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Doctor in Philosophy (PhD) Thesis

Plant secondary compounds in small ruminant feeding in stall-fed and pastoral system in the Mediterranean

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Dedicated to all those who made this possible…….

Thanks

Luce su Luce! Dio guida verso la luce se lui vuole …….”

Sura Noor 24:37

La lumière propre du visage vient de la chandelle de l'esprit.

Djalâl-ud-Dîn Rûmî

Table of Contents

Abstract

The utilization of alternative feed resources such as agro-industrial by-products or rangelands may contribute to reduce carbon footprint and improve sustainability of livestock farming systems.My thesis aimed at investigating how animals can be made to ingest alternative feed resources containing higher amounts of Plant Secondary compounds (PSCs) than usual forages and concentrates. PSCs are present in agroindutrial by products and in the natural vegetation of rangelands in the Mediterranean area. They are often associated with anti-nutritive properties such as reduced palatability and protein digestion but can also have positive effects especially on health. Two systems of lamb meat production were investigated, namely stall-fed in Siciliy and pastoral in Morocco.

In the stall-fed system, we investigated the potential of replacing barley by two locally available agro industrial by- products containing polyphenols, namely carob pulp and dehydrated citrus pulp (DCP). The animals were 90 days of age Comisana lambs assessed during fattening. Food intake pattern and blood metabolic profile were monitored. It was found that ingestion of a diet containing 35% of carob pulp or DCP resulted in comparable performance, feed efficiency and carcass weight and yield as compared to animals ingesting a cereal based diet (control). The daily feed consumption pattern was markedly affected by the inclusion of carob pulp in the diet.Control group ate more than 40% of the whole daily intake in the first 90 min of feeding compared with only 33% in the carob groups but the overall feed intake was unchanged. The highest level of DCP inclusion in the diet also produced a different rate of feed consumption, as compared to the control group. In terms of blood metabolic profile, carob ingesting animals had lower cholesterol, higher urea and higher NEFA compared to the control whilst with DCP ingestion none of the parameters measured were affectedPertaining to the blood protein profile, carob pulp addition gave minor effects, only a tendency for the albumin to globulin (AG) ratio to rise has been observed; in the case of DCP inclusion in lambs diet, the serum albumin and the AG ratio were higher compared to the control; but the values were not a a level to cause metabolic distress.

In the pastoral system, we collected the local ecological knowledge (LEK) of shepherds and analysed the possible relationship between the grazing management implemented and the PSCs content of rangeland plants. Three sites were investigated in the Middle Atlas of Morocco, around the towns

of Boulemane, Guigou and El Ksabi. Thirty-five farmers were encountered to describe local farming systems, and eleven shepherds were selected, which were subsequently interviewed *in situ* to collect their LEK about plant species, seasonality, grazing strategies and animal ingestion behaviour.In parallel, based on the interviews, samples of plants ingested were collected and the chemical composition in terms of nutrients and PSCs i.e. total phenols and tannins. It was found that each site could be associated to a type of grazing system and that the rangelands were classified based on the most predominant plant. The amount of LEK of the shepherds increased with experience and involvement in decision making. Only a handful of plants were considered as important in the opinion of shepherds and plants were classified as a function of their role in the daily intake (e.g. fodder, apetizer, …). Polyphenols were relatively low in most of the plants assayed ranging from 0.7 %DM in *Festuca* sp. to 4.0%DM in *Quercus rotundifolia*. Shepherds identified the plants which differed in circumstantial palatability and designed the grazing circuits to stimulate the intake of a diversity of plants. Due to the government concern about rangeland over-utilization and the increasing reliance on conserved feed, there is a risk for the pastoral systems to become less dependant on rangelands resources and for the LEK of shepherds to be lost over generations.

The study demonstrated that PSCs have a central role in both stall-fed and pastoral system of livestock prodcution and in each case. Strategies are available to increase their intake and thereby enhance the use of agro-industrial by products in the former and PSCs rich plants in the latter. This will contribute in reducing carbon footprint, improve the sustainability of the systems and at the same time maintain animal well-being.

