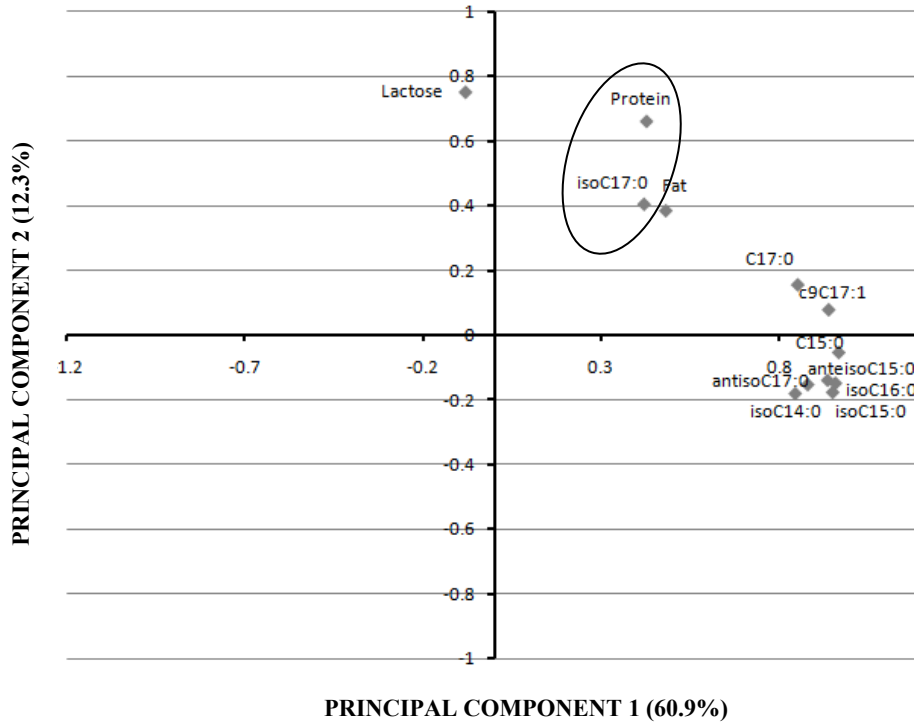
This could suggest a contribution of relatively low amounts of dietary fat to greater milk OBCFA concentrations. However, when integrating milk fatty acid data of all samples from the different farms over the complete Cuban sampling period, in a principal component analysis, variation within OBCFA concentration was shown to evolve quite independently from external fatty acids or fatty acids which are de novo synthesized in the mammary gland (see further, Figure 5.4). Hence, differences in milk OBCFA concentrations between milk of temperate and tropical regions, still needs some further investigation.

### **5.3. Individual OBCFA as specific biomarkers**

Dietary nutrient composition could explain some variation in levels of OBCFA. However, iso C17:0 showed a somewhat different pattern as compared with the other OBCFA.

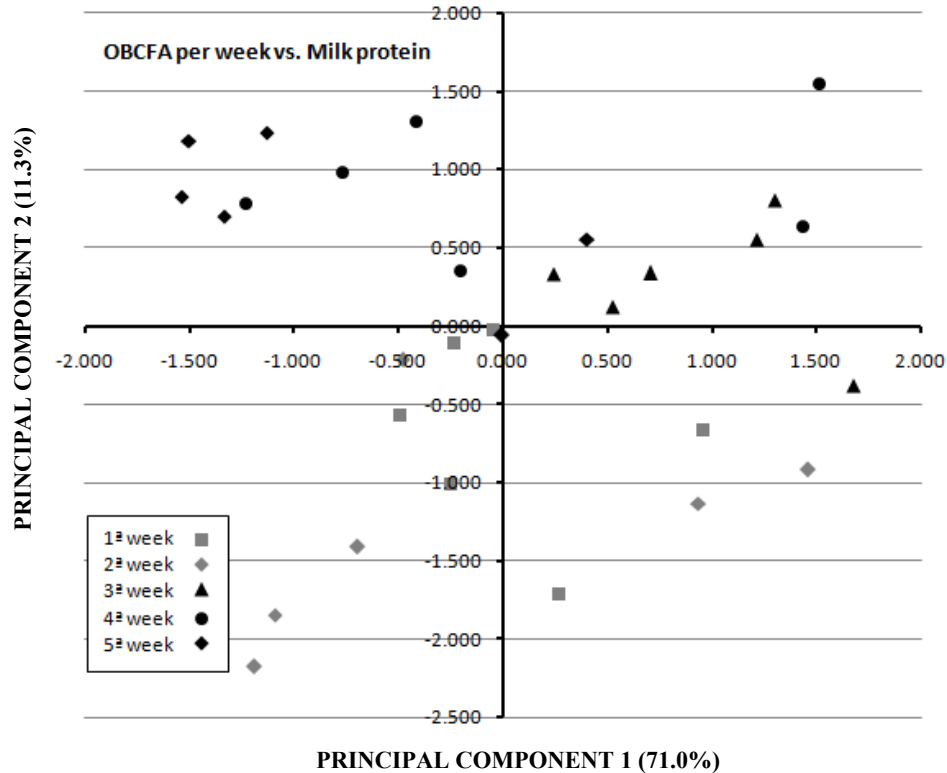
According to Sauvant and Bas (2002), increased content of iso C17:0 could give an indication of a higher amount of aminoacids available in the diet and partially fermented in the rumen, since iso C17:0 comes from branched-chain precursors i.e. branched chain volatile fatty acids from fermentation of amino acids (valine, leucine and isoleucine). Inversely, lower amounts of iso C17:0 might indicate limited amino acids fermentation and hence eventually a lack of rumen degradable protein.

