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PALATABILITY OF CONCENTRATES FED TO SHEEP

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A mia madre….

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TABLE OF CONTENTS

LIST OF TABLE

Chapter 1

Chapter 2

Chapter 3

Chapter 4

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 3

CHAPTER 1

INTRODUCTION

PHYSIOLOGICAL BASIS OF PERIPHERAL SENSES

Generality

Peripheral senses represent the connection between the internal state of an animal and the environment surrounding it. They play a fundamental role in driving the animal in its environment and ensuring its survival.

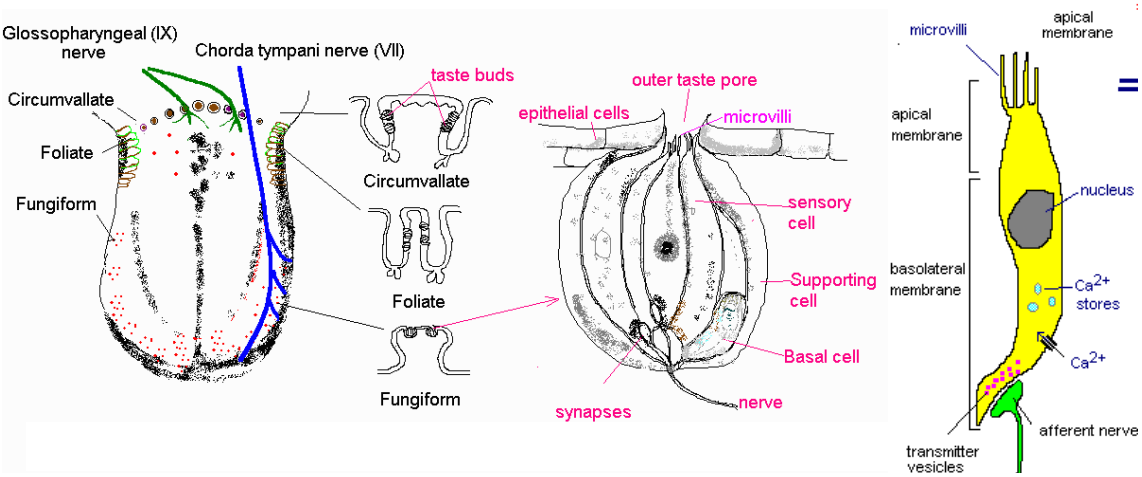
The peripheral senses are commonly distinguished in five apparatuses which are able to distinguish different stimuli: vision, sound, somatosensory, taste and smell. Even if each sensory system is able to distinguish different types of stimuli, they all converge in the brain, where interact to produce a sensation that allows to understand how the external ambient is interacting with the animal. This sensation will drive the animal to react to the stimulus in the best way possible. The effects that feeds can generate in the animal involve mostly the following senses: smell, taste and somatosensing. However, the vision is probably also involved especially in the recognition of feed characteristics.

Taste

In the common language, the word "taste" is often used to describe sensations arising from the oral cavity. However, it is important to distinguish that taste sensations are triggered from feed molecules which act on specific biological structures, defined as chemosensory gustatory system. Along with taste sensations, food usually evokes simultaneously other sensations. These are named in their wholeness as "*flavor"*, *w*hich includes a complex feeling network consisting of a combination of taste, smell, appearance, texture, temperature, mouth feel and past experience (Goff and Klee, 2006). Although it is not always easy to perceive all these sensations separately, the non-gustatory components are sensed by different systems: olfaction and somatosensory organs. The cells which sense the taste are named Taste Receptor Cells (TRCs), which are organized in structures called taste buds, located within the gustatory papillae.

Taste buds are, depending on the species, groups of 50-150 TRCs (Chandrashekar et al., 2006), classified into four cell types, from type I to type IV cells, based on their ultrastructural and morphological features (Lindemann, 1996). These biological structures are supported by several layers of support cells surrounding a central pore, where apical ends of the TRCs are exposed to the oral cavity and interact with taste stimuli, usually water-soluble chemicals (Bachmanov and Beauchamp, 2007). Just below the taste bud apex, taste cells are joined by tight junctional complexes that prevent gaps between cells. Taste buds are typically assembled in special structures in the surrounding epithelium, termed gustatory papillae, which differ for their form and for their location on the tongue surface (Figure 1., Jacob, 2008). The fungiform papillae contain 1-5 taste buds, depending on the species, and are located on the anterior side of the tongue; the foliate papillae contain dozen to hundreds taste buds, depending on the species, and are placed on the posterior lateral sides of the tongue; the circumvallate papillae contain, depending on the species, hundreds to thousands taste buds, and are found in the back of the tongue (Jacob, 2008). Most of the

taste papillae are located on the tongue surface, but there is also a substantial number of non-lingual taste papillae in the palate, oropharynx, larynx, epiglottis, and the upper esophagus (Bachmanov and Beauchamp, 2007). Recently, some taste receptors were also found in the gastro intestinal tract (Dyer et al., 2005). The different disposition of taste papillae are commonly believed to be linked to different taste sensible regions. However, on the contrary, recent molecular and functional data have revealed that all areas of the tongue surface can detect all five different tastes (sweet, bitter, sour, salty, umami) (Chandrashekar et al., 2006).



A. Tongue **B. Taste papillae** **C. Taste bud** **D. Taste receptor cell**

Figure 1. Human tongue with the position of the different types of taste papillae (A-B). Taste bud (C) and scheme of a taste receptor cell (D) (Jacob, 2008).

Taste transduction

The current consensus is that taste sensation can be divided into five qualities: bitter, sour, salty, sweet and umami (i.e. savory) (Bachmanov and Beauchamp, 2007).

Each taste communicates different information about food quality to the animals. Bitter taste often indicates the presence of toxins or spoiled food; salty taste indicates the presence and concentration of minerals in the food. Sweet taste is associated with the presence of carbohydrates, whereas the umami taste indicates the presence of the amino acid L-glutamate, which in turn indicates the presence of proteins in the food. Finally, sour taste is associated to the acidity of the food and, together with bitter taste, warns against the intake of potentially noxious and/or poisonous chemicals. Acid sensing is also important in other processes such as the monitoring of CO_2 levels in the blood (Lahiri and Forster, 2003). Although it may appear oversimplified, all the vast array of taste sensations that can be felt by each individual derives from the combination of these five basal tastes which allow the animals to recognize the composition of foods.

Mammalian taste transduction has been reviewed by several authors (Scott ,2005; Chandrashekar et al., 2006; Bachmanov and Beauchamp, 2007)) who explained that the systems dedicated to recognize this five stimuli are the TRCs, as previously mentioned. According to the same authors, taste receptors function as chemoreceptors that interact with taste stimuli, or ligands, to initiate an afferent signal transmitted to the brain, which results in taste perception. Reception of taste qualities that human describe as sweet, umami and bitter involves proteins from the T1R and T2R families. Some proteins belonging to these families have been proposed as receptors for salty and sour tastes. T1R and T2R receptors belong to a superfamily of Guanine nucleotide binding protein-coupled receptors (GPCRs) (Bachmanov and Beauchamp, 2007), which are heterotrimeric proteins associated to the cell membrane, composed of three subunits: α, β and γ and involved in many signal transduction complexes (Preininger and Hamm, 2004). However, since some other taste stimuli can penetrate cell membranes (i.e. sodium, protons and some bitter compounds) and interact with intracellular targets to activate TRCs,. it is not so clear what would be a taste receptor for such ligands.

A large number of proteins has been proposed to act as taste receptors, however not all of them are accepted by the scientific community (Bachmanov and Beauchamp, 2007).

The modality of taste detection has not been definitively demonstrated. One of the most accepted ways has been named as "*labelled line model"* (see the review of Chandrashekar et al., 2006). This model affirms that each of the five basal tastes is recognized by specific cells, which express specific receptors. This is in contrast with the common believes of the two past decades saying that every taste cell was able to recognize every taste. Therefore, according to the *"labelled line model"*, sweet, bitter, salt, umami and sour are felt by specific receptors.

Sweet, umami and bitter tastes

Sweet and umami perception is mediated by a small family of three GPCRs: T1R1, T1R2 and T1R3.

T1Rs constitute the common base for the perception of sweet and umami tastes and this crucial role has been demonstrated both in rats and humans (Li et al., 2002). In fact, T1Rs are expressed in subsets of TRCs, and their combinations define three cell types: TRCs co-expressing T1R1 and T1R3 (T1R1+3 cells), TRCs co-expressing T1R2 and T1R3 (T1R2+3 cells) and TRCs containing T1R3. Functional expression studies demonstrated that the combination of T1R3 and T1R2 (T1R2+3) forms a heteromer T1R2+3 which is a sweet receptor and that these cells are the sweet-sensing TRCs (Chandrashekar et al., 2006).

The umami taste is the taste of proteins. In humans only two amino acids (monosodium glutamate - MSG - and aspartate) evoke the sensation of umami (e.g. the taste of meaty broths for humans), even if other mammals can feel a larger range of amino acids. Different studies on taste cell expression of rats, reviewed by Chandrashekar et al., (2006), affirmed that T1R1 and T1R3 combine to form the amino acid receptor T1R1+3 and that cells expressing these proteins are the umami taste receptors. Thus, GPCRs are very flexible proteins which

deeply change their selectivity by changing their combination of sub-units (T1R2+3 = sweet; T1R1+3 = umami).

Sweet and umami receptors share a common base to detect different stimuli but each of them recognizes a limited subset of compounds, strictly connected to the nutritional requirements of the organism. In contrast, bitter receptors have to recognize a very large set of compounds and have to prevent the ingestion, even in very small quantities, of noxious molecules. Even if these compounds may have a different chemical composition, they all evoke a common sensation known as "bitter". Therefore, bitter receptors are probably codified by a large number of genes which allow the receptors to recognize a large number of compounds evoking only one sensation. However, the bitter receptors are not able to distinguish among the different classes of compounds and can exclusively alert the organism not to eat a certain feed.

Bitter perception is mediated by a large family of approximately 30 highly divergent GPCRs, the T2Rs, which are selectively expressed in subsets of TRCs that differ from those involved in sweet and umami perception. A large number of T2Rs function as bitter taste receptors and several of them have distinctive polymorphisms associated with significant variations in sensitivity to selective bitter tastants. This can explain why a very broad range of chemical compounds which are not structurally related can evoke a unique sensation. (Chandrashekar et al., 2006). In fact, most of T2Rs are expressed in the same TRCs. This means that individual T2R-expressing cells may function as broadly tuned sensors for all bitter chemical compounds but cannot distinguish among the different chemical compounds evoking the bitter sensation (Chandrashekar et al., 2006). In addition to the activation of T2Rs receptor proteins, some bitter compounds can interact with ion channels in the cell membrane or with intracellular targets. Thus, these proteins may also function as receptors for these compounds (Bachmanov and Beauchamp, 2007). Although being expressed in separate subsets of cells, signals for sweet and umami tastes pass through a common

pathway to transduce tastant recognition into cell activation (Chandrashekar et al., 2006).

Salt and sour tastes

Numerous studies show that salt and sour taste perception implies the direct entry of Na^+ and H^+ into the receptor cells through specialized membranes (see reviews by Scott (2005); Chandrashekar et al., 2006; Bachmanov and Beauchamp, 2007). In the case of salt perception, TRCs activation is believed to be mediated by the entry of Na^+ through amiloride-sensitive Na^+ channels. Even if the identity of the salt 'receptor' is still unknown, it has been demonstrated that salt perception and salt signal transduction is not mediated by G-protein (Wettschureck and Offermanns, 2005).

The perception of sour taste is still being discussed. A broad range of cell types, receptors and mechanisms have been proposed to be responsible for sour taste (Table 1.1), including amiloride-sensitive cation channel (ACCN1); HCN1 and HCN4 (members of a family of hyperpolarization-activated nucleotide-gated (HCN) channels); and several two-pore domain potassium leak conductance channels from the K_2P family (PDKD2L1).

The review by Chandrashekar et al. (2006) suggested, on the basis of gene expression pattern and pharmacological analysis, that TASK-1 is the most likely candidate for sour taste receptor, although other K_2P channels cannot be excluded.

In contrast, Bachmanov and Beauchamp (2007) suggested that the most recent genetic and functional studies demonstrate that a member of the TRP ion-channel family, PKD2L1, demarcates sour-sensing TRCs. In fact, PKD2L1 is selectively expressed in a population of TRCs distinct from those mediating sweet, umami and bitter tastes, and can be considered the most believable candidate to function as sour receptor. These findings are supported by the fact that engineered mice

deprived of the PKD2L1 cells are not able to sense the sour taste (Huang et al., 2006)

Table 1.1. Tastant selectivity of candidate mammalian taste receptors (Chandrashekar et al., 2006; modified).

Tastant quality	Receptor(s)	Class of tastants	Example of tastants
Sweet	T1R2+ T1R 3	Sugar	Sucrose, fructose, glucose, maltose
		Artificial sweetener	Saccharin, aspartame
		D-amino acids	D-Phenylalanine, D-alanine, D-serine
		Sweet protein	Monellin, thaumatin, Curculin
Umami	T1R1+ T1R 3	L-amino acids and peptides	L-Glutamate, L-AP4, glycine*, L-amino acids*
	mGluR4 mGluR1	Amino acids	
	N-methyl-D - aspartatetype glutamate		
Bitter	T2R5	toxic/noxious compounds	Cycloheximide
	T2R4 T2R8 T2R44		Denatonium
	T2R16		Salicin
	T2R38		PTC
	T2R43 T2R44		Saccharin
	Unknown	Other toxic/noxious compounds	Quinine, strychnine, atropine
Salty	Na^+ channels	Minerals and electrolytic balance	NaCl and other salts
Sour	PKD2L1	Acids, pH	Citric acid, tartaric acid, acetic acid, hydrochloric acid
	ACCN1		
	HCN1, HCN4		
	TASK1 and others		

*Preferentially activates mouse but not human receptors.

Smell

Smell, together with view, is the first sense that gives the animal important information regarding the surrounding environment. For example, a predator (e.g. a dog) can smell the prey from many km of distance, and in this case smell is much more efficient than view to provide information essential for survival.
This kind of biological chemical detectors has to be able to recognize and distinguish among hundred of thousands of low molecular mass chemical compounds and the brain has to be able to associate a specific effect to each compound or mixing of chemical compounds. For example, more than 7000 flavor volatiles have been identified and catalogued from foods and beverages and a single fruit or vegetable synthesizes several hundred volatiles (Goff and Klee, 2006). The ability to manage this sophisticated discriminatory task is the result of an evolution of thousand of years during which each animal species developed its specific capability to recognize smells strictly connected to their specific life environment and behavior. Even if each animal species has its own smell capability and specificity, every animal, from the simplest organism to the most complex, shares a common organization of the olfactory system which is divided in two sub-systems:

- ✓ *Common or main olfactory system*

  This is an "open" system because it is not possible to predict which kind of chemical compound it will run into. This system is used by animals to find food, detect predator and prey, and to mark territory.

- ✓ *Second or accessory olfactory system*

  This system is directly connected to the reproduction needs, being important for species survival. It has developed for the specific task of finding a receptive mate. It is also known as *vomeronasal system* has developed following a specific evolutionary exigency to recognize liquid-borne compounds named "*pheromones"*, which act as sexual signals (Sánchez-Andrade et al., 2005) This type of signal provides information regarding not

only the position of the other sex, but also its reproductive state and sexual availability. This signal is also important to regulate animal social-behavior (Firestein, 2001)

Organization and physiology of the olfactory system in vertebrates has been recently reviewed by Firestein (2001). This review showed that the fundamental cells of the olfactory system are the olfactory sensory neurons (OSNs). These neurons represent the direct connection among volatile compounds and the brain. The OSNs of the mammals are located in the upper side of the nasal cavity in groups of 6-10 millions to form special cartilaginous structures named "turbinates". Each OSN is a bipolar neuron composed by a single dendrite extended up to the surface where it ends forming a knob from which 20-30 really thin cilia project; the latter are the real site of the sensory transduction. Signals are transported through a very thin axon which ends in a region called "olfactory bulb". Every axon coming from a specific receptor ends in a specific region of the olfactory bulb called "glomerulo". Glomeruli are spherical structures formed by the conjunction among the incoming axons of the OSNs and the dendrites which project from the mitral cell in the bulb. Then, the mitral cell receives the information coming from the OSN and projects this information to other regions of the brain.

The olfactory receptors (ORs) are formed by proteins similar to those of the taste receptors (GPCRs), but more than 1,000 genes express the GPCRs of the ORs, making them the largest gene family of the genome. About 60% of these genes are thought to be pseudogenes.

ORs are highly specific and the same receptor can recognize a different molecule depending on the species considered. For example, in the case of a receptor known as I7, the mouse and rat hortologues showed a differential response with one being more sensitive to octanal and the other to heptanal. Among the 15 amino acids that differ in the two genes, a single residue in

transmembrane domain 5 (valine or isoleucine) was found to be sufficient to confer this different sensitivity.

Role of fruit and vegetable smells and flavors in animal nutrition behavior

The relationship between the volatile fraction of vegetables and the perception of nutritious or healthy compounds has been reviewed by Goff and Klee (2006). These authors, based on several studies, hypothesized that mammals can discriminate among vegetables by recognizing in their volatile fraction (flavor) the presence of nutritious or healthy compounds. In fact, olfaction is a very sophisticated apparatus that evolved in thousand of years during which each animal species has developed its specific ability to recognize the smell characteristics of their specific habitat. An important part of the habitat of animals is composed of vegetables which not only constitute the diet of herbivores but also provide important nutrients to other animal categories. Plants are capable of synthesizing tens to hundreds of thousands of primary and secondary metabolites with diverse biological properties and functions. Plant organic volatile compounds generated from both primary and secondary metabolites are generally low molecular lipophilic compounds. Although a single fruit or vegetable synthesizes several hundred volatiles, only a small subset generates the flavor fingerprint that helps animals and humans to recognize appropriate food and to avoid poor or dangerous food choices. Flavor preferences begin to develop before birth and develop rapidly during the first month of life (Hudson and Distell, 1999; Schaal et al., 2000; Mennella et al., 2001). Flavor preference requires several feeding experiences in order to develop (Rozin, 1990; Villalba and Provenza, 1997a), whereas flavor aversion can be learned much more rapidly and depends on the concentration of toxin present in the feed (Garcia et al., 1955; Rozin, 1990; Launchbaugh and Provenza, 1994).

The review of Goff and Klee (2006) used tomato as an example, but, in reality, each vegetable or fruit produces many different volatile compounds with the aim

to facilitate seed production and dispersal. Thus, plants need to produce attractive flavor to facilitate seed dispersal and frequently they produce volatiles (or volatile precursors) which have antimicrobial or health-promoting activities. Thus, flavor volatiles can be perceived as positive nutritional signals. The impact of a chemical on flavor perception is determined by both its concentration and the odor threshold (the ability to sense it). Flavor threshold is highly variable and is often very low for the most important volatiles present in fruit. In addition, frequently only a small number of volatiles produced from a fruit has a positive impact on flavor perception. It is interesting to see how domestication programs of fruit species have affected their volatile composition. Breeding programs have historically focused on yield, color, shape and disease resistance. In contrast, flavor, which is a complex, multigenic trait, has not been a high priority for them. Therefore, selection for characters other than flavor had negative consequences on fruit flavor and may have influenced feed perception by animals.

As explained in detail by Goff and Klee (2006), plant volatiles derive from chemical reactions which involve compounds having healthy effects on humans. In fact, in tomato fruit the most abundant volatiles are derived from catabolism of essential fatty acids. These volatiles are associated with flavors described as "tomato", "green" or "grassy". They are derived from linoleic acid (hexanal) and linolenic acid (cis-3-hexanal, cis-3-hexenol, trans-2-hexanal) via lipoxygenase activity and are, therefore, indicators of the presence of free fatty acids classified as essential to the human diet. The six-carbon aldehydes and alcohols derived from omega-3-linolenic acid are also important flavor components of diverse groups of plant products including apple, sweet cherry, olive, bay leaf and tea. Breakdown of linoleic acid generates decadienoate esters important for pear flavor as well as butanoate esters and hexanol important for banana flavor. Essential fatty acids are also degraded to lactones in peaches, apricots, and coconuts. Many of the fruit aliphatic esters, alcohols, acids, and carbonyls are derived from essential fatty acids (Goff and Klee, 2006). It is interesting to note that some of these compounds (free fatty acids) that have beneficial effects on

human health can have, in contrast, a noxious effect on ruminants because they are toxic for rumen microbes (Palmquist and Jenkins, 1980; Chalupa et al., 1984).

A second class of volatiles that contribute positively to tomato flavor is derived from the essential amino acids leucine, isoleucine and phenylalanine, thus being indicative of its content of free amino acids. These volatiles (2- and 3-methylbutanal, 3-methylbutanol, phenylacetaldehyde, 2-phenylethanol, methyl salicylate) are important flavor constituents of many fruits. Some non essential amino acids are metabolized to volatiles; most notably cysteine is the precursor of allicine, an important flavor component of garlic which has antibacterial and antifungal activities (Goff and Klee, 2006).

An important characteristic of fruit flavor volatiles is that even if few of them are produced in detectable quantities throughout fruit development, most of them are mainly associated with fruit ripening. The specific appearance of these volatiles during fruit ripening and their relative absence from vegetative tissues suggests a role in signaling ripeness and attracting seed dispersal organisms.

Thus, tomato, as well as other flavored fruit, produces a set of signals indicating fruit ripening and their nutritional and healthy properties.

Unlike ripening fruits, vegetables produce most of the volatiles sensed as flavor only after their cells are disrupted. This disruption mixes substrates with enzymes responsible for generating flavor volatiles. For example, garlic, onions and mustards, as well as, some other vegetables, produce the volatiles allyl isothiocyanate and allicine after cellular disruption. These volatile flavor compounds exhibit antimicrobial activity when present in a variety of foods. In human nutrition the spices are a category of foods common in all the population. The synthesis of volatiles in popular spices suggests that flavor perception is linked with specific health properties. For example, curcumin, a major flavor volatile of the spice turmeric, is reported to have both anti-inflammatory and anti-tumor activities. Many spices with flavors appreciated in a variety of cultures are reported to have antimicrobial activities, including allicine from

garlic, and thymol, borneol, isoborneol, eugenol, allyl isothiocyanate, and cavracol from rosemary, sage, clove, mustard, chili pepper, and thyme. Thus, due to these flavor properties, spices can be sensed by humans as an indicator of good preservation of the foods and as a source of healthy compounds (Goff and Klee, 2006)

Behavioral studies reported in the review of Goff and Klee (2006) support the hypothesis of a connection among sensory perception, flavor preferences and health benefits. For example, rodent feeding studies demonstrate that preferences for bitter or other undesirable flavors can be learned when those flavors are associated with desirable nutrients. Herbivores learn to consume toxin-containing plants with additional foods that neutralize their toxic effects (Villalba and Provenza, 2002, 2005). Learned and innate preferences follow different pathways in the brain. In fact, mutant mice in which olfactory sensory neurons in a specific area of the olfactory epithelium are ablated, by targeted expression of the diphtheria toxin gene, lacked innate responses to aversive odorants, even though they were capable of detecting them and could be conditioned for aversion with the remaining glomeruli. These results indicate that, in mice, aversive information is received in the olfactory bulb by separate sets of glomeruli: those dedicated for innate and those for learned responses (Kobayakawa et al., 2007).

Goff and Klee (2006) concluded their review affirming that there is a correlation between health and the volatiles that contribute to the positive perception of foods. These flavor signals of fruit have evolved (at least in part) to provide positive information to seed-dispersal organisms. In many fruit, almost every important volatile is derived from an essential nutrient. Not all desirable volatiles derive from essential nutrients, nor all volatiles derived from essential nutrients are viewed as desirable among the different animal species. Essential fatty acids, as some other compounds, can be metabolized to produce off flavors in certain circumstances such as the off-flavors generated by the lipoxigenase activity during soybean processing. Despite few exceptions, essential nutrient-derived aromas are are correlated positively with their precursors.

Somatosensing

The sense of touch, named as somatosensing, provides a wide range of information including the sensation of balance and coordination, pressure and vibration, pain and temperature. This sense, in addition to taste and smell, plays a role in food selection, giving information about temperature, creaminess and, in general, physical properties of food. In addition, the sense of touch also acts as an initial alert system that signals when there are potentially dangerous or damaging environmental conditions.

The somatosensing system will be briefly described here, based on the review by Patapoutian et al. (2003). The neurons that sense these distinct stimuli are located in the dorsal root ganglia (DRG) and within cranial nerve ganglia such as trigeminal ganglion. The DRG neurons are specialized and they can be classified on the basis of the kind of stimulus they detect as proprioceptors, low-threshold mechanoreceptors, and cells that sense pain and/or temperature. Proprioceptors are sensory terminals that are present in muscles, tendons and joint capsules, and receive information about the movements and position of the body. Nociceptive (pain) neurons detect noxious thermal, mechanical (high threshold) or chemical stimuli. Thermosensitive neurons detect temperature either in the noxious range or in the innocuous range. The DRG neurons are pseudounipolar: one cell process travels long distances reaching peripheral tissues such as the skin and muscle, where it detects sensory stimuli, whereas another branch relays this information to the dorsal horn of the spinal cord. Whereas the peripheral branches of proprioceptive and low-threshold mechanosensitive neurons terminate in specialized organs in the skeletal muscle and skin, the axons of temperature- and pain-sensing neurons travel to the epidermal end of the skin and terminate as free nerve endings. On the basis of their conduction velocities, both temperature- and pain-sensing neurons can be of either types: small-diameter, slowly conducting unmyelinated C-fibers or larger-diameter, more rapidly conducting, thinly myelinated Aδfibres. However, recent studies of other sensory

modalities have shown that the sensory ion channel can be either directly gated by the sensory stimulus or activated indirectly through a signaling pathway that involves GPCRs activation. For thermosensation, the process is thought to begin through specific receptor proteins that are located within the free nerve ending in the skin (Patapoutian et al., 2003). A more recent system proposes the involvement of the direct activation of thermosensitive excitatory transient receptor potential (TRP) ion channels sensible to either cold or hot stimulus (Lumpkin and Caterina, 2007).

At present, six temperature-sensitive TRP channels have been described (see the review of Lumpkin and Caterina, 2007), which together cover almost the entire range of temperature that mammals are able to sense. In particular, four TRP channels belonging to the TRPV subfamily are activated by heating, with characteristic activation temperature ranging from warm temperatures (> 25°C for TRPV-4; > 31°C for TRPV-3) to heat (> 43°C for TRPV-1) and noxious heat(> 52°C for TRPV-2). Differently, TRPM8 and TRPA-1 are activated by cooling (< 28°C for TRPM-8; < 18°C for TRPA-1).

Thermoreceptors and mechanoreceptors give important information in food selection, because the first respond to stimuli such as cool, minty and spicy and the second give information about physical properties such as creaminess, thickness and others.

Cool, minty and cold

The cooling sensation of mint-derived menthol is well known (see the review of Patapoutian et al., 2003). In fact, several studies of skin cold-receptive fields indicate a strong correlation between menthol and cold sensitivity of individual free nerve endings. Menthol also modulates the activity of cool-induced currents recorded from lingual and nasal induced currents.. Menthol and cooling stimuli are transduced through a non-selective cation channel that is located within the cutaneous peripheral projections of DRG neurons. Such channel, TRP melastin 8

(TRPM-8), is activated by chemical cooling agents (such as menthol) or when temperatures drop below 26 °C, suggesting that it mediates the detection of cold thermal stimuli by primary afferent sensory neurons (Patapoutian et al., 2003).
Recently, Bautista et al. (2007) showed that cultured sensory neurons and intact sensory nerve fibers from TRPM-8 deficient mice showed strongly diminished responses to cold. These animals also showed a clear behavioral deficit in their ability to discriminate between cold and warm surfaces, or to respond to evaporative cooling. At the same time, TRPM-8 mutant mice were not completely insensitive to cold as they avoided contact with surfaces below 10°C. This work demonstrated an essential and predominant role of TRPM-8 in thermosensation over a wide range of cold temperatures, validating the hypothesis that TRP channels are the principal sensors of thermal stimuli in the peripheral nervous system. The same role of TRPM-8 in cold sensation was recently confirmed by Colburn et al. (2007), who showed that sensory neurons derived from TRPM-8 null mice lacked detectable levels of TRPM-8 mRNA and protein and that the number of these neurons responding to cold (18 °C) and menthol (100 μM) was greatly decreased. Furthermore, Dhaka et al. (2007) showed that mice lacking TRPM-8 had severe behavioral deficits in response to cold stimuli.

Hot and spicy

The studies reviewed by Patapoutian et al. (2003) indicate the presence of two types of sensory fibers classified on the basis of their temperature response threshold. Some fibers respond to a moderate threshold (~ 43°C), whereas a smaller percentage respond to a high threshold (~ 52°C). The 43 °C point is within a range at which we perceive a shift from innocuous to noxious heat. Responsiveness to capsaicin (the hot ingredient of chili peppers), a vanilloid compound, was shown to be a primary pharmacological trait of a main

subpopulation of heat-sensitive neurons, particularly those with small to medium-sized fibers that are activated at 45 °C.

The cloned receptor TRPV-1, which belongs to the TRP family, has been proposed as the channel that mediates inception at the pain threshold (≥ 42°C). In fact, heterologous expressions of TRPV-1 resulted in capsaicin-gated currents similar to the responses that are evoked in sensory neurons by the same currents. Importantly, TRPV-1 is also activated by noxious temperatures equal to or higher than 42°C Patapoutian et al. (2003).

Mechanotransduction

Touch receptors respond to pressure, stretch or air movements. These receptors are responsible for some sensations in the mouth such as creaminess, hardness, fragility, and all sensations linked to the physical characteristics of feed. The mechanisms of perception and transduction have been recently reviewed by Lumpkin and Caterina (2007). These authors established that the perception of painful touch is initiated by high-threshold C- and Aδ-nociceptors that can be polymodal or solely mechanoreceptive; while light touch is sensed by Aβ afferents with low mechanical thresholds. C-fibers are involved also in light touch sensing and seem to be involved in social interaction such as the maternal bonding.

Even if the mechanism of transduction is unknown, it seems that the transduction channels are activated directly by mechanical stimuli. Three theories have been proposed to explain the activation of ion channels (Lumpkin and Caterina, 2007). The first affirms that their activation is stretch-activated; a second theory affirms that ion channels require a link with extracellular protein or cytoskeletal protein to be activated; while the third theory affirms that the ion channels are indirectly activated through the mediation of protein in the lipid bilayer. One limitation of indirect mechanisms is that they are intrinsically slower than direct mechanical gating. In fact, direct gating was first proposed for hair cells because of their

remarkable transduction speed (about 40 μs). Because this delay is sufficient to accommodate vesicle fusion, transmitter diffusion and activation of ligand-gated ion channels, this timescale alone cannot rule out indirect coupling models (Lumpkin and Caterina, 2007).

Regulation of food intake and peripheral senses

Peripheral senses play a fundamental role in the control of food intake, as confimed by recent neurobiological studies on the involvement of peripheral senses in processes regulating food intake in mammals (e.g. see review by Morton et al., 2006). In fact, Morton et al. (2006) demonstrated that with the aid of cognitive, visual and olfactory cues, food must first be identified and distinguished from a large range of nutritious and potentially toxic environmental constituents. Subsequently, flavor information must be associated with both short- and long-term signals regarding nutritional state. The integration of this information will influence the animal in his decision to eat and will regulate the quantity of food eaten. The regulation of the quantity of food eaten is a very complex mechanism not fully understood. The common knowledge is that feed intake is regulated by two integrated systems, the *short* and *long* term system of regulation of food intake each involving a set of signals which communicate to the brain the nutritional status of the body. All the mechanism acts to maintain the "energy homeostasis" and thus adjusts the intake over time to maintain stable the amount of body fuel reserves stored as fat (Morton et al., 2006)

The long-term regulation of food intake acts in order to maintain a stable amount of body fat reserves. The theory of the adiposity negative feedback is based on the assumption that circulating signals inform the brain of changes in body fat reserves, and that, in response to this input, the brain regulates the energy balance in order to stabilize the body fat content (Kennedy, 1953). The most important long-term signal regulators are leptin (a strong suppressor of food intake and thereby inducing weight loss) and insulin (another hormone which suppress energy intake). The role of these two hormones in the long-term regulation of food intake is due to their characteristics. In fact, insulin and leptin concentration in the blood is proportional to the body fat reserves and these molecules can enter the brain. Numerous insulin and leptin receptors have been found in many regions of the brain involved in the regulation of food intake (Havel, 2001). Their

action on the regulation of food intake has been confirmed because both leptin (Klok et al., 2007) and insulin (Schwartz et al., 2000) promote weight loss by acting in these brain regions, while lack of these neuronal signals increases food intake and body weight gain (Schwartz et al., 2000).

However, other gastrointestinal (GI) hormones (ghrelin, Peptide YY_{3-36} (PYY_{3-36})) and nutrients (amino acids, fatty acids) seem to be involved in the long-term regulation of food intake (Havel, 2001). For example, PYY_{3-36} is a hormone that reduces food intake and plasma levels of PYY_{3-36} decline in advance of meals. On the contrary, ghrelin is a powerful orexigenic (appetite stimulator) hormone (Wren et al., 2001) produced primarily in the stomach whose concentration peaks before meals and rapidly decreases after the beginning of meals (Cummings and Overduin, 2007).

Among the nutrient-related signals implicated in the homeostatic control of feeding, free fatty acids are involved because they exert an effect similar to those of insulin in several areas of the brain involved in the energy homeostasis process, by favoring intracellular accumulation of long-chain fatty acyl-CoA (LCFA-CoA). It has been proposed that the intracellular LCFA-CoA accumulation signals nutrient abundance, whereas the enzyme AMP-activated protein kinase (AMPK) senses nutrient insufficiency. This system feels the availability of energy through the ratio AMP/ATP. When this ratio reaches a critical level, AMPK is activated to increase the activation of substrates and then the production of ATP. The activation of AMPK causes an increase of food intake. The AMPK activation is inhibited by insulin and leptin action but is stimulated by ghrelin (Minokoshi et al., 2004, Andersson et al., 2004 cited by Morton et al., 2006). Therefore, altered signaling of AMPK can affect the feeding effects of both insulin-leptin and ghrelin (Morton et al., 2006).

Long time regulation of food intake can be influenced by sensorial characteristics of food. There is some evidence suggesting a possible influence of peripheral senses on this process. For example, Nombekela and Murphy (1995) tested the effect of feeding a sweetened or unsweetened total mix ration (TMR) on total dry

matter intake in dairy cows during the entire lactation period. Sucrose application did not have any effect on dry matter intake during lactation. Nevertheless, cows fed the sweetened TMR ate more than those fed the unsweetened TMR at the beginning of the lactation, which is the most critical period for a dairy cow, when the maximization of dry matter intake is fundamental to reach high milk production levels.

A more recent work (Thomas et al., 2007) conducted on calves suggested a possible effect of peripheral senses on long term regulation of feed intake. In this case, three groups of calves were fed a calf starter which differed for the flavor added to the water: water without any flavor added (control), water with orange flavor and water with vanilla flavor. Dry matter intake and daily weight gain for the group fed water with orange flavor was higher than those of the other two groups. The results of this work indicate the possibility of enhancing the long term food intake by increasing the hedonic perception of food.

The "satiety" signals coming from the gut are transmitted, via vagal afferent fibers, to the nucleus tractus solitarius in the hindbrain, which is the same area of the brain participating in gustatory, satiety and visceral sensation. Obviously, the maintenance of the homeostatic equilibrium involves the adjustment of intake on a meal-to-meal basis, and thus it needs a system of control which regulates the meal size and frequency and interacts with the long term control of food intake (Morton et al., 2006).

The short term regulation of food intake implies the triggering of the sense of satiation which in mammals is activated by gastric distension and by the release of GI factors like cholecystokinin (CKK).

CCK is an intestinal hormone produced in response to increasing concentration of nutrients (especially lipids and proteins) in the intestine lumen (Cummings and Overduin, 2007). Moreover, CCK is found and produced in many areas of the central nervous system involved in the regulation of feeding behavior and its action as food regulator works through the activation of CCKA receptors subtype. In fact the antagonist of the subtype receptor CCKA., increase the intake

in monkeys, moreover, rats with altered CCKA receptors showed an higher energy intake respect to normal rats (Havel, 2001). CCK acts on short term regulation of food intake and, in fact, its effects ragarding reduction of food intake are compensated by the action of long-term regulators of food intake (leptin and insulin), whose concentration are decreased by increased concentrations of CCK (Havel, 2001) The perception of food reward begins with the acquisition of information regarding the food flavor through the peripheral senses. This information is transmitted to different areas of the brain which collectively permit the discrimination of the feeds on the basis of their flavor (which includes taste, smell, and texture). The "secondary taste neurons" is an area of the brain located in the orbifrontal cortex where taste, visual, olfaction and cognitive information are integrated. The responses of these cells are "hunger-dependent" because their activity decreases during the meal consumption. This fact implies that the activity of these cells is regulated by satiety signals, which regulate the beginning and the termination of meals. Maybe also body fat related-signals are involved; in fact, both leptin and insulin inhibit the brain reward circuitry, because low concentration of these hormones augments food palatability, whereas high concentration of both diminishes it.