Riassunto

L'utilizzo di risorse alimentari alternative, come i sottoprodotti agroindustriali o i pascoli naturali, possono contribuire a ridurre la carbon footprint ed a migliorare la sostenibilità dei sistemi di allevamento. Il mio lavoro di tesi ha avuto lo scopo di investigare come gli animali possano essere alimentati con fonti alimentari alternative, contenenti composti secondari delle piante (Plant Secondary Compounds - PSCs) in quantità più elevate che nei comuni foraggi e mangimi concentrati. I PSCs sono presenti nei sottoprodotti agroindustriali e nella vegetazione spontanea dei pascoli del bacino del Mediterraneo. Essi sono spesso associati a proprietà antinutrizionali, come la scarsa appetibilità e la ridotta digestione delle proteine, tuttavia possono avere effetti positivi, specialmente sulla salute. Sono stati messi allo studio due sistemi di produzione di carne di agnello, l'uno realizzato in Sicilia con alimentazione in stalla e l'altro in Marocco con animali al pascolo.

Nel sistema di alimentazione in stalla, è stato investigato il potenziale di sostituzione dell'orzo con due sottoprodotti industriali contenenti polifenoli e disponibili a livello locale, cioè polpa di carrube e pastazzo di agrumi essiccato (dehydrated citrus pulp-DCP). Gli animali di 90 giorni di età, di razza Comisana, sono stati valutati durante le prove di ingrasso. Sono stati monitorati l'assunzione degli alimenti e il profilo metabolico del sangue. È stato riscontrato che le diete contenenti il 35% di polpa di carrube o DCP hanno dato luogo a performance di crescita, efficienza alimentare, peso della carcassa e resa paragonabili a quelli ottenuti dagli animali alimentati con dieta a base di cereali (controllo). La sequenza alimentare giornaliera è stata sensibilmente influenzata dall'inclusione della polpa di carrube nella dieta. Il gruppo controllo ingeriva più del 40% dell'intera assunzione giornaliera nei primi 90 minuti, mentre gli animali dei gruppi carruba ingerivano solo il 33%, tuttavia l'ingestione totale non variava tra i gruppi. Anche la dieta con più alti livelli di DCP ha prodotto un diverso tasso di consumo alimentare rispetto al gruppo controllo. Il profilo metabolico del sangue degli animali alimentati con carrube ha mostrato un più basso tasso di colesterolo e un maggiore contenuto di urea e di NEFA rispetto al gruppo controllo, mentre l'ingestione di DCP non ha influenzato nessuno dei parametri misurati. Per quanto riguarda il profilo proteico del sangue, l'aggiunta di polpa di carrube non ha sortito effetti rilevanti, ad eccezione di una tendenza all'incremento del rapporto albumina/globulina (AG); nel caso dell'inclusione di DCP nella dieta degli agnelli, i valori dell'albumina del siero e del rapporto AG erano più alti di quelli riscontrati nel gruppo controllo; tuttavia i valori non erano a livelli tali da causare stress metabolico.

Nel sistema di allevamento al pascolo sono state messe a frutto le conoscenze ecologiche a livello locale (local ecological knowledge-LEK) dei pastori ed è stata analizzata la possibile relazione tra il sistema di pascolamento messo a punto e il contenuto di PSCs delle piante pabulari. Sono stati stati studiati tre siti nel Medio Atlante del Marocco, intorno alle città di Boulemane, Guigou ed El Ksabi. Sono stati intervistati trentacinque allevatori per descrivere il sistema di allevamento locale e sono stati scelti undici pastori, che sono stati successivamente intervistati in situ per raccogliere le loro LEK sulle specie di piante, sulla stagionalità, sulle strategie di pascolamento e sul comportamento alimentare degli animali. In parallelo delle piante che, secondo i pastori intervistati, sono normalmente pascolate sono stati raccolti campioni sui quali sono state condotte analisi per determinarne la composizione chimica in termini di nutrienti e PSCs, come fenoli totali e tannini.È stato riscontrato che ogni località può essere associata a un sistema di pascolamento e che i pascoli erano classificati sulla base della pianta predominante. La quantità di LEK dei pastori è aumentata con l'esperienza e con il coinvolgimento nel processo decisionale. Poche piante erano considerate importanti dai pastori ed esse erano classificate in funzione del loro ruolo nella razione giornaliera (ad esempio foraggi, appetizzanti, ecc.). Il contenuto in polifenoli era relativamente basso nella maggior parte delle piante analizzate, variando dallo 0,7% sulla sostanza secca in Festuca spp. al 4,0% in Quercus rotundifolia. I pastori hanno identificato le piante più appetibili e hanno progettato i circuiti di pascolo per stimolare l'assunzione di una maggiore varietà di piante. A causa delle preoccupazioni governative sull'eccessivo sfruttamento dei pascoli e della crescente dipendenza dagli alimenti conservati, c'è il rischio che i sistemi di allevamento tradizionali scompaiano e che il LEK dei pastori vada perduto nel corso delle generazioni.