As we suggested milk protein to largely depend on microbial protein (see 5.2.2.1.), lower availability of rumen degradable protein could impair milk protein secretion. This was rested by integration of both milk protein (and fat) as well as milk OBCFA concentrations in a principal component analysis (Figure 5.2). Indeed, a positive association between milk protein and iso C17:0 was observed.



**Figure 5.2: Loading plot of a principal component (PCA), describing the relationship among odd- and branched-chain fatty acids (g/100g milk fatty acids) and main compounds of Cuban dairy milk (fat, protein and lactose, in %m/m) in bulk milk samples from 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010)**

Our results also showed differences in distribution of OBCFA concentrations according to the type of predominant dietary feed compound used during the experiment (grass vs CT var. 115). These differences are mainly related with the amount of iso C17:0. This result would suggest that CT var. 115 induces higher proportions of iso C17:0 and which might reflect higher amounts of amino acids in the diet as described above. This agrees with the result of the chemical composition of natural grasses compared with *Pennisetum purpureum* var. CT-115 (80g/kg DM vs. 93g/kg DM). Furthermore, positive values for natural grasses during the last 2 weeks of the experiment could be explained as an effect of increased forage quantity and quality of these grasses at the end of August compared with July (Figure 5.3). This result is in agreement with the schematic representation of Lima (2011a) described above (see 2.1.2.).

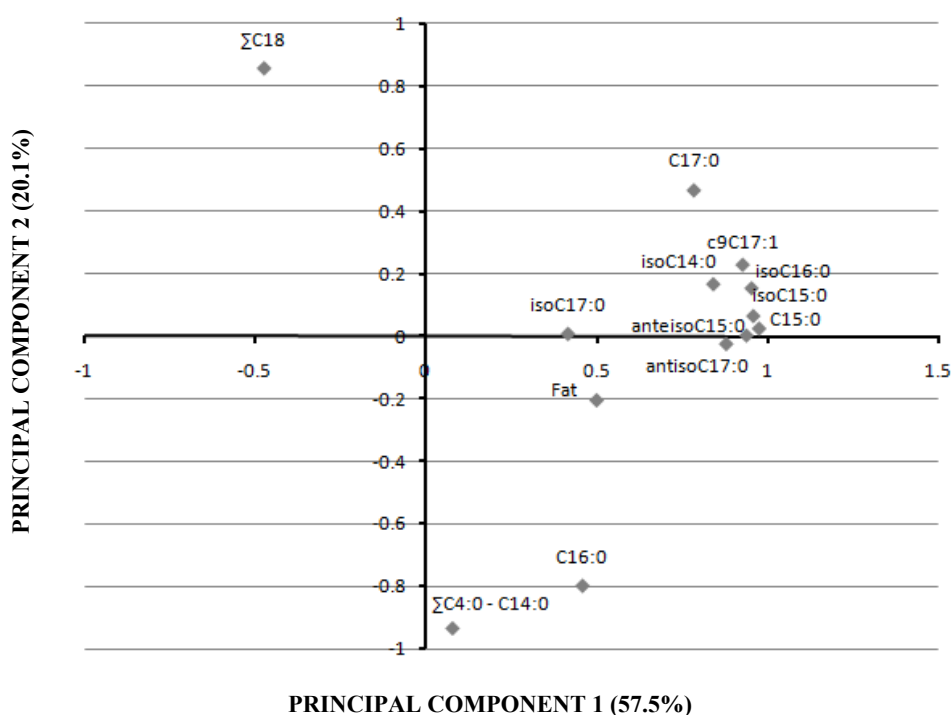


**Figure 5.3: Score plot of a principal component analysis based on milk odd- and branched-chain fatty acids concentrations (g/100g milk fatty acids) in milk fat obtained from 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010)**