More recent works on neurobiology suggest a new explanation for the relationship between food reward and regulation of food intake. For example, Baly et al. (2007) demonstrated that leptin and different isoforms of its receptors are expressed in the olfactory mucosa of rats and that leptin is synthesized locally in that mucosa. In addition, immunoreactivity was detected on cilia membranes, where odorants bind to their receptors. Interestingly, fasting significantly enhanced transcription of both leptin and its receptors in rat olfactory mucosa, suggesting that leptin can be a strong regulator of olfactory function, acting as a neuromodulator of the olfactory message in the cilia of olfactory mucus. Based on the consideration that fasting and satiation can modulate the olfactory detection in rats (Aimé et al., 2007), Julliard *et al.* (2007) conducted an interesting experiment demonstrating that orexin increased and leptin decreased

olfactory sensitivity. This confirmed their hypothesis that leptin and orexin (a strong stimulator of food intake secreted in the hypothalamic neurons and released in the olfactory bulb) are involved in the signaling between hypothalamus and olfactory mucosa and that these hormones act on olfactory sensitivity very similarly to they way they act on fasting and satiety. Therefore, orexin and leptin appear to be important factors in the interdependency of olfaction and food intake.

Then, regulation of food intake starts before the first bit of food is ingested. The effects of vision or only of the hearing of signals related to food administration are well known as Pavlov's reflexes (Pavlov, 1902). In brief, this mechanism implies the activation of the receptors by food-related stimuli and is more related to neural than nutrient stimulation; because of that the phenomenon is named cephalic-phase. One of the most known effects of this type of responses is the cephalic-phase insulin response, which consists in a quick release of insulin after oral stimulation that in humans reaches the peak after 4 min and returns to the normal values 8–10 min after the insulin release; also glucagon and pancreatic polypeptide are released after the stimulus, but their effects are not well understood. The magnitude of the cephalic-phase response is thought to be related to the palatability of the food, but this effect is still debated. However, Teff (2000) have demonstrated that high palatable foods tend to increase the insulin and the pancreatic polypeptide release compared to less palatable foods.

Another much known cephalic-phase response is the increase of salivary flow. This effect can be explained with the multiple role of the saliva in the digestive processes such as the maintenance of the integrity of the oral cavity, the initiation of the digestive processes, the facilitation of swallowing, the buffer effect in the oral cavity and the regulation of rumen pH (Mattes, 2000).

FEED REWARD AND RUMINANT NUTRITION

Generality

To be consumed, a feed has first to be recognized as edible, in order to provide information about its content of healthy or compounds. The role of some senses (i.e. sight, smell, touch and taste) in the feeding behavior of ruminants has been studied and reviewed by several authors (Arnold, 1970; Goatcher and Church, 1970; Demarquilly, 1978; Church, 1979; Grovum, 1988) with the aim to individuate which variables affect feed palatability. However, the concept of palatability is still controversial. The word palatability usually designates those characteristics of a feed that invoke a sensory response in the animal (Greenhalgh and Reid, 1971) and is considered to be the corollary of the animal's appetite for the feed (Baumont,1996).

Scientific research on animal nutrition, which has focused on other aspects, has probably not given enough attention to the role of senses in the processes of regulation of feed intake. In fact, none of the published feed intake prediction systems takes into account the sensory response to the feed as a factor acting in these processes (Baumont, 1996). This could be due to the fact that the effects of senses on feed intake regulation are not clear, and that even the definition of palatability is not so clear. Different authors interpret palatability differently and, in several cases, the word "palatability" is not completely accepted by the scientific community, because it is not clear if it takes into consideration the influence of a different physiological state of the animal on the sensorial perception of the same feed.

The various definitions of palatability can be summarized as follows. Greenhalgh and Reid (1971) and Church (1979) defined palatability as the "dietary characteristics or conditions which stimulates a selective response by the animal"; thus palatability was considered as an inherent characteristic of the feed as affirmed by Hodgson (1979).

For Matthews (1983), the palatability of a feed is interchangeable with preference for the feed. It is determined by the taste, smell, appearance, temperature and texture of the feed.

Forbes (1986, 1995) claimed that palatability cannot be considered solely as a quality of the feed, since it depends on the experience and metabolic status of the animal considered. Thus, palatability of a feed is not absolute and depends on the state of hunger of the animal (Gallouin and Le Magnen, 1987).

Jarrige (1988) affirms that palatability of the feed is the corollary of the appetite of the animal, which is the stimulation to eat awaked by the feed. From this point of view, eating rate, especially at the beginning of the meal, is a good criterion to determine the animal's appetite, and palatability includes all the physical (plant bearing, spines, etc) and chemical (odor, taste, etc) characteristics of the feed that act on appetite.

Mertens (1996) defined palatability as a characteristic of feeds that is associated with gustatory, olfactory, or visual acceptability by animals. Although palatability is a feed characteristic, it is in part the result of learned behavior of the animal. Therefore, it should be related with differences in intake that are not explained by differences in fill or nutrient availability of the feed. Moreover, Mertens (1996) distinguished between feed palatability and feed preference (or selection), defining the last as a specific indication of palatability that is related to the relative acceptability of feed components when the animal is given a choice. Although preference gives information about differences among feeds, it may not affect intake when a single feed is offered (Black et al., 1989, cited by Mertens, 1996).

However, Mertens (1996) does not mention physical characteristics of the plant; thus, it is not clear if the physical characteristics that determine, for example, ease of prehension and ease of mastication are components of palatability or not.

For animals fed indoors, it is well known that the same hay in long, chopped or ground form is not eaten at the same rate and amount (Jarrige et al., 1995). This is an important aspect, because Villalba and Provenza (1998) found that lambs

ingested barley and alfalfa at higher rates when feeds were offered whole than when they were ground, which was consistent with previous findings (Black and Kenney, 1984; Kenney and Black, 1984).

Taking in account all these different opinions, the point of view of other authors such as Rolls (1986) and Provenza (1995, 1996) seems more acceptable in that palatability is defined as the interrelationship between flavor and post-ingestive effects, influenced by feed's chemical characteristics, animal's nutritional state and past experience with feed.

Methods to measure palatability

A method commonly accepted to measure feed palatability in ruminants has not been defined yet, probably because this is a relatively recent field of study in animal nutrition. One of the biggest problems in measuring feed palatability is to distinguish the effects of feed reward and the post-ingestive consequences of ingestion that feed stimulate in the animal. In fact, as already mentioned, feedback influences liking for a flavor and factors such as food's chemical characteristics, animal nutritional state and its past experiences with the food influence flavor-feedback preferences. Another fact which causes difficulties in measuring feed palatability is the different roles of smell and taste on feed perception and feed-back process. In fact, olfaction is involved in animal protection from predators and from the assumption of toxic feeds, thus acting in a "short-time" protection. Differently, taste is strictly connected to the process of learning because it is directly connected with neurons coming from bowels and is involved in the feed-back process of learning, which requires a time scale of minutes or hour. In summary, the association of taste, smell and feed-back sensations determines the rejection of a feed in a process of strengthening. This problem is more important when the objective is to determine the effect of the first impact of feed sensorial properties on the animal. For example, when a feed is firstly offered to an animal, it is often refused (phenomenon named "feed

neophobia") but, at the same time, even when the animal is already familiar with certain feeds, some feeds can be accepted whereas others can be refused. Probably, the sensorial signals of the feed can act in different ways depending on the nutritional status of the animal or the presence of some odorous compounds can induce the animal not to eat the feed, independently from its nutritional value.

Therefore, an ideal measure of palatability, intended as a measure of the effect of senses on feed intake, should not be influenced by the consequences of the previous ingestion of feeds (Matthews, 1983) nor by the post-ingestive consequences of intake (Grovum and Chapman, 1988). If these parameters are not satisfied, differences in voluntary intake cannot be attributed only to palatability as they result from the sensory response and the digestive, metabolic and hormonal events following meals. To overcome these difficulties, recording intake during the first minutes following exposure to the feed reduces the risk of confounding palatability with post-ingestive factors. Initial eating rate, which can double from one forage to another, may thus be a good criterion for evaluating the sensory response invoked by a feed and thus its palatability (Baumont, 1996). Another technique for avoiding post-ingestive effects is sham-feeding, in which the ingested feed is diverted from the digestive tract through an oesophageal fistula. This technique was used on sheep for palatability trials by Grovum and Chapman (1988). However, Forbes (1995) noted that sham-feeding can affect supply of minerals in the rumen because of the interruption of saliva flux, thus causing disequilibrium on the mineral balance and affecting animal's preference.

In conclusion, the sensorial response caused by feeds can be measured through intake and intake rates at the beginning of meals. This can be done when a feed is present alone or in a situation of free choice in which feed preferences are determined.

Control of feed intake and palatability

As mentioned in the previous paragraph, intake is regulated by complex mechanisms that control initiation and cessation of feeding behavior, the metabolism and expenditure of nutrients and the stability of body weight in the animal. Changes in animal requirements or in the diet energy or metabolizable content cause the activation of several mechanisms of feed intake control, which drive animal intake in the appropriate direction (Forbes, 2003)

One of the main objectives of ruminant nutritionists has been for a long time to find a method to predict animal productivity based on the nutritive value of feeds, the genetic value of animals (potential productivity), and the environmental characteristics in which the animal are bred. As a result, a large number of models have been developed to predict animal performance based on simulation of digestive and metabolic processes. However, even if the degree of accuracy reached by these models has greatly increased, the estimation of production responses has not been as successful. The major cause of this gap among what models predict and the real performance of ruminants are the difficulties to predict intake (Mertens, 1996).

For example, with forage based-diets, beef cattle, as other ruminants, increase their DM intake as the rate and extent of digestion increase. This phenomenon can be attributed to a physical limit to intake. NDF has been used to account for the bulkiness of feeds, because of its slow rate of digestion and correlation with forage intake. However, there is a large variability in NDF intake of forages, which strongly suggests that bulk is not the only factor affecting forage intake. In fact, even if energy- and bulk- sensing mechanisms are involved, many other factors are also involved in the control of intake, including nutrients, diseases and environmental conditions (Forbes, 2003).

The variables taken into account vary among the several models of feed intake prediction currently available. These models vary from those based on an exclusive signal, select the most limiting factor and use it as the exclusive

regulator of feed intake, to those more inclusive, which integrate the combination of different factors in a single effect. The most used models of intake prediction by ruminant nutritionists aim to predict the intermediate (i.e. among days) intake regulation. The objective of these models is to predict as accurately as possible the feed intake into time ranges determined by the physiological status of the animal (i.e. beginning of lactation, second part of lactation, dry) in order to formulate optimal rations. The intermediate feed intake regulation (among days) is driven by energy needs, and is composed by the addition of daily intake, which fluctuates around a mean value that represents the long term requirements related to the homeostatic regulation of body energy status. This fluctuation is related to genetic potential, body reserves, growth, reproduction and production. In the long term, body energy stores act as a buffer, compensating the energy deficit and storing the energy surplus. The fluctuation of intake normally registered day by day is the result of the feed intake regulation within days. For example, sometimes it can happen that an animal can eat over its normal limit during a meal and subsequently limit the intake in the following meal. The mechanisms involved in the daily regulation of feed intake (among hours) require signals that provide rapid and direct regulation of feed intake (i.e. rumen fill). Thus, factors other than those previously cited can act in the mechanism of the short-term regulation of feed intake. Part of the short term variation in feed intake could be explained by the fact that the mechanism of regulation is not fast enough to make the animal stop eating before overeating occurs. Furthermore, learned behavioral components related to management and feed palatability could act and influence the quantity of feed eaten during a meal (Mertens, 1996). However, all the prediction models of feed intake base their prediction balancing nutrient requirements, nutritive values of feeds and body energy stores. Even if their prevision accuracy has improved during the last decades, the residual error is still large and suggests, as mentioned before, that other factors, not considered in the models, can be involved in the regulation of feed intake. Palatability is one of the

most likely factors to influence animal behavior and, thus, feed intake regulation in short and medium time and also animal behavior in a longer time.

Palatability of forages and concentrates

Grazing animals have to distinguish between a large range of different cultivated and wild grasses. It is well known that grasses contain not only nutrients but also a high number of toxic compounds. Therefore herbivores have to be able to select between these compounds and distinguish the potentially toxic feeds and, at the same time, satisfy their nutritional requirements through intake of these grasses. The way how ruminants select their diet involves mechanisms related to sensorial perception and the feedback of post-ingestive effects of feed on animal health.

The young animals can select among forages by learning processes in which the mother and relatives teach them which grass species are eatable, so that they can carefully experience new forages and learn by their post-ingestive effects.

Four models of food selection have been proposed, as described in detail in the review of Provenza, 1995). In brief, the first model "euphagya" affirms that the animals can select among feeds by an innate capacity to smell the presence of nutrients, and healthy and toxic compounds. This model, even if not completely accepted, seems interesting, because of the recent evidences that demonstrate the capacity of mammals to select feeds containing aromas deriving from healthy compounds (see the review of Goff and Klee, 2006), the presence of a region deputed to the innate recognition of volatile compounds in the olfactory mucosa of rat (Kobayakawa et al., 2007) and the discovery that some hormones known to be involved in the regulation of feed intake are synthesized also in the olfactory mucosa and play a role in the regulation of feed intake by regulating the sensibility of the olfactory mucosa (Baly et al., 2007*;* Julliard et al., 2007). The second model "hedyphagia" also relates the smell and taste senses to the capacity of animals to select among feeds. The proposed model affirms that animals can

distinguish immediately feeds that “taste good” from those that “taste bad”, thus avoiding toxic feeds by this mechanism. The third model (body morphophysiology and size) proposes an evolutionary mechanism in which animals evolve their morphology and physiology based on the types of feeds available in their environment and therefore they have a different ability to ingest forages that differ in physical and chemical characteristics. The fourth model, which is the most accepted in the scientific community, is the learning process by post-ingestive consequences. There are numerous scientific evidences supporting this theory that demonstrate the ability of animals to select among forages with the aim to meet their nutrient requirements and minimize intake of toxic compounds (Provenza, 1995; Baumont et al., 2000). However, some trials suggest that animals have an innate sense to drive their chose to select among different feeds (Kobayakawa et al., 2007).

Acquisition of flavor preferences

It has been demonstrated in many experiments that ruminants can acquire preferences for flavors paired with several nutrients. The nutrients which create the strongest linkage among flavor and post-ingestive consequences are protein and energy sources. However even feeds low in protein or energy seem to be able to generate post-ingestive consequences that can create the formation of preference (Villalba et al., 2008).

- *Acquisition of preference for flavors paired with protein*

Villalba and Provenza (1997a) investigated the formation of preferences in lambs for flavors paired with different sources of protein containing different levels of nitrogen. Lambs formed a dose-dependent preference for flavors paired with nitrogen sources. In fact, lambs receiving urea as nitrogen supplier formed a preference for flavor associated with the lowest dose and avoided flavor

associated with the highest dose, whereas lambs receiving casein or gluten as protein suppliers formed a preference for the highest doses of these supplements and avoided the lowest dose. This is a demonstration that the formation of preferences is linked to post-ingestive effects that nutrients create in the organism. The fact that animals avoided the highest dose of urea can be explained by the potential toxic effect generated by urea. This negative effect was connected by the animals to the flavor associated with the high dose of urea; thus animals reject this feed when they have the possibility to choose. On contrary, the lowest doses of casein and gluten may have created a post-ingestive effect too weak to be tightly connected to the associated flavor. Another interesting aspect of this work was the study of the persistence of flavor preference over time. In fact, in a weekly preference test conducted for two consecutive weeks after the end of the experiment, it was demonstrated that lambs maintained the same preferences showed previously. It whould be interesting to evaluate if the flavor preference can persist for longer time intervals.

Arsenos et al. (2000a) studied how a delayed-type of learning could account for the conditioned feeding responses of sheep towards novel feed flavors associated with post-ingestive consequences. The post ingestive consequences were created through the administration of two different doses (15 *vs* 75 g) of casein at different moments (long delay or short delay). Sheep were then adapted to associate novel flavors to either high or low protein content. Afterwards, sheep were submitted to three treatments which differed for the duration of delay between flavor exposure and casein administration. The results showed that there was no effect of time on the conditioned responses towards flavored feed, but there was a strong association between flavor and casein administration. The results of this experiment suggest that sheep develop a strong association among flavor and nutritional stimulus (post-ingestive feedback effect) also when the post-ingestive consequences are significantly delayed from flavor exposure.

In another work, Arsenos et al. (2000b) studied the relationship between the conditioned responses of sheep toward food flavors associated with the administration of ruminally degradable protein (RUP) and ruminally undegradable but readly digestible protein (DUP) sources. Sheep preferred flavors associated with DUP compared to flavors associated with RUP. However the animals did not exhibit difference on flavor preferences when flavors associated with DUP were compared with flavors associated with RUP+DUP. The results of this experiment reinforced the belief that sheep are able to select their diet on the basis of protein degradability.

- *Acquisition of preference for flavors paired with energy and other nutrient sources*

Following an idea similar to those previously stated, Villalba and Provenza (1997b) investigated the formation of flavor preferences for poor feeds associated to flavors and to intraruminal administration of different doses of starch. Lambs showed a strong preference for flavors paired with administration of starch compared to those not paired with starch and this preference persisted during the following 8 weeks.

Burritt and Provenza (1992) demonstrated that lambs created preference even for flavors paired with water solution containing glucose. In that experiment, after a period of 10 days of conditioning, when a water solution containing either glucose or saccharin paired either with orange and grape flavors was offered to two groups of lambs, lambs were free to choose among two solutions without sweeteners but flavored. The results showed that they selected those solutions associated with the flavor previously paired with glucose. These results suggest that lambs were able to associate the flavors with the positive post-ingestive effects of glucose and learned to select this flavor because they received a good sensation from solutions paired it. In fact, before the beginning of the trial, the preference of lambs for solutions containing the two sweeteners but, in this case,

without flavors was tested. In this case, lambs did not exhibit any preference for either sweetened solutions, indicating that they were not innately able to distinguish the effects of ingestion of this solutions.

Sutoh et al. (2007) tested the effect of propionate and acetate on the reinforcement of feed flavor preferences in wethers, in three experiments. In the first one, whethers were divided in two groups: half of each group received clove-flavored straw and the other half cardamom-flavored straw. During straw ingestion, each group of wethers received intramesenteric infusion of sodium propionate or a saline solution. On even numbered days, infusion and flavours exposure were switched between groups. At the end of this conditioning period (12 days), in a preference test performed for 2 days, animals showed a clear preference for straw flavored with propionate infusion.

In the second experiment, it was tested if sheep whethers develop a preference for feed flavor paired with intramesenteric infusion of sodium acetate. Sheep whethers developed a dose-dependent preference for flavors paired with intramesenteric solution of sodium acetate. The results of both experiments suggest that both sodium propionate and sodium acetate act as preference-reinforcing signals.

In the third experiment, Sutoh et al. (2007) compared the effect of flavor preference-reinforcing signals between sodium propionate and sodium acetate. In this case, three different trial were conducted to determine which compound (propionate vs acetate), develop the strongest flavor-preference signals. In all trials, the authors found that propionate was more efficient than acetate as flavor-reinforcing signal.

The above discussed results support the hypothesis of Villalba and Provenza (1997b) that lambs develop preferences and aversion for feeds along a continuum that depends on the amount of volatile fatty acids supplied.

Formation of flavor preference is normally believed to be linked to those feeds which create strong post-ingestive effects as those with high energy or protein content. In fact, the experiments previously illustrated demonstrated that the

formation of flavor preference associated with protein and energy was dose dependent. However, it has been demonstrated that flavor preference can be formed also for those aliments without energy or protein content such as minerals (Villalba et al., 2008). Indeed, even if lacking of nutritional value, these elements are fundamental in animal nutrition and their absence in the diet can generate severe problems regarding animal health and animal production, because they are involved in cellular processes and are fundamental constituents of milk. Villalba et al. (2008) studied the capacity of lambs fed diets with unbalanced minerals to develop flavor preferences for feeds differing in mineral content. Two groups of lambs were fed a diet low in P but rich in Ca or high in P but rich in Ca. Subsequently, during a short conditioning period (6 days), lambs received three grape pomace feeds flavored with three different aromas and enriched of NaCl, NaH_2PO_4 or $CaCO_3$. After this conditioning period, lambs were subjected to preference tests (15 min) during which they were free to choose among the three flavored grape pomace feeds. The results showed that lambs receiving diets low in P preferred to eat grape pomace with the flavor associated with high P content, whereas lambs fed diet low in Ca preferred to eat that rich in Ca. This behavior demonstrated the capacity of these animals to discriminate among feeds with different mineral concentrations. This capacity was connected to the ability of lambs to associate the different flavor of the feed with the post-ingestive consequences. In fact, before the beginning of the experiment, preference tests among the flavors applied to the grape pomace used in this experiment were performed and lamb groups were subsequently paired for this variable.

Preference for flavor is not constant over time, being characterized by dynamic changes related to the duration of exposure (Early and Provenza, 1998). Animals previously exposed to a flavor decreased preference for it and acquired preference for alternative flavors even after only 1 d of exposure. This was demonstrated by three experiments where lambs fed a flavored feed containing an adequate content of nutrients during the morning preferred to eat the same feed but flavored with another aroma. The degree of preference was related to the

energy content of feeds. In fact, lambs fed a flavored feed containing 90% TDN in the morning preferred to eat aftwerwards an alternative feed containing 110% TDN and being differently flavored. The opposite behavior was observed on lambs initially fed the 110% TDN feed. This could be explained by the formation of a sensory satiety which induces the animals to eat feeds alternatively flavored (Early and Provenza, 1998). Interaction among senses and post-ingestive effects calibrate the selection of feeds to maintain the homeostatic equilibrium. In fact, also lambs fed high digestible flavored feed in the morning prefered slowly digestible feed flavored with a different aroma in the evening (Early and Provenza, 1998).

Acquisition of flavor aversion

Similarly to the way ruminants develop flavor preferences for feeds in relation to their content of nutrients, they avoid flavor paired with feeds rich in toxin or any type of substances that create malaise sensations. These feeds can be characterized by excess of toxins or excess of nutrients.

The capacity of animals to sense the post-ingestive effects of feed intake is very quick. After the first bit of feed is ingested, the blood flow in the ruminal artery increases within one min and reaches the maximum flow in 15 min (Barnes et al., 1986). Propionate and acetate concentration in the ruminal artery increase after 15 min from the beginning of meal (De Jong, 1981). This means that ruminants are able to sense post-ingestive effects of meals within 15 min depending on ruminal degradability of the feeds ingested. Then also malaise can be quickly felt by the animal and associated with the feed flavor, in order to avoid further ingestion of that feed.

Goats limit intake of twigs that contain tannins within 1 hour (Provenza et al., 1994), whereas sheep learn to refuse feeds containing LiCl in the same range of time (Provenza et al., 1993)

In a study on feed intake regulation, Burrit and Provenza (2000) conducted several trials in which lambs were offered: i) feed containing toxin A, ii) feed containing toxin B, iii) feed with toxin A and feed with toxin B simultaneously in two different feeders, iv), feed without toxin. Each trial differed for the type of toxins tested as follows: i) trial 1: amygdaline vs. LiCl; ii) trial 2: LiCl vs. LiCl; iii) trial 3: sparteine vs. saponin; and iv) trial 4: oxalate vs. nitrate. The results were not clear and varied with type of toxins tested. In some cases, lambs fed two feeds containing each a different toxin ate more than lambs fed a feed containing a single toxin, whereas in others the opposite behavior was observed. Even if the results were influenced by the nature of the toxin offered to the lambs, it was observed that lambs fed feeds containing toxins (single or not) ate less than lambs offered feeds without toxins. This showed that lambs selected their diet in order to limit the intake of toxic feeds.

The degree of flavor aversion for feeds paired with toxins and the generalization of the aversion to the paired flavor apparently does not depend on the intensity of the applied flavor but is strictly related to the concentration of the toxin (Launchbaugh and. Provenza, 1994). That is why aversion to a food increases with the severity of the illness and decreases as the delay between food ingestion and illness increases (Garcia et al., 1974, du Toit et al., 1991, Ralph and Cheney 1993, cited by Provenza, 1995). Refusal of a flavor paired with toxins by lambs is generalized to other feeds joined by a common flavor (Launchbaugh and. Provenza, 1994).

Formation of flavor aversion is strictly related to smell. In fact, lambs exposed to novel aroma and subsequently receiving an intraruminal infusion of LiCl, further refuse familiar feeds even if they do not contain any toxin (Provenza et al., 2000).

Considering the large number of meals consumed by an animal throughout its life and the relatively low incidence of toxicosis that occur, it is likely that toxicosis may happen because of failure in the feedback and sensory system (Provenza et al., 1992). It is interesting to note that animals learn to self-medicate

through the feedback system. In fact, lambs eating feeds containing toxic substances learn after a conditioning time to prefer alternative feeds containing specific compounds that rectify the state of malaise afterwards. The post-ingestive feedback acts in this way to maintain the homeostatic equilibrium by balancing the intake of toxins with the intake of alternative feeds that contrast the bad effects of toxins (Villalba et al., 2006a).

Effects of feeds volatile compounds on feed palatability

The previous paragraph presented evidences of the formation of preferences or aversion for flavors which were associated to positive or negative post-ingestive effects, respectively. It was demonstrated how ruminants select feeds based on feed flavor and that the acceptance or avoidance of a flavor is quickly learned through the feed-back mechanism. However, some scientific evidence suggests a role of feed chemical compounds on feed palatability and on the formation of feed preference. One class of the compounds ascribed to affect feed palatability are the terpenes. In some studies, it was examined how single or mixed of terpenes affected feed palatability in ruminants.

Estell et al. (1998) studied the effects of six volatile compounds (camphor, limonene, cis-jasmone, β-caryophyllene, borneol, α-pinene) at different concentrations (0, 0.5, 1.2 and 10 times higher than the concentration normally found in tarbush plants) on intake of alfalfa pellets by lambs. The volatile compounds were selected from terpenes that were related to tarbush consumption by sheep (Estell et al., 1994) and diet selection (Estell et al., 1996). The treated alfalfa pellets were fed to lambs in a 20 min-interval test. The addition of camphor and α-pinene had a negative effect on alfalfa pellet consumption, whereas the other compounds did not influence the intake of alfalfa pellets. In a similar experiment, Estell et al. (2000) tested other single terpene compounds (p-cymene, α-humulene, 1,8-cineole, 3-carene, or sabinene), following the same procedures. None of the tested terpenes had effects on the consumption of alfalfa

pellets by lambs. Subsequently, Estell et al. (2005, 2007) tested the effects of other single terpenes on the intake of alfalfa pellets and found that individual terpenes did not affect feed palatability either. Differently, other results indicated that ethanol extract, hexane extract and ether extracts of *Flourensia cernua*, containing different classes of terpenes, applied to alfalfa pellets at the same concentration measured on the plant reduced intake of alfalfa pellets in lambs during a 20 min test (Estell et al., 2001). These results suggest that the presence of different terpens in the same feed can affect palatability of alfalfa pellets in lambs, whereas the presence of single compunds does not affect it. Maybe the effect of the extract on feed palatability is related to interactions among individual compounds. Although not tested in these experiments, it seems that interactions among flavor compounds components and feed matrix can stimulate a feed perception that cannot be tested by studying the effects of single compounds alone. In fact, it was demonstrated that dietary monoterpenes affect feeding patterns in lambs because intake of alfalfa pellet treated with monoterpenes decreases during the first hour of exposure (Dziba and Provenza, 2008). Villalba et al. (2006b) demonstrated that terpenes (camphor, 1,8-cineole, methacroleine and p-cymene) added to diets based on beet pulp or barley meal, at the same concentration found on big sagebrush, depressed intake of lamb fed beet pulp based diets and influenced preference because lambs preferred beet pulp than barley when offered a choice. Moreover, addition of terpenes increased *in vivo* digestibility of beet pulp based diets but not of barley based diets.

In addition to terpenes, it was demonstrated that other flavor compounds can affect feed palatability and then feed preference and intake. De Rosa et al. (2002) compared preference of goats for straw pellets flavored with water, ryegrass extract or clover extract. The straw pellet preference was tested in a 3 min cafeteria trial test, in order to avoid the formation of post-ingestive effects. The preference for pellets flavored with the ryegrass extract was the highest, to the preference for clover flavored straw pellets was intermediate, whereas that for water straw was the lowest. These results indicate an effect of flavor on feed

preference also in a condition of absence of differences in post-ingestive effects. Similar results were found by Distel et al. (2007) providing a highly nutritive feed in lambs. In this case, intake was measured when natural hay, fed *ad libitum*, was unflavored or flavored with different aromas (garlic, oregano or basil). Feed intake increased of 10% ($P < 0.07$) in lambs fed flavored hay compared to lambs fed unflavored natural hay. These results indicate an effect of feed perception on intake of feed. However, in a second experiment in which flavoured and unflavored alfalfa hay were tested, the same authors did not find effects of flavor on intake of alfalfa hay. This was attributed to the fact lambs had been fed a diet based on alfalfa hay before the beginning of the second trial.

Robertson et al. (2006) studied the effects of different flavors on the intake of poor feed by mature castrated male sheep and goats. In this experiment, 8 different flavors were tested with a cafeteria trial by applying flavor on barley straw pellets. Animals were first adapted to the flavors. Subsequently they were exposed to the free choice among flavors in a 30 min cafeteria trial test where flavored straw pellets were offered in pairs. Sheep and goats showed a preference for flavored straw in relation to unflavored straw. When comparing the two species, sheep were more sensitive to flavored feeds, exhibiting a greater preference within flavor than the other species. However, the type of flavors preferred by sheep and goats were similar. Sheep showed preference for truffle, garlic, onion, apple, caramel, maple orange, whereas goats showed preference for truffle, onion, apple, and garlic. This work indicates an effect of these flavors on intake of straw pellets with low nutritional value and suggests the evaluation of these flavors as intake enhancer for sheep and goats. It is still to be demonstrated the effectiveness of these flavors as feed enhancer when they are applied to more nutritive feeds and when they interact with the natural flavors of the feed.

Another strategy used to improve feed palatability is to apply grass extract to the feeds, in order to simulate the odor of a familiar and highly palatable feed. Dohi et al. (1996, 1997) demonstrated that methanol extracts of perennial ryegrass stimulated the feeding behavior of goats, suggesting the presence of specific

stimulant compounds in the herbage. These results suggest that the basic and less volatile chemicals in perennial ryegrass stimulate feeding in sheep in the absence of other cues, and that ryegrass contains intake stimulants that should be extracted and used as hay intake enhancer. In a similar trial, high grain concentrate extracts (diethyl ether or methyl extracts) were used in place of ryegrass extract (Dohi and Yamada, 1997). Sheep and goats showed a preference for diethyl ether extract treated hay in comparison with untreated hay, whereas they did not show a preference for methyl extract hay compared to untreated hay.

Influence of aromas on acceptance or refusal of new feeds by ruminants

Quick changes of diet often cause refusal of new feeds. The avoidance of a new or unfamiliar feed is very common in ruminants (Chapple et al., 1987; Provenza et al., 1995) and has been commonly associated with generation of fear sensation (Launchbaugh et al., 1997; Boissy, 1995). However, Herskin et al. (2003) found that exposure to new feeds did not cause an increase in hearth rate in cattle. According to Wong and McBride (1993), refusal of new feed seems to be connected with feed reward because it is related to flavor neophobia and is probably the result of an innate protective mechanism which ensures the animal to have the possibility to learn through the post-ingestive consequences, i.e. the effects of the ingestion of new feeds on health and nutritional status. The way how flavor acts on acceptability of new feeds by ruminants is still unknown. Villalba and Provenza (2000) tried to better understand how ruminants learn to select the most nutritious feeds when fed frequently a large range of different feeds. One experiment dealt with the process of formation of flavor preferences by intraruminal infusion of starch (at constant or adjusted doses) to lambs previously fed a novel flavored feed. Lambs receiving a constant dose of starch (150 g/d) did not develop a preference for flavored feed paired with starch infusion, whereas lambs receiving an infusion of starch proportional to the quantity of flavored feed, previously ingested, developed a strong preference for

the flavored feed. In another experiment, it was demonstrated that lambs previously fed a flavored feed (coconut flavored milo) for a long time (51 d) preferred in following preference tests flavored straw to unflavored straw. On the contrary, lambs that had not received flavored milo for 51 d did not exhibit any preference for flavored straw in subsequent preference tests. An additional treatment applied the lambs fed flavored feed consisted of submitting one group to intraruminal infusion of starch. After the treatment, these lambs ate more flavored straw compared to those which had not received the starch infusion and to lambs infused with a toxin (LiCl), and these differences persisted even when the infusion of starch was suspended. These results suggest that i) formation of the flavor preference is tightly influenced by learning through post-ingestive feedback, and ii) the strength of the preference is proportional to the intensity of the post-ingestive effect. The generalization process is driven by the post-ingestive effects too, and lambs can rapidly change from a familiar feed to a novel one based of a common flavor among feeds (Villalba and Provenza (2000).
To better understand the connection among sensorial properties, toxicity and the formation of feed preference in lambs, Provenza et al. (2000) conducted three experiments. In the first experiment, lambs were briefly (60 s) exposed to a familiar feed added with a novel flavor, without tasting it, and then they were immediately submitted to an intraruminal infusion of LiCl which induced toxicosis. Afterwards, the lambs that had sniffed the flavored feed and then received the LiCl doses ate less flavored feed than those that did not receive the LiCl doses. In the second experiment, it was tested if an unpleasant herb flavor (extracted from *Astragalus bisculatus*) could induce feed rejection in lambs. Following the same procedure of the first experiment, all lambs were exposed to the flavor of *Astragalus bisculatus* (i.e. sniffing the flavor), and then one group received intraruminal *Astragalus bisculatus* doses, whereas the other group did not. The results showed that lambs that had received *Astragalus bisculatus* as well as those that had not received it exhibited feed refusal. In the third experiment, it was tested in which way the degree of familiarity with the odor of

the *Astragalus bisculatus* along with toxicosis induced by intraruminal infusion of LiCl affected the preferences of lambs for feed added or not with the flavor of *Astragalus bisculatus.* Lambs which had experienced the flavor of *Astragalus bisculatus* for 8 days did not form preferences among feed flavored with the flavor of *Astragalus* bisculatus or not flavored. Lambs that had the same long time experience with the *Astragalus bisculatus* flavor but received LiCl after the sniffing of this flavor, refused the feed paired with the same flavor. Lambs with 1 day of experience with the same flavor without receiving the LiCl dose did not reject the flavor, wheareas those which had received the dose of LiCl refused the feed flavored with *Astragalus bisculatus.* These results suggest that lambs do not have an ancestral capacity to recognize toxic feeds only by flavor transmission but, on the contrary, they form preferences only by experiencing the post-ingestive effects caused by the feeds. In order to limit this problem, Provenza et al. (1995) conducted two experiments dealing with two different strategies to encourage sheep to eat a novel feed. The first strategy was to increase the fasting effect through feeding sheep a restricted diet. The animals used in this experiment were orphan lambs, in order to exclude the effect of transmission of feed knowledge by the mother. In this experiment, the effects of two levels of feed restriction (750 *vs* 1500 g/d of alfalfa pellets) on acceptance of a novel feed were studied. After 10 days of feed restriction, lambs were fed 600 g of a novel feed (split peas) for 15 min/day for 4 days. Lambs increased the intake of the novel feed over time but there was not effect of feed restriction on intake. The second experiment tested the effects of increasing familiarity with a flavor (onion) on intake of a novel feed. In order to make lambs become familiar with onion, they were exposed to onion flavor for 2, 7 or 12 days. With 7 days of exposure there were no difference on intake of the novel feed but, in the first 2 days of offering of the novel feed, lambs ate more novel feed with onion than novel feed without onion. With 12 days of exposure to onion, lambs ate more novel feed flavored with onion than that without added onion. Therefore, it

seems that lambs need at least 12 days of exposure to become enough familiar with a flavor so that they are encouraged to eat a novel feed.

As previously showed, feed neophobia can be attenuated by the addition of a familiar flavor to feeds. This effect was confirmed by Van Tien et al. (1999), who found a faster acceptance of a new feed when paired with a familiar flavor or smell (grass) in sheep, and by Launchbaugh et al. (1997) who found that lambs fed onion-flavored barley or rice twice a day for 4 days ate more onion-flavored rice than lambs who had never experienced rice nor onion flavor. Acceptance of new feeds can be increased also by repeated exposure to new feeds. In fact, lambs offered 4 different new feeds for 3 consecutive days ate more the 4th new feed than the first new feed, indicating that generalization of experience with new feeds can reduce feed neophobia (Launchbaugh et al., 1997). Similarly, in the study of Simitzis et al. (2007), lambs were fed oregano oil flavored diet when they were from 15- to 55-day-old. In subsequent tests starting from the end of that period, lambs showed a preference for oregano oil flavored after 3, 5, 7, 9 months and 1 year; being the strength of the preference significantly higher after 9 months. The previous exposure to a flavor can be effective on the acceptability of new feeds even when it happens before birth. In fact, lambs born from ewes exposed to oregano oil flavored diets during pregnancy (50th – 130th day) exhibited a preference for oregano oil flavored feeds when they were 3-; 4-, 5-; 6- , and 7.5-month-old (Simitzis et al., 2008). These effects are not an exclusive characteristic of ruminants, and can be generalized for other mammals. In fact, a similar behavior was found by Cheney and Miller (1997) in rats. In this case, rats were forced to familiarize with mint or strawberry flavor by adding these aromas to water. Afterwards rats were offered a mint-flavored feed and rats who had drunk strawberry or plain water ate less mint-flavored feed than those who had drunk mint-flavored water. This suggested that linking the familiarity to a flavor with the flavor of the new feed can improve feed intake during the first days of exposure to new feeds.