Lo studio ha dimostrato che i composti secondari hanno un ruolo centrale sia nell'allevamento in stalla sia al pascolo. Sono disponibili strategie per aumentare l'intake e in questo modo incrementare, innanzitutto, l'uso di sottoprodotti agroindustriali e, in secondo luogo, l'uso di piante ricche in PSCs. Questo potrà contribuire alla riduzione della carbon footprint, al miglioramento della sostenibilità dei sistemi di allevamento e, allo stesso tempo, al mantenimento del benessere animale.

Résumé

En alimentation animale l'utilisation des ressources alimentaires alternatifs tels les parcours ou les sous-produits de l'agro-industrie peut contribuer à atténuer l'empreinte de carbone des systèmes d'élevage et améliorer leur durabilité. Ma thèse a pour objectif celui d'étudier le mode selon lequel les animaux peuvent ingérer ces ressources alimentaires alternatives qui contiennent des concentrations élevées en composes secondaires par rapport aux concentres et fourrages utilisés habituellement. Dans la région méditerranéenne, il est reconnu que les composés secondaires sont présents dans les sous-produits agro industriels ainsi que dans la végétation naturelle des parcours. Ils sont souvent associés à des propriétés anti-nutritifs tels que la réduction de l'appétence et la digestion des protéines, mais peuvent aussi avoir des effets positifs, notamment sur la santé. Deux systèmes de production d'agneau de viande ont été étudiés ; un système en stabulation dans la Sicile centrale et un système pastorale au Maroc.

Dans le système de stabulation, nous avons étudié le potentiel de remplacer l'orge par deux sous-produits qui contiennent les polyphénols et qui sont disponibles localement, il s'agit de la pulpe de caroube et de la pulpe d'agrumes déshydratée (PAD). Des agneaux de race Comisana de 90 jours d'âge , durant la phase d'engraissement, les paramètres tel que la prise alimentaire et le profil métabolique hématique ont été contrôlés. Il a été constaté que l'ingestion d'une ration contenant 35% de pulpe de caroube ou DCP a donné lieu à des performances comparables en terme d'efficacité alimentaire, poids et rendement carcasse par rapport aux performances d' animaux ingérant une alimentation à base de céréales. Le pattern de consommation journalière d'aliments a été sensiblement affecté par l'inclusion de pulpe de caroube. Le groupe témoin a mangé plus de 40% de l'ensemble de l'apport quotidien durant les 90 premières minutes suivant la distribution d'aliments par rapport à seulement 33% pour le groupe de caroube mais l'apport global d'alimentation était inchangée. Le pourcentage le plus élevé de PAD dans la ration a donné lieu a un taux de prise alimentaire différent par rapport au groupe contrôle .En ce qui concerne le profil métabolique, les animaux alimentés a base de caroube avaient un taux de cholestérol plus bas mais l'urée et le NEFA supérieurs par rapport aux animaux du groupe témoin, par contre aucun de ces paramètres n'as été changé par l'ingestion de PAD. En addition, par rapport au profil protéique du sang, l'addition de caroube a eu des effets mineurs, on a observé une tendance que le rapport

albumine globuline (AG) soit plus élevé, cette tendance a été plus marquée par l'inclusion de PAD par rapport au contrôle, mais sans arriver aux niveaux de stress métabolique.