**Legend:** Score plot shows distribution and clustering according to milk protein content and the week of milk sampling

However, our results seem contradicting with other studies. Vlaeminck *et al.* (2006a) reported that highest amounts of anteiso C17:0 to be linked to a decreased dietary crude protein and with higher milk OBCFA content. Other unexpected reports suggested that iso C17:0 increased by 360% (Singh *et al.*, 2004) compared with the control diet when fish oil (Shingfield *et al.*, 2003), fish and sunflower oil (Jones *et al.*, 2005) or marine algae (Singh *et al.*, 2004) were supplemented, whereas these feed resources don't supply rumen degradable protein. However, these historical data on iso C17:0 and anteiso C17:0 associations with dietary protein should be considered with caution as they might be erroneous through a former problem of coelution with iso C17:0 and anteiso C17:0 and C16:1 cis and trans isomers during GC analysis. In the current study, precautions were taken to avoid this, i.e., analysis by two different temperature programs as reported by

Stefanov *et al.*, (2010). As we already discussed, iso C17:0 behaved somewhat differently from other OBCFA and was particularly associated with variation in milk protein and fat content. This association might be more straight forward for milk protein as compared with milk fat, regarding the origin of milk iso C17:0. In relation to milk fat content, reduction in milk fat could be hypothesized to have a dual origin: either indication of general decrease of microbial fermentation e.g. due to a decrease in rumen degradable amino acids which could result in a decrease of acetate and butyrate or an indication of limited amounts of dietary fat. Both factors are explored a bit more (Figure 5.4).

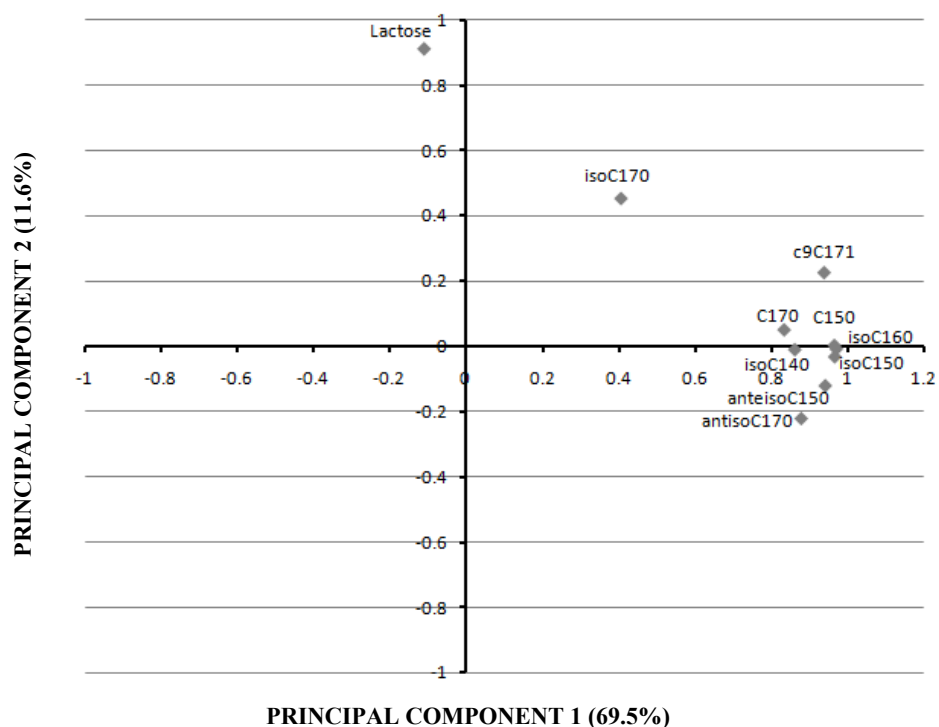


**Figure 5.4: Loading plot of a principal component (PCA), describing the relationship among odd- and branched-chain fatty acids and milk fatty acids groups originating from de novo synthesis ( $\Sigma C4:0 - C14:0$ ) in the mammary gland, dietary origin eventually after rumen metabolism ( $\Sigma C18$ ) or dual origin (C16:0) (g/100g milk fatty acids) vs. milk crude fat (%m/m) in bulk milk samples from 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010)**

Milk fatty acids originating from de novo synthesis in the mammary gland ( $\Sigma C4:0 - C14:0$  and partially C16:0), show a strong negative correlation with PC2, opposite to milk fatty

acids from dietary origin eventually after rumen metabolism ( $\Sigma C18$ ) which showed a strong positive relation. Milk fat evolved quite independently and only showed a weak relation with PC2 and somewhat according to PC1.