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CHAPTER 2

PALATABILITY OF CONCENTRATES FED TO LAMBS AND EWES

ABSTRACT

The aim of this research was to evaluate the palatability of the most common ingredients used to produce concentrate mixes for dairy sheep. Thus, an experiment was carried out to measure the palatability of 14 feeds commonly used for concentrate formulation: soybean meal 44; soybean meal 49; soybean hulls; corn grains; canola meal; wheat grains; beet pulps; dehydrated alfalfa; sunflower meal; oat grains; pea grains; wheat brans; corn middlings; and corn gluten meal. The sensorial effects of these feeds were tested in 6-min palatability tests on 14 female lambs and 14 multiparous dry ewes, following a 14 (days) x 14 (feeds) Latin square design. The lambs showed the following rank of feed palatability, in decreasing order of dry matter intake (DMI): soybean meal 49 (24.5 g/d), wheat grains (22.8 g/d), pea grains (17.4 g/d), corn grains (14.9 g/d), soybean hulls (13.1 g/d), beet pulps (11.9 g/d), wheat brans (11.4 g/d), soybean meal 44 (10.9 g/d), corn middlings (7.5 g/d), canola meal (5.0 g/d), sunflower meal (2.8 g/d), corn gluten meal (1.7 g/d), corn gluten meal (1.7 g/d), dehydrated alfalfa (0.4 g/d), and oat grain (0.0 g/d). The ewes showed more clear preferences, with the DMI of four feeds (beet pulps = 62.8 g/d; wheat grains = 56.4 g/d; pea grains = 56.3 g/d; corn grains = 52.7 g/d) being much higher ($P<0.05$) than that of the other feeds. The same trends were observed when the DMI Ratio (i.e. mean DMI of the experimental period of each experimental feed/mean DMI of barley meal during the last 4 days of the adaptation period) was considered. Lambs showed a higher DMI Ratio than ewes ($P < 0.05$) for soybean meal 49 (55.0 vs. 3.4 %), soybean hulls (29.8 vs. 5.5 %), soybean meal

44 (24.1 vs. 7.0 %), whereas the contrary occurred for beet pulps (24.9 vs. 65.8 %), corn gluten (4.7 vs. 27.4 %), and oat grains (0.4 vs. 10.1 %). For lambs, the rank of rate of intake among feeds was similar to that observed for DMI, except that: i) pea grains, wheat brans and, even more strikingly, soybean meal 44 were eaten at a faster rate than their DMI would have suggested; and ii) soybean hulls and, even more strikingly, wheat grains were eaten at a slower rate than their DMI would have suggested. As for lambs, for ewes the rank of the rate of intake among feeds was similar to that observed for DMI, except that: i) pea grains and all feeds with significantly lower DMI were eaten at a faster rate than their DMI would have suggested; and ii) beet pulps, wheat grains and corn grains were eaten at a slower rate than their DMI would have suggested. The rank among feeds within sheep category regarding mean level of intake on a BW basis (mg/kg BW) and on a metabolic weight basis (mg/kg$^{0.75}$ BW) was similar to that observed for DMI, due to the limited variations in BW within category. In conclusion, the intake of the experimental feeds by lambs varied from high to low values in a continuum, without clear cuts, suggesting that their choices were markedly influence by sensorial perceptions (smell and/or flavour) and, to a lesser extent, by previous feeding experiences. In contrast, the ewes showed a marked preference for 4 feeds often supplied as single ingredients (beet pulps and wheat, pea, and corn grains), which were probably identified by the ewes, and low intake or almost total rejection for the other feeds, which included several feeds commonly used for sheep feeding but rarely used as single ingredients.

Key words: palatability, concentrate, sheep, lambs, ewes.

INTRODUCTION

In dairy sheep farms, the ewes are normally fed on pasture, whereas concentrates are supplied only during the two daily milkings. Therefore, the ewes have to eat the concentrates in a short time, which varies around 3 min depending on the model of the milking machine used and on the organization of the farm (Pazzona, 1999 cited by Caria 2006). During this period of time, they have to recognize the feed they are given and to decide if they are going to eat it or not. Thus concentrate feed palatability can play an important role in this decision. Indeed, it has been often observed that when a new feed or feed mix is supplied at milking, for some days the ewes either refuse to eat the new feed or reduce their intake. This causes economical damage to both the farmer, due to milk yield losses, and the feed mills, which have to deal with complains from their customers. For this reason, it is quite common that sheep are fed the same concentrate throughout lactation, even if this is not nutritionally advisable, because requirements for lactation and quality of pastures change considerably over time. In addition, feed mills have difficulties in changing the ingredients of a feed mix that become too expensive over time or that are not more available.

Provenza et al. (1995) found that at least 12 days are required to make a lamb familiar with a novel feed. Afterwards the mechanisms of learning and the strong hunger convince the animal to eat the new feed. However, this period is too long to avoid production losses. The velocity in which sheep accept to eat a new feed has been related to their level of experience. In fact, through a feedback system, animals learn to recognize the nutritional value of feeds by connecting their sensorial properties with their post-ingestive consequences (Provenza, 1995). However, when sheep decide to eat or to refuse a new feed, just after being exposed to it, their decision is probably more based on its sensorial properties (smell and taste) than on its post-ingestive effects. The palatability of feeds seems to be negatively affected by some intrinsic sensorial properties of feeds such as those given by the presence of terpenes (Estell et al., 1998; Villalba et al.,

2006; Dziba and Provenza, 2008). Some feeds, such as canola meal, are known to contain compounds perceived as unpalatable by the animals (Frederick et al., 1988), whereas Others, such as oat grains, can affect the sensorial properties of milk (Kim Ha and Lindsay, 1991). Despite the importance of these aspects in ruminant nutrition, the effects of sensorial properties of the most common concentrate feeds on palatability are little known. This is particularly important for dairy sheep, which receive the concentrates separately from the other dietary ingredients of their diet and have little time available to eat them.

Thus, the aim of this research was to evaluate the palatability of the most common ingredients used to produce concentrate mixes for dairy sheep.

MATERIALS AND METHODS

The experiment was carried out at the AGRIS research centre of Bonassai (Olmedo), located in the North-West of Sardinia (Italy), and testing the palatability of 14 concentrate feeds (Table 1) on 14 Sarda multiparous dry ewes and 14 Sarda female lambs.

Before the beginning of the experiment, the flock of mature ewes were fed on pasture and supplemented with commercial pellets, whereas the lambs were fed, indoors, commercial pellets (Consorzio Agrario di Sardegna, Cagliari, Italy) composed of: barley meal, corn grains, soybean meal, wheat grains, beet pulps, alfalfa dehydrated meal, wheat brans, molasses (DM 87.5%, crude fiber 9.4%, CP 18.9%, DM basis) and alfalfa hay.

After selecting the ewes and lambs to be used in the experiment, they were confined in two different pens and fed for six days a mixture of alfalfa and ryegrass hay and supplemented with a mixture of barley meal plus urea and commercial pellets. During this pre-experimental period the proportion of commercial pellets and alfalfa hay in the diet was gradually decreased until only ryegrass hay and a barley meal plus urea mixture were fed. Animals had also

access to water and to a block of minerals and vitamins *ad libitum*. The ration was designed to cover the requirements of both lambs and ewes.

After the pre-experimental period, an adaptation period started (13 days in total), during which the following daily *routine* was applied:

- at 7:00 a.m. barley and hay refusals were took off from the two collective pens (one for lambs, one for ewes);
- at 8:00 a.m. lambs and ewes, in sequence, were trained: 1) to spontaneously enter an individual pen with a manger containing two steel bowls with 100 g of barley meal each; 2) to stay there for 6 minutes for the palatability test (to be effectively conducted during the experimental period); in this time the animals were left alone, in order not to be disturbed, but they could see the other animals; 3) to leave the pen and go to an adjacent collective pen at the end of the palatability test. In that pen the animals received ryegrass hay *ad libitum*, in order to limit the post-ingestive effects of barley;
- After all animals had finished the routine described above, they were brought back to the original collective pen and fed rationed amounts of ryegrass hay and barley meal mixed with urea, in the amounts and proportions described in Tables 2 and 3.

The sequential entry of the two groups was inverted each day, in order to limit differences in fasting time between lambs and ewes. The adaptation period ended once the intake during the 6 min palatability test had become sufficiently stable for some days (day 13 of the adaptation period).

Afterwards a 14 d experimental period started. During this period the same daily *routine* used during the adaptation period was followed, except that instead of barely meal the animals received during the 6 min palatability test 200 g (divided in two bowls) of one of the 14 different finely ground experimental feed ingredients (Table 1). The experiment was based on a 14 (feeds) x 14 (days/animals) Latin Square design with 14 animals per category (lambs and ewes).

When the experiment was completed, both lambs and ewes were supplemented twice a day with a basal diet composed of ryegrass hay and a mixture of barley meal plus urea.

Animals

The 14 female lambs were selected from the farm flock to have similar age (mean ± SD, 72 ± 9 d), body weight (BW, 16.9 ± 1.3 kg) and weaning days (32 ± 8 d).
The 14 mature dry ewes were also selected from the farm flock to have similar body weight (BW, mean ± SD, 50.6 ± 3.1 kg), body condition score (BCS, 3.1 ± 0.2) and age (4 ± 1 year).

Feeds

Fourteen feeds were tested in the experiment: soybean meal 44 (SN); soybean meal 49 (HP); soybean hulls (SH); corn grains (CG); canola meal (RC); wheat grains (WG); beet pulps(BP); dehydrated alfalfa (AH); sunflower meal (SM); oat grains (OG); pea grains (PG); wheat brans (WB); corn middlings (HO); and corn gluten meal (GL).
The feeds used were finely ground with a 1 mm screen size, by using a hammer mill, to reduce the effects of the physical form on their palatability.

Measurements

The amount of feeds consumed in the collective pen was measured as group intake, by subtracting the orts from the daily supply. The amount of feed consumed during the 6 min palatability tests was measured individually as the difference between the weight of feed offered and that left in each bowl after 6 minutes.

The feeding behavior of the animals during the 6 min tests was recorded with a digital camera (Sony DCRSR32E; Sony, Japan). After the end of the experiment, the videos were analyzed to measure the time that each animal spent eating during the whole 6 min test. The intake rates for each feed were then calculated as the amount of feed consumed divided by the time spent eating. Dry matter intake (DMI) of each feed was expressed as daily mean per animal (g/d per head), as mean level of intake on a BW basis (LI-BW; mg/kg BW) and on a metabolic weight basis (LI-MW; mg/kg$^{0.75}$ BW). For the 6 min palatability test, the ratio between the DMI of each experimental feed and the individual mean DMI of barley meal during the last 4 days of the adaptation period (DMI Ratio) was also calculated.

Samples of the feeds offered were collected during the experiment and stored at -20 °C until chemical analyses were performed. The body weight of lambs and ewes and the body condition score (BCS) of ewes were measured three times during the experimental period: immediately before the beginning of the experimental period (2nd of April2008); at half of the experimental period (15th of April 2008); and immediately after the end of the trial (24th of April 2008).

Chemical analyses

Feed and hay samples were analyzed for DM, ash, NDF, ADF, ADL (Van Soest et al., 1991), CP, and ether extract (AOAC, 1990). The non-fiber carbohydrate (NFC) concentration was calculated as [100-NDF-CP-EE-ash], where EE = ether extract, estimated by feed tables.

Statistical analyses

The data derived from the 6 min palatability tests were subjected to statistical analysis by using the **"**Proc GLM" of SAS (1990) on the basis of the following model (SAS, 1990):

$Y= \mu + \alpha_i + \beta_j + \gamma i + \delta i + \varepsilon_{ijk}$

μ = overall mean,

α_i = fixed effect of feeds,

β_j = fixed effect of animals,

δi = fixed effect of time,

ε_{ijk} = random error.

Treatment means were separated by using the test of Tukey at a threshold of $P < 0.05$.

The relationship between DMI of feeds and experimental days were analyzed by simple and multiple linear regression by using the "Proc REG" (SAS, 1990).

Comparisons between lambs and ewes eating data were carried out for each experimental feed by applying a one-way analysis of variance with two levels (lambs and ewes).

RESULTS

Composition of the basal diets and of the experimental feeds

The chemical composition of each ingredient and of the whole basal diet is reported in Tables 1 and 2. The chemical composition of the experimental feeds (Table 1) was highly variable, especially in terms of content (%) of CP (mean ± SD, 26.2 ± 18.5), NDF (36.4 ± 14.9) and ADF (19.4 ± 14.1).

Body weight and BCS variations

The mean weight of lambs increased from 16.9 kg at the beginning of the experimental period to 18.7 kg at its end (22 days in total). Thus, the mean weight gain of the lambs measured during the experimental period was of 0.08 kg/day. The weight of the ewes was constant during the experimental period, and ranged from 50.6 ± 3.1 kg at the beginning of the experimental period to 50.5 ± 4.4 kg at the end of the same period. Their BCS was also stable, ranging from 3.1 ± 0.2 at the beginning of experimental period and 3.0 ± 0.2 at the end of the same period.

Intake

The mean intake of barley meal during the palatability tests of the adaptation period (Figure 1) increased gradually during the first 8 days and then stabilized during the last four days, during which it was equal to 48 ± 6 g/d and 115 ± 3 g/d for lambs and ewes, respectively. The mean daily total DMI intake of the basal diet was similar between lambs and ewes (Table 3). However, lambs had much higher intake of barley meal and lower intake of hay compared with the ewes (190 ± 4 g/d vs. 76 ± 23 for lambs and ewes, respectively; Table 3). As a result the lambs had higher intake of CP and NFC and lower intake of fiber than the ewes (Table 3).

The mean intake of each experimental feed (Table 4) varied substantially within each sheep category.

The rank of feed palatability by the lambs was, in decreasing order of intake: soybean meal 49 (24.5 g/d), wheat grains (22.8 g/d), pea grains (17.4 g/d), corn grains (14.9 g/d), soybean hulls (13.1 g/d), beet pulps (11.9 g/d), wheat brans (11.4 g/d), soybean meal 44 (10.9 g/d), corn middlings (7.5 g/d), canola meal (5.0 g/d), sunflower meal (2.8 g/d), corn gluten meal (1.7 g/d), dehydrated alfalfa (0.4 g/d), and oat grains (0.0 g/d) . The ewes showed more clear preferences,

with four feeds (beet pulps = 62.8 g/d; wheat grains = 56.4 g/d; pea grains = 56.3 g/d; corn grains = 52.7 g/d) having much higher ($P<0.05$) DMI than the others (Table 4).

The same trends were observed when the DMI Ratio (mean DMI of the experimental period of each experimental feed/mean DMI of barley meal, last 4 days of the adaptation period) was considered (Table 4). Lambs showed a DMI Ratio higher than ewes ($P < 0.05$) for soybean meal 49 (55.0 vs. 3.4 %), soybean hulls (29.8 vs. 5.5 %), soybean meal 44 (24.1 vs. 7.0 %), while the contrary occurred for beet pulps (24.9 vs. 65.8 %), corn gluten (4.7 vs. 27.4 %), and oat grains (0.4 vs. 10.1 %).

The number of lambs that never ate the feeds varied considerably, going from 0% for corn grains to 71% for dehydrated alfalfa (Table 5).

For lambs, the rank of the rate of intake among feeds (Table 5 and Figure 2) was similar to that observed for DMI, except that:

- pea grains, wheat brans and, even more strikingly, soybean meal 44 were eaten at a faster rate than their DMI would have suggested;
- soybean hulls and, even more strikingly, wheat grains were eaten at a slower rate than their DMI would have suggested.

The number of ewes that never ate the feeds varied considerably, going from 0% (pea grains, what grains and beet pulps) to 50% (dehydrated alfalfa and soybean meal 49) (Table 5).

As for lambs, for ewes the rank of the rate of intake among feeds was similar to that observed for DMI (Table 5 and Figure 3), except that:

- pea grains and all feeds with the lowest DMI were eaten at a faster rate than their DMI would have suggested;
- beet pulps, wheat grains and corn grains were eaten at a slower rate than their DMI would have suggested.

The rank among feeds within sheep category regarding LI-BW and LI-MW was similar to that observed for DMI, due to the limited variations in BW within category. The feeds that determined higher ($P < 0.05$) LI-BW in lambs compared

to ewes were (Table 6): soybean meal 49 (1379 vs. 69 mg/kg BW, for lambs and ewes respectively), soybean hulls (757 vs. 128 mg/kg BW), soybean meal 44 (611 vs. 142 mg/kg BW). The ewes showed higher LI-BW than lambs for corn gluten meal (92 vs. 504 mg/kg BW for lambs and ewes, respectively) and oat grains (0.0 vs. 203 mg/kg BW). Similar contrasts were observed when LI-MW instead of LI-BW was considered (Table 6). In addition, beet pulps showed significantly higher LI-MW in ewes than in lambs.

The chemical component of the feeds that had the strongest relationship with LI-MW was the content of NFC (R^2 = 0.39; Figure 4). Another important variable was the EE of the feeds. This lead to the following multiple regression equation, obtained pooling lambs and ewes data:

LI-MW = 1189 + 34.8 NFC - 384 EE (R^2 adj.= 0.51; P<0.001).

There was no association between DMI and experimental days for any of the feeds tested, except for corn gluten meal, whose intake increased as the experiment progressed both in lambs (r = +0.57; P<0.03) and in ewes (r = +0.63; P<0.02), and for corn middlings, whose intake by ewes increased as the experiment progressed (r = +0.53; P<0.05). The DMI tended (P<0.10) to increase as the experiment progressed when the lambs were fed pea grains (r = +0.50), soybean hulls (r = +0.48), corn grains (r = +0.47), and oat grains (r = +0.26).

DISCUSSION

Basal diets, experimental feeds and weight variations

The composition of the ingredients of the basal diet and of the whole basal diet corresponded to the values normally considered to constitute a diet supply able to cover the requirements of the lambs and the ewes (Table 1 and 2). The fact that lambs had higher protein and NFC intake, even though their DMI of the basal diets was similar to that of the ewes, was probably the result of their higher intake of barley and urea mix, to cover their high growth requirements. The lower NDF concentration in the diet of lambs than in that of ewes probably accounted for their lower rumen capacity and NDF digestibility.

The composition and intake of basal diets was sufficient to ensure a mean growing rate of the lambs of 80 g/d. This is a quite low growth rate for lambs in the third month of life. (they started the experiment with a men age of 72 d and completed it at 103 d). Bussu (2002) and Golosio (2006) observed that Sarda lambs with a mean age of 75 days had a growth rate around 150 g/d. Moro (2000) found, with lambs with a comparable age of those used in this trial, a growth rate of about 100 g/d. The low growth rate of the lambs involved in this trial might be explained by the source of CP used. Indeed, urea, which was chosen as a CP source to avoid the utilization of other CP sources that were going to be used in the experiment, might not have stimulated adequate amino acid supply.

The ration was adequate for the requirements of mature ewes, which had a stable BW and BCS during the experiment.

The highly variable composition of the experimental feeds (Table 1) was expected considering that cereals, protein meals and digestible fiber sources were included.

Animal training

Before the experiment started, it was forecasted that one of the biggest difficulties would have been to teach to the animals the daily routine of the palatability tests, which required that they entered alone into the individual pen, consumed the concentrate in the bowls, and finally moved from the individual pen to the adjacent collective pen. However, the animals learned the routine quite quickly. In fact, both lambs and ewes started to eat a reasonable quantity of barley meal in the individual pen already after the 3rd day of the adaptation period (Figure 1) and achieved a stable intake after 9 days only.

Feed intake during the palatability tests

The intake of experimental feeds showed clear differences on feed palatability among lambs and among ewes and between lambs and ewes.

Lambs

The lambs were chosen from the flock on the basis of their age, aiming to have the youngest of them, with little feeding experience. At the beginning of this experiment the lambs were (mean days ± S.D.) 72 ± 9 days old and had been weaned for 32 ± 8 days. This means that their feeding experience with solid feeds was limited to the feeds described in the Materials and Methods section.

The rank of lambs' feed preference showed no clear trends in favor of protein sources, starch sources, or fiber sources. Thus, it seems that the animals were not looking for a specific nutrient. The lack of clear trends also suggests a high degree of curiosity by the lambs, probably because they did not have enough experience to associate the smell or the taste of the feeds supplied with any specific post-ingestive effect. Indeed, lambs seems to create their flavor preference on the basis of the post-ingestive effects of the feeds eaten (Burritt

and Provenza, 1992; Villalba and Provenza, 1997a,b; Arsenos et al., 2000a,b; Atwood et al., 2001). In our experiment, the lambs were separated from the mother after weaning and could not learn from them which feed to prefer or discard; being thus forced to learn by experience the association between feed flavor and post-ingestive consequences (Villalba and Provenza, 2000). Therefore, lambs could not take advantage of the fact that, as demonstrated by Provenza (1995), information regarding feed characteristics and danger can be socially transmitted between generations, especially through the strong relationship between mother and offspring. Considering their lack of experience, it is surprising that the lambs exerted a certain degree of preference, especially in favor of soybean meal 49, soybean meal 44, wheat grains, corn grains, beet pulps. This might be explained by the fact that these feeds were all included components in the pellets fed to the lambs before the beginning of the trial. Therefore it is possible that the lambs were familiar with the flavor associated with these feeds and, consequently, with their associated post-ingestive consequences. However familiarity cannot be the only reason for the observed acceptance or refusal of the feeds tested, because dehydrated alfalfa, which was almost completely refused during the palatability tests (Table 4), was also included in the pellets. In addition, alfalfa hay was fed to the lambs before the adaptation period started.

Pea grains, which were also highly preferred by lambs, were not present in the formulation of the pellets used after weaning. Therefore, this preference for pea grains can be explained in two ways: 1) high intrinsic palatability, due to its sweet taste; 2) the lambs might have become familiar with this feed, very commonly used for dairy sheep feeding, before birth (Hudson and Distel, 1999; Schaal et al., 2000; Mennella et al., 2001) or during the weaning period, before the beginning of the trial. Pea grains were also characterized by high intake rate, being equal to that of soybean meal 44 and the second after soybean meal 49 (Table 5 and Figure 2).

Some feeds were almost completely refused by all lambs (Table 4 and Figure 2). In particular, the very low intake of dehydrated alfalfa and of oat grains is hard to explain, considering their widespread use in animal nutrition. The total rejection of dehydrated alfalfa by lambs and ewes suggests the presence of some off-flavors in our samples, possibly produced during the high temperature dehydration process of alfalfa. It should be remarked that the dehydrated alfalfa present in the pellets used after weaning and that tested in this experiment had different origin. The compete rejection of oat grains might be associated with its high content of short chain free fatty acids, which have been associated with off-flavors in milk (Kim Ha and Lindsay, 1991).

The fact that one of the most preferred feeds by lambs was the soybean meal 49 might be explained by the high protein requirements of this category of animals. However, the amount of soybean meal 44 eaten was not as high as that of soybean meal 49 and the lambs refused other feeds with high protein content such as the corn gluten meal and canola meal. Therefore, other factors were probably involved in these responses.

Even though each lamb tested each feed only once, it was expected that as the experiment progressed the overall daily intake of the lambs would have increased, as a result of the fact that the lambs would have got used to the continuous change in feed. However, this phenomena was significant only for corn gluten meal, while only a weak trend was observed for other four feeds (pea grains, soybean hulls, corn grains, and oat grains). No clear explanations can be given on this regard.

Ewes

The DMI of experimental feeds by the mature dry ewes showed a clear difference between two groups of feeds, one composed of four feeds with very high palatability (beet pulps and wheat, pea, and corn grains), the other composed of the rest of the feeds, which had low palatability (Table 4). The DMI

of each of the 4 very palatable feeds was at least 3 times higher than that of the other feeds. The preference of the ewes can be at least partly explained by their feeding experience. In fact, the most palatable feeds are among the most commonly used for the feeding of sheep in Sardinia. They are also often supplied alone, without being mixed with other feeds. For this reason, they could probably be easily identified by the ewes and associated with positive flavor or post-ingestive effects. Beet pulps are often used for their richness in digestible fiber and for their low protein content, both for sheep grazing young pastures rich in protein and poor in ruminable fiber and for mid-late lactation ewes to improve the lactation persistency (Cannas et al., 2002). Also pea grains, wheat grains and corn grains are commonly used in the AGRIS flock, from which the ewes were selected, as ingredients of mixed feedstuffs, or as single ingredient. However, it should be pointed out that also some of the least preferred feeds by ewes, such as soybean meal 44 and 49, soybean hulls, sunflower meal, wheat brans, oat grains and dehydrated alfalfa, are frequently used. Among the ingredients tested, canola meal, corn gluten meal, and corn middlings are probably the least used in the local feed mills. A common characteristic of the least preferred feeds is that, with the exception of dehydrated alfalfa, they are usually included in feed mixes and not used alone. Thus, one possible explanation for their refusal was that the ewes might have had difficulties to associate their flavor with post-ingestive effects.

Rates of intake were measured in our trial because they are considered as one of the best indicator of the degree of liking of a feed by indicating the wish to eat the feed (Baumont, 1996). Even the analysis of intake rates showed that ewes strongly refused the low palatability group of feeds, even though their rate of intake was proportionally higher than their DMI (Figure 3).The latter observation may suggest that these feeds were at first appreciated by the animals (fairly high intake rate) but quickly evoked negative short term post-ingestive effects. Many of them were protein meals (soybean meal 44 and 49, sunflower meal, canola meal, corn gluten meal) and might have induced a negative intake feedback through excessive ammonia formation. This might have not happened when pea

grains were used because this feed, even though being often considered a protein source, is richer in starch than in protein. The other very palatable feeds (beet pulps, wheat grains and corn grains) are all very low in CP content (Table 1). The fact that the basal diet contained urea, a very soluble source of CP, might have exacerbated the negative effects of high protein feeds with low NFC content. However, this explanation might not be appropriate to explain the low palatability of oat grains and soybean hulls. The former showed low intake also by lambs, probably for the presence of short chain free fatty acids, as already discussed. Soybean hulls, instead, where eaten in fairly large amounts by the lambs (Table 4 and Figure 2). Similarly to beet pulps, which were the most palatable feed in this trial, soybean hulls are very rich in pectin (Ipharraguerre and Clark, 2003) and are a common ingredient of feed mixes for dairy sheep (Cannas et al., 2002). Several experiments have showed that the presence of soybean hulls in high concentrations (between 40% and 60% of dietary DM) was associated with very high intake both in total mixed rations (Boe et al., 2004) and in pelleted diets (Cannas et al., 2003; Boe, 2007). Thus, not clear reasons can be given for their low palatability with adult ewes in this trial.

Since most of the feeds were probably familiar to the ewes, at least as components of pellets or concentrate mixes, we can hypothesize that at least for the feeds with medium-low protein content the choices of the ewes were driven more by the sensorial effects that the feeds evoked than by their post-ingestive effects. For example, since oats were finely ground in our experiment, this treatment might have generated enough heat to induce the production of off-flavours. As reported by Molteberg et al. (1996), the heat process make oats develop intense rancid odor and flavor and bitter taste, especially when they are heat-treated with their hulls (Molteberg et al., 1996).

Comparison between lambs and ewes

The comparisons between lambs and ewes were based on their DMI Ratio, intake rates, LI-BW, and LI-MW.

The results of the DMI Ratio, showing that the lambs ate more soybean meal 49, soybean hulls and soybean meal 44 and less beet pulps, corn gluten meal and oat grain than the ewes ($P < 0.05$, Table 4) were substantially confirmed by the LI-BW and LI-MW analysis (Table 6). The results of intake rates showed that the lambs had an higher intake rate only of soybean meal 49 than the ewes, while the ewes showed a faster ingestion of pea grains, wheat brans, beet pulps, wheat grains, corn middlings, corn gluten and oat grains than the lambs. These results can be attributed more to the differences of body size among lambs and ewes than to palatability effects. Indeed, it is well known that young animals have a slower eating rate than adult ones (Van Soest, 1994).

The fact that lambs preferred soybean by-products (meals and hulls) could be explained by their high protein requirements for growth, but many other protein meals available were not chosen. For this reason, it is more likely that lambs were more familiar with soybean products, which were present in the pellets they used after weaning, than with the other protein sources.

It is possible that the high preference for soybean hulls, not used in the above mentioned pellets, was due to a process of generalization, which increases the ingestion of a novel feed when it is characterized by a flavor similar to that of a known feed (Launchbaugh and Provenza, 1994).

The higher preference of the ewes for beet pulps compared to the lambs can be explained by the fact that the former were already accustomed to eating this feed. Beet pulps are often included in concentrate mixes and, even more frequently, are supplied alone in the barn manger before bringing the ewes to the pasture or indoors at the end of the grazing time (at night).

Both lambs and ewes ate very little corn gluten meal and oat grains. Therefore, the statistically higher preference for these 2 feeds exerted by the ewes compared to the lambs might not have an important biological implication.

One of the most interesting results of this experiment was that some feeds were refused by both lambs and ewes. Dehydrated alfalfa, sunflower meal, and canola meal were eaten in very small amounts by both sheep categories. Low intake was also observed for corn gluten meal and corn middlings. The reason for their rejection cannot be associated with the degree of feeding experience. Thus, the most likely explanation is that these feeds evoked negative sensorial perceptions in both sheep categories. The source of these negative perceptions should be explored by an appropriate characterization of the compounds present in these feeds that might affect their flavor or taste. The fact that the content of NFC was the chemical component of the feeds with the strongest relationship with LI-MW (Figure 4) could suggest that it affected the taste of feeds, and both lambs and ewes tended to prefer the sweetest feeds. However, the correlation between the two variables was weak and cannot explain the high palatability of the feeds with low NFC, i.e. soybean meal 49 and 44, showed by the lambs. Differently, ewes showed a strong preference for beet pulps, pea grains and wheat grains, which all are feeds rich in NFC. Thus, the palatability behavior observed in this experiment cannot be fully explained by the chemical contents of the feeds and is probably also associated with the feeding experience and the sensorial properties of the feeds.

CONCLUSIONS

The results of the palatability tests showed clear differences between the feeds chosen by the lambs and those chosen by the ewes.

Lambs showed preferences for feeds previously experienced in their life (i.e. soybean meal 49, soybean meal 44, corn grains). However they also ate in substantial (e.g. pea grains) or small (e.g. corn gluten meal, sunflower meal, and canola meal) amounts feeds they had never eaten before. They also totally refused an experienced feed (dehydrated alfalfa) and a not previously experienced feed (oat grains). Thus, the intake of the experimental feeds by lambs varied from high to low values in a continuum, without clear cuts. These results suggest that their choices were markedly influenced by sensorial perceptions (smell and/or flavor) and, to a lesser extent, by previous feeding experiences. In contrast, the ewes showed a marked preference for 4 feeds often supplied as single ingredients (beet pulps and wheat, pea, and corn grains), which they could probably identify clearly, and low intake or almost total rejection for the other feeds, including several feeds commonly used for sheep feeding but rarely used as single ingredients. This suggests that the choice of the ewes was markedly influenced by the sensorial properties of the feeds or that they were less prone than lambs to experience novel flavors.

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Table 1. Chemical composition of the ingredients of the basal diet and of the experimental feeds.

Ingredients	*Chemical composition (% of DM)*							
	DM	CP	NDF	ADF	ADL	Ash	EE	NFC*
Basal diet								
Ryegrass hay	86.7	10.3	61.1	35.0	7.1	10.0	2.0	16.6
Barley	87.2	11.8	11.9	7.2	0.3	2.1	2.0	72.2
Urea	99.0	281.0	-	-	-	-	-	-
Experimental feeds								
Alfalfa, dehydrated	89.0	16.3	58.4	43.7	8.6	11.5	2.2	11.6
Beet pulps	88.9	10.0	50.6	26.2	6.9	4.2	0.7	34.5
Corn gluten meal	90.4	69.9	21.5	7.9	4.3	1.3	2.6	4.7
Corn grains	87.8	8.5	26.9	4.6	0.4	1.1	3.9	59.6
Corn middlings	87.3	17.8	27.7	7.6	1.7	4.1	3.7	46.7
Oat grains	89.3	11.0	43.3	24.9	3.4	6.3	5.2	34.2
Pea grains	86.7	22.3	19.1	7.5	1.5	4.3	1.9	52.4
Canola meal	87.1	38.3	35.2	26.0	14.4	8.0	2.8	15.7
Soybean hulls	88.7	14.9	60.9	44.9	9.5	5.1	2.1	17.0
Soybean meal 44	87.8	43.7	23.5	17.4	3.0	6.9	1.3	24.6
Soybean meal 49	87.6	51.5	23.5	9.6	2.0	7.3	1.8	15.9
Sunflower meal	89.4	32.6	52.6	34.4	11.7	7.1	2.9	4.8
Wheat brans	86.6	17.2	43.8	13.1	3.3	5.3	3.6	30.1
Wheat grains	86.3	12.6	22.4	3.8	0.6	1.7	1.9	61.4

* calculated on a DM basis as [100-NDF-CP-EE-ash], where EE = ether extract.
- = not measured

Table 2. Ingredients and chemical composition of the diets fed to lambs and ewes during the adaptation and the experimental period.

	Lambs	Ewes
	Ingredients (% of DM)	
Ryegrass hay	71	83
Barley meal	28	16
Urea	0.9	0.6
	Chemical composition (% of DM)	
DM	87	87
CP	13	12
NDF	47	53
ADF	27	30
ADL	5	6
Ash	8	9
EE	2	2
NFC*	30	24

* calculated on a DM basis as [100-NDF-CP-EE-ash], where
EE = ether extract.

Table 3. Mean feed and nutrient intake of the ingredients of the basal diet during the experimental period (14 days).

	Lambs	Ewes
	Feed intake, g/d per head	
Ryegrass hay	368 ± 54	500 ± 17
Barley meal	190 ± 4	76 ± 23
Urea	6.2 ± 4	2.6± 0.8
	Nutrients intake, g/d per head	
DM	565	578
CP	74	67
NDF	263	304
ADF	151	175
ADL	29	34
Ash	43	50
EE	11	11
NFC*	170	139

* calculated on a DM basis as [100-NDF-CP-EE-ash], where
EE = ether extract.
SD = standard deviation among days.

Table 4. Mean DMI and mean DMI Ratio (mean DMI of the experimental period of each experimental feed/mean DMI of barley meal, last 4 days of the adaptation period) of each feed, ranked in decreasing order of lamb preference, fed to lambs and ewes during the 6 min tests.

Feed	DMI (g/d per head)		DMI Ratio (%)	
	Lambs	Ewes	Lambs	Ewes
Soybean meal 49	24.5 [a]	3.8 [b]	55.0 [a]	3.4 [b]
Wheat grains	22.8 [a]	56.4 [a]	51.3	55.6
Pea grains	17.4 [ab]	56.3 [a]	39.2	60.2
Corn grains	14.9 [abc]	52.7 [a]	35.2	54.1
Soybean hulls	13.1 [abcd]	6.3 [b]	29.8 [a]	5.5 [b]
Beet pulps	11.9 [abcd]	62.8 [a]	24.9 [b]	65.8 [a]
Wheat brans	11.4 [abcd]	18.6 [b]	28.0	19.6
Soybean meal 44	10.9 [abcd]	7.1 [b]	24.1 [a]	7.0 [b]
Corn middlings	7.5 [bcd]	18.0 [b]	16.4	19.1
Canola meal	5.0 [bcd]	2.7 [b]	10.4	2.6
Sunflower meal	2.8 [cd]	6.2 [b]	6.1	6.1
Corn gluten meal	1.7 [cd]	14.8 [b]	4.7 [b]	27.4 [a]
Dehydrated alfalfa	0.4 [d]	1.5 [b]	1.3	1.4
Oat grains	0.0 [d]	9.8 [b]	0.4 [b]	10.1 [a]
SEM	2.1	6.2	0.05	0.06
P (day) <	0.005	0.4	0.001	0.07
P (animal) <	0.001	0.001	0.001	0.001
P (feed) <	0.001	0.001	0.001	0.001
P (sheep category)	-		0.05	

- analysis not performed

[a,b,c,d] Letters indicate differences within columns for DMI and between columns for DMI Ratio ($P < 0.05$)

Table 5. Percentage of animals that refused the feeds and intake rate (g/s) of the different experimental feeds, ranked in decreasing order of lamb feed preference, fed to lambs and ewes during the 6 min tests.