Dans le système pastoral, nous avons pu recueillir les connaissances écologiques locales (CEL) de bergers ainsi qu'analyser la relation entre la gestion des pâturages en œuvre et le contenu CFP de plantes des pâturages. L'étude a intéressé trois sites du Moyen Atlas Marocain, aux alentours des villes de Boulemane, Guigou et El Ksabi. Trente-cinq agriculteurs ont été rencontrées pour décrire les systèmes agricoles locaux, et onze bergers ont été sélectionnés et ont été interviewé in situ pour recueillir leur LEK sur les espèces végétales, la saisonnalité, les stratégies de pâturage et le comportement d'ingestion des animaux.

En parallèle, sur la base des interviews, des échantillons de plantes ingérées ont été recueillies et la composition chimique en terme de nutriments et PSC (phénols totaux et tanins) ont été déterminés. Il a été constaté, que chaque site pourrait être associé à un type de système de pâturage et que les pâturages ont été classés en fonction de la plante la plus prédominante. Le montant de la connaissance de berger a augmenté avec son expérience et son implication dans la prise de décision. Selon les bergers, seulement quelques plantes ont été considérés comme importantes et ceux-ci ont été classés en fonction de leur rôle dans l'apport quotidien (par exemple fourrage, apéritif, ...). Dans la plupart des plantes analysées le contenu en phénols a été relativement faible allant de 0,7% MS pour *Festuca sp* à 4,0% MS pour *Quercus rotundifolia*. Les bergers ont identifié les plantes qui diffèrent dans l'appétence circonstancielle et ont conçu les circuits de pâturage pour stimuler l'apport de plusieurs plantes. En raison de la préoccupation du gouvernement pour la sur exploitation des parcours et la dépendance croissante sur les aliments concentrées, les systèmes pastoraux risquent de devenir encore moins utilisées et que la connaissance des bergers de ne pas être as transmise aux générations futures.

L'étude a démontré que les plantes qui contiennent des composés secondaires jouent un rôle central soit dans le système pastoral qu'en stabulation, et dans chaque cas des stratégies qui permettent d'augmenter leur prise alimentaire par les animaux existent ; à travers l'amélioration de leur utilisation dans le premier système, et l'augmentation de l'emploi des sous-produits agro-industriels dans le second. Ceci contribuera à réduire l'empreinte environnementale, améliorer la durabilité des systèmes tout en respectant le bien-être animal.

Introduction

Introduction

1.Farming systems will need to adapt, and especially change their feeding practices, in order to meet the issues of feeding the human population while preserving the environment.

It is estimated that the human population, which is around 7 billion today, will reach 8 billion by 2030 (FAO, 2009). The demand for animal products is expected to increase, e.g. meat production would need to increase by over 200 million tonnes to a total of 470 million tonnes in 2050, 72 percent of which would be produced in the developing countries, which is at 58 percent today (Alexandratos *et al.* 2012). Agricultural production and livestock rearing need to change in order to be able to meet the higher demand of animal products and at the same time ensure the sustainability of agro ecosystems (Bruinsma, 2003). Agriculture and farming systems are taking a heavy toll on the natural resources and there is an urgent need to rethink and thereby optimise the use of natural resources (FAO, 2009). In the next decades, farmers are expected to produce more (in terms or amount or quality of animal products), at minimal cost, while reducing the inputs and minimising the impacts on the environment. Coupled with the increasing unpredictability of climatic conditions and the extreme volatility of inputs and outputs prices, farmers are increasingly becoming vulnerable and need to innovate continuously and develop resilient and sustainable mean of production (Delgado *et al.* 1999 ; FAO, 2011).

The nature of the challenges is contrasting whether we are in the developed or developing world, although some issues are common to both. In the former, the concerns are mainly ecological, i.e. reducing the polluting contribution of the agricultural sector especially in terms of methane gas emissions, pesticides residues and soil contaminants. In the developing world, the challenge is more economical, i.e. increase productivity to reduce the amount of imported agricultural products and at the same time implement sustainable production systems

Feeding is the main input in almost all livestock production systems and its importance is illustrated by its use in classifying livestock production system (Herrero *et al.* 2013). Globally, ruminants would consume 3.7 billion tons (of which 48% grass-based forages), which is more than threefold the 1

billion ton consumed by monogastrics (pigs and poultry) (Herrero *et al.* 2013). There is a dichotomy in livestock production depending on the dominant type of feed used which is either grazed herbage (associated with extensive pasture-based systems) or conserved forage and concentrate (associated with intensive systems). These two types of systems exist both in the developed and in the developing world, although the intensive model dominates in "developed" areas.