Finally, in relation to milk lactose content, we observed an independent performance of lactose values compared to OBCFA in milk from Cuban dairy cattle (Figure 5.5), although a possible relation between C15:0 and/or C17:0 and lactose was expected, since propionate is derived from glucose and a link between propionate and C15:0 and/or C17:0 is suggested.



**Figure 5.5: Loading plot of a principal component (PCA), describing the relationship among odd- and branched-chain fatty acids (g/100g milk fatty acids) and milk lactose (%m/m) in bulk milk samples from 6 dairy farms of “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the Cuban rainy season (July and August 2010)**

#### **5.4. Effect of mineral supplementation in OBCFA concentrations**

The essentiality and role of mineral elements in rumen microbe metabolism have already been emphasized in detail in several reviews dealing with sulfur or with other major minerals and trace elements (Durand *et al.*, 1987). However, in this study milk OBCFA concentrations were not significantly different between three levels of mineral supplementation. Furthermore, the only significant difference was observed in relation to the two dairy farms evaluated (“La Carmita” and “Aleman”) as we explained previously. Nevertheless, according to Durand *et al.* (1980), the amounts of minerals needed to meet microbial requirements are often assessed in terms of concentrations in the rumen medium or of total dry matter content in the diet, which could suggest that higher levels of mineral supplementation may produce significant effects. On the other hand, García *et al.* (2010) reported trace mineral deficiencies of Cu, Zn and Fe in the soil and in serum levels in animals of south-central province of Villa Clara e.g. the average value of Cu was below 11.77mmol/l which is the critical deficiency limit for dairy cows and 75% of the animals (cows and heifers) were diagnosed with hypocupremia. Hence, although no rumen microbial impairment was suggested from changes in milk OBCFA concentrations, mineral supplementation might be recommended for animals under Cuban conditions in terms of animal rather than microbial requirements.

## CHAPTER 6

### Conclusion

Local availability of feed resources in “Desembarco del Granma” Cooperative (Santa Clara - Cuba) was based on *Dichantium spp.* and *Pennisetum purpureum var. CT-115*. As no information was available related to OBCFA in milk produced in tropical conditions we interpreted our results stepwise. First, we compared to milk OBCFA concentrations in milk from cows under temperate conditions. OBCFA proportions showed higher levels in Cuban milk from native Cuban cows or crossbreeds as compared to milk from Holstein Friesian cows in temperate regions. Moreover, increased proportions of iso-fatty acids were also observed in relation to the sum of microbial OBCFA, especially for iso C14:0 and iso C15:0, which is indicative of high levels of lignocellulosic materials and low levels of fermentable carbohydrates in central Cuban cows’ diets. Nevertheless, as all milk odd- and branched-chain fatty acids (except anteiso C17:0), including both branched and odd chains, were influenced positively under central Cuban conditions, we suggested that the origin of this increase could be either an indication of general increase in microbial activity or an indication of limited amounts of dietary fat. However, variation within OBCFA concentration was shown to evolve quite independently from external fatty acids or fatty acids which are de novo synthesized in the mammary gland. Further, a positive relation between milk protein and iso C17:0 was observed. Similarly, CT var. 115 induced higher proportions of iso C17:0 compared to natural grasses and it was reflected in higher levels of milk protein. We suggested that iso C17:0 could be an indicator of limited amounts of rumen degradable protein. In addition, OBCFA were not influenced by different levels of mineral supplementation but were linked with differences between farms. Finally, milk OBCFA could be used as biomarker for rumen function under Cuban conditions. However, differences in milk OBCFA concentrations between milk of temperate and tropical regions needs some further investigation and more research is still necessary to determine OBCFA concentrations in milk of dairy cows: monitoring should be performed during the whole year and in different tropical environments as well as under more controlled experimental conditions e.g. using fistulated dairy cows.