Feeds	Animal which did not eat (% of the total)		Intake rate (g/s)	
	Lambs	Ewes	Lambs	Ewes
Soybean meal 49	14	50	0.35 ^{Aa}	0.12 ^{Bef}
Soybean meal 44	14	29	0.28 ^{ab}	0.24 ^{cde}
Pea grains	7	0	0.28 ^{Bab}	0.52 ^{Aa}
Corn grains	0	7	0.22 ^{abc}	0.34 ^{bcd}
Wheat brans	14	7	0.18 ^{Bbcd}	0.28 ^{Acde}
Beet pulps	29	0	0.17 ^{Bbcde}	0.49 ^{Aab}
Soybean hulls	21	36	0.16 ^{bcde}	0.05 ^{f}
Wheat grains	21	0	0.14 ^{Bbcdef}	0.39 ^{Aabc}
Canola meal	43	43	0.09 ^{cdef}	0.06 ^{f}
Corn middlings	14	14	0.07 ^{Bcdef}	0.21 ^{Acdef}
Sunflower meal	29	7	0.05 ^{def}	0.15 ^{ef}
Corn gluten meal	29	0	0.01 ^{Bef}	0.28 ^{Acde}
Dehydrated alfalfa	71	50	0.00 ^{Bf}	0.04 ^{Af}
Oat grains	50	14	0.00 ^{Bf}	0.18 ^{Adef}
SEM	-	-	0.03	0.04
P (day) <	-	-	0.001	0.10
P (animal) <	-	-	0.001	0.001
P (feed) <	-	-	0.001	0.001
P (sheep category)	-		0.05	

^{A,B} Capital letters indicate differences between columns ($P < 0.05$)
^{a,b,c,d,e,f} Small letters indicate differences within column ($P < 0.05$)
- = not calculated

Table 6. Mean DMI level of intake on a BW (LI-BW; mg/kg BW) and metabolic weight basis (LI-MW; mg/kg $BW^{0.75}$) of each feed, ranked in decreasing order of lamb preference, fed to lambs and ewes during the 6 min tests.

Feed	LI-BW mg/kg BW		LI-MW mg/kg $BW^{0.75}$	
	Lambs	Ewes	Lambs	Ewes
Soybean meal 49	1379 ^{Aa}	69 ^{Bb}	2829 ^{Aa}	188 ^{Bb}
Wheat grains	1289 ^{a}	1130 ^{a}	2640 ^{a}	3001 ^{a}
Pea grains	941 ^{ab}	1138 ^{a}	1949 ^{ab}	3016 ^{a}
Corn grains	820 ^{abc}	1049 ^{a}	1691 ^{abc}	2790 ^{a}
Soybean hulls	757 ^{Aabcd}	128 ^{Bb}	1543 ^{Aabcd}	339 ^{Bb}
Beet pulps	686 ^{abcd}	1267 ^{a}	1400 ^{Babcd}	3360 ^{Aa}
Wheat brans	653 ^{abcd}	374 ^{b}	1334 ^{abcd}	991 ^{b}
Soybean meal 44	611 ^{Aabcd}	142 ^{Bb}	1255 ^{Aabcd}	377 ^{Bb}
Corn middlings	421 ^{bcd}	361 ^{b}	866 ^{bcd}	958 ^{b}
Canola meal	289 ^{bcd}	54 ^{b}	589 ^{bcd}	144 ^{b}
Sunflower meal	155 ^{cd}	122 ^{b}	320 ^{cd}	326 ^{b}
Corn gluten meal	92 ^{Bcd}	504 ^{Ab}	191 ^{Bcd}	1346 ^{Ab}
Dehydrated alfalfa	23 ^{d}	30 ^{b}	46 ^{cd}	80 ^{b}
Oat grains	0 ^{Bd}	203 ^{Ab}	0 ^{Bd}	533 ^{Ab}
SEM	119	124	243	330
P (day) <	0.002	0.46	0.002	0.44
P (animal) <	0.001	0.001	0.001	0.001
P (feed) <	0.001	0.001	0.001	0.001
P (category) <	0.05		0.05	

^{A,B} Capital letter indicates differences between lambs and ewes on LI-BW or LI-MW ($P < 0.05$)

^{a,b,c,d} Small letters indicate differences within columns ($P < 0.05$)

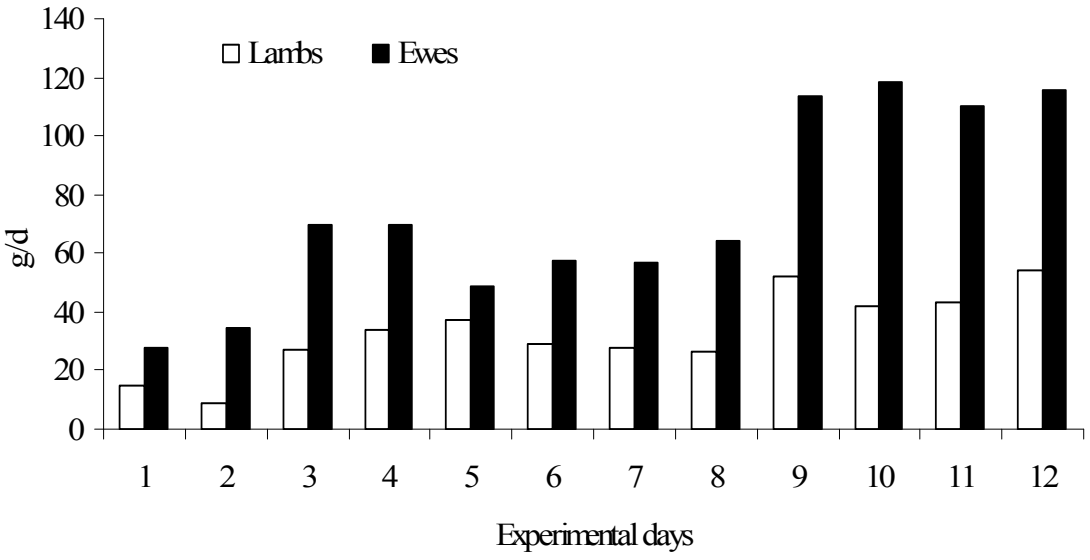

Figure 1. Daily barley intake of lambs and ewes during the 6 min palatability tests during the adaptation period.

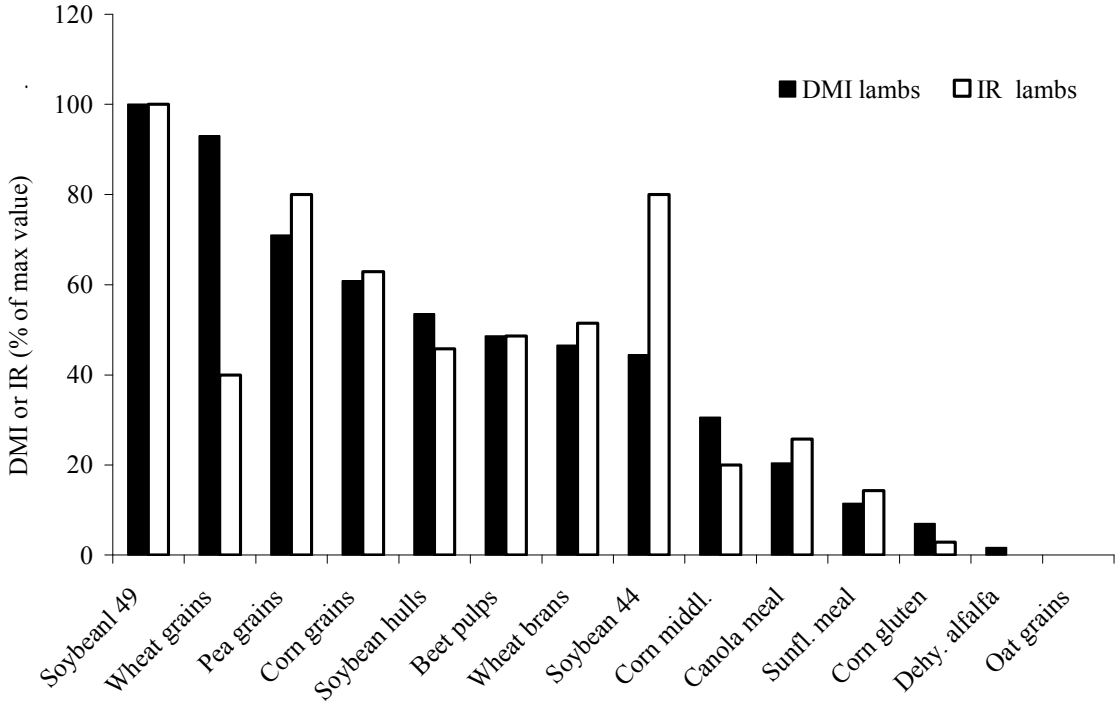

Figure 2. DMI and intake rate (IR) of each of the feeds used in the palatability tests, expressed as proportion of the value observed for the feed with the highest DMI or IR. Measurements on lambs.

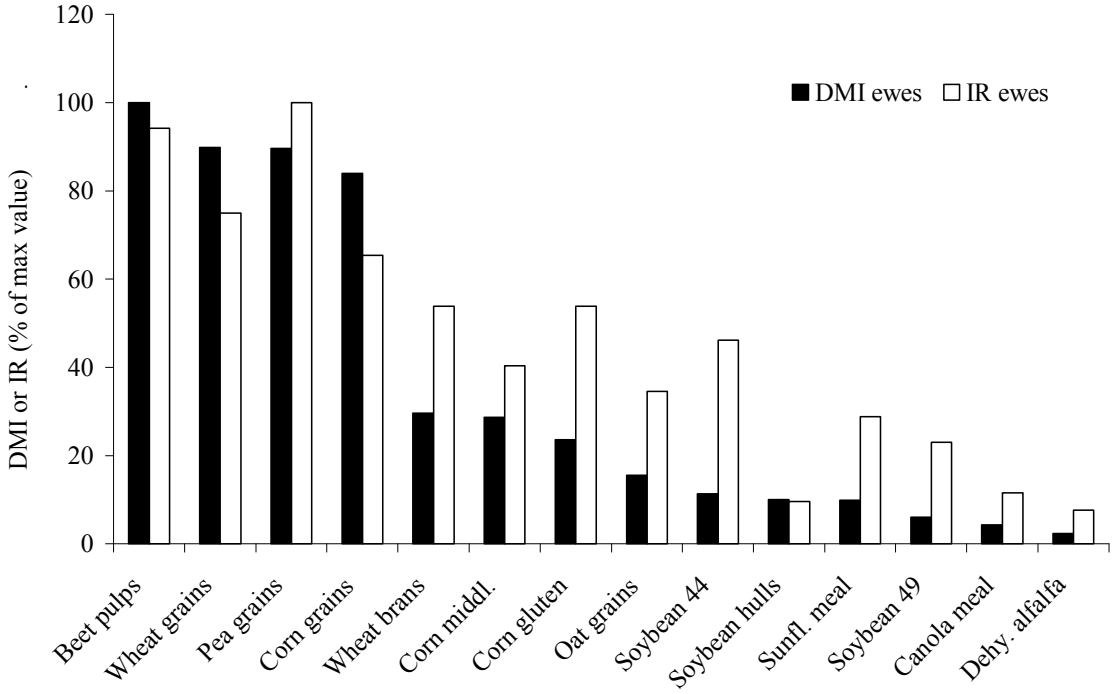

Figure 3. DMI and intake rate (IR) of each of the feeds used in the palatability tests, expressed as proportion of the value observed for the feed with the highest DMI or IR. Measurements on ewes.

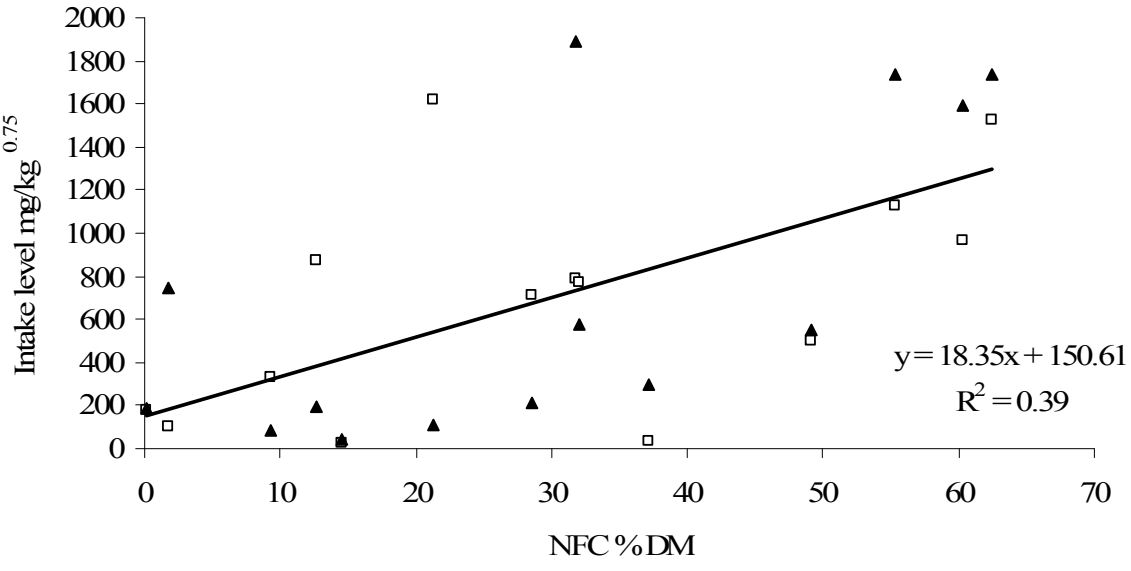

Figure 4. Relationship between NFC content of the experimental feeds and level of intake on a metabolic weight basis (LI-MW; mg/kg $BW^{0.75}$) of lambs (□) and ewes (▲).

CHAPTER 3

THE USE OF FLAVOURS TO IMPROVE THE PALATABILITY OF CANOLA MEAL AND OAT GRAINS FED TO LAMBS AND EWES

ABSTRACT

The aim of this study was to test the possibility of enhancing the palatability of canola meal and oat grains, by adding to them synthetic flavours, using two sheep categories (young female lambs and dry multiparous ewes) with different levels of feeding experience. For this reason, two experiments were carried out. In the first experiment the palatability of canola meal supplied alone (control) or in combination with 13 different flavours was studied in inexperienced animals (lambs) or experienced ones (mature ewes). The second experiment resembled the first one except for the use of oat grains instead of canola meal. The flavours tested were: 1 - sweet taste product with stevia, licorice and fenugreek notes; 2 - sweet taste product with licorice and fenugreek notes; 3 - sweet flavour and taste with anisic and toasted notes of licorice; 4 - sweet flavour and taste characteristic of natural sugar beet molasses; 5 - sweet flavour and taste with pleasant orange note characteristic of juice; 6 - sweet flavour and taste with pleasant apple note; 7 - sweet flavour and taste with pleasant creamy coconut and vanilla bottom; 8 - saccharine free sweetener nucleus; 9 - savoury taste product; 10 - fresh onion flavour with a savoury fraction; 11 - pleasant combination of cereals notes with slight toasted character; 12 - combination of fatty and roasted notes characteristic of argan oil; and 13) herbal flavour with bitter alfalfa note.

The sensorial effects of canola meal and oat grains (controls) and flavour combinations were evaluated in 6 min palatability tests on 14 female lambs and 14 multiparous dry ewes, following a 14 (days) x 14 (feeds) Latin square design.

In experiment 1, the lamb mean dry matter intake (DMI) did not differ among treatments, with the DMI of two treatments being numerically higher and of all

others being lower than the control. In the case of ewes, the intake of canola meal flavoured with products 12 and 2 was statistically higher ($P < 0.05$) than that of canola meal flavoured with products 6 and 9. However, none of the treatments was significantly different from the control. For many treatments, intake increased significantly as the experiment progressed. In particular, there was a significant relationship between DMI and time for the treatments 1 to 5 for lambs and for all treatments, except for the number 12, for ewes. The slopes of the regressions were always numerically higher for ewes than for lambs, but the differences in slope became significant only for the treatments 4 and 5. In the case of ewes, for several treatments (i.e. 5, 7, 8, 4 and 9, in decreasing order), the association was very high, with treatment 5 having the highest value ($r^2 = 0.85$).

In experiment 2, the mean DMI of lambs had a fairly high variability among treatments, even though the differences were not significant. In the case of mature ewes the variability among treatments was much smaller than that observed for lambs and was not significant either. In lambs the time effect (increased intake as the experiment progressed) was significant only for the treatment 6. In the case of ewes, time effect was significant for a larger number of treatments (in decreasing order of association: 6, 3, 1 and 2, 5, 4, 12, control, 8). The slopes of the regressions were always numerically higher for ewes than for lambs, with the highest value observed for the diet 1 of the ewes. The degree of association was in general smaller in experiment 2 than in experiment 1.

In conclusion, the two experiments showed that some flavours favoured the adaptation of the animals to the initially unpalatable feeds tested, being the ewes more prone to adapt to these feeds than the lambs.

Key words: palatability, sheep, flavours, canola meal, oat grains

INTRODUCTION

In dairy sheep farms, the ewes are usually fed on pasture and concentrate supply occurs only during the two daily milkings. Since milking sheep is a fast process, it is important that the concentrates supplied during milking are palatable and eaten quickly. Concentrate mixes, or some of their ingredients, are often changed by the farmers to adapt the concentrate composition to the variations in the stage of lactation of the animals or in the composition or availability of the pasture. In addition, feed mills often need to substitute the ingredients that become too expensive or that are not available any more. When novel feeds are rejected by animals, important production losses (Ortega-Reyes et al., 1992), proportional to their production level,, occur.

The avoidance of a new or unfamiliar feed is very common in ruminants and is normally due to generation of fear sensation (Chapple et al., 1987; Provenza et al., 1995). However, recently Herskin et al. (2003) found that exposure to new feeds did not cause an increase in hearth rate in cattle, suggesting that new or unfamiliar feeds do not cause fear but are rejected for other reasons instead.

Feed refusal of new feeds is connected with feed reward and is related with a phenomenon called food neophobia (Launchbaugh et al., 1997), which is probably the result of an innate protective mechanism that ensures the animal the possibility to associate the flavor of new feeds with the post-ingestive consequences of their ingestion on their health and nutritional status(Provenza, 1995). However, the way flavors act on the acceptability of new feeds by ruminants is still unknown.

In a previous trial (Chapter 2 of this Dissertation) on the palatability of a variety of feeds, commonly used in feed mills, in lambs and dry ewes, we found a broad range of responses, which differed depending on the previous experience of the animals. However, some of the feeds were refused by both categories of sheep, among which we chose one protein source, canola meal, and a energy source, oat grains to conduct the successive study here presented. Both feeds are important

ingredients in ruminant nutrition. In particular, canola meal is important because of its good amino acidic composition, but its use is often limited by its low palatability (Morand-Fehr, 2003); whereas oat grains are a very common starchy feed, which is often used both at a farm and feed mill level to substitute barley. A way to enhance the acceptability and palatability of these feeds could be to improve their sensorial properties by adding flavors which remind aroma and/or tastes well-known and liked by the animals (Launchbaugh et al., 1997) or flavors which have been associated with intake increase in ruminants (Nombekela and Murphy, 1995; Thomas et al., 2007).

Thus, the aim of this study was to test the possibility of enhancing the palatability of canola meal and oat grains by adding to them synthetic flavors. This was done on two sheep categories (young female lambs and dry multiparous ewes) to study possible interactions between palatability of the tested flavors and the different levels of feeding experience of the animals.

MATERIALS AND METHODS

This study consisted of two experiments.

In the first experiment the palatability of canola meal supplied alone (control) or in combination with 13 different flavours was studied in inexperienced animals (female lambs) or experienced ones (mature ewes).

The second experiment resembled the first one except that oat grains instead of canola meal were used.

Experiment 1

The experiment was carried out at the AGRIS research centre of Bonassai (Olmedo) located in the North-West of Sardinia (Italy) with 14 Sarda multiparous dry ewes and 14 Sarda female lambs. Before the beginning of the experiment the selected ewes and lambs were confined in two collective pens and fed a mixture of barley meal, urea and ryegrass hay. Animals had access to water and to a block of minerals and vitamins *ad libitum*. The adaptation period started on May 22nd and lasted 9 days in total, during which the following daily *routine* scheme was applied:

- at 7:00 a.m. barley and hay refusals were took off from the colletive pens (one for lambs, one for ewes);
- at 8:00 a.m. lambs and ewes, in sequence, were trained: 1) to spontaneously enter an individual pen with a manger containing two steel bowls with 100 g of barley meal each; 2) to stay there for 6 minutes for the palatability test (to be conduceted int the experimental period); in this time the animals were left alone, not being disturbed but being able to see the other animals; 3) to go to an adjacent collective pen at the end of the test, where they received ryegrass hay *ad libitum*, in order to limit the post-ingestive effects of barley;
- after all animals had finished the activities described above, they were brought back to their original collective pen and fed rationed amounts of

ryegrass hay, put in a hay rack, and barley meal mixed with urea, supplied in a manger.

The sequential entry of the two groups was inverted each day in order to limit a possible fasting effect.

After the adaptation period, the experimental period started, lasting 14 days in total. During this period the daily *routine* scheme was applied as explained before but in place of the barley meal, 200 g of canola meal were supplied, during the 6 min palatability test, alone or after being mixed with 13 different flavors (Table 1). The feeds were offered in rotation following a 14 (canola meal alone or canola meal + 13 flavors) x 14 (days) completely randomized Latin square design for each sheep category. The animals were not disturbed during the 6 min time, being left alone in the experimental pen. When the experiment was completed, both lambs and ewes were supplemented twice a day with a basal diet composed of a mixture of barley meal plus urea and ryegrass hay until the adaptation of the experiment 2 started. The basal diet was supplied in amounts that could cover the maintenance and growth requirements of the lambs and the maintenance requirements of the ewes.

Animals

Fourteen female lambs used in a previous concentrate palatability experiment (Chapter 2 of this Dissertation) were also used in this experiment. Their body weight at the beginning of the experiment was equal to 21.0 ± 2.3 kg.

Fourteen multiparous mature dry ewes used in the same previous concentrate palatability experiment (Chapter 2 of this Dissertation) were also used in this experiment. Their body weight at the beginning of the experiment was equal to 49.6 ± 4.2 kg and their BCS was 3.0 ± 0.2.

Feeds and flavors

Canola meal tested in this experiment was the same used in the previous concentrate palatability trial (Chapter 2 of this Dissertation). Thirteen flavors (Table 1), produced by LUCTA SA (Barcelona, Spain) and provided in form of powder were tested, being named with numbers as follows: 1 - sweet taste product with stevia, licorice and fenugreek notes; 2 - sweet taste product with licorice and fenugreek notes; 3 - sweet flavour and taste with anisic and toasted notes of licorice; 4 - sweet flavour and taste characteristic of natural sugar beet molasses; 5 - sweet flavour and taste with pleasant orange note characteristic of juice; 6 - sweet flavour and taste with pleasant apple note; 7 - sweet flavour and taste with pleasant creamy coconut and vanilla bottom; 8 - saccharine free sweetener nucleus; 9 - savoury taste product; 10 - fresh onion flavour with a savoury fraction; 11 - pleasant combination of cereals notes with slight toasted character; 12 - combination of fatty and roasted notes characteristic of argan oil; and 13 - herbal flavour with bitter alfalfa note.

Experimental flavors (Table 1) were composed of taste enhancers (flavors 1, 2, 8, 9), aroma and taste enhancers (flavors 3, 4, 5, 6, 7), and aroma enhancers (flavors 10, 11, 12, 13).

The canola meal was finely ground with a 1 mm screen size by using a hammer mill, to reduce the effects of the physical form on their palatability.

The flavors were mixed with the canola meal according to the following scheme: the flavour (105 g) was mixed with 7 kg of canola meal into a sac. The sac was vigorously shaken for 5 minutes, in order to ensure a homogeneous incorporation of the flavour in the feed. The day before the beginning of the experimental period, the 100 g of flavoured and unflavoured canola feeds were weighed, placed in small plastic bags and preserved in a refrigerator (4°C). Every morning before the beginning of the trial the flavoured feeds were taken out from the fridge, to reach room temperature, and then used in the trial. The chemical composition of the ingredients of the basal diet and of the canola meal are

summarized in Table 2. The quantity of basal diet supplemented to each sheep category during the whole trial was balanced to satisfy their requirements (Table 3).

Measurements

The amount of feed consumed by each animal was measured as the difference between the weight of feed offered and that remained after 6 minutes (the time spent in the individual pen). The feeding behaviour of the animals during the 6 min test was recorded with a digital camera (Sony DCRSR32E; Sony, Japan). After the end of the experiment, the videos were analysed to measure the time each animal spent eating each feed during the 6 min test. The intake rates for each feed were then calculated as the amount of feed consumed divided by the time spent eating each feed. Dry matter intake (DMI) of each feed was expressed as daily mean per animal (g/d per head), as mean intake per body weight (mg/kg BW) (LI-BW) and as mean intake per metabolic weight (mg/kg$^{0.75}$ BW) (LI-MW). Moreover, it was calculated the ratio among intake of each experimental feed and the individual mean DMI of barley meal during the last 4 days of the adaptation period (DMI Ratio). Regression between DMI and experimental days was calculated both for lambs and ewes. Samples of feed on offer were collected once and stored until analyses at -20 °C. The body weight of lambs and ewes and the body condition score (BCS) of ewes were measured three times during the experiment: immediately before the beginning of the experimental period (23th May 2008); at the half of the experimental period (7th June 2008); and at the end of the experimental period (13th June 2008).

Chemical analyses

Feed and hay samples were analyzed for DM, ash, NDF, ADF, ADL (Van Soest et al., 1991), CP and ether extract (AOAC, 1990). The non-fiber carbohydrate

(NFC) concentration was calculated as [100-NDF-CP-EE-ash], where EE = ether extract.

Statistical analyses

Data on nutrient intakes were analysed using the **"**Proc GLM" on the basis of the following model (SAS, 1990):

$Y= \mu + \alpha_i + \beta_j + \gamma i + \delta i + \varepsilon_{ijk}$

μ = overall mean,

α_i = fixed effect of flavor,

β_j = fixed effect of animal,

δi = fixed effect of time

ε_{ijk} = random error.

Treatment means were separated by using the test of Tukey at a threshold of $P < 0.05$.

Relationships between DMI of each flavored feed and time were analyzed by simple linear regression by using the "Proc REG" (SAS, 1990). Comparisons among slopes and intercepts of these regression were performed by analysis of covariance using the "Proc. GLM" (SAS, 1990).

Comparisons between lambs and ewes DMI Ratio, Intake rate, LI-BW, LI-MW of flavored canola meal were done by one way analysis of variance.

Experiment 2

This experiment was carried out with the same methods described for experiment 1, except that ground oat grains were used instead of canola meal. The adaptation period started on June 22nd and lasted 6 days in total, while the experimental period started at the end of the adaptation period and lasted 14 days. The same fourteen female lambs coming from the experiment 1were used. Their body weight (BW) at the beginning of the experiment was 24.7 ± 2.3 kg (mean ± SD).

The same fourteen multiparous mature dry ewes coming from the experiment 1 were used. Their body weight at the beginning of the experimental period was 50.8 ± 5.2 kg (mean ± SD) and their BCS was (3.0 ± 0.3 (mean ± SD).

The body weight and BCS were measured three times during the experimental period: during the first days of the experimental period (30th June 2008); at the half of the experimental period (4th July 2008); and at the end of the experimental period (10th July 2008).

RESULTS

Experiment 1

Basal diet composition and intake and body weight variations

The composition of the single ingredients and of the whole basal diets (Tables 2 and 3) corresponded to the planned values.

The mean daily DMI intake of the basal diet was higher for the lambs than for the and ewes, as planned (Table 4). Lambs had much higher intake of barley meal (293 ± 3 g/d vs. 87 ± 1 for lambs and ewes, respectively; Table 4) and lower intake of ryegrass hay (460 ± 15 g/d vs. 531 ± 37 for lambs and ewes, respectively; Table 4) than the ewes. As a result, the lambs had higher intake of CP and NFC compared with the ewes, whereas the intake of fiber was similar between both categories (Table 4).

The weight of the lambs ranged from 21.0 ± 2.3 kg (mean ± SD) at the beginning of the experiment 1 to 23.0 ± 2.2 kg at the end of the experimental period. Thus, their mean weight gain during the experimental period was 0.1 kg/day. Ewe's weight was constant during the experimental period, ranging from 49.6 ± 4.2 kg at the beginning of the experimental period to 50.8 ± 5.2 kg at the end of the same period. The BCS of ewes was also constant, being 3.0 during the whole experimental period.

Palatability tests

During the last 4 days of the adaptation period (Figure 1), the individual intake of barley meal during the 6 minutes of the palatability test was relatively constant, and ranged from 64.1 ± 6.1 g/d (mean ± SD), for lambs, to 87.3 ± 12.4 g/d, for ewes.

The lamb mean DMI (Table 5) did not differ among treatments (canola meal fed alone, i.e. control, or combined with each of the 13 flavours), with the DMI of two treatments being numerically higher and of all others being lower than the control.. In the case of ewes, the intake of canola meal flavoured with products 12 and 2 was statistically higher ($P < 0.05$) than that of canola meal flavoured with products 6 and 9 (Table 5). However, none of the treatments was significantly different from the control. In addition, no significant differences in DMI Ratio were observed between the two sheep categories.

The intake rates did not differ among treatments within sheep category (Table 6). These rates were always numerically higher in ewes than in lambs, even though only for treatments 13 and 8 differences became significant ($P < 0.05$).

For lambs neither LI-BW nor LI-MW (Table 7) differed significantly among feeding treatments. For mature ewes, the differences observed in LI-BW and LI-MW reflected those already commented for DMI. Comparisons between lambs and ewes regarding LI-BW and LI-MW (Table 7) showed that lambs had higher ($P < 0.05$) LI-BW (1620 vs. 757 mg/kg BW) and LI-MW (3554 vs. 2015 mg/kg $BW^{0.75}$) than ewes for the canola meal flavored with product 9.

For many treatments there was a significant time effect, with an increase in intake as the experiment progressed (increasing number of experimental days). In particular, once two outliers had been excluded (the same two lambs or ewes showing zero intake for all treatments), there was a significant relationship between DMI and time for the treatments 1 to 5 for lambs and for all treatments, except for number 12, for ewes (Table 8). The slopes of the regressions were always numerically higher for ewes than for lambs, but the differences in slope

became significant only for the treatments 4 and 5 (Table 8). In the case of ewes, for several treatments (i.e.. 5, 7, 8, 4 and 9, in decreasing order), the association was very high, with treatment 5 having the highest value ($r^2 = 0.85$; Figure 3).

Experiment 2

Basal diet composition and intake and body weight variations

The composition of the single ingredients and of the whole basal diets (Tables 2 and 3) corresponded to the planned values and was the same of experiment 1. As in experiment 1, the mean daily DMI intake of the basal diet was higher for the lambs than for the ewes (Table 4), as a result of the much higher intake of barley meal (320 ± 18 g/d vs. 100 ± 1 for lambs and ewes, respectively; Table 4) and lower intake of ryegrass hay (556 ± 20 g/d vs. 639 ± 8 for lambs and ewes, respectively; Table 4) than those of the ewes. As a result, the lambs had higher intake of CP and especially of NFC compared with the ewes, whereas the intake of fiber was similar between both categories (Table 4).
The weight of the lambs ranged from 24.7 ± 2.3 kg (mean ± SD) at the beginning of the trial to 25.4 ± 2.3 kg at the end of the experimental period. Thus, the mean weight gain of the lambs measured during the experimental period was 0.08 kg/d. Ewe's weight was constant during the experiment, ranging from 50.8 ± 5.2 kg (mean ± SD) at the beginning of the experimental period to 51.0 ± 5.7 kg at its end. The BCS of ewes did not change and was around 3.0 during the experiment.

Palatability tests

During the last 4 days of the adaptation period, the individual intake of barley meal during the 6 minutes spent into the individual pen for the palatability test (Figure 2) was relatively constant and similar to that observed in experiment 1, ranging from 53.8 ± 9.8 g/d (mean ± SD), for lambs, to 91.4 ± 12.1 g/d, for ewes.

The mean DMI of lambs had a fairly high variability among treatments (ground oat grains fed alone, i.e. control, or combined with each of the 13 flavours), but the differences were not significant (Table 9). For mature ewes the variability among treatments was much smaller than that for lambs and was not significant either (Table 9).

The mean DMI of oat grains by lambs during the experiment was very low (4.4 g/d) compared to that of ewes (50.4 g/d). As a result, the DMI Ratio was much higher ($P < 0.05$) for all treatments in mature ewes than in lambs.

Even when LI-BW or LI-MW where compared between the two categories, lambs had much lower DMI than ewes (Table 10).

The intake rate of lambs was very low for all treatments (results not shown), due to the very low DMI observed during the palatability test. For the ewes, there were no significant differences among the treatments (Figure 4).

In lambs the time effect (increased intake as the experiment progressed) was not significant, even after the values of the same two lambs with zero intake had been discarded for all treatments, except for a significant and low coefficient of determination ($R^2 = 0.34$) for the treatment 6 (Table 11). In the case of adult ewes, time effect was significant, after the values of the same two ewes with zero intake had been discarded for all treatments, for a larger number of treatments (in decreasing order of association: 6, 3, 1 and 2, 5, 4, 12, control, 8; Table 11). The slopes of the regressions were always numerically higher for ewes than for lambs (Table 11), with the highest value being observed for the diet 1 of the ewes. The degree of association was in general smaller in experiment 2 than in experiment 1 (Table 11 vs. Table 8).

DISCUSSION

Basal diet composition and intake and body weight variations

The basal diet supplied in the two experiments had the same chemical composition. However, in experiment 2 the amount supplied (the diets were rationed) was slightly higher than in experiment 1 to account for the higher requirements of the growing lambs. Some extra feed was also given to the ewes in experiment 2.

In both experiments the daily intake of the basal diet, the CP and the NFC was higher in the lambs than in the ewes. This is consistent with the higher requirements in energy and protein of lambs than ewes.

The lambs started the adaptation period of experiment 1 at a mean age of 145 d, with an average BW of 21.0 kg, and completed experiment 2 at a mean age of 194 d, with an average BW of 25.4 kg. Thus, in the 21 days (adaptation and experimental periods) of the experiment 1, the mean growth rate of lambs was 100 g/d and during the 21 days of the experiment 2, it was even lower, being equal to 80 g/d.

These values are lower than those found by Moro (2000) and Bussu (2002) on lambs of comparable age fed *ad libitum*. The low growth rate of the lambs involved in this trial might be explained by the fact that urea was used as a CP source because this might not have provided an adequate amino acid supply.

The BW and the BCS of mature ewes was constant during the two experiments, suggesting that the ration covered their maintenance requirements.

In both experiments the daily routine was learned quickly both by ewes and lambs, which were already familiar with this type of activity since they had participated at the experiment described in the Chapter 2 of this Dissertation.

Barley intake in the 6 min palatability test was almost constant during the last 4 days of the adaptation period for both experiments. However, lambs' barley intake during the adaptation period of the experiment 2 was lower than during the

adaptation period of the experiment 1. This may be a result of the high barley intake as a component of the basal diet during the experiment 2. It is possible that the high environmental temperatures that occurred during the experiment 2 favoured the intake of the basal diet, which occurred mostly at night, and discouraged the intake during the palatability tests, which occurred in the morning when environmental temperatures were high. It is well known that heat stress can severely limit the intake in ruminants and modify their feeding schedule, favouring the intake during the cooler hours (Morand-Fehr and Doreau, 2001). Mature ewes did not show differences between the two experiments regarding barley intake during the palatability test carried out in the adaptation periods. Probably ewes were less affected by heat stress because: i) their intake of basal diet was, as a proportion of BW, much lower (1.24% of BW) than that of the lambs (3.45% of BW); it is well known that heat stress increases, for the same environmental conditions, as DMI increases (Morand-Fehr and Doreau, 2001); and ii) ewes had much lower barley grain intake in the basal diets than lambs (Table 4).

Palatability tests

The 13 experimental flavors tested in the two experiments covered a broad spectrum of aromas, tastes and combinations of the two (Table 1), so that they could stimulate only the taste, only the smell or both.

Experiment 1

In the case of lambs no differences occurred during the palatability tests due to the treatments for all the variables considered (DMI, DMI Ratio, intake rate, LI-BW and LI-MW). In general, the intake of the 14 feeds during the palatability tests were lower (35.2 g/d) than that observed for barley during the adaptation period (62.9 g/d), with the DMI ratio ranging from 45% (treatment 5) to 72%

(Treatment 12). However, these values are much higher than that observed for canola meal when the palatability of different concentrates was compared (10.4%, corresponding to a DMI of 5 g/d; Table 4 of Chapter 2). It is clear that for the lambs canola meal was already a known feed when experiment 1 started. A tendency for a further adaptation occurred during the experiment for the Control (canola meal without flavours), since its DMI slightly, even though not significantly, increased as the experiment progressed ($P<0.13$ with all data; $P<0.08$ when two ewes with zero intake were excluded; data not shown). This was not true for the treatments 1 to 5 (Table 8), which showed a significant effect of time over DMI in the palatability tests. All these treatments included enhancers of the sweet perception, with taste (treatments 1 and 2) or with both aroma and taste (treatments 3, 4, 5) enhancers. The DMI of these feeds in the last 4 experimental days was higher than their average calculated in the whole experiment (Figure 5).

The ewes showed a higher DMI, LI-BW and LI-MW for the canola meal flavored with the products 2 and 12 than for canola meal flavored with the products 9 and 6 (Tables 5 and 7). The low palatability of flavor 9 (savoury taste product) for ewes, differently from what observed for lambs, could be explained by the low protein requirements of ewes, since savoury taste is usually associated with protein flavour (Chandrashekar et al., 2006). Thus flavour 9 was probably more attractive for the lambs because of their high protein requirements for growth. In contrast to flavour 9, no clear explanations for the low palatability of flavour 6 for ewes can be given.