Interestingly, although the challenges differ depending on the context, a more efficient use of local feed sources which cannot serve for human nutrition (e.g. agro-industrial by-products or rangeland) responds both to the issues of economic and environmental sustainability(Oltjen *et al.* 1996, Tilman *et al.* 2002, Devendra C and Leng RA 2011). In intensive livestock systems, strategies to improve productivity and reduce pollution are likely to focus on efficiency of use of feed resources as this would not only increase unit production per unit feed but also reduce waste and methane gas produced per unit feed and therefore is determining for animal performance and productivity (Devendra *et al.* 2011). Devendra *et al.*(2011) also suggested that strategies should focus on maximising use of available biomass which includes forage resources, crop residues as well as agro-industrial by-products (AIBP) like citrus pulp, spent grains and non-conventional feed which are feeds that are not traditionally used in animal feeding like oil palm leaves, palm press fibre, cassava foliage, spent brewer's grains, sugar cane bagasse (Devendra, 1992). The use of local feed resources bring also the added advantage of reducing the carbon footprint to the system. Concerning pasture-based production system, the rationality is the same, i.e. improving efficiency and therefore strategies would focus on devising means to incorporate as much grazed forage as possible in the diets of ruminants. This implies ingestion of less palatable plant species, which nevertheless may contribute to the nutritional requirements and even provide added advantage such as improved health and better performance by virtue of their chemical composition especially in terms of bioactives (Provenza *et al.* 2009). Optimising the use of rangelands vegetation would enable to improve the sustainability of many pastoral systems (FAO, 2009). However, because of the complexity of the herder-animal-plants relationship and the significant influence of climatic, edaphic and geographical factors, it is important first to elucidate these relationships and only after, based on that knowledge can means be identified and implemented to improve rangelands resources use.

2.In Mediterranean areas, the utilization of alternative feed resources such as rangelands or agro-industrial by-products might be constrained by the presence of plant secondary compounds.

The Mediterranean area is a region of about 700 million people (Rancourt *et al.* 2008) and includes all countries who has at least part of their coast in the Mediterranean sea and traditionally was associated to all those countries where olive trees can be grown. The region is characterised by a relatively dry and hot climate with more than 65% of the rainfall concentrated in the winter months (Bolle Hans-Jürgen, 2003). Schematically, the Mediterranean region may be divided into the northern European part and the southern African part. In terms of livestock production, there is a contrast in terms of species reared, as the north is more cattle oriented whilst the south is more dedicated to small ruminants (Alary *et al.* 2002. This is mainly attributed to the feed resources availability which is limited in the southern part mainly due to the low rainfall (<500mm) resulting in relatively arid areas and thereby the vegetation is more conducive for small ruminant keeping. Farmers in these arid areas follow pastoral-based animal production systems, which are an age-old tradition on both sides of the sea (Rancourt *et al.* 2008). One potent characteristic of such systems is their use of locally available resources, which increases their resilience compared to other systems, which are more dependent on non-local resources and global markets (Neely *et al.* 2009). Another important characteristic of the Mediterranean area is the potentiality that it has in terms of alternative feed resources. The region has a thriving agro-industry, which is among the biggest in the world for a number of produce like olives, and oranges, which provide a considerable quantity and range of agro-industrial by products such as carob pulp, olive cake, citrus pulp (www.feedipedia.com). These by-products may be potentially used by the livestock sector but unfortunately, this potential is far from being tapped fully for a number of reasons (Ben Salem *et al.* 2004). A reason for which they are scarcely incorporated in livestock rations is their chemical composition especially in terms of content of Plant Secondary Compounds (PSCs). The Mediterranean region by virtue of its biodiversity of its rangelands, know-how of its farmers and quantity and variety of alternative feeding resources make that there is high potential for the development of livestock production systems which can be both sustainable and ethical (Meynard *et al.* 2011).