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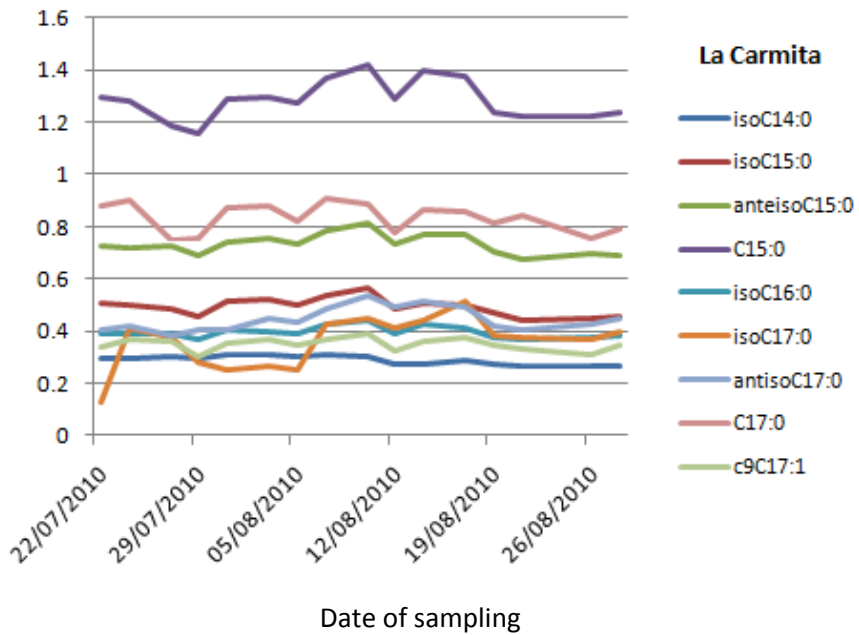
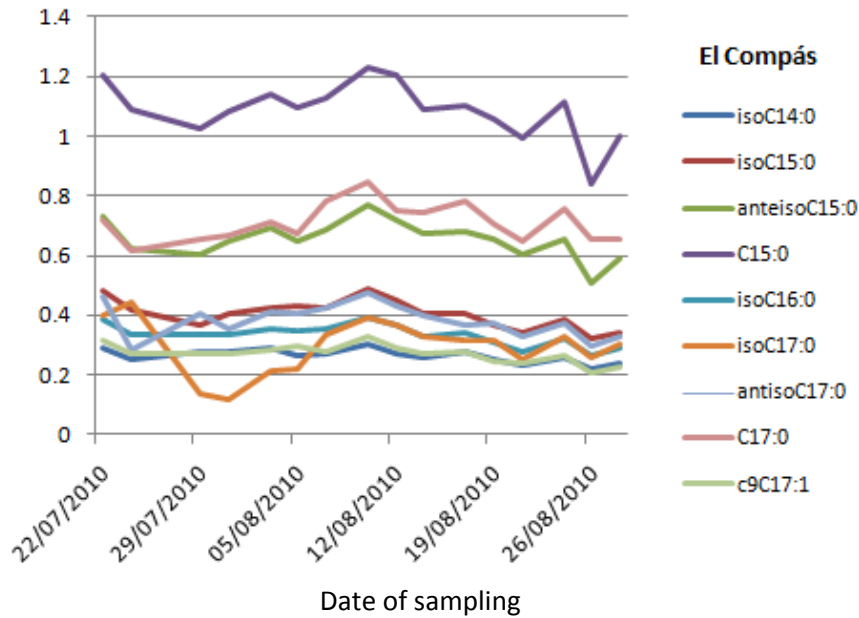
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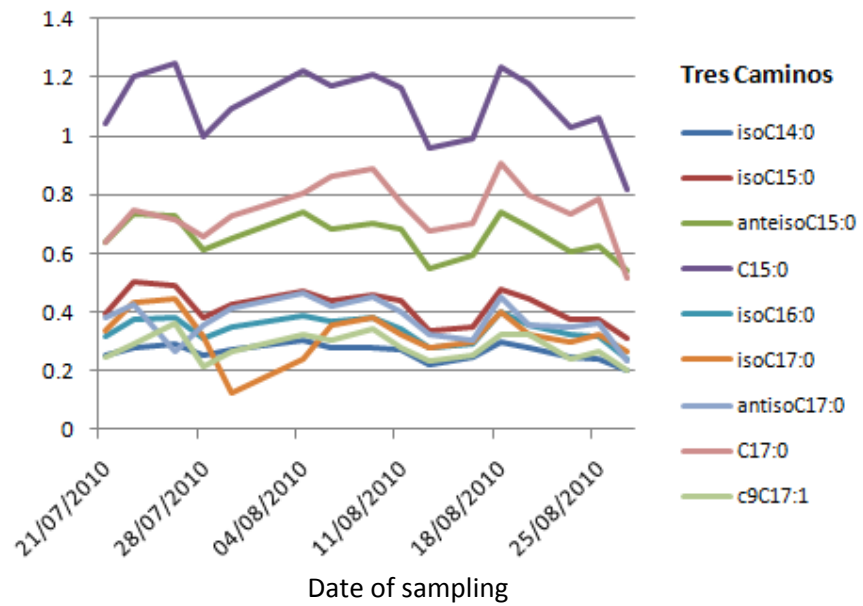