The average intake of the 14 feeds during the palatability tests was lower (53.9 g/d) than that observed for barley during the adaptation period (88.7 g/d), with the DMI ratio ranging from 56% (treatment 6) to 97% (treatment 11). What is really surprising is that the average DMI measured in this experiment is much higher than that observed for canola meal when the palatability of different concentrates was compared (DMI = 2.7 g/d; DMI Ratio 2.6%; Table 4 of Chapter 2). It is clear that the ewes quickly adapted to canola meal, since all feeds tested

in the palatability tests included it, in contrast to the experiment of Chapter 2 in which each ewe had canola meal only once.

Looking at the analysis of regressions (Table 8), it appears that the DMI of the ewes in the palatability tests for all treatments, except for the n. 12, was affected by the experimental days, with increasing intake as the experiment proceeded (Figure 3 for treatment 5). In lambs this phenomena was observed only for five treatments and the slopes, which indicate the intensity of the effect, were much lower than those observed for the mature ewes. Considering the importance of the time effect on the ewes, the mean DMI in the last 4 experimental days was calculated for each treatment and compared with the corresponding mean DMI in the 14 days of the experiment. It appeared that for some of the treatments (especially 2, 3, 4, 5) the DMI in the last 4 days of the experiment was much higher than that of the Control. Thus, this suggests that, over time, the ewes adapted to canola meal, which was the base of all treatments, but ewes having the flavours 2, 3, 4, 5 increased their intake more than those fed all other treatments, including the control (Figure 6).

This suggests that the ewes showed a faster learning process towards a relatively new feed than the lambs, as if ewes were more sensitive to the post-ingestive effects of the diet. The importance of post-ingestive effects on the learning process was stressed by Villaba and Provenza (2000).

The treatment 12 showed the highest numerical DMI both in lambs and in mature ewes (Table 5). The fact that DMI of this treatment was not affected by time suggests that this aroma enhancer (Table 1) was attractive to the animals since the first days it was used. This result is particularly interesting because the increase of DMI during the first days of exposure to a novel feed was the main objective of this trial.

Experiment 2

During the palatability tests of the experiment 2, the lambs had very low DMI of oat grains (4.4 g/d), regardless if flavours were applied or not. This confirms the results of the palatability experiment of Chapter 2, in which lambs did not eat oat grains at all. No significant differences associated with the feeding treatments were observed, even though there were large differences in numerical terms (from 0.2 g/d to 8.2 g/d). In contrast, the ewes which, , ate very little oat grains (DMI: 9.8 g/d, DMI Ratio: 10.1%), in the experiment of Chapter 2, showed high mean intake (50.4 g/d) during experiment 2, similarly to what occurred with canola meal.

The low intake of lambs might have been caused by a low palatability of oat grains. Molteberg et al. (1996) reported the formation of rancid odours and flavor and the formation of bitter taste in oat grains physically processed with their hulls. Since the oats used in experiment 2 were ground with their hulls, the same deterioration just described might have occurred. In addition, rancidity development might have been helped by the high environmental temperatures that occurred during the experiment. However, it is hard to understand why the lambs did not adapt to this feed whereas the mature ewes did.

The low DMI of the lambs might have been caused by factors other than palatability. In fact, the experiment 2 was carried out during the summer, from June 27th to July 10th, in correspondence of high temperatures, which probably can explain the low intake during the palatability tests. Indeed, even during the adaptation period the lambs showed lower intake of barley meal in the palatability tests in comparison to the experiment 1. Heat stress might not have affected the mature ewes because their level of DMI intake of the basal diet was much lower (1.46% of BW) than that of lambs (3.52% of BW).

Only in the case of the treatment 6 the lambs increased their DMI over time, as the experiment progressed (Table 11). The slope and the coefficient of determination were both low. In contrast, in the case of mature ewes intake in the

palatability tests increased as the experiment proceeded in nine of the 14 treatments tested, including the control. The slopes were high, indicating a quick variation of intake over time.

It is interesting to notice that, when the mean DMI in the last 4 experimental days was compared with the corresponding mean DMI in the 14 days of the experiment, some of the treatments (especially 2, 3, 4, 5) had a much higher DMI in the last 4 days of the experiment than the other treatments, including the control (Figure 7). These flavours were the same that induced the highest DMI in the last 4 experimental days for the canola meal based diets.

Again, the ewes showed a faster learning process towards a relatively new feed than the lambs.

Interestingly, the highest R^2 was measured for flavor 6 on both lambs and ewes, suggesting a more uniform reaction of the animals to this treatment than to the others (Table 11).

General discussion

The products added to either canola meal (experiment 1) or oat grains (experiment 2) act in different ways, by stimulating only the taste, only the smell or both. Most of the taste and taste + aroma enhancers were constituted by compounds enhancing the perception of sweet. This is consistent with the results of Nombekela and Murphy (1995), who found that cows fed at the beginning of the lactation with sweetened TMR ate more feed than those fed the same TMR not sweetened. However, differently from the experiment of Nombekela and Murphy (1995), in our experiments the palatability tests lasted only 6 min per day, to minimize post-ingestive effects.

The aroma enhancers included aromas which have shown to be attractive for sheep, such as those of onions, cereals, herbal, savoury, in order to enhance the attraction for the two unpalatable feeds. Robertson et al. (2006) found that sheep and goats preferred onion flavored straw than unflavored straw. Many authors

(Burritt and Provenza, 1992; Villaba and Provenza, 1997a,b; Arsenos et al., 2000a,b; Atwood et al., 2001) showed that ruminants can develop preference for flavors associated with proteins. This should have been particularly important for the lambs used for the experiments, which had high protein requirements.

In reality the flavours did not improve the intake of feed, calculated in the whole 14 experimental days, compared to the control in both experiments. The only noticeable aspect was that in experiment 1 (canola meal based treatments) the treatment 12 (canola meal + the aroma enhancers 12) showed the highest numerical DMI both in lambs and in mature ewes (Table 5). However, the analysis of the regression of the DMI over the experimental days showed some interesting general patterns in both experiments:

- both for lambs and ewes, the DMI of canola meal and oat grains observed in the two experiments was much higher than that measured comparing different pure ingredients (Chapter 2);
- the ewes increased their DMI linearly as the experiment progressed for most of the treatments. This did not occur or occurred to a small extent on the lambs;
- in ewes the DMI of the feeds associated to the treatments 2, 3, 4, and 5 was higher than that for the Control and for the other treatments. The DMI of these treatments was 40% higher or more than the average values for the whole experiment. The same feeds also showed the highest DMI, in the final days of the palatability tests, when canola meal was fed to lambs but not when oat grains were fed.

The positive effects of some of the flavours that gave the highest intake at the end of the experiments, is supported by some previous evidence. In particular, the treatment 5 was characterized by the flavor of orange. Thomas et al. (2007) found that calves receiving orange flavor or vanilla flavor in their beverage water had higher DMI and growing rates respect to calves who drunk water without added flavors. In addition, Robertson et al. (2006) found that both sheep and goats preferred orange flavored straw than unflavored straw in a 30 min palatability test.

Regarding the linear increase in DMI observed in both experiments for the ewes and, to a smaller extent, for the lambs fed canola meal, Provenza et al. (1995) found that at least 12 days are required to make a lamb familiar with a novel feed. The two experiments described in this chapter lasted 14 days, confirming the minimum time-span suggested by these authors. The increase in intake as the experiment progressed probably occurred because, despite the presence of the flavours, the animals recognised the presence of the two feeds tested (canola meal and oat grains), having then adapted to these feeds.

This implies that for the treatments which did not cause an intake increase over time, the flavours were able to confound the animals covering the flavour of the feed used. Since each animal tested each combination of feed and flavour only once, these combinations were seen by the animals as novel feeds, so no adaptation occurred. A further implication of this reasoning is that the lambs seemed to be more confused by the presence of the flavours than the ewes, since in only few cases the DMI of the lambs increased as the experiment progressed. This is an interesting hypotheses that may be tested in further studies.

CONCLUSIONS

The experiments conducted to overcome the low palatability observed in Chapter 2 for some of the ingredients tested, namely canola meal and oat grains, showed that some of the flavours tested had a noticeable effect on their intake. This effect increased over time as the experiment progressed.

Another important finding was that the ewes showed a faster learning process, as the experiment progressed, towards a relatively new feed than the lambs, as if ewes were more responsive to the post-ingestive effects of the diets or more able to identify the feeds used even when flavours were added to them.

Whereas the ewes seemed to be able to eat large amounts of the two feeds initially perceived as unpalatable as they became acquainted with these feeds, the lambs adapted to canola meal but maintained a strong aversion for oat grains.

The reasons of these differences in behaviour and the mechanisms underlying the higher adaptation of the animals to the two feeds tested when some flavours were used need to be further explored and understood.

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Table 1. Description of the flavours applied to canola meal and fed to lambs and ewes during the palatability tests of the experiments 1 and 2.

Product	*Description*
	Aroma enhancers
10	Fresh onion flavour with a savoury fraction.
11	Pleasant combination of cereals notes with slight toasted character.
12	Combination of fatty and roasted notes characteristic of argan oil.
13	Herbal flavour with bitter alfalfa note.
	Taste enhancers
1	Sweet taste product with stevia, licorice and fenugreek notes.
2	Sweet taste product with licorice and fenugreek notes.
8	Saccharine free sweetener nucleus.
9	Savoury taste product.
	Aroma and taste enhancers
3	Sweet flavour and taste with anisic and toasted notes of licorice.
4	Sweet flavour and taste characteristic of natural sugar beet molasses.
5	Sweet flavour and taste with pleasant orange note characteristic of juice.
6	Sweet flavour and taste with pleasant apple note.
7	Sweet flavour and taste with pleasant creamy coconut and vanilla bottom.

Table 2. Chemical composition of the ingredients of the basal diet and of the experimental feeds used during the experiments 1 and 2.

	Chemical composition (% of DM)							
	DM	CP	NDF	ADF	ADL	Ash	EE	NFC*
Basal diet ingredients								
Ryegrass hay	86.7	10.3	61.1	35.0	7.1	10.0	2.0	16.6
Barley	87.2	11.8	11.9	7.2	0.3	2.1	2.0	72.2
Urea	99.0	281.0	-	-	-	-	-	-
Experimental feeds								
Canola meal	87.1	38.3	35.2	26.0	14.4	8.0	2.8	15.7
Oat grains	89.3	11.0	43.3	24.9	3.4	6.3	5.2	34.2

* calculated on a DM basis as [100-NDF-CP-EE-ash], where EE = ether extract.
- not measured

Table 3. Ingredients and chemical composition of the diets fed to lambs and ewes during the experiments 1 and 2.

	Experiment 1		*Experiment 2*	
	Lambs	Ewes	Lambs	Ewes
	Ingredients (% of DM)			
Ryegrass hay	65	85	66	86
Barley meal	34	14	33	14
Urea	0.8	0.8	0.8	0.8
	Chemical composition (% of DM)			
DM	87	87	79	76
CP	13	13	13	13
NDF	44	54	39	47
ADF	25	31	22	26
ADL	5	6	3	4
Ash	7	9	6	7
EE	2	2	2	2
NFC*	34	22	40	31

* calculated on a DM basis as [100-NDF-CP-EE-ash], where EE = ether extract.

Table 4. Mean feed and nutrients intake from the components of the basal diets fed to lambs and ewes during the experiments 1 and 2.

	Experiment 1		*Experiment 2*	
	Lambs	Ewes	Lambs	Ewes
	Feed intake, g of DM/d			
Ryegrass hay	460 ± 15	531 ± 37	556 ± 20	639 ± 8
Barley meal	293 ± 3	87 ± 1	320 ± 18	100 ± 1
Urea	5.8± 0.06	4.9± 0.04	5.9± 0.3	5.0± 0.05
	Nutrient intake, g/d			
DM	758	623	882	744
CP	98	79	119	99
NDF	331	334	342	349
ADF	191	192	192	194
ADL	35	38	25	27
Ash	55	55	51	51
EE	15	12	18	15
NFC*	259	143	352	230
	Level of intake, % of BW			
DMI	3.45	1.24	3.52	1.46

*calculated on a DM basis as [100-NDF-CP-EE-ash], where EE = ether extract.

Table 5. Experiment 1. Mean DMI and mean DMI Ratio (mean DMI during the experimental period/mean DMI of barley meal during the last 4 days of the adaptation period) of lambs and ewes during the 6 min tests, fed canola meal alone (control) or canola meal mixed with 13 different flavours. Data ranked in decreasing order of lamb DMI.

Treatment	DMI (g/d head)		DMI Ratio (%)	
	Lambs	Ewes	Lambs	Ewes
12	41.1	65.1 [a]	72.2	79.9
11	40.1	55.8 [ab]	70.4	96.9
Control	38.6	61.3 [ab]	68.2	70.6
6	38.5	38.8 [b]	58.6	55.7
1	38.2	60.3 [ab]	69.5	67.4
9	37.6	38.0 [b]	64.3	62.4
10	36.9	54.0 [ab]	61.8	91.4
2	35.1	64.1 [a]	62.2	73.1
8	35.0	43.4 [ab]	59.7	80.4
13	32.8	61.7 [ab]	60.1	73.8
4	32.1	55.3 [ab]	52.1	62.7
3	29.6	60.2 [ab]	53.5	67.9
7	29.4	43.4 [ab]	48.6	87.2
5	27.8	52.6 [ab]	45.3	60.6
SEM	1.1	6.3	0.02	0.03
P (day) <	0.001	0.001	0.001	0.001
P (animal) <	0.001	0.001	0.001	0.001
P (flavor) <	NS	0.001	NS	NS
P (category)	NS		NS	

[a,b] Letters indicate differences within column ($P < 0.05$)
NS = not significant ($P > 0.05$)

Table 6. Intake rate (g/s) of canola meal alone (control) or canola meal mixed with 13 different flavours during the 6 min tests. Data ranked in decreasing order of lamb intake rates.

Treatment	Intake rate (g/s)	
	Lambs	Ewes
1	0.23	0.28
10	0.22	0.31
2	0.21	0.29
5	0.21	0.23
Control	0.19	0.28
6	0.19	0.24
9	0.19	0.27
12	0.19	0.25
13	0.19 [b]	0.28 [a]
3	0.17	0.29
4	0.17	0.24
7	0.17	0.49
11	0.17	0.27
8	0.16 [b]	0.28 [a]
SEM	0.006	0.020
P (day) <	0.001	0.001
P (animal) <	0.001	0.04
P (flavor) <	NS	NS
P (category) <	0.05	

[a,b] Letters indicate differences between lambs and ewes ($P < 0.05$)
NS = not significant ($P > 0.1$)

Table 7. Experiment 1. Mean DMI level of intake on a BW (LI-BW; mg/kg BW) and metabolic weight basis (LI-MW; mg/kg $BW^{0.75}$) of canola meal alone (control) or canola meal mixed with 13 different flavours fed to lambs and ewes during the 6 min tests.

Treatment	LI-BW mg/kg BW		LI-MW mg/kg $BW^{0.75}$	
	Lambs	Ewes	Lambs	Ewes
12	1806	1287 ^{a}	3938	3428 ^{a}
11	1754	1093 ^{ab}	3830	2919 ^{ab}
1	1730	1216 ^{ab}	3751	3225 ^{ab}
6	1730	775 ^{b}	3752	2059 ^{b}
Control	1663	1235 ^{ab}	3644	3274 ^{ab}
10	1625	1046 ^{ab}	3543	2802 ^{ab}
9	1620 ^{A}	757 ^{Bb}	3554 ^{A}	2015 ^{Bb}
2	1564	1299 ^{a}	3401	3440 ^{a}
8	1516	858 ^{ab}	3319	2286 ^{ab}
13	1459	1229 ^{ab}	3173	3267 ^{ab}
4	1425	1107 ^{ab}	3103	2939 ^{ab}
3	1314	1215 ^{ab}	2858	3220 ^{ab}
7	1283	849 ^{ab}	2801	2270 ^{ab}
5	1225	1022 ^{ab}	2668	2734 ^{ab}
SEM	50	51	109	135.4
P (day) <	0.001	0.001	0.001	0.001
P (animal) <	0.001	0.001	0.001	0.001
P (flavor) <	NS	0.001	NS	0.001
P (category) ≤	0.05		0.05	

^{A,B} Capital letters indicate differences between lambs and ewes on LI-BW or LI-MW ($P < 0.05$)
^{a,b,c,d} Small letters indicate differences within columns ($P < 0.05$)
NS = not significant ($P > 0.1$)

Table 8. Experiment 1. Regression of DMI of canola meal, fed alone (control) or mixed with 13 different flavours, on the experimental days. Two animals with very low DMI were discarded for each sheep category.

	Lambs (2 outliers discarded)				*Ewes (2 outliers discarded)*			
Treat	Slope	Intercept	R^2	*P* <	Slope	Intercept	R^2	*P* <
Control	-	-	-	NS	11.0	-5.0	0.55	0.05
1	4.6	12.9	0.34	0.05	7.3	19.1	0.37	0.05
2	4.9	9.2	0.52	0.01	7.9	20.8	0.48	0.05
3	4.6	3.4	0.49	0.05	9.1	7.8	0.62	0.01
4	4.9 [B]	4.6 [A]	0.38	0.05	10.9 [A]	-11.2 [B]	0.73	0.001
5	4.1 [B]	5.3 [A]	0.38	0.05	11.6 [A]	-19.1 [B]	0.85	0.001
6	-	-	-	NS	7.9	-11.7	0.64	0.01
7	-	-	-	NS	7.9	-9.7	0.81	0.001
8	-	-	-	NS	8.1	-7.9	0.77	0.001
9	-	-	-	NS	6.6	-4.1	0.69	0.01
10	-	-	-	NS	6.9	12.2	0.34	0.05
11	-	-	-	NS	7.9	9.8	0.39	0.05
12	-	-	-	NS	-	-	-	NS
13	-	-	-	NS	6.3	28.3	0.32	0.06

[A,B,] Capital letters indicate significant differences ($P < 0.05$) in slope or intercept between lambs and ewes fed the same feed during the 6 min palatability tests.
Treat = treatment

Table 9. Experiment 2. Mean DMI and mean DMI Ratio (mean DMI during the experimental period /mean DMI of barley meal, last 4 days of the adaptation period) of lambs and ewes during the 6 min tests, fed oat grains alone (control) or mixed with 13 different flavours. Data ranked in decreasing order of lamb DMI.

Treatment	DMI (g/d head)		DMI Ratio (%)	
	Lambs	Ewes	Lambs	Ewes
3	8.2	48.7	13.5 ^{B}	81.1 ^{A}
2	8.0	41.7	12.7 ^{B}	65.6 ^{A}
1	7.9	42.6	12.0 ^{B}	72.5 ^{A}
Control	6.6	43.1	11.4 ^{B}	74.4 ^{A}
6	5.3	45.9	5.6 ^{B}	83.9 ^{A}
12	4.7	53.1	6.4 ^{B}	94.1 ^{A}
11	4.3	55.8	6.3 ^{B}	97.8 ^{A}
10	3.9	58.6	7.3 ^{B}	104.6 ^{A}
13	3.7	52.4	6.3 ^{B}	92.4 ^{A}
7	3.2	53.6	4.3 ^{B}	98.7 ^{A}
5	2.4	49.4	8.0 ^{B}	86.8 ^{A}
4	1.7	48.6	3.4 ^{B}	75.5 ^{A}
8	1.4	55.6	2.8 ^{B}	105.6 ^{A}
9	0.2	57.1	0.6 ^{B}	99.6 ^{A}
SEM	0.68	1.5	0.01	0.03
P (day) <	NS	0.001	NS	0.001
P (animal) <	0.001	0.001	0.001	0.001
P (flavor) <	NS	NS	NS	NS
P (category) <	-		0.05	

[a,b,] Letters indicates differences between rows ($P < 0.05$)
NS = not significant ($P > 0.1$)
- analysis not performed.

Table 10. Experiment 2. Mean DMI level of intake on a BW (LI-BW; mg/kg BW) and metabolic weight basis (LI-MW; mg/kg $BW^{0.75}$) of oat grains alone (control) or mixed with 13 different flavours fed to lambs and ewes during the 6 min tests.

	LI-BW mg/kg BW		LI-MW mg/kg $BW^{0.75}$	
Flavor	Lambs	Ewes	Lambs	Ewes
3	369 [b]	953 [a]	803 [b]	2544 [a]
2	356 [a]	840 [a]	773 [a]	2226 [a]
1	345 [a]	816 [a]	750 [a]	2189 [a]
Control	295 [a]	865 [a]	639 [a]	2297 [a]
6	220 [b]	903 [a]	484 [b]	2407 [a]
12	217 [b]	1008 [a]	468 [b]	2713 [a]
11	199 [b]	1071 [a]	429 [b]	2872 [a]
13	169 [b]	998 [a]	367 [b]	2683 [a]
10	154 [b]	1115 [a]	346 [b]	2998 [a]
7	128 [b]	1025 [a]	281 [b]	2752 [a]
5	83 [b]	944 [a]	191 [b]	2534 [a]
4	64 [b]	933 [a]	144 [b]	2501 [a]
8	60 [b]	1055 [a]	132 [b]	2839 [a]
9	9 [b]	1110 [a]	19 [b]	2967 [a]
SEM	31.2	25.9	67.6	71.2
P (day) <	NS	0.00	NS	0.00
P (animal) <	0.001	0.00	0.00	0.00
P (flavor) <	NS	NS	NS	NS
P (category)	0.05		0.05	

[a,b] Letters indicate differences between lambs and ewes when fed the same feed in the 6 min palatability tests($P < 0.05$)
NS = not significant ($P > 0.1$)

Table 11. Experiment 2. Regressions of DMI of oat grains, fed alone (control) or mixed with 13 different flavours, on the experimental days. Two animals with very low DMI were discarded for each sheep category.

	Lambs (2 outliers discarded)				*Ewes (2 outliers discarded)*			
Treat	Slope	Intercept	R^2	P <	Slope	Intercept	R^2	P <
Control	-	-	-	NS	6.3	7.0	0.33	0.05
1	-	-	-	NS	7.4	-2.0	0.43	0.05
2	-	-	-	NS	7.1	0.1	0.43	0.05
3	-	-	-	NS	7.0	11.2	0.49	0.05
4	-	-	-	NS	6.8	9.6	0.39	0.05
5	-	-	-	NS	7.1	11.6	0.41	0.05
6	1.27	-4.5	0.34	0.05	5.6	24.3	0.50	0.05
7	-	-	-	NS	-	-	-	NS
8	-	-	-	NS	5.8	32.4	0.26	0.09
9	-	-	-	NS	-	-	-	NS
10	-	-	-	NS	-	-	-	NS
11	-	-	-	NS	-	-	-	NS
12	-	-	-	NS	5.6	23.1	0.34	0.05
13	-	-	-	NS	-	-	-	NS

NS = not significant (P > 0.1)
Treat = treatment

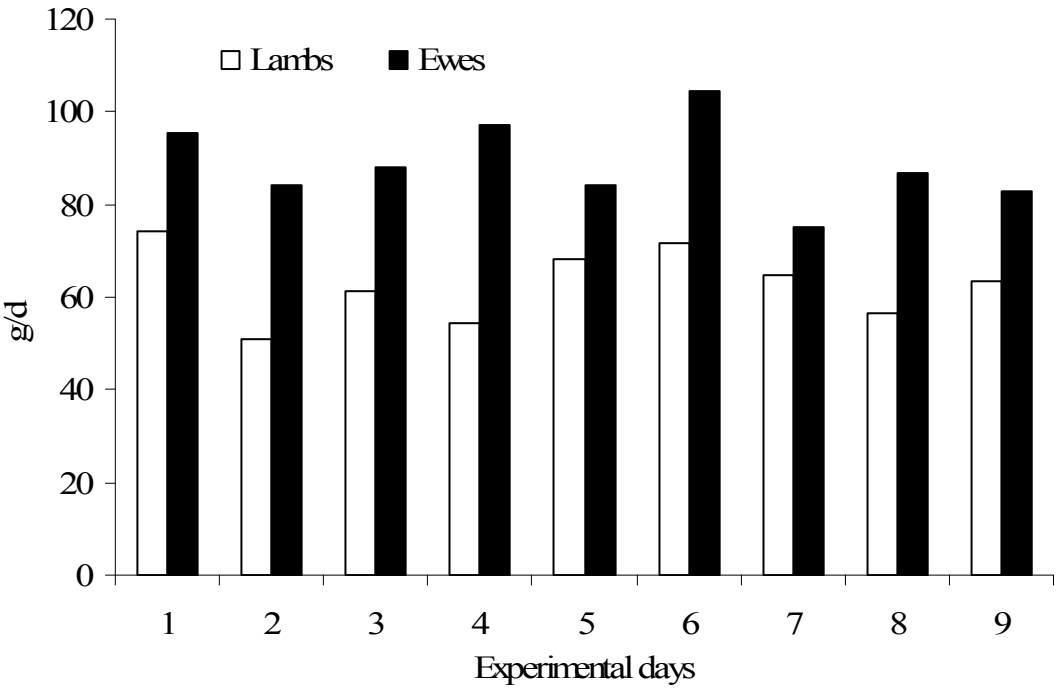

Figure 1. Experiment 1. Daily barley intake of lambs and ewes during the 6 min palatability tests during the adaptation period.

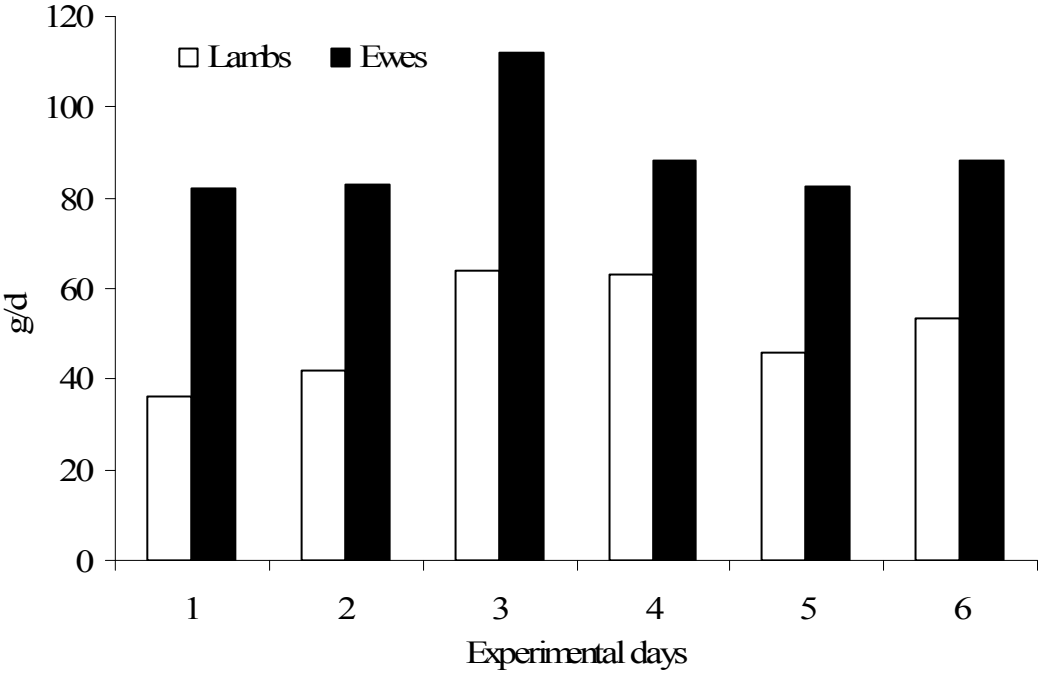

Figure 2. Experiment 2. Daily barley intake of lambs and ewes during the 6 min palatability tests during the adaptation period.

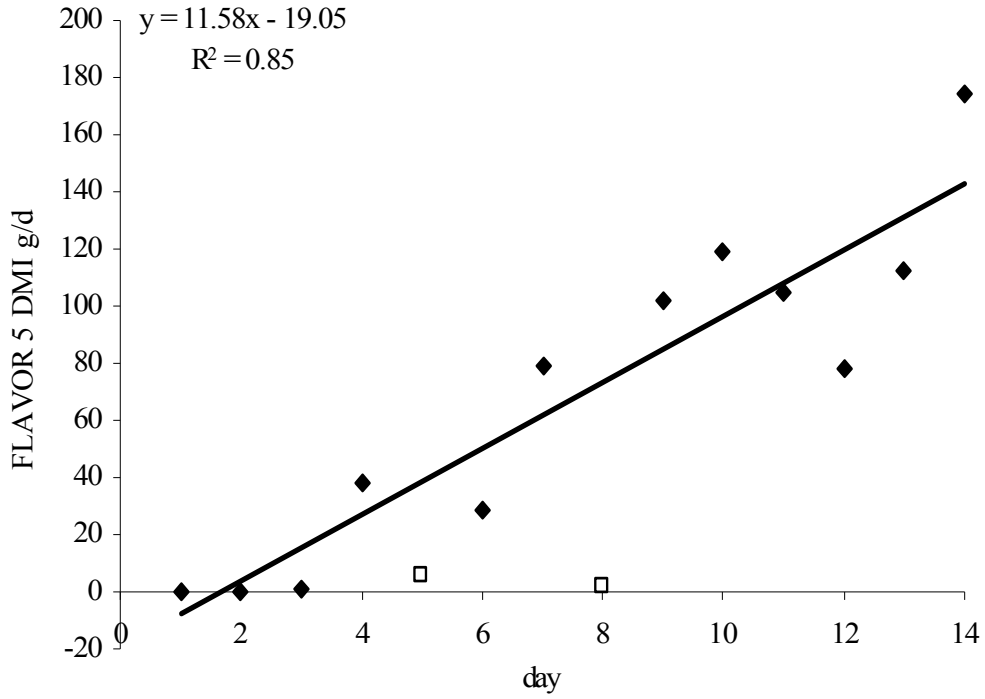

Figure 3. Experiment 1. Variation of ewes' DMI of canola meal flavoured with flavor n. 5 (sweet flavour and taste with pleasant orange note characteristic of juice) during the 14 days of experimental period, excluding the same two ewes discarded for the other treatments (□).

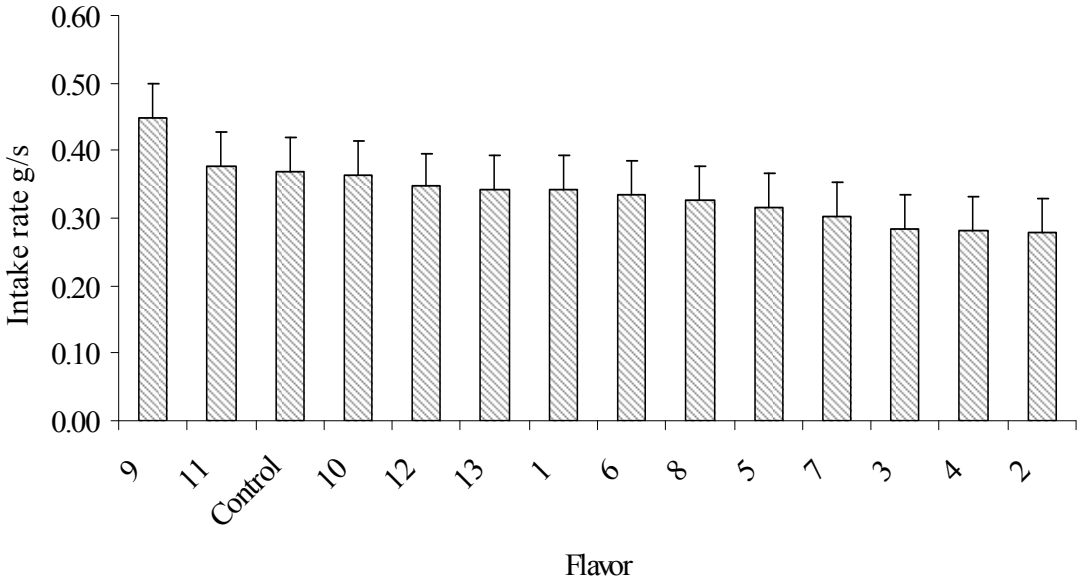

Figure 4. Experiment 2. Intake rate (g/s) of oat grains supplied alone (Control) or mixed with 13 different flavors fed to the ewes during the 6 min palatability tests.

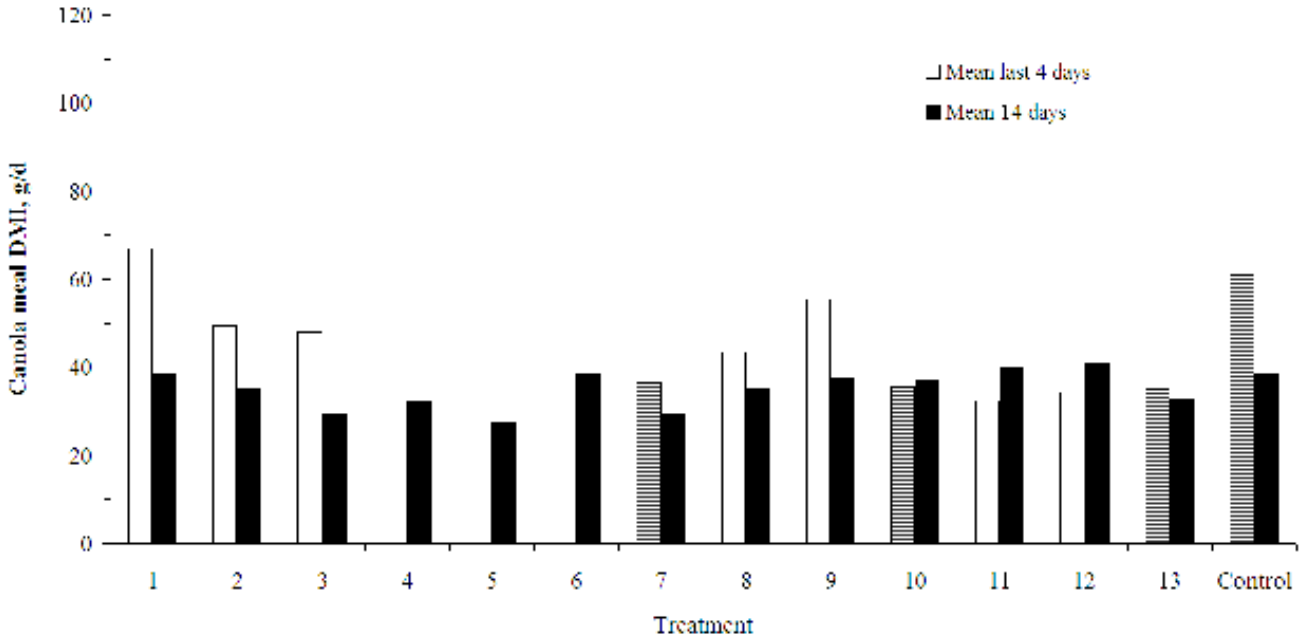

Figure 5. Experiment 1: canola meal based diets fed to lambs. Mean DMI of the last 4 experimental days of the palatability tests compared with the mean DMI in the whole experiment (14 days). The means of the last 4 days are reported with columns with stripes when the regression between DMI and experimental days was not significant. The horizontal line indicates the whole experiment mean of the control.

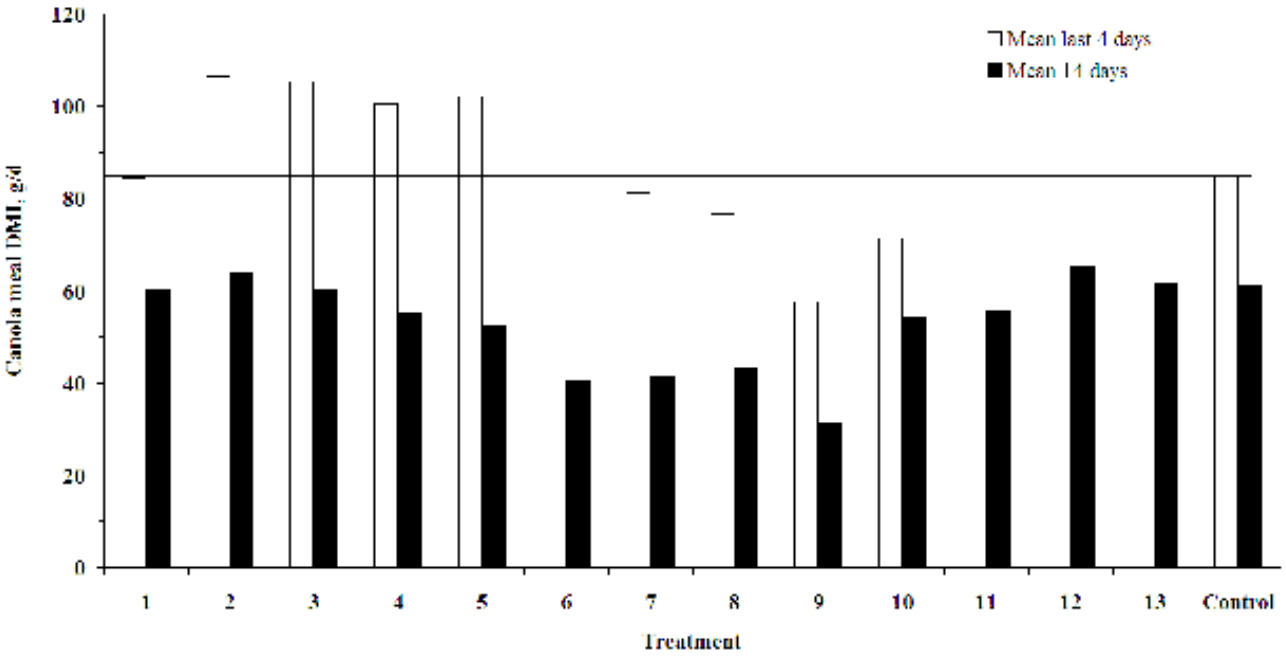

Figure 6. Experiment 1: canola meal based diets fed to ewes. Mean DMI of the last 4 experimental days of the palatability tests compared with the mean DMI in the whole experiment (14 days). The means of the last 4 days are reported with columns with stripes when the regression between DMI and experimental days was not significant. The horizontal line indicates the mean of the last 4 days of the control.