3. Plant secondary compounds (PSCs) can modify animal intake and performance

Plant secondary compounds are a very diverse group of phytochemicals, which comprises substances such as tannins, saponins, alkaloids, essential oils and others. The name derives from the fact that their roles in plant metabolism were unknown for a long period and thereby they were considered as not indispensable for the survival of the plants compared to primary compounds (e.g. proteins and carbohydrates). Among PSCs, tannins have been studied most. Tannins are polyphenolic compounds which have the ability to bind with proteins and polysaccharides (Kumar and Vaithiyanathan, 1990; Leinmuller *et al.* 1991) in the gut of ruminants. Tannins are ubiquitous in nature (McLeod, 1974; Perevolotsky, 1994) and can be found at variable concentrations in the feeds ingested by ruminants in the Mediterranean area (Frutos *et al.* 2002). The importance of tannins in ruminant nutrition especially in the Mediterranean region is derived from the fact that they can have a profound effect on ingestion, productivity, health and product quality (Frutos *et al.* 2004, Durmic *et al.* 2012, Priolo *et al.* 2007).

Intake of very high levels of tannins may cause toxicity (Waghorn, 2008) and that is why tannins have been generally been regarded as a toxin rather than a nutrient. Generally, tannins are considered as toxins with detrimental effect on animal performance and health: reduced feed intake, reduced protein and dry matter digestibilities, lower liveweight gains, decrease in milk production and wool growth (Waghorn 2008). These negative effects are due mainly to the interference by these compounds in the metabolism like e.g. the binding of tannins with proteins reducing their availability. However, besides these anti-nutritional and toxic effects, a number of positive effects on health in particular has been reported (Min *et al* 2003; Crozier *et al.* 2009) like improved milk yield and reduced parasite load (Lisonbee, 2008). The main benefit of tannins in animal nutrition results from the ability of tannins to bind with proteins. Some tannins bind with proteins in the feeds and prevent their degradation in the rumen making them eventually available in the intestine. These proteins are called as by-pass proteins. As a result, these animals will absorb more amino acids present in the diet compared to those fed on iso-nitrogenous; non-tannin containing feeds (Mueller-Harvey, 2006). Ruminants have an inherent capacity to adjust their

behaviour and intake based on the tannins content and factors such as exposure, both when and where, contribute to this ability (Du toit *et al.* 1991; Iason *et al.* 2006). In addition, factors such as the concentration and type of tannins as well as interaction with other chemical compounds may also have a significant effect on intake. Frutos *et al*, 2004 suggest that although there is no general agreement, nevertheless there might be a threshold level of tannins below which the positive effects are more apparent and beyond which detrimental results might occur. Though, these authors explain that due to the very complex nature of the metabolism involved it has been very difficult to devise guidelines on the inclusion of tannin-rich species in ruminant nutrition.

Tannins are increasingly becoming important in ruminant nutrition especially since the last decade (Waghorn, 2008). There are a number of reasons, which can be identified, but two main issues appear to be paramount when dealing with the issue of tannins, each pertaining to sometimes-antagonistic production systems. One is the issue in terms of quality of products of animal origins, i.e. milk and meat. The potential utilisation of tanniniferous plant species in pasture management for self-medication for bloats and parasite control (Lisonbee LD., 2008) and abatement of methane emissions (Longo *et al.* 2007) are mainly relevant to production systems in developed countries. They might offer the opportunity to produce a product of high quality while minimising use of drugs and chemicals and reducing negative impacts on environment. The other issue, especially in the tropics, is the utilisation of alternative feeding resources (not in competition with human nutrition), which should enable to improve the overall agricultural productivity of a given region. In Mediterranean regions, increasing the proportion of rangeland vegetation in the diet can contribute to reducing land encroachment.