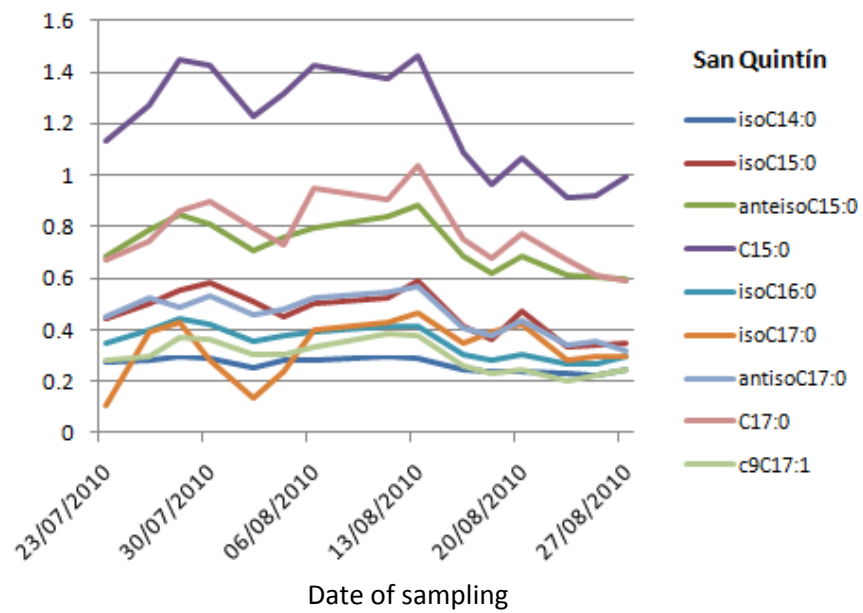
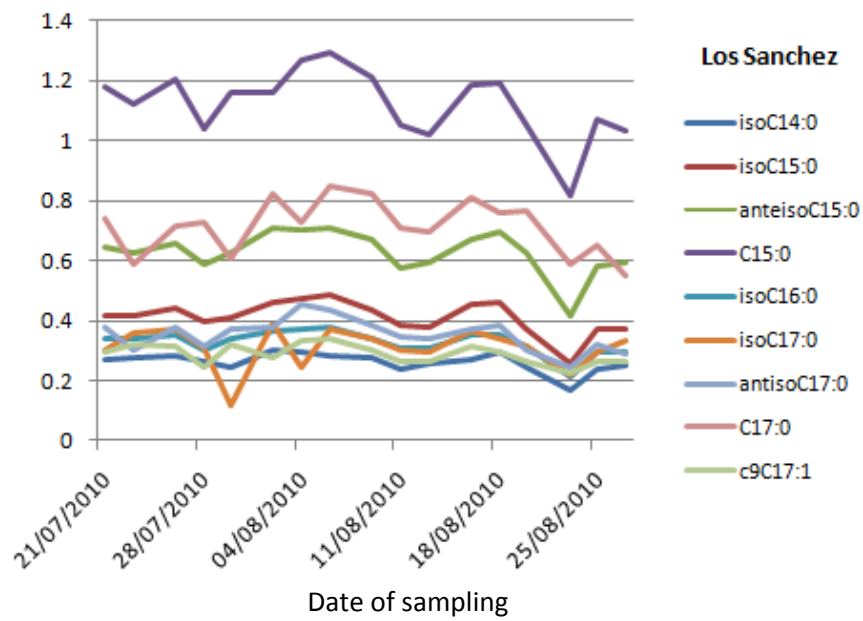
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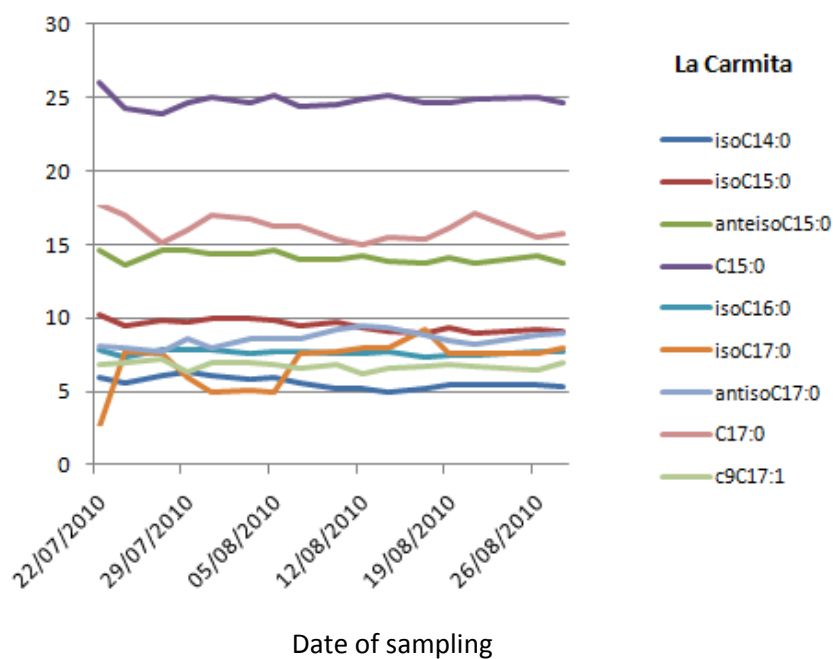
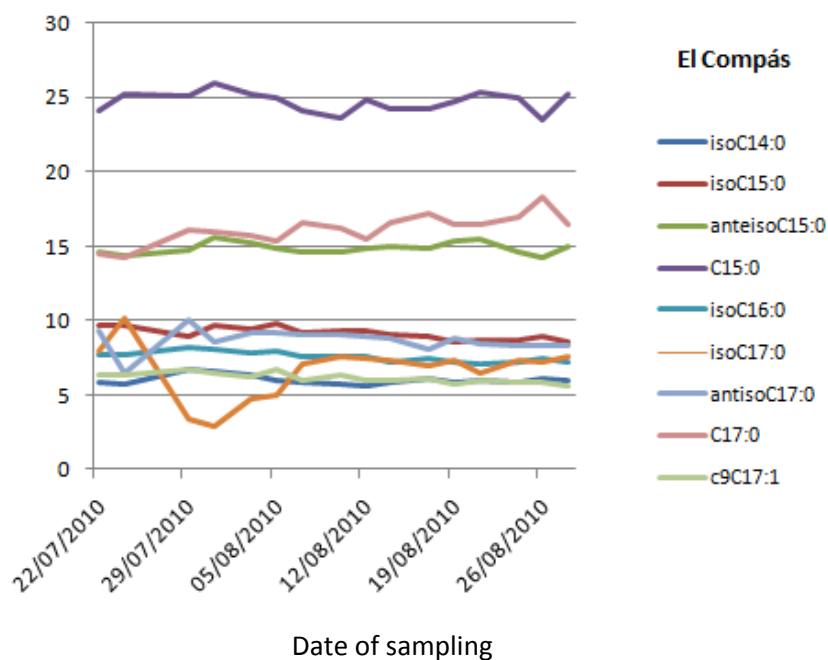
**Appendix 1: Temporal changes in the concentration of milk odd and branched fatty acid (g/100g milk fatty acids) in milk fat, sampled at “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the rainy season (July and August 2010)**