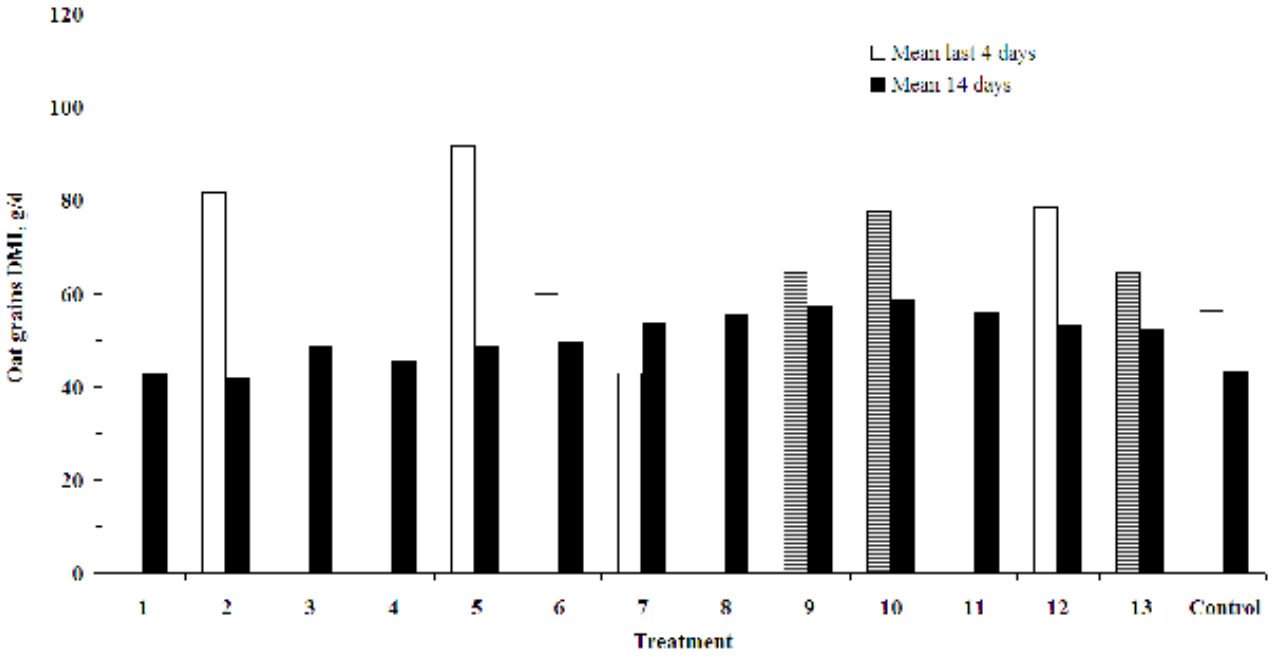

Figure 7. Experiment 2: oat grains based diets fed to ewes. Mean DMI of the last 4 experimental days of the palatability tests compared with the mean DMI in the whole experiment (14 days). The means of the last 4 days are reported with columns with stripes when the regression between DMI and experimental days was not significant. The horizontal line indicates the mean of the last 4 days of the control.

CHAPTER 4

VOLATILE ORGANIC COMPOUNDS AND PALATABILITY OF CONCENTRATES FED TO LAMBS AND EWES

ABSTRACT

The aromatic characteristics of concentrate feeds seem to affect feed intake in ruminants. In a previous study, the palatability of several feeds, mostly concentrates, was tested in lambs and ewes. In this study the volatile profile of the feeds used in that was measured by gas chromatography-olfactometry and mass spectrometry and associated to the compound identified with this analyses with their palatability.

The rank of total volatile organic compounds (VOCs) found in the feeds was, in decreasing order: beet pulps (31 VOCs), oat grains (28 VOCs), dehydrated alfalfa (24 VOCs), soybean hulls (23 VOCs), soybean meal 44 (20 VOCs), corn gluten meal (17 VOCs), sunflower meal and barley meal (16 VOCs), corn middlings and soybean meal 49 (15 VOCs), wheat brans (14 VOCs), faba beans (13 VOCs) and, finally, corn grains, pea grains and wheat grains (6 VOCs). Beet pulps, which were among the feeds preferred by both lambs and ewes, were characterized by a pleasant aroma because they were the richest of aldehydes (11 VOCs) and poor of sulphur compounds (2 VOCs). Dehydrated alfalfa and sunflower meal, which were commonly refused by lambs and ewes, were both rich of sulphur compounds (5 VOCs), whose unpleasant notes probably affected the palatability. Soybean meal 44, which was refused by the ewes, was characterized by a rich aroma profile but probably by a negative note, due to the presence of methanamine, which gave an off-flavour identified as rotted fish odour. Oat grains, which were also refused by lambs and ewes, were characterized by pleasant flavours due to their richness of aldehydes (10 VOCs) and terpenes (7 VOCs). The oat grains refusal was probably due to the presence,

among the terpenes, of a unique compound (a-pinene), which is known to negatively affect intake of alfalfa pellets in lambs. Corn gluten meal, which was refused by lambs and ewes, was characterized by the presence of six sulphur compounds, which gave unpleasant notes of garlic and cooked potato to the feed and probably negatively affected the palatability of this feed.

Key words: volatile organic compounds, gas chromatography olfactometry, palatability, sulphur compounds.

INTRODUCTION

Despite the important effects that feed flavour can have on feed palatability (Burritt and Provenza, 1992; Villaba and Provenza, 1997a,b; Arsenos et al., 2000a,b; Atwood et al., 2001) and quality of animal products (Ha and Lindsay, 1991; Moio et al., 1993a,b; Nudda et al., 2002; Carpino et al., 2004) only a few studies were carried out on the effects of flavours on ovine feeding behaviour (Arsenos and Kyriazakis, 2001). A recent feed palatability study (Chapter 2) was carried out on Sarda female lambs and multiparous dry ewes. Fourteen feeds, chosen from those more commonly present in feed mixes for ruminants, were subjected to short term palatability tests, so that post-ingestive effects could be minimized. The results showed clear differences on palatability among feeds, and between lambs and ewes. In fact, lambs preferred some feeds (i.e. soybean meal 49 and wheat grains) and refused others (i.e. canola meal, sunflower meal, oat grains, and dehydrated alfalfa). The ewes, instead, preferred beet pulps, pea grains, wheat grains and corn grains, and refused other feeds, such as canola meal, sunflower meal, oat grains, and dehydrated alfalfa. Thus, some feeds were refused both by lambs and ewes, despite no negative post-ingestive effects could be associated with them. It is likely that the palatability of the feeds was associated to the presence of chemical compounds that affects their flavour.

Thus, the objectives of this research were: i) to study the volatile profile of the feeds used in the experiment described in Chapter 2 by gas chromatography-olfactometry and mass spectrometry, ii) to associate the compounds identified with these analyses with the palatability of the feeds previously measured.

MATERIALS AND METHODS

Samples

Fifteen different feeds, most of them concentrates, were selected for the analyses. The samples included all the feeds used in the experiment described in the Chapter 2, with the exclusion of canola meal, that was not available at the moment of the analyses. Faba beans, not used in the experiment above mentioned because substituted by canola meal, was also analysed. Thus, the aroma profile of the canola meal and the palatability measurements of faba beans could not be measured. The samples were finely ground, stored at room temperature in sterilized containers and then sent to the flavour laboratory of the Corfilac (Ragusa, Italy) to be analyzed. The feeds selected were those most commonly used as ingredients for ruminant feeding and were denominated as follows: dehydrated alfalfa, barley meal, beet pulps, corn gluten meal, corn grains, corn middlings, faba beans, oat grains, pea grains, soybean hulls, soybean meal 44, soybean meal 49, sunflower meal, wheat brans, and wheat grains. In the previous trial of palatability (Chapter 2), canola meal was used instead of faba beans, due to organizational problems. Thus, the aroma profile of the canola meal and the palatability measurements of faba beans are not available.

Extraction of volatile organic compounds

Volatile organic compounds (VOCs) were extracted by static headspace with a solid phase microextraction (SPME) fiber with a 50/30 μm divinylbenzene/carboxen/PDMS coating (Supelco, Bellefonte, PA). Fiber was pre-conditioned before initial use, by inserting them into the injector port of a gas chromatography olfactometer (GC/O) for 3 h at 270 °C, and reconditioned between extractions at the same temperature for 5 min, followed by 10 min at ambient temperature. For each extraction, 5 g of grated concentrate sample were put into a 22 mL vial and conditioned in a bath for 1 h, at 37°C. During this time, the equilibrium between of the VOCs between the gas-phase and the sample was established. A syringe holding the SPME fiber was then fit into place, the stopcock opened and 1 cm of the fiber was exposed to the static headspace of the sample for an additional 30 min, to establish the equilibrium of the VOCs between the gas-phase and the solid-phase of the adsorbent. The syringe was then removed from the septum and the volatiles analyzed by GC/O.

Gas chromatography and olfactometry

GC/O analysis was performed by a single sniffer, previously trained using the procedure and the standard compounds described by Marin et al. (1988). These standards consisted of a group of 22 compounds used to evaluate olfactory acuity and to determine if a sniffer has specific anosmia for certain odors. After extraction of volatiles, the fiber was desorbed into a modified Hewlett Packard 6890 gas chromatograph (Datu Inc., Geneva, NY) characterized by a fused-silica capillary column HP-1, 12 m, 0.32 mm i.d., 0.52 μm film thickness. Splitless injection was performed at 250 °C; the oven temperature program was: 35 °C for 3 min, 6 °C/min to 190 °C, then 30 °C/min to 225 °C and 225 °C for 3 min. He was used as carrier gas and the column flow rate was 1.9 mL/min. The eluted compounds were mixed with humidified air using a method described by Acree

and Barnard (1994) and the sniffer was continuously exposed to this source for 30 min. The time of response to individual odours perceived by the sniffer was recorded by Charmware software (v.1.12, Datu Inc., Geneva, NY). Response times were converted into retention indices (RI) for each VOC and displayed by the software as a series of peaks in an "aromagram". RI values were calculated relative to a series of normal alkanes (C_7 – C_{18}) previously injected into the FID port of the same gas chromatograph.

Gas Chromatography Mass Spectrometry

The SPME fiber was re-exposed to the static headspace of the same sample and then desorbed into a gas chromatography mass spectrometer (Hewlett Packard 6890), characterized by cross linked methyl siloxane capillary column HP-1, 25 m, 0.20 mm i.d., and 0.11 μm film thickness, using the same GC/O temperature program. Retention times (RT) of volatile compounds were calculated relative to the same series of normal alkanes (C_7 – C_{18}) used in GC/O, that had been previously injected into the GC/MS. This procedure permitted a direct comparison between RI values obtained from GC/O and RT values obtained from GC/MS.

RESULTS AND DISCUSSION

The results of the gas chromatography olfactometry qualitative analysis of the fifteen animal feed samples showed a quite large variability in the number of volatile organic compounds (VOCs) in their aroma profile (Table 1). The total number of VOCs detected in the feeds was, in decreasing order: beet pulps (31 VOCs), oat grains (28 VOCs), dehydrated alfalfa (24 VOCs), soybean hulls (23 VOCs), Soybean meal 44 (20 VOCs), corn gluten meal (17 VOCs), barley meal and sunflower meal (16 VOCs), corn middlings and soybean meal 49 (15 VOCs), wheat brans (14 VOCs), faba beans (13 VOCs) and, finally, corn grains,

pea grains and wheat grains (6 VOCs). The principal chemical classes of VOCs found in the feeds are showed in Table 2.

Among the feeds tested, lambs showed a preference for soybean meal 49, wheat grains, pea grains, corn grains, soybean hulls, beet pulps, wheat brans, and soybean meal 44 (Chapter 2). The ewes showed a more clear preference for beet pulps, wheat grains, pea grains, and corn grains (Chapter 2).

Beet pulps.

The aroma profile of the beet pulps, which was among the preferred feeds by both lambs and ewes (Chapter 2), was mostly composed of aldehyde, terpene, ketone, lactone and sulphur chemical classes and, to a smaller part, by pyrazine (Table 2). In particular, beet pulps had two lactones, i.e. γ-heptalactone and γ-nonalactone, that give peach fruity notes (Table 3), two heterocyclic compounds, i.e. methyl cinnamate and safrole with sweet and spice notes, respectively (Table 4), one pyrazine, i.e. ethyl dimethyl pyrazine which gives burnt/nutty notes (Table 3), and one alcoholic compound, identified as a unique compound among the other feeds, (Z)-3-hexenol with green notes (data not showed). In addition, two unique not identified compounds which gave rancid notes were detected in this sample (data not showed). Among the tested feeds, beet pulps samples presented the highest number of aldehydes (11 aldehyde compounds), followed by oat grains (10 aldehyde compounds) (Table 5). The origin of aldehydes is mainly due to the degradation of amino acids available in the feeds. However, feeds with a higher content of crude protein (i.e. corn gluten, corn middlings, pea grains soybean meal 44 and 49, sunflower meal) had a lower number of aldehydes compounds than beet pulps and oat grains (Table 5), which are characterized by high contents of NDF and NFC and were thus expected to have a low content of aldehydes compounds. Therefore, in the case of beet pulps and oat grains, the aldehyde compounds could be originated from the oxidation process of unsaturated free fatty acids in these samples. In general, aldehyde

compounds (Table 5) gave pleasant notes to the feeds, such as green, orange, nutty, hay, fried oil, oil and vanilla notes, except for the mercapto acetaldehyde and heptanal which conferred, respectively, garlic and rancid notes. The 2-undecenal was detected as a unique compound in beet pulps, characterized by fruity notes. Moreover, terpene compounds (Table 6) influenced positively the flavour of beet pulps, giving wood, green, spice and characteristic beet notes to the sample. Besides, (E)-linalool oxide and (Z)-dihydrocarvone were found as unique terpene compounds both giving pleasant notes (green) to the beet pulp feed. The terpene compounds likely originate from the degradation of the carotenoids precursors present in the feed (Lewinsohn et al., 2005). The sulphur compounds found in the beet pulps sample were only two (Table 7). These compounds, which gave garlic and popcorn notes, could be originated from the degradation of amino acids in the sample. In summary, the general aroma profile of beet pulps was very pleasant and this fact could explain why both lambs and ewes showed a high intake level of this feed (Chapter 2).

Dehydrated alfalfa and sunflower meal.

Both lambs and ewes refused almost completely dehydrated alfalfa and sunflower meal (Chapter 2). Dehydrated alfalfa showed a richer aroma profile than sunflower meal (24 vs. 16 VOCs, respectively, Table 1). Dehydrated alfalfa aroma profile was characterized by a high number of sulphur compounds (5 sulphur compounds, Table 7) that mainly gave garlic notes, due to the presence of methyl ethyl sulphide, thiophene, dimethyl trisulphide, cooked potato notes, due to the presence of the methional, and meat notes due to methyl dithiofurane. Moreover, a note of popcorn originated from a pyrrole (2-acetyl-1-pyrroline) was also found in the dehydrated alfalfa (data not shown). This chemical class was also the most representative in sunflower meal as well (Table 7), which showed the same number of sulphur compounds (5 VOCs) and the same unpleasant notes characterizing the alfalfa sample. Therefore, the low

palatability of dehydrated alfalfa and sunflower meal was likely due to the negative effect of sulphur compounds.

Soybean meal 44, the soybean meal 49 and soybean hulls.

Ewes found unpalatable soybean meal 44, soybean meal 49 and soybean hulls (Chapter 2). The soybean meal 44 was characterized by a rich aroma profile mainly represented by aldehyde, sulphur compounds and ketones and, to a lower extent, by amine, lactone, pyrazine and terpene (Table 2). Only some sulphur compounds determined unpleasant notes like garlic, due to the presence of thiophene and dimethyl trisulphide, meaty notes from methyl thiofurane, and cooked potato notes from methional (Table 7). A negative effect of the aroma profile of soybean meal 44 was due to the presence of the methanamine that gave an off-flavour identified as rotted fish odour (Table 4). This compound found in the soybean meal 44 sample had a high percent value (90%) of duration during the sniffing run reported in the aromagram. Besides, a-thujene (floral notes) was detected like a unique terpene compound (Table 6), but its value of percent of duration during the sniffing run reported in the aromagram was low (42%) (data not showed), and for this reason it could not have affected the palatability of this soybean meal 44. Probably the ewes disliked this feed because of more than one interacting factors was present. In fact, during the palatability tests (Chapter 2), ewes clearly refused all those feeds, including soybean meal 44, that are normally used only as component of mixed pelleted feeds but not as unique concentrate supplements during the milking. Thus, the ewes might have had difficulties to associate their flavour with their post-ingestive effects. This fact, associated with the off-flavor determined by the presence of methanamine, might explain the low palatability of soybean 44 for the ewes. The presence of methanamine can also explain why the DMI of soybean meal 44 tended to be lower than that of soybean meal 49; the later being the most preferred feed by lambs (Chapter 2).

The refusal of the soybean meal 49 by the ewes was likely due to the reasons already explained for the refusal of soybean meal 44 and, in addition, to the presence of different sulphur compounds, which was the main chemical class characterizing this feed (Table 2). These compounds (Table 7) gave soybean meal 49 unpleasant garlic, potato and meat notes which, together with the low protein requirements of the ewes and their low familiarity with the soybean aroma, probably determined the low DMI of this feed by this category of animals.

The soybean hulls showed a richer aromatic profile with respect to soybean meal 44 and 49 (Table 1). In fact, it was formed by 23 VOCs, which included one alcohol (nonanol), eight aldehydes (2-hexenal, which was a unique compound, heptanal, octanal, (Z)-2-octenal, (E)-2-nonenal, (Z)-2-nonenal, 2,4-nonadienal and (E,E)-2,4-decadienal) (Tables 2 and 5); four ketones (2,3-butanedione, 1-octen-3-one, octanone, 3,5-octadien-2-one) (Tables 2 and 8); one lactone (γ-heptalactone) (Tables 2 and 3); one pyrazine (ethyl dimethyl pyrazine) (Tables 2 and 3); two sulphur compounds (thiophene and dimethyl trisulfide) (Tables 2 and 7); three terpenes (Table 2 and 6); one alcohol; and three not identified compounds (data not showed). In the case of soybean hulls, only heptanal, thiophene, dimethyl trisulfide and two of the not identified volatile compounds produced bad odours and could have influenced the choice of this feed.

Oat grains.

Both lambs and ewes found the oat grains unpalatable. The oat grains aroma profile was characterized by very pleasant flavours like green, orange, nutty, hay, mushroom, peach, sweet, spice, pine, lemon and coconut notes, principally due to their richness of aldehydes (Tables 2 and 5) and terpenes (Tables 2 and 6). Only one sulphur compound (methyl ethyl sulphide) (Table 7) and a not identified compound, perceived at low percent duration during the sniff run, conferred off-flavours, respectively, like garlic and rancid notes. However, oat

grains was the feed with the highest content of terpenes (7 in total; Table 2). Some studies demonstrated that terpenes negatively affected feed palatability in lambs (Estell et al., 1996; Villalba et al., 2006; Dziba and Provenza, 2008). In fact, among the terpenes of oat grains a unique compound, a-pinene, was found (Table 6), which influenced negatively and linearly intake of alfalfa pellets by lambs (Estell et al., 1998). Thus, even if in this analysis terpenes were associated with pleasant notes in oat grains, it is not possible to exclude that a-pinene may have affected negatively the palatability of this feed.

Corn gluten meal.

In the palatability tests, the lambs showed a very low intake of corn gluten meal while ewes ate a significantly higher quantity of this feed compared to the lambs. However, also for the ewes this feed was not included among the preferred ones (Chapter 2). The aroma profile of corn gluten meal showed a lower number of volatile compounds than the oat grains (17 vs. 28 VOCs, respectively, for corn gluten and oat grains, Table 1). However, the corn gluten meal aroma profile was characterized by the presence of four sulphur compounds (Table 2) perceived in high percent of duration (70-80%) during the sniff runs. These compounds gave to the feed unpleasant notes of garlic and cooked potato (Table 7). Thus, these bad characteristics probably affected negatively the palatability of corn gluten meal.

CONCLUSIONS

In this preliminary trial, the feeds differed in their aroma profile for the total number of VOCs as well as for the types of chemical classes of volatile compounds present. Some interesting findings regarded especially the class of the sulphur compounds, which seemed to influence negatively the palatability of the tested feeds in both lambs and ewes. Moreover, the class of terpenes seemed to negatively affect the palatability of oat grains. However, it was not possible to find for all feeds a clear and definite relationship between the intrinsic aromatic characteristics of the feeds and their palatability. In fact, it seems that preferences of lambs and ewes can be affected more by their feeding experience and requirements than by the aromatic properties of the feed. Therefore, more studies are needed to deeply investigate the interactions between the sensorial characteristics of concentrate feeds and their effects on feed intake in ruminants.

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Table 1. Total number of volatile compounds found in each feed sample.

Feed	**Total Volatile Compounds (*)**
Beet pulps	31
Oat grains	28
Dehydrated alfalfa	24
Soybean hulls	23
Soybean meal 44	20
Corn gluten meal	17
Barley meal	16
Sunflower meal	16
Corn middlings	15
Soybean meal 49	15
Wheat brans	14
Faba beans	13
Corn grains	6
Pea grains	6
Wheat grains	6

(*) data include the non identified compounds

Table 2. Principal chemical classes of volatile compounds found in each feed sample.

Chemical class	**Samples analyzed (tot *)**														
	AD	**BA**	**BP**	**CG**	**CM**	**FB**	**GL**	**HP**	**OG**	**PG**	**SH**	**SM**	**SN**	**WB**	**WG**
Aldehyde	6	8	11	3	5	2	7	4	10	2	8	5	6	4	5
Amine	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Ketone	3	3	4	1	2	2	2	2	3	1	4	3	4	2	1
Ester	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Lactone	1	2	2	0	0	0	0	1	1	0	1	0	1	0	0
Pyrazine	1	0	1	0	0	0	0	2	0	0	1	0	1	1	0
Sulphur	5	1	2	0	5	4	4	5	2	1	2	5	4	5	0
Terpene	3	0	5	1	0	1	1	0	7	1	3	1	1	1	0

AD: alfalfa, dehydrated; BA: barley meal; BP: beet pulps; CG: corn grains; CM: corn middlings; FB: faba beans; GL: corn gluten meal; HP: soybean meal 49; OG oat grains; PG: pea grains; SH: soybean hulls; SN soybean meal 44; SM: sunflower meal; WB: wheat brans; WG: wheat grains.

* Not identified compounds are not showed.

Table 3. Ester, lactone and pyrazine compounds extracted from the feed samples by SPME technique.

Compounds	Chemical class	Descriptor	LRI [a]	Ident [b]	AD	BA	BP	CG	CM	FB	GL	HP	OG	PG	SH	SM	SN	WB	WG
isobutyl acetate	ester	fruit	773	PI	*														
ethyl methylbutyrate	ester	fruit	844	PI												*			
ethyl octenoate	ester	fruit	1206	PI									*						
TOTAL					**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**1**	**0**	**0**	**0**
γ- heptalactone	lactone	peach	1248	MS	*	*	*					*	*		*		*		
γ- nonalactone	lactone	peach	1365	PI		*	*												
TOTAL					**1**	**2**	**2**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	**0**	**1**	**0**	**0**
ethyl dimethyl pyrazine	pyrazine	burnt, nutty	1086	PI	*		*					*			*		*	*	
diethylmethyl pyrazine	pyrazine	nutty	1160	PI								*							
TOTAL					**1**	**0**	**1**	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**1**	**0**	**1**	**1**	**0**

[a] LRI, Linear Retention Indices, capillary column HP -5. [b] Identification: MS (Wiley library); PI (Internet Data Base: flavornet)
AD: dehydrated alfalfa; BA: barley meal; BP: beet pulps; CG: corn grains; CM: corn middlings; FB: faba beans; GL: corn gluten meal; HP: soybean meal 49; OG: oat grains; PG: pea grains; SH: soybean hulls; SN: soybean meal 44; SM: sunflower meal; WB: wheat brans; WG: wheat grains.

Table 4. Amine and eterocyclic compounds extracted from the feed samples by SPME technique.

Compounds	Chemical class	Descriptor	LRI [a]	Ident [b]	AD	BA	BP	CG	CM	FB	GL	HP	OG	PG	SH	SM	SN	WB	WG
methanamine	amine	rotted fish	618	MS						*						*	*		
TOTAL					**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**0**
phenylacetic acid	eterocyclic	sweet	1263	PI									*						
safrole	eterocyclic	spice	1273	PI		*	*						*						
methyl cinnamate	eterocyclic	sweet up	1374	PI			*												
TOTAL							**1**	**2**						**2**					

[a] LRI, Linear Retention Indices, capillary column HP -5. [b] Identification: MS (Wiley library); PI (Internet Data Base: flavornet)
AD: dehydrated alfalfa; BA: barley meal; BP: beet pulps; CG: corn grains; CM: corn middlings; FB: faba beans; GL: corn gluten meal; HP: soybean meal 49; OG: oat grains; PG: pea grains; SH: soybean hulls; SN: soybean meal 44; SM: sunflower meal; WB: wheat brans; WG: wheat grains.

Table 5. Aldehyde compounds extracted from the feed samples by SPME technique.

Compounds	Chemical class	Descriptor	LRI [a]	Ident [b]	AD	BA	BP	CG	CM	FB	GL	HP	OG	PG	SH	SM	SN	WB	WG
mercapto acetaldehyde	aldehyde	garlic	674	PI							*								
hexanal	aldehyde	green	798	PI,MS	*	*	*		*	*	*	*	*			*	*	*	*
2-hexenal	aldehyde	green	890	PI											*				
heptanal	aldehyde	rancid	899	PI,MS	*		*								*				
(Z)-2-heptenal	aldehyde	orange	901	MS			*						*						
octanal	aldehyde	orange	1002	PI,MS	*	*	*		*				*		*	*	*		
(Z)-2-octenal	aldehyde	nutty	1058	PI,MS		*	*		*				*		*		*		*
nonanal	aldehyde	orange	1104	PI,MS			*						*						
(E)-2-nonenal	aldehyde	green	1147	PI,MS	*	*	*	*			*	*	*	*	*	*	*	*	*
(Z)-2-nonenal	aldehyde	hay	1157	PI	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
2,4-nonadienal	aldehyde	fried oil	1215	PI	*	*	*	*	*		*	*	*		*	*	*	*	*
2-decenal	aldehyde	oil	1250	PI									*						
(E,E)-2,4-decadienal	aldehyde	fried oil	1318	PI		*	*				*		*		*				
2-undecenal	aldehyde	fruit	1354	PI			*												
vanillin	aldehyde	vanilla	1384	PI		*					*								
TOTAL					**6**	**8**	**11**	**3**	**5**	**2**	**7**	**4**	**10**	**2**	**8**	**5**	**6**	**4**	**5**

[a] LRI, Linear Retention Indices, capillary column HP -5. [b] Identification: MS (Wiley library); PI (Internet Data Base: flavornet)AD: dehydrated alfalfa; BA: barley meal; BP: beet pulps; CG: corn grains; CM: corn middlings; FB: faba beans; GL: corn gluten meal; HP: soybean meal 49; OG: oat grains; PG: pea grains; SH: soybean hulls; SN: soybean meal 44; SM: sunflower meal; WB: wheat brans; WG: wheat grains.

Table 6. Terpene compounds extracted from the feed samples by SPME technique.

Compounds	Chemical class	Descriptor	LRI [a]	Ident [b]	AD	BA	BP	CG	CM	FB	GL	HP	OG	PG	SH	SM	SN	WB	WG
a-thujene	terpene	floral	931	PI,MS													*		
a-pinene	terpene	resin, pine	943	PI									*						
dihydrolinalool	terpene	wood	1053	PI				*		*				*	*	*			
trans-sabinene hydrate	terpene	nutty	1110	PI			*						*		*				
menthone	terpene	green	1125	PI											*				
(E)-linalool oxide	terpene	green	1174	PI			*												
(Z)-dihydrocarvone	terpene	green	1193	PI			*												
geranial	terpene	lemon	1278	PI									*						
p-menthenethiol	terpene	sweet	1280	PI									*						
methyl geranate	terpene	floral	1305	PI							*							*	
dihydrocarvyl acetate	terpene	solvent	1334	PI	*														
d-elemene	terpene	coconut	1346	PI									*						
eugenol	terpene	spicy	1367	PI									*						
a-copaene	terpene	spice	1382	PI,MS	*		*						*						
geosmin	terpene	beet,earth	1410	PI,MS			*												
b-patchoulene	terpene	rose, plant	1412	MS	*														
TOTAL					**3**	**0**	**5**	**1**	**0**	**1**	**1**	**0**	**7**	**1**	**3**	**1**	**1**	**1**	**0**

[a] LRI, Linear Retention Indices, capillary column HP -5. [b] Identification: MS (Wiley library); PI (Internet Data Base: flavornet)

AD: dehydrated alfalfa; BA: barley meal; BP: beet pulps; CG: corn grains; CM: corn middlings; FB: faba beans; GL: corn gluten meal; HP: soybean meal 49; OG: oat grains; PG: pea grains; SH: soybean hulls; SN: soybean meal 44; SM: sunflower meal; WB: wheat brans; WG: wheat grains.

Table 7. Sulphur compounds extracted from the feed samples by SPME technique.

Compounds	Chemical class	Descriptor	LRI [a]	Ident [b]	AD	BA	BP	CG	CM	FB	GL	HP	OG	PG	SH	SM	SN	WB	WG
methyl ethyl sulfide	sulphur	garlic	631	PI	*		*		*	*	*	*	*			*		*	
thiophene	sulphur	garlic	668	PI	*		*		*	*	*	*			*	*	*	*	
methyl furanthiol	sulphur	meat	865	PI												*			
methional	sulphur	cooked potato	908	PI	*				*		*	*				*	*	*	
dimethyl trisulfide	sulphur	garlic	967	PI,MS	*				*	*	*	*			*	*	*	*	
ethyl dimethyl thiazole	sulphur	mushroom	1080	PI		*							*						
methyldithiofurane	sulphur	nutty,meat	1175	PI	*				*	*		*		*			*	*	
TOTAL					**5**	**1**	**2**	**0**	**5**	**4**	**4**	**5**	**2**	**1**	**2**	**5**	**4**	**5**	**0**

[a] LRI, Linear Retention Indices, capillary column HP -5. [b] Identification: MS (Wiley library); PI (Internet Data Base: flavornet)
AD: dehydrated alfalfa; BA: barley meal; BP: beet pulps; CG: corn grains; CM: corn middlings; FB: faba beans; GL: corn gluten meal; HP: soybean meal 49; OG: oat grains; PG: pea grains; SH: soybean hulls; SN: soybean meal 44; SM: sunflower meal; WB: wheat brans; WG: wheat grains.

Table 8. Ketone compounds extracted from the feed samples by SPME technique.

Compounds	Chemical class	Descriptor	LRI [a]	Ident [b]	AD	BA	BP	CG	CM	FB	GL	HP	OG	PG	SH	SM	SN	WB	WG
2,3-butanedione	ketone	butter	640	MS	*		*		*	*	*	*			*	*	*	*	
heptanone	ketone	solvent	889	PI													*		
1-octen-3-one	ketone	mushroom	980	PI,MS	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
(Z)-1,5-octadien-3-one	ketone	geranium	983	PI	*														
octanone	ketone	orange	998	PI		*	*								*				
3,5-octadien-2-one	ketone	orange	1094	PI,MS		*	*						*		*	*	*		
undecanone	ketone	peach	1295	PI									*						
TOTAL					**3**	**3**	**4**	**1**	**2**	**2**	**2**	**2**	**3**	**1**	**4**	**3**	**4**	**2**	**1**

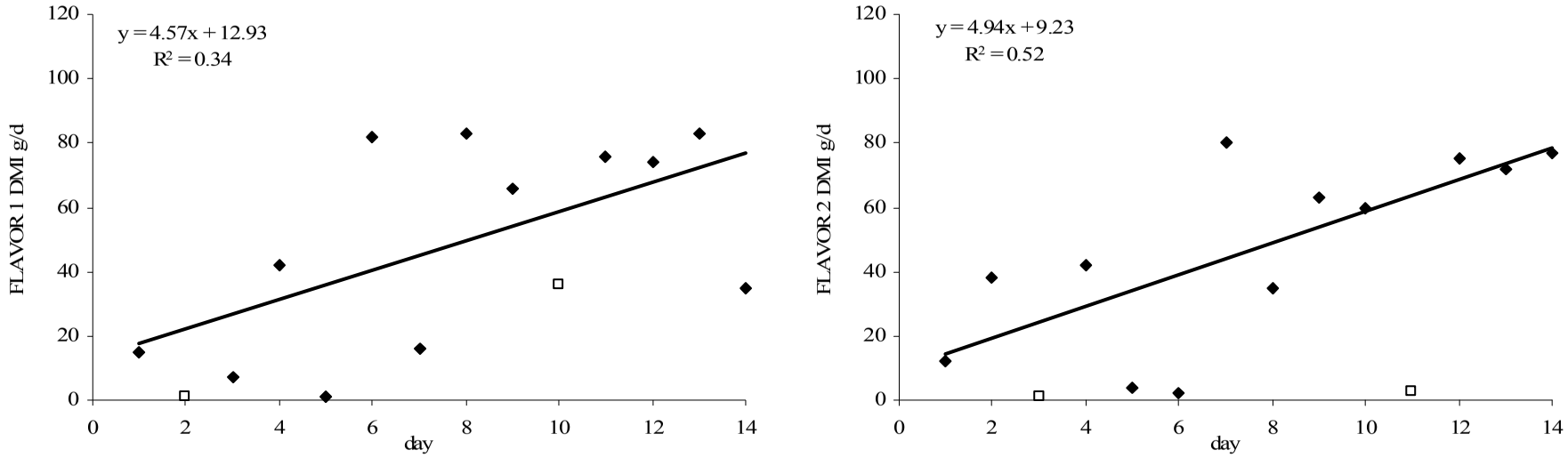
[a] LRI, Linear Retention Indices, capillary column HP -5. [b] Identification: MS (Wiley library); PI (Internet Data Base: flavornet)
AD: dehydrated alfalfa; BA: barley meal; BP: beet pulps; CG: corn grains; CM: corn middlings; FB: faba beans; GL: corn gluten meal; HP: soybean meal 49; OG: oat grains; PG: pea grains; SH: soybean hulls; SN: soybean meal 44; SM: sunflower meal; WB: wheat brans; WG: wheat grains.

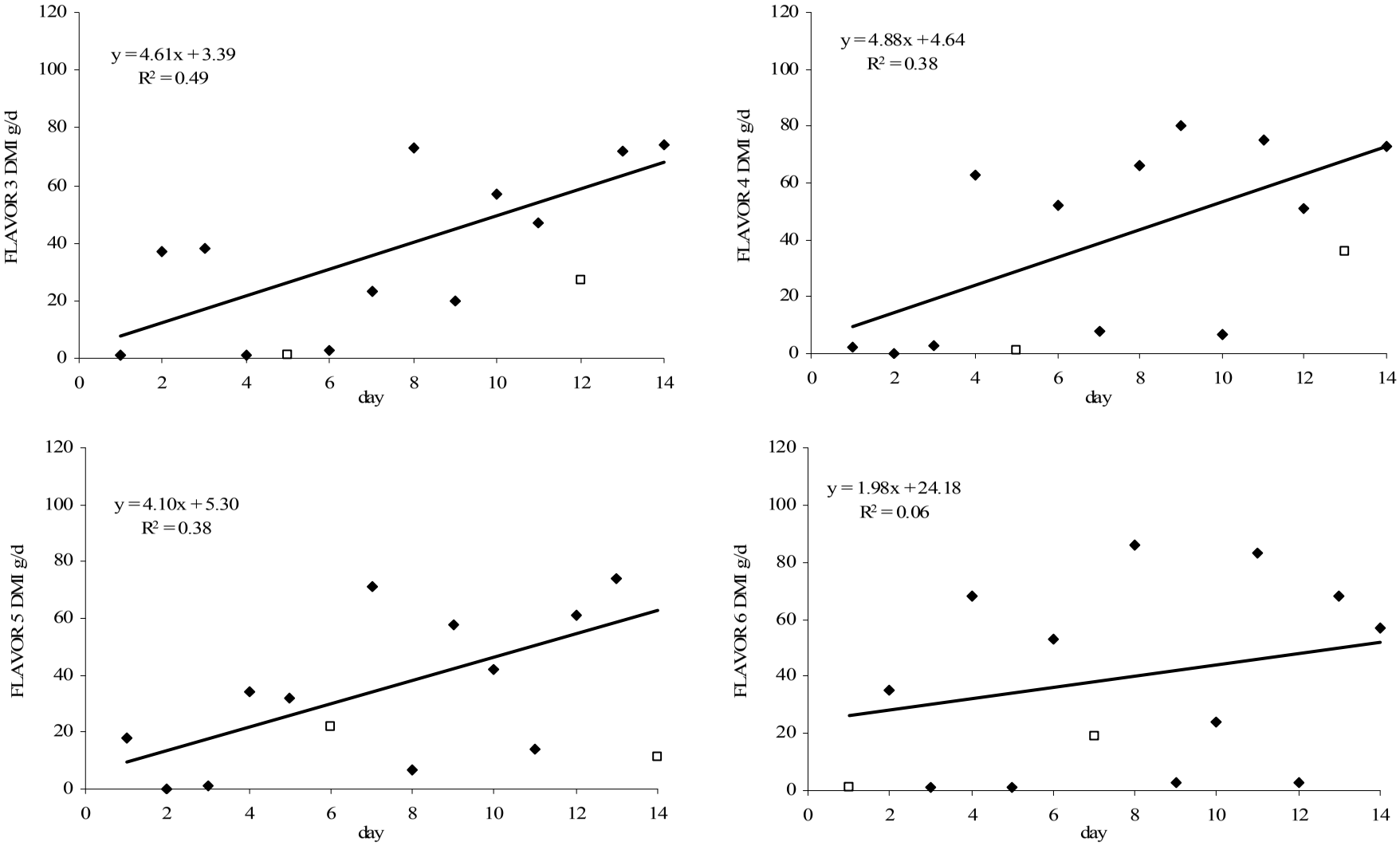
PALATABILITY OF CONCENTRATES FED TO SHEEP

APPENDIX A

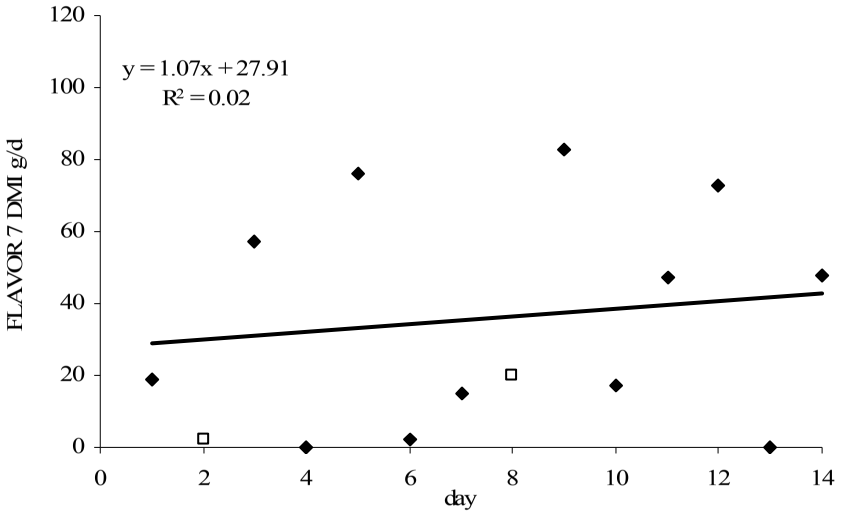
Figures of individual measurements

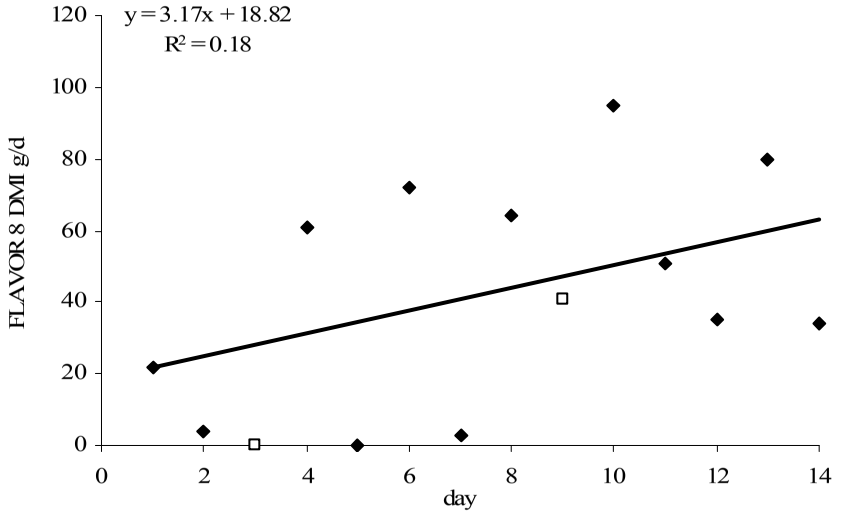
A – Chapter 3 - Experiment 1. Variation of lambs DMI of canola meal flavored with 13 flavors or without added flavors (Control) during the 14 days of experimental period, excluding the two ewes with the lowest mean DMI (□) during the experimental period.

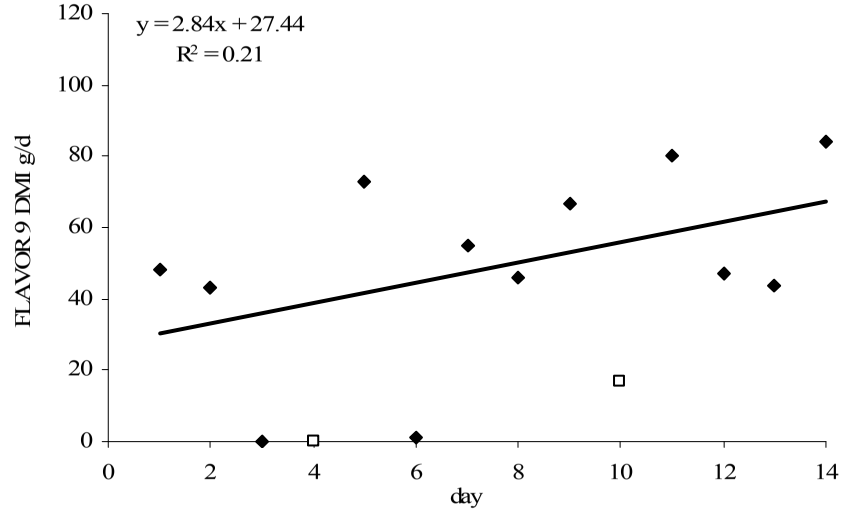


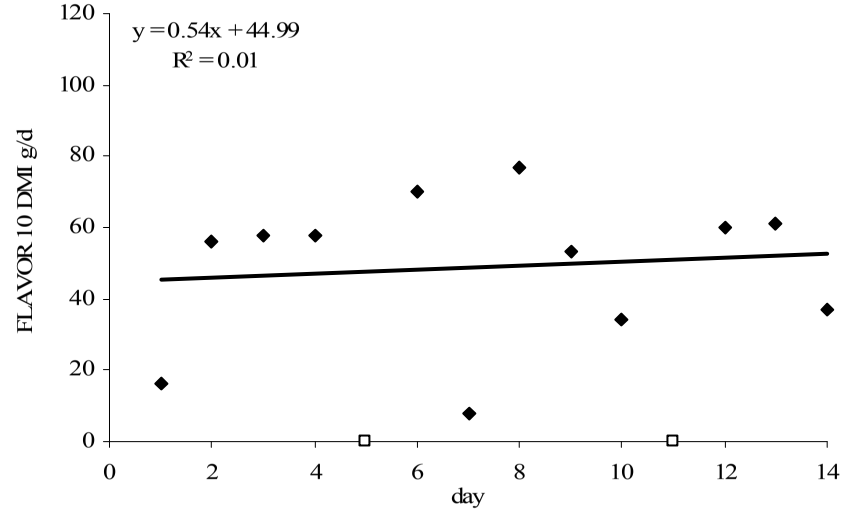

y = 4.61x + 3.39
R² = 0.49
FLAVOR 3 DMI g/d
day
y = 4.88x + 4.64
R² = 0.38
FLAVOR 4 DMI g/d
day
y = 4.10x + 5.30
R² = 0.38
FLAVOR 5 DMI g/d
day
y = 1.98x + 24.18
R² = 0.06
FLAVOR 6 DMI g/d
day

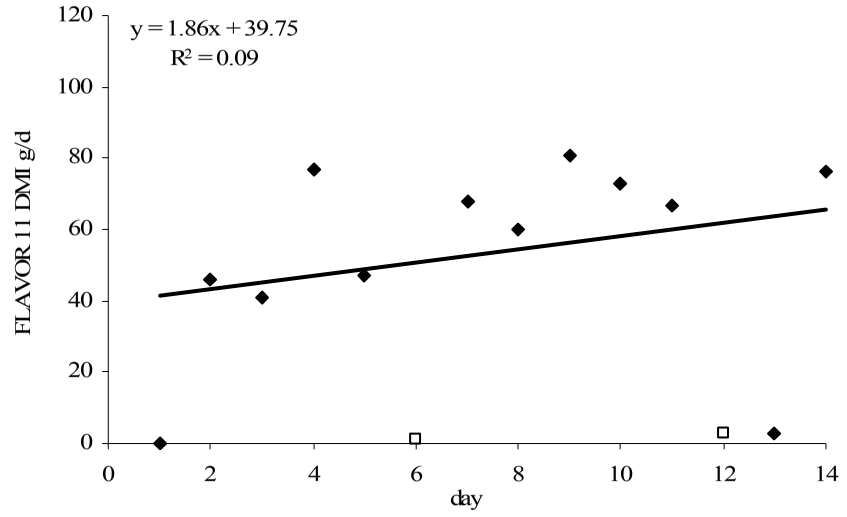
II

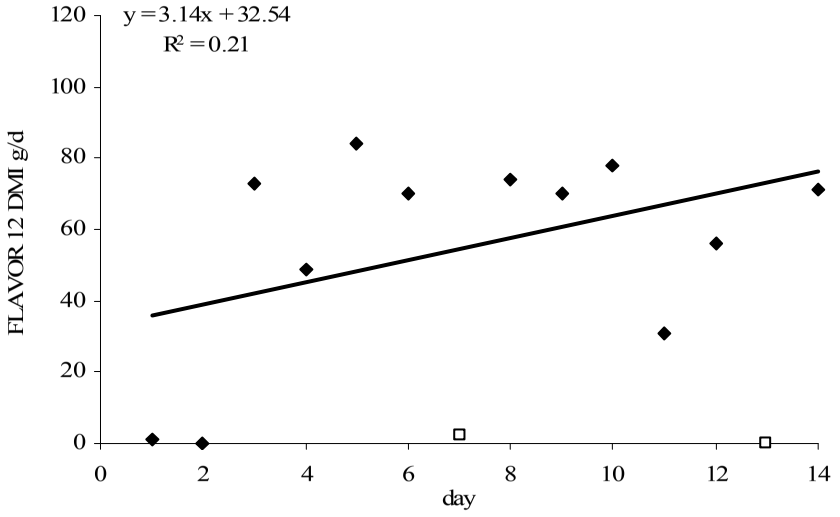

y = 1.07x + 27.91
R² = 0.02
FLAVOR 7 DMI g/d
day

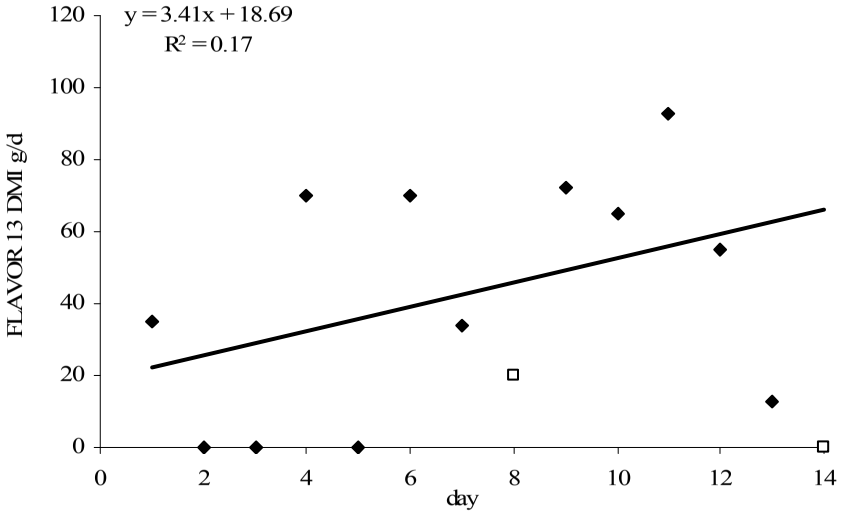

y = 3.17x + 18.82
R² = 0.18
FLAVOR 8 DMI g/d
day

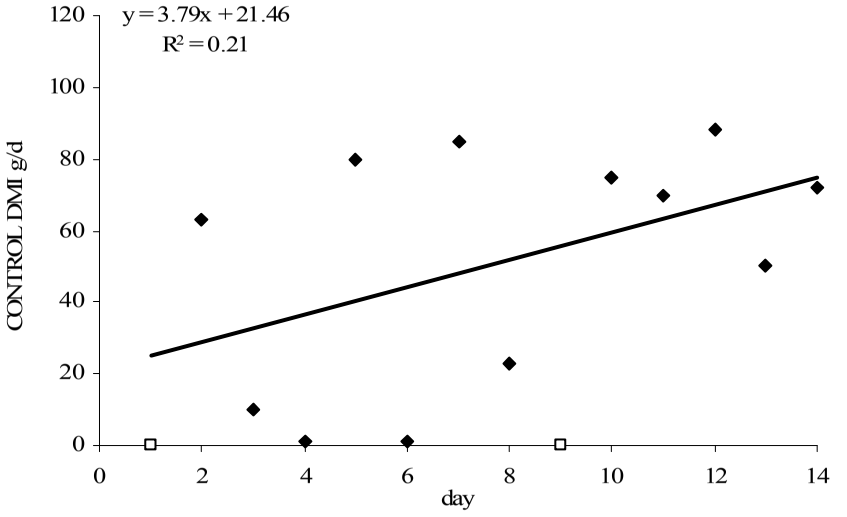

y = 2.84x + 27.44
R² = 0.21
FLAVOR 9 DMI g/d
day


y = 0.54x + 44.99
R² = 0.01
FLAVOR 10 DMI g/d
day

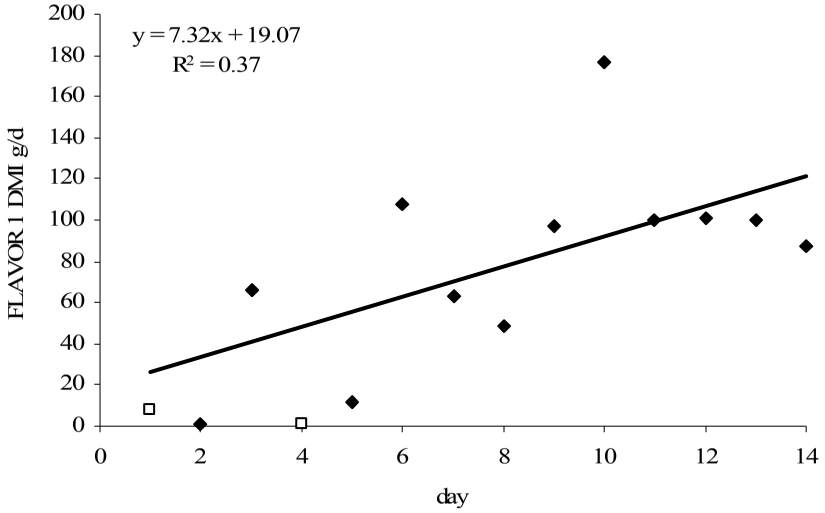

y = 1.86x + 39.75
R² = 0.09
FLAVOR 11 DMI g/d
day

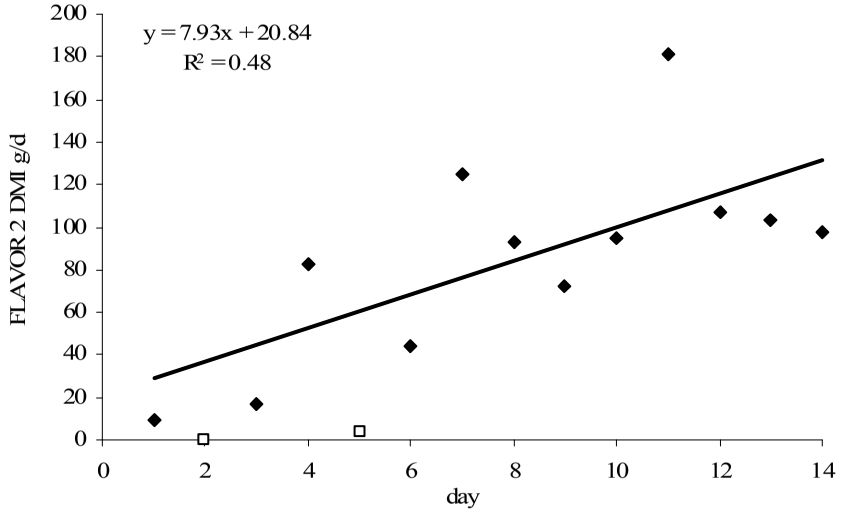

y = 3.14x + 32.54
R² = 0.21
FLAVOR 12 DMI g/d
day

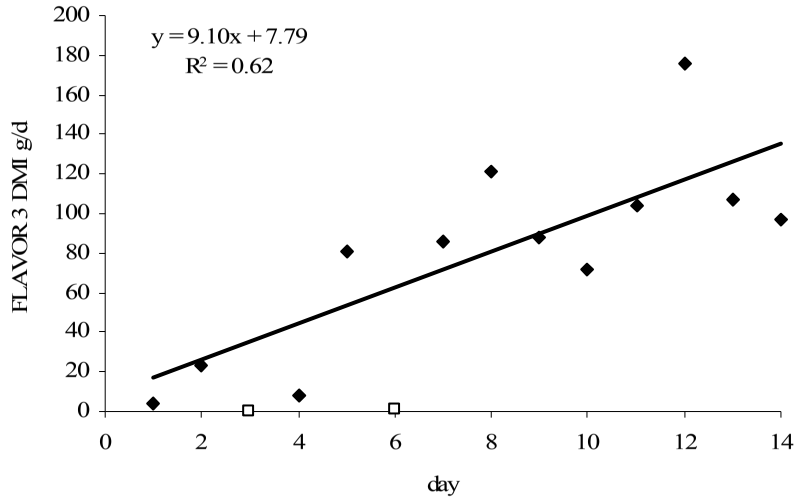

y = 3.41x + 18.69
R² = 0.17
FLAVOR 13 DMI g/d
day

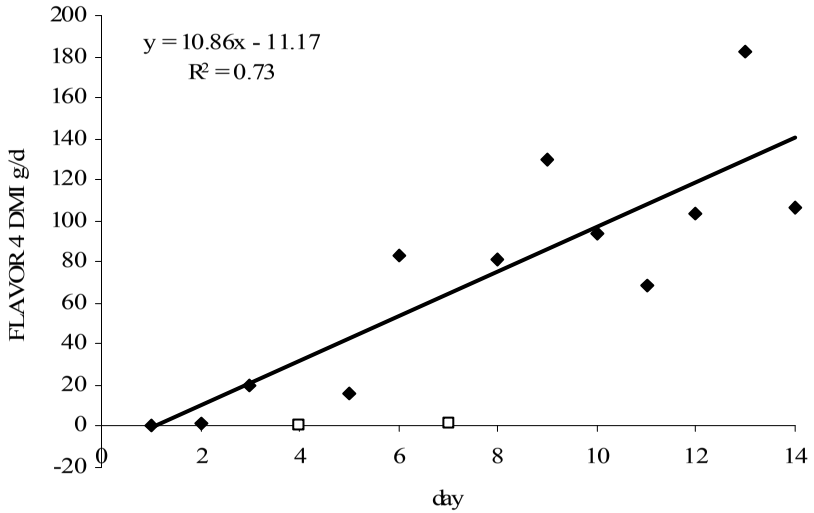

y = 3.79x + 21.46
R² = 0.21
CONTROL DMI g/d
day

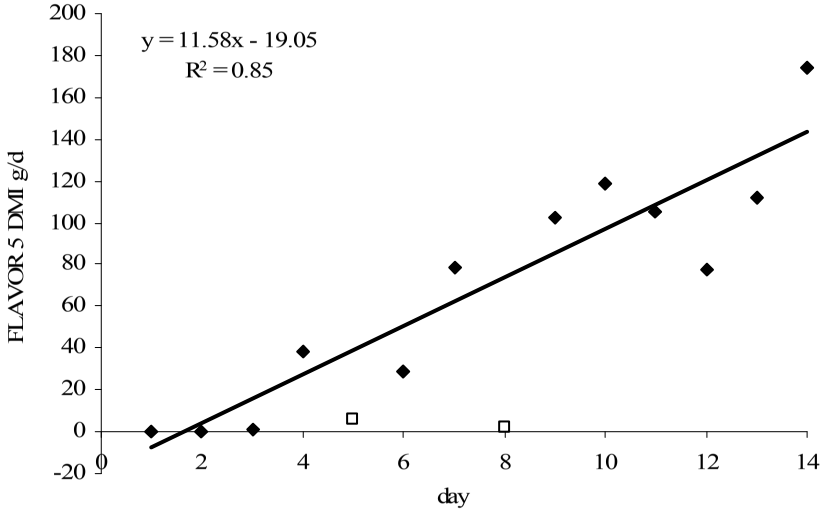
B – Chapter 3 - Experiment 1. Variation of ewes DMI of canola meal flavored with 13 flavors or without added flavors (Control) during the 14 days of experimental period, excluding the two ewes with the lowest mean DMI (□) during the experimental period.