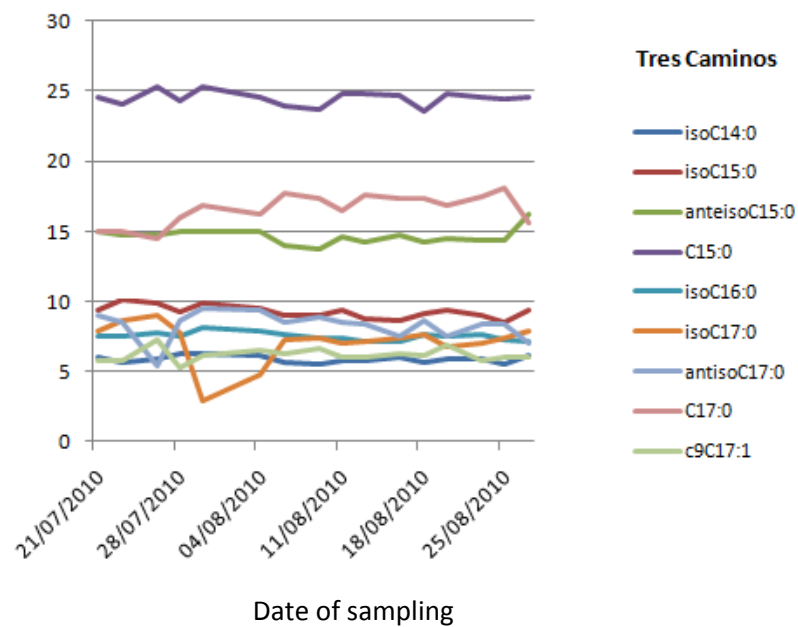
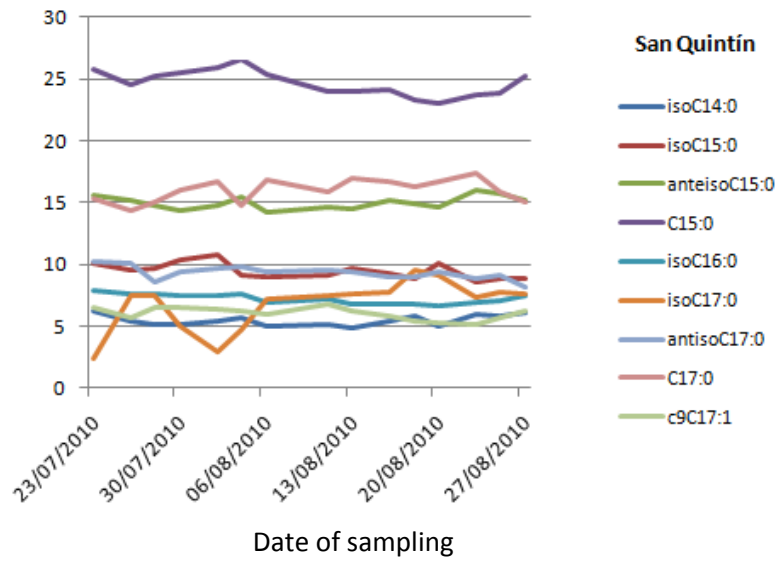
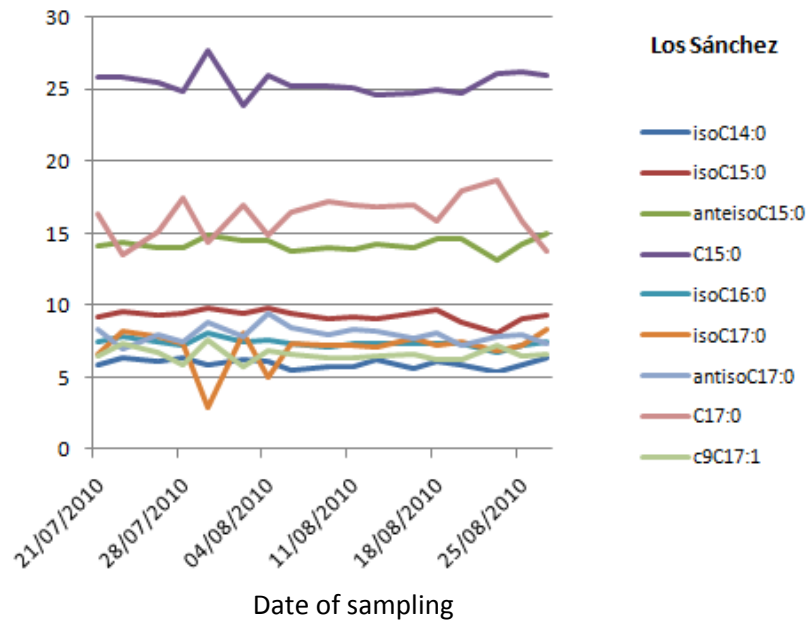






**Appendix 2: Temporal changes in the concentration of milk odd and branched fatty acid (g/100g OBCFA) in milk fat, sampled at “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the rainy season (July and August 2010)**





**Appendix 3: Temporal changes in the concentration of milk fatty acids groups originating from de novo synthesis ( $\Sigma$ C4:0 – C14:0) in the mammary gland, dietary origin eventually after rumen metabolism ( $\Sigma$ C18) or dual origin (C16:0) vs. milk C15:0, sampled at “Desembarco del Granma” Cooperative (Santa Clara - Cuba) during the rainy season (July and August 2010)**