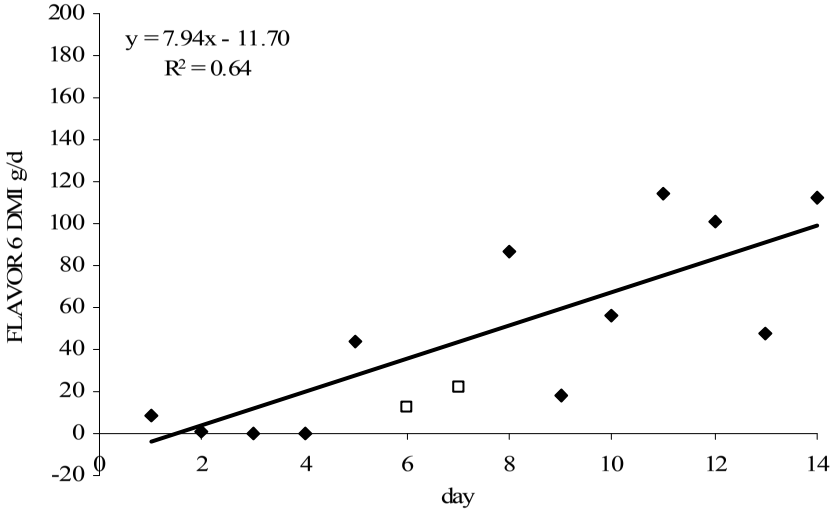


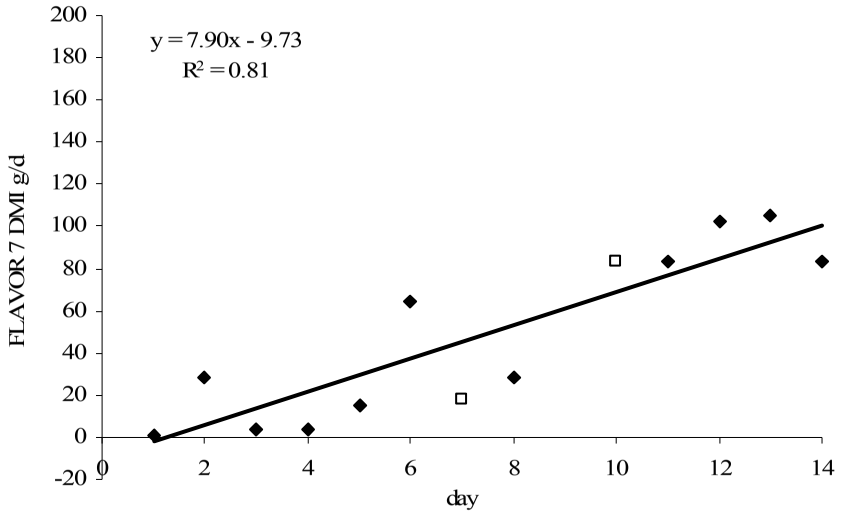


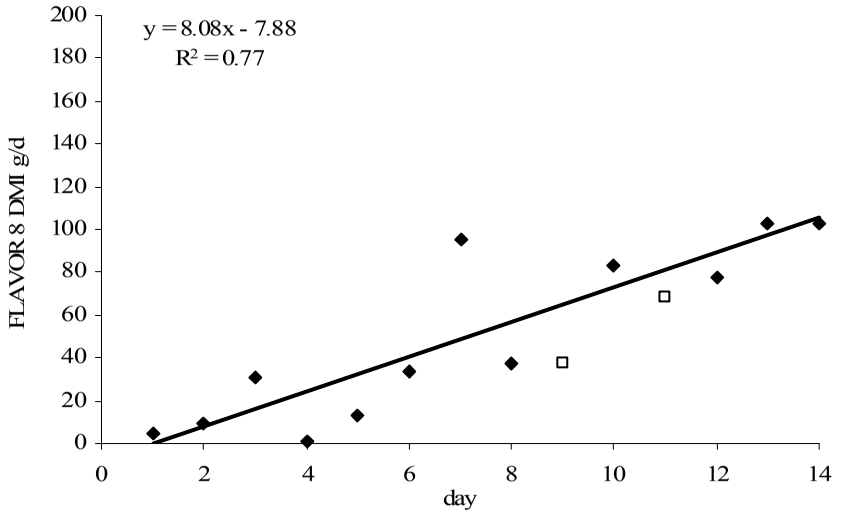

y = 9.10x + 7.79
R² = 0.62
FLAVOR 3 DMI g/d
day

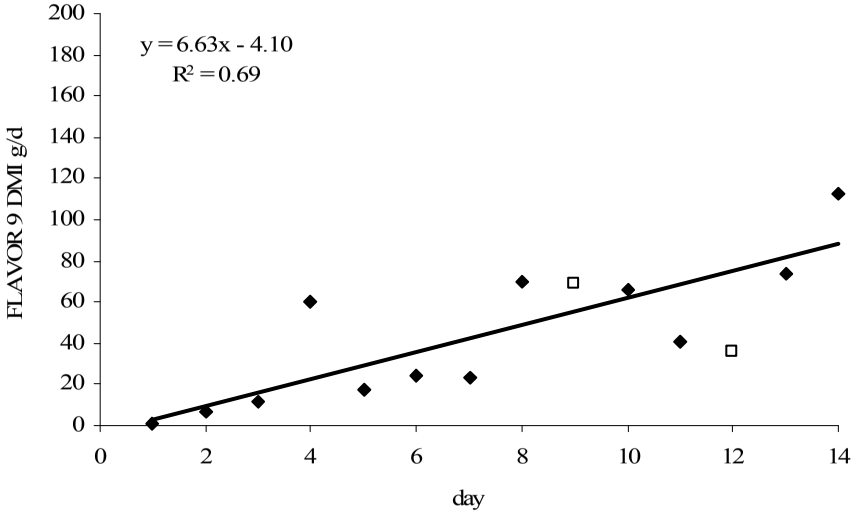

y = 10.86x - 11.17
R² = 0.73
FLAVOR 4 DMI g/d
day

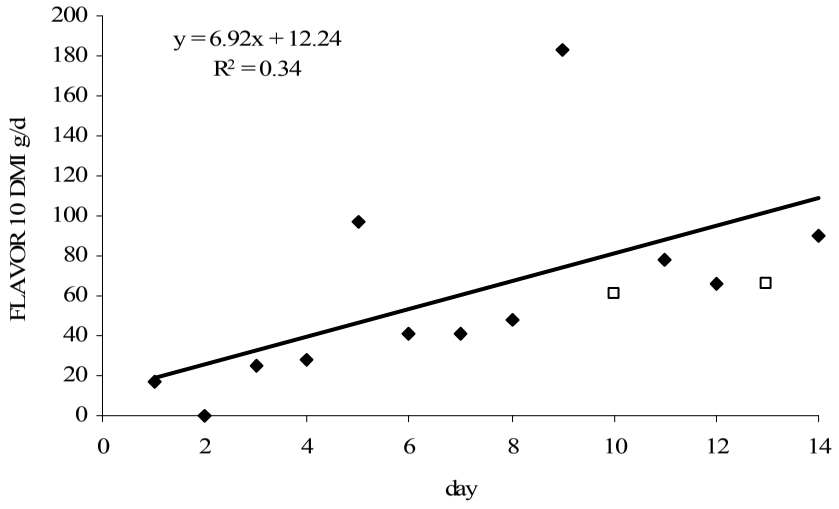

y = 11.58x - 19.05
R² = 0.85
FLAVOR 5 DMI g/d
day

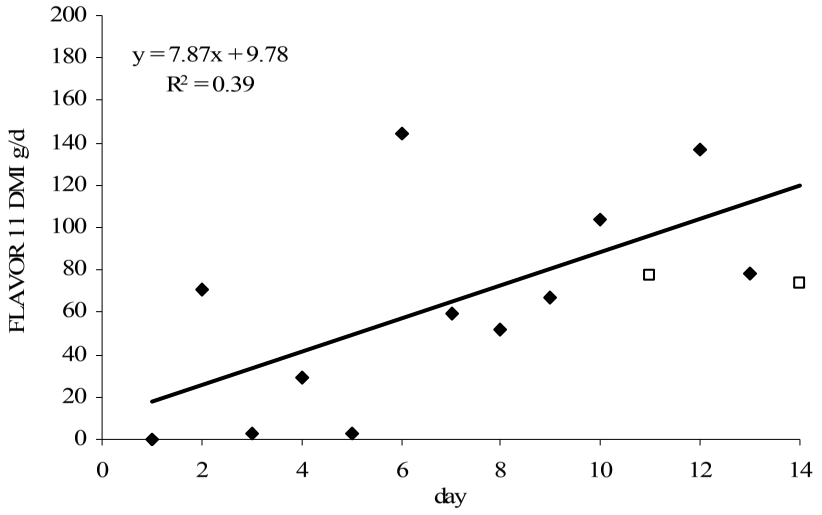

y = 7.94x - 11.70
R² = 0.64
FLAVOR 6 DMI g/d
day

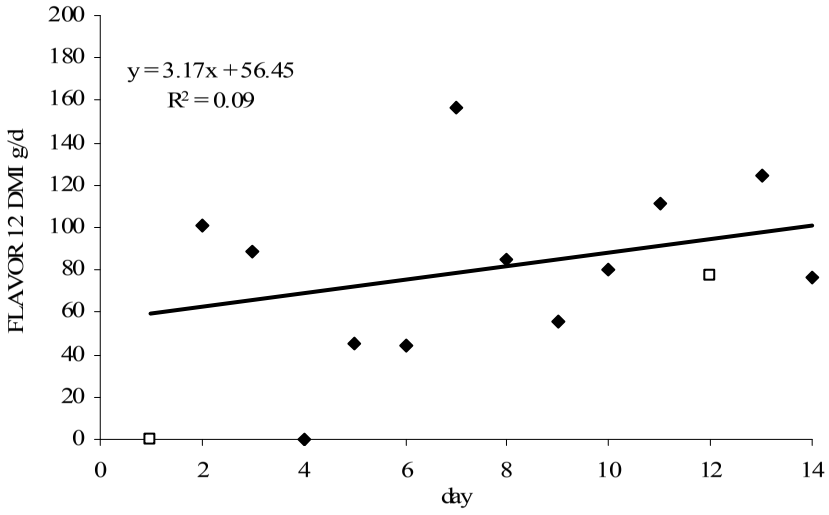

y = 7.90x - 9.73
R² = 0.81
FLAVOR 7 DMI g/d
day

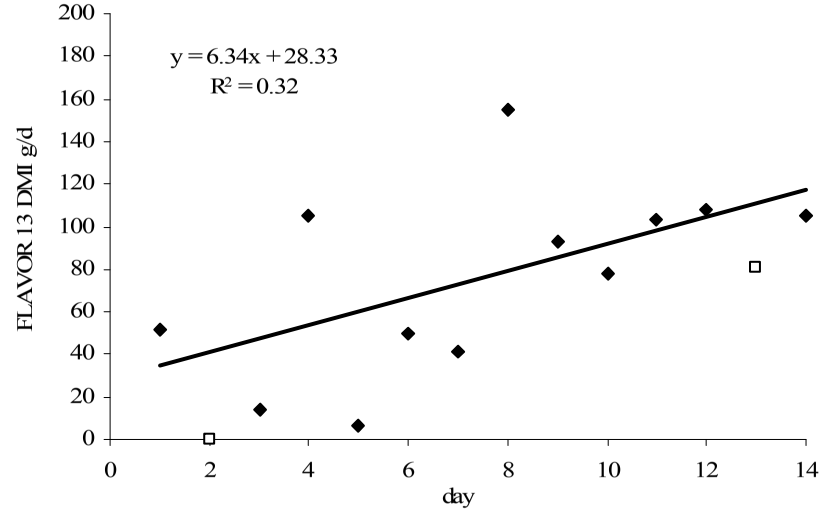

y = 8.08x - 7.88
R² = 0.77
FLAVOR 8 DMI g/d
day

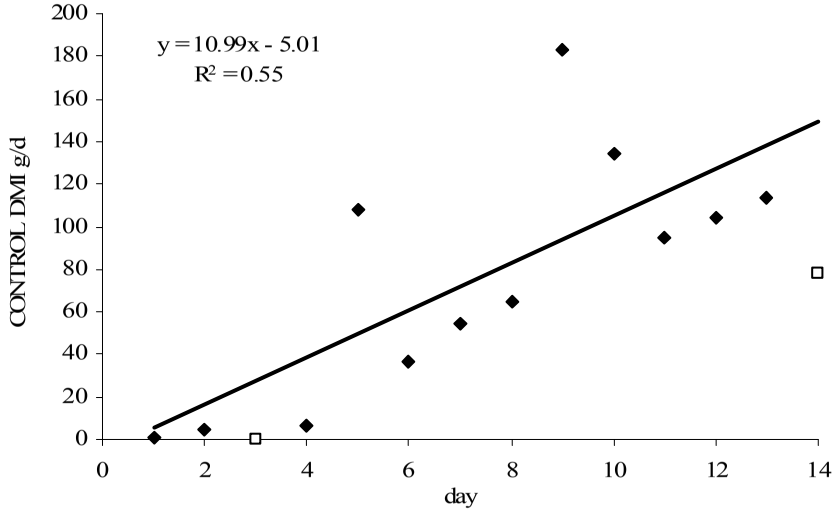

y = 6.63x - 4.10
R² = 0.69
FLAVOR 9 DMI g/d
day


y = 6.92x + 12.24
R² = 0.34
FLAVOR 10 DMI g/d
day

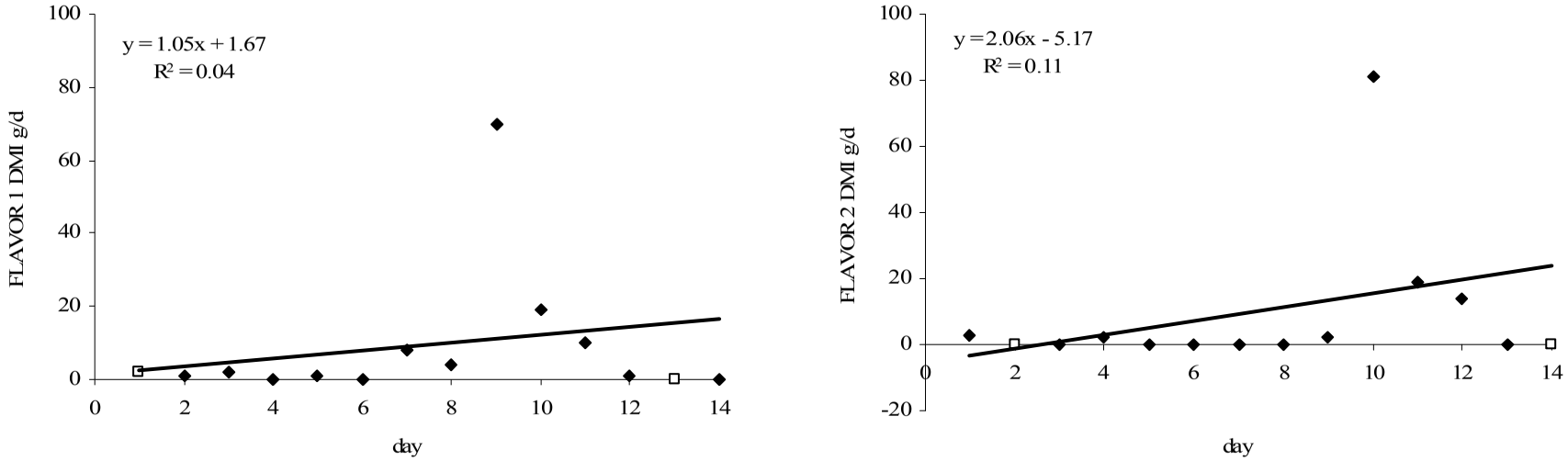

y = 7.87x + 9.78
R² = 0.39
FLAVOR 11 DMI g/d
day

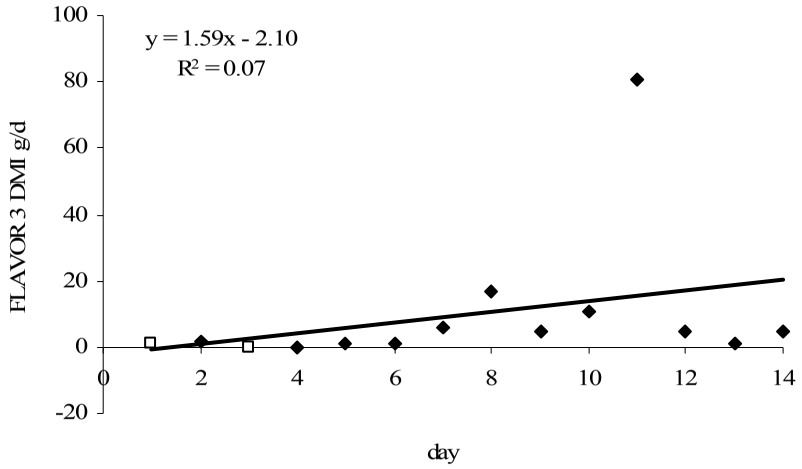

y = 3.17x + 56.45
R² = 0.09
FLAVOR 12 DMI g/d
day

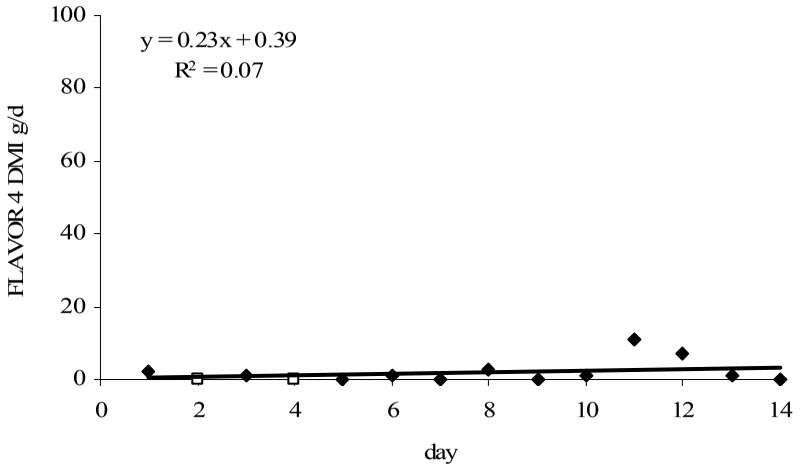

y = 6.34x + 28.33
R² = 0.32
FLAVOR 13 DMI g/d
day

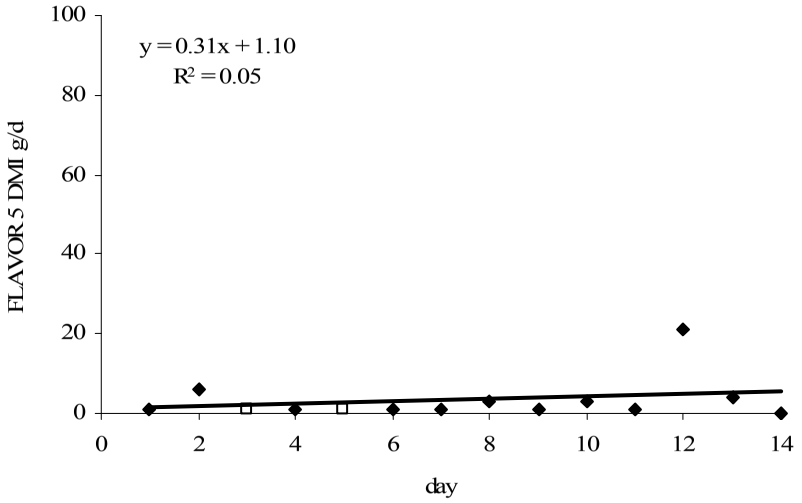

y = 10.99x - 5.01
R² = 0.55
CONTROL DMI g/d
day

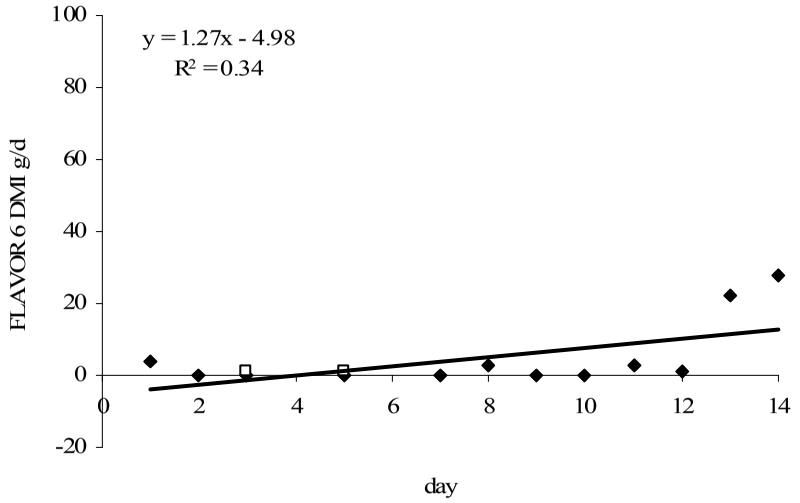
C – Chapter 3 - Experiment 2. Variation of lambs DMI of oat grains flavored with 13 flavors or without added flavors (Control) during the 14 days of experimental period, excluding the two ewes with the lowest mean DMI (□) during the experimental period.

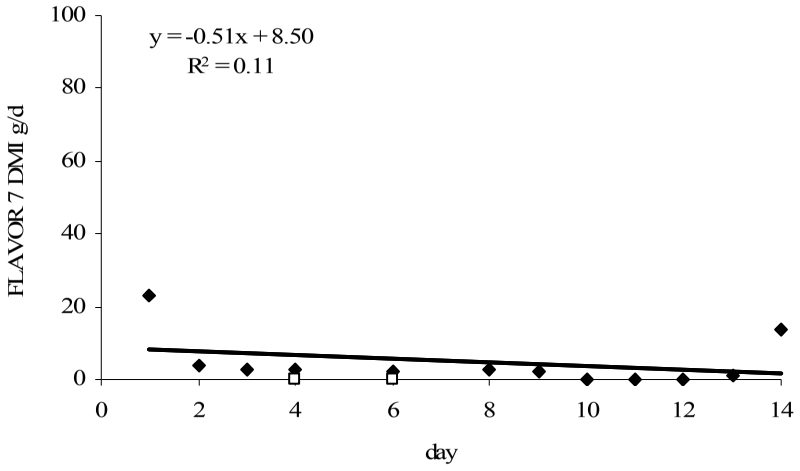


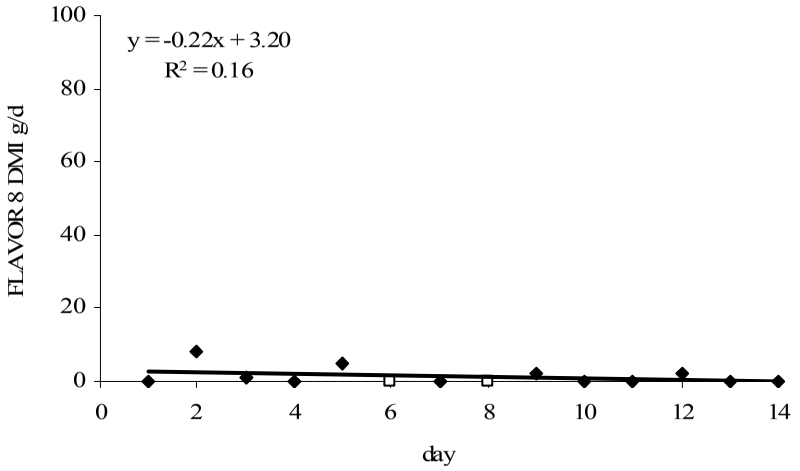

y = 1.59x - 2.10
R² = 0.07
FLAVOR 3 DMI g/d
day

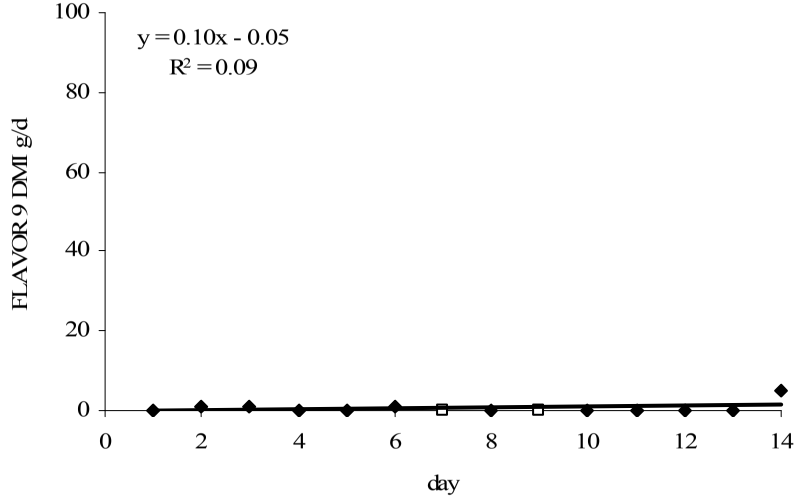

y = 0.23x + 0.39
R² = 0.07
FLAVOR 4 DMI g/d
day

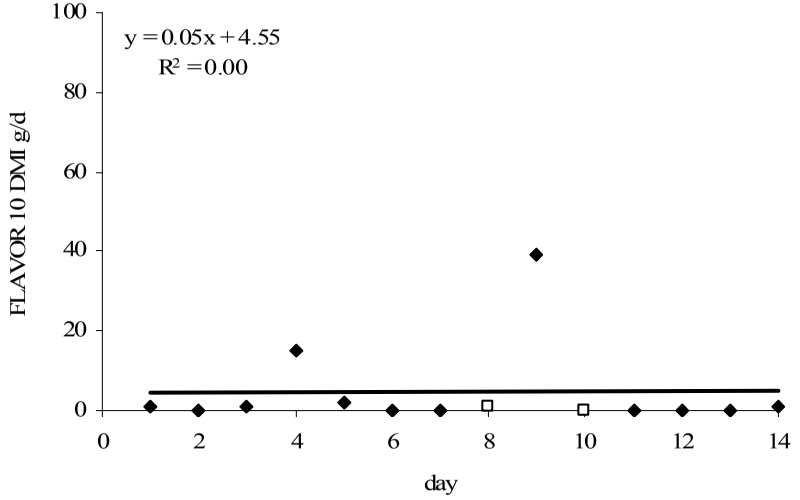

y = 0.31x + 1.10
R² = 0.05
FLAVOR 5 DMI g/d
day

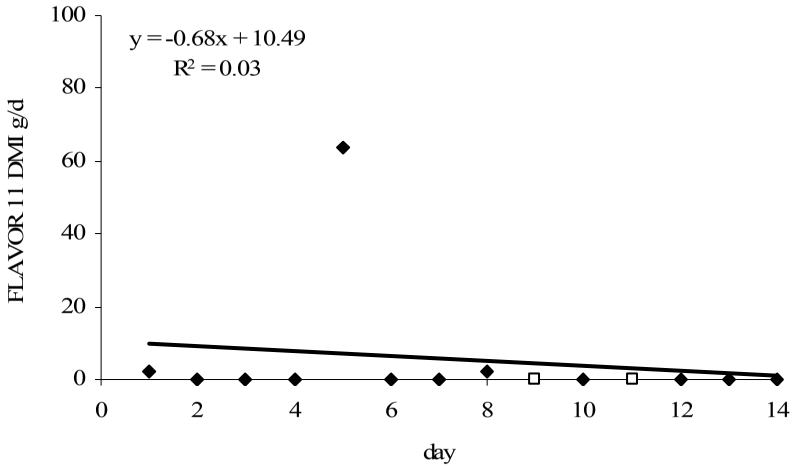

y = 1.27x - 4.98
R² = 0.34
FLAVOR 6 DMI g/d
day

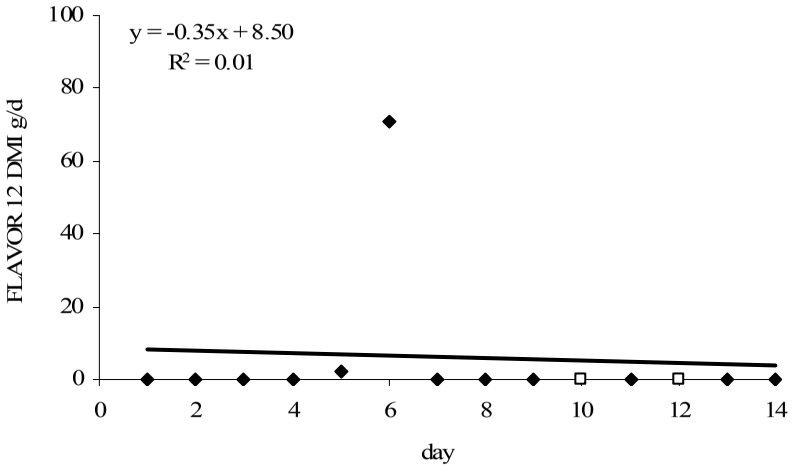

y = -0.51x + 8.50
R² = 0.11
FLAVOR 7 DMI g/d
day

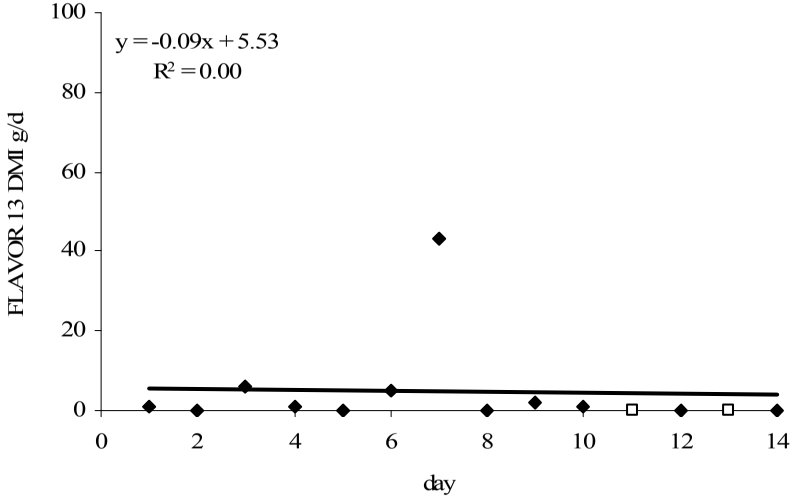

y = -0.22x + 3.20
R² = 0.16
FLAVOR 8 DMI g/d
day

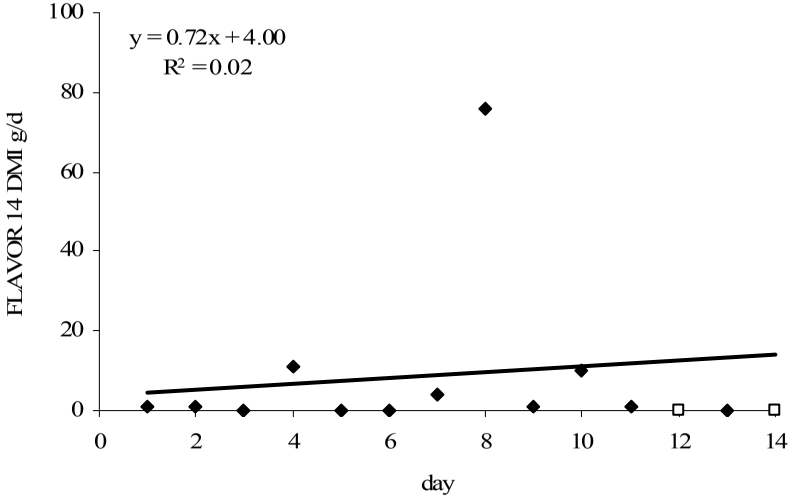

y = 0.10x - 0.05
R² = 0.09
FLAVOR 9 DMI g/d
day


y = 0.05x + 4.55
R² = 0.00
FLAVOR 10 DMI g/d
day

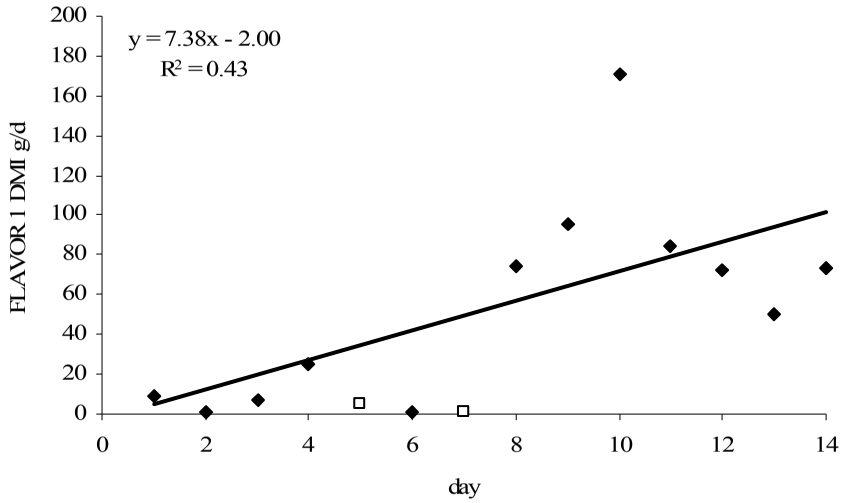

y = -0.68x + 10.49
R² = 0.03
FLAVOR 11 DMI g/d
day

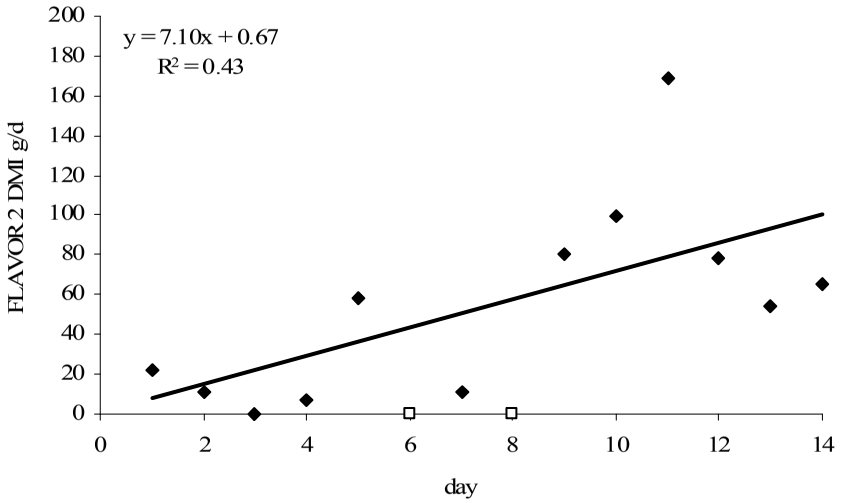

y = -0.35x + 8.50
R² = 0.01
FLAVOR 12 DMI g/d
day

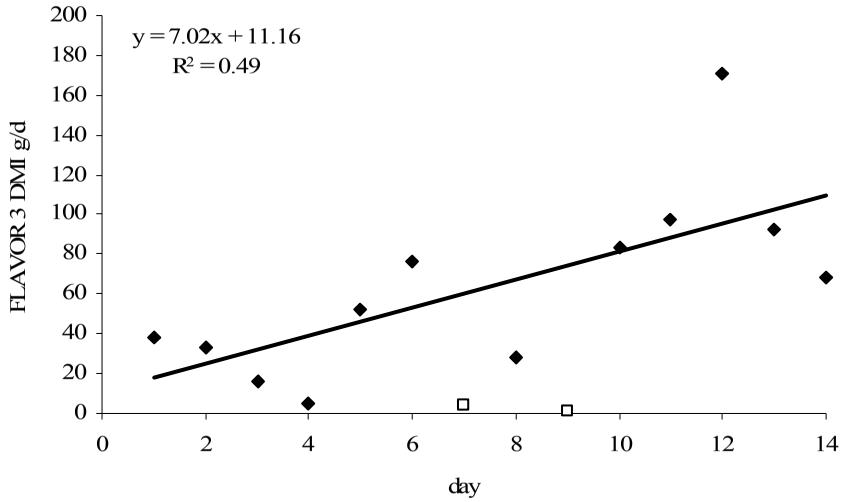

y = -0.09x + 5.53
R² = 0.00
FLAVOR 13 DMI g/d
day

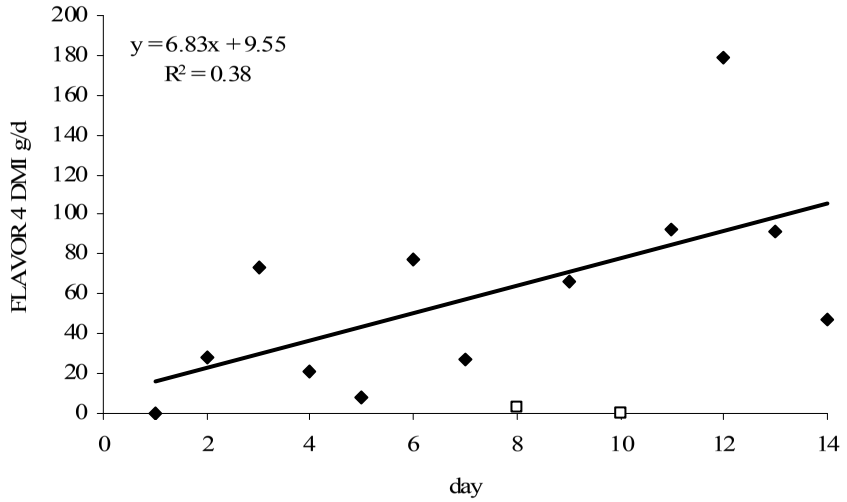

y = 0.72x + 4.00
R² = 0.02
FLAVOR 14 DMI g/d
day

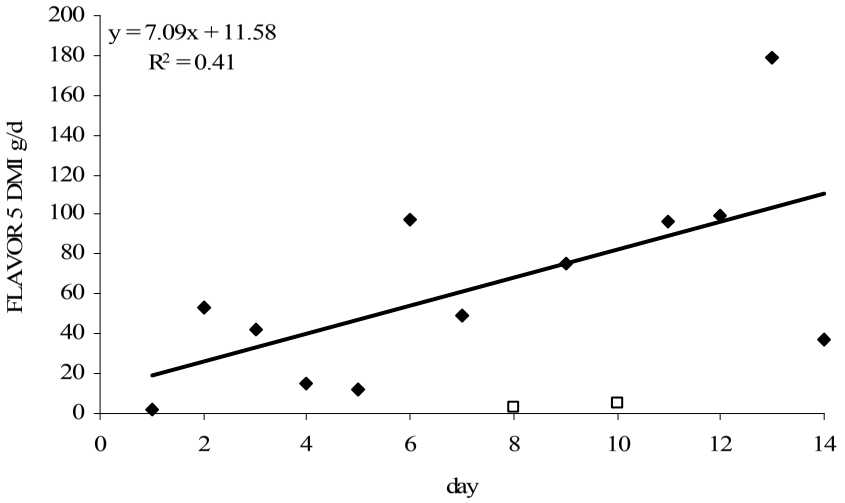
D – Chapter 3 - Experiment 2. Variation of ewes DMI of oat grains flavored with 13 flavors or without added flavors (Control) during the 14 days of experimental period, excluding the two ewes with the lowest mean DMI (□) during the experimental period.

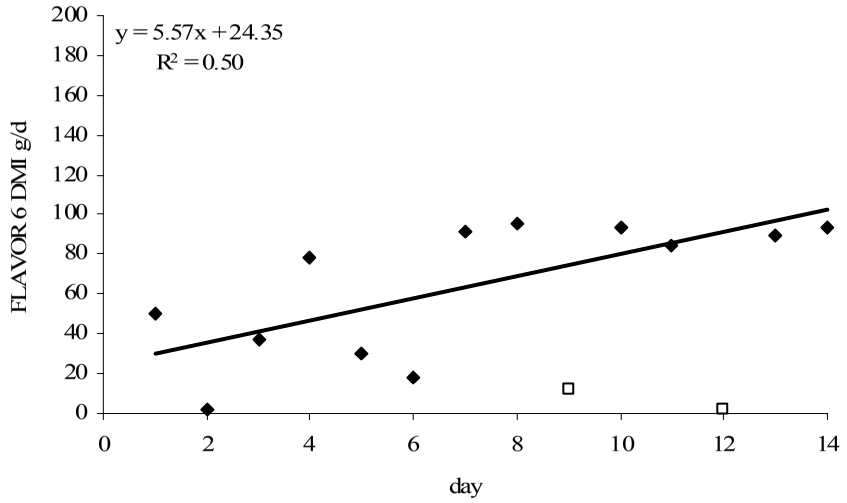


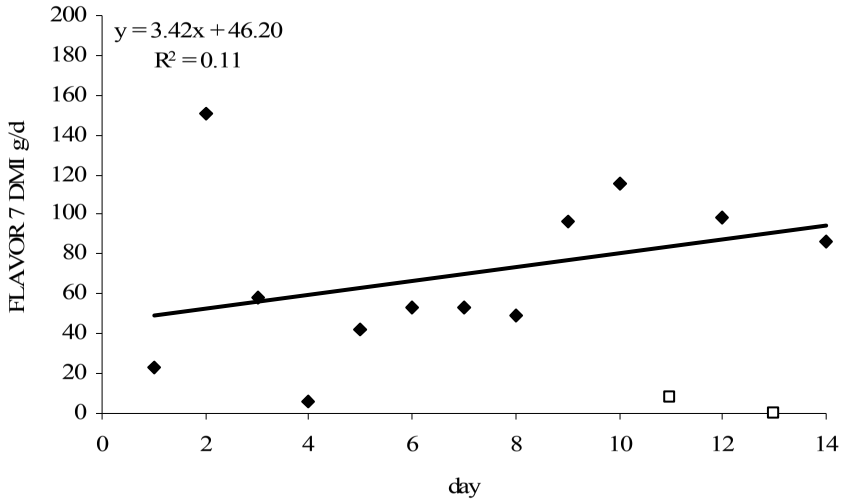


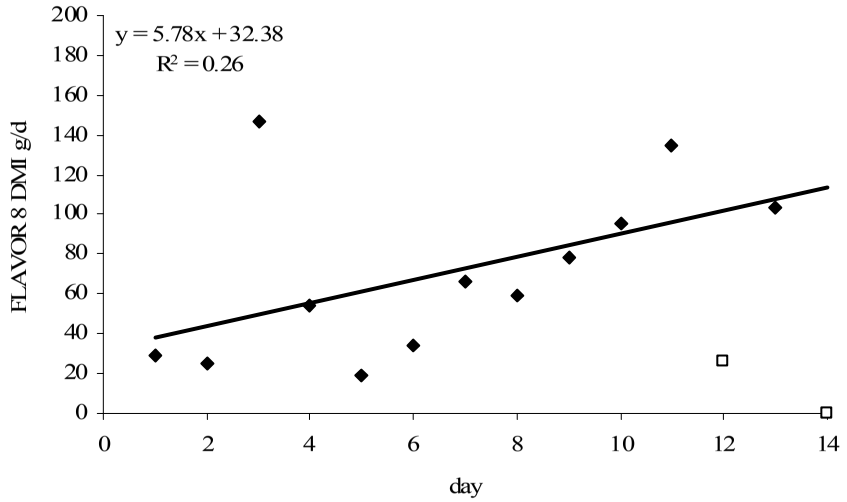

y = 7.02x + 11.16
R² = 0.49
FLAVOR 3 DMI g/d
day

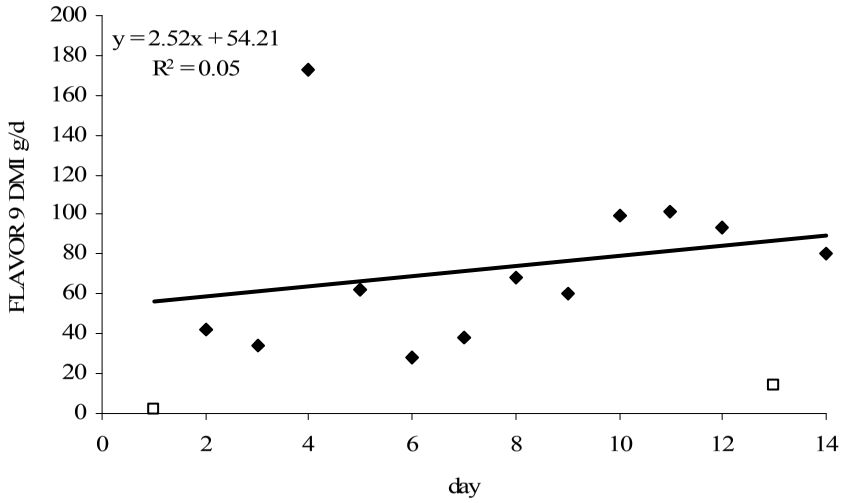

y = 6.83x + 9.55
R² = 0.38
FLAVOR 4 DMI g/d
day

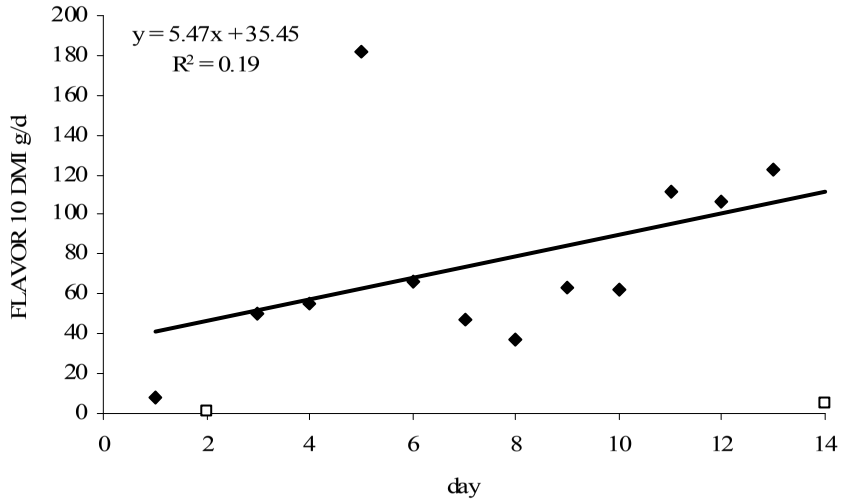

y = 7.09x + 11.58
R² = 0.41
FLAVOR 5 DMI g/d
day

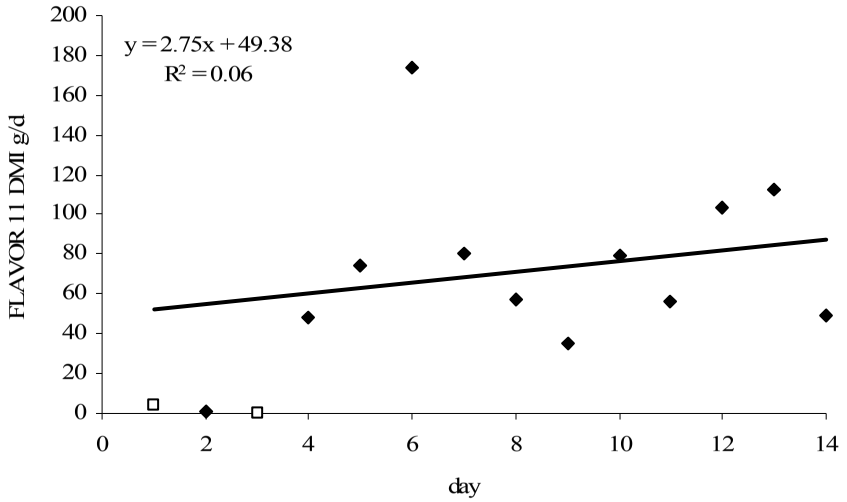

y = 5.57x + 24.35
R² = 0.50
FLAVOR 6 DMI g/d
day

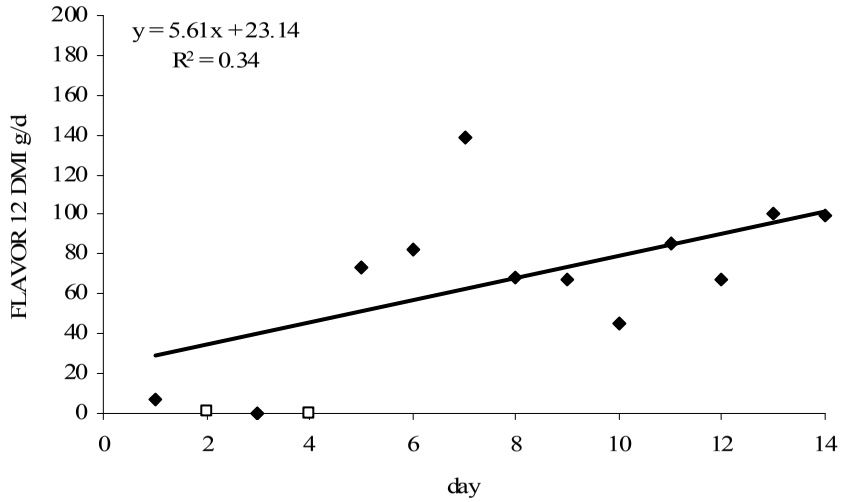

y = 3.42x + 46.20
R² = 0.11
FLAVOR 7 DMI g/d
day

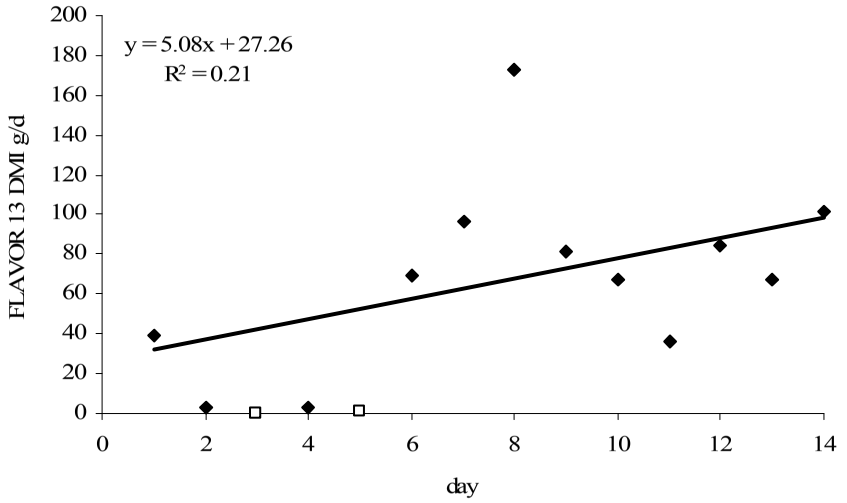

y = 5.78x + 32.38
R² = 0.26
FLAVOR 8 DMI g/d
day

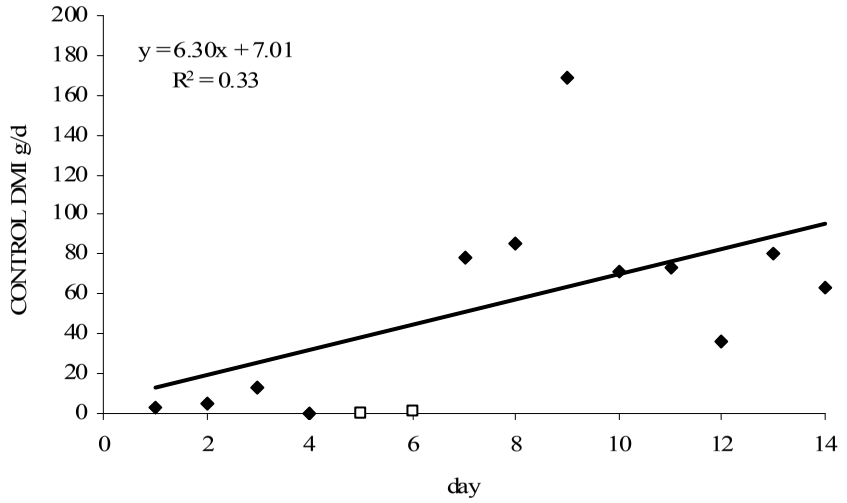

y = 2.52x + 54.21
R² = 0.05
FLAVOR 9 DMI g/d
day


y = 5.47x + 35.45
R² = 0.19
FLAVOR 10 DMI g/d
day


y = 2.75x + 49.38
R² = 0.06
FLAVOR 11 DMI g/d
day


y = 5.61x + 23.14
R² = 0.34
FLAVOR 12 DMI g/d
day


y = 5.08x + 27.26
R² = 0.21
FLAVOR 13 DMI g/d
day


y = 6.30x + 7.01
R² = 0.33
CONTROL DMI g/d
day