In rangelands, degradation implies a reduced availability of traditionally ingested species, resulting in a challenge to shepherds try to feed their animals on the same changed resources that they have at their disposal. The intake of any forage species depends on its availability , physical characteristics and the ability of the herd to graze and digest them (Baumont *et al.*2000). Villalba et al. 2010 reported how PSCs modify ingestion modify animal ingestion behaviour, as the animals modulate the ingested forage profile to maintain ruminal function so as to avoid metabolic disorders form

arising. Such behaviour of the herd would entail a modification of the vegetation profile in the rangelands subsequently change the ecology of the rangelands.

4. In stall: the inclusion of PSCs rich agro-industrial by-products in the diet of ruminants

PSCs comprises such a diverse group of chemicals which interact with ruminant metabolism (Frutos, 2004) in so many ways that it is difficult to pinpoint a mode of action and predict what effects they may have (Muelley-Harvey, 2006). Provenza (2003) inferred that PSCs limit the intake of nutrients more when the animals are dealing with a single feed. Whilst in case of mixed diets, there are interactions among the PSCs which could have a net positive result on intake and performance (Tilman, 1982; Provenza, 2003). Indeed, Makkar (2003) suggested to "formulate a mixture of PSCs with the aim that they will interact among them and thereby nullify each other" as one of the strategies pertaining to tannins rich ingredients in stall fed system. Generally for animals fed in stall, the ration is not a single ingredient but rather a mixture so as to meet the nutritional requirements of the animal. Thus, the PSC-rich feeds need to be studied within the framework of a whole diet, and not as single feeds. For example, tannins are known to bind with proteins (Aharoni *et al.* 1998; Frutos *et al.* 2004), carbohydrates (Schofield *et al.* 2001) and saponins (Freeland *et al.* 1985). Interaction with these diverse constituents of a diet, may strengthen, nullify or complement the effects of tannins (Lisonbee 2009). Therefore various PSCs can be complementary such that their toxicities may be abated through interactions from each other (Lisonbee 2009) thereby resulting in display of their beneficial effects meanwhile avoiding their deleterious effects. This is what is aimed at when including alternative feed resources which are generally rich in PSCs in diets of ruminants.

In the Mediterranean area there are many agro-industrial by-products that could find a valuable use in the livestock production systems by their inclusion in concentrates, in total mixed ration or even in feed blocks (Ben Salem and Nefzaoui 2003; Molina-Alcaide *et al.* 2010). Agro-industrial by-products arising from olive oil, wine, carob, citrus juice and artichoke supply chain are known to contain PSCs. Pertaining olive cake, for example, the recent methods of olive oil extraction produce by-products containing low levels of lignin and remarkable levels of residual oil, antioxidants and bioactive phenols (Servili *et al.* 2011). Artichoke by-products (leaves, external bracts and stems) produced by the artichoke processing industry represent about 80–85% of the total biomass of the plant and could be used as a source of inulin but also of phenolics (Lattanzio *et al.* 2009). Pandino *et al*. (2013) have characterized the qualitative and quantitative polyphenol profile of leaves and floral stem from two Sicilian varieties identifying materials of interest to the pharmaceutical industries. These two examples put into evidence that agro-industrial by-products could represent raw material for the production of food additives and nutraceuticals. The challenge is for the livestock sector therefore is in finding in these new areas

another way for their valorisations.These two examples put into evidence that agro-industrial by-products i) are often rich in secondary compounds and ii) contain PSCs that could represent raw material for the production of food additives and nutraceuticals. The challenge for the livestock sector therefore is in finding in these new areas another way for their valorisations.

5. At pasture, the Local Ecological Knowledge (LEK) of the farmer or shepherd might determine the grazing management and ultimately the diet of the animals

Rangelands are important sources of forages for the rearing of small ruminants particularly in the arid regions (Allen-Diaz *et al.* 1996). They provide cheap forage but also support ecological services (carbon sequestration, biodiversity preservation, water quality – Havstad et al. 2007). In the Mediterranean region, rangelands are often degraded, either by under-utilisation causing shrub encroachment and increased fire risk (southern Europe) (Flamant *et al.* 1999) or by over-utilization resulting in the disappearance of perennial species and the development of low-palatable, invasive species and bare ground, with consequent erosion, soil end fertility losses (northern Africa) (Davis, 2005). In Mediterranean rangelands, many typical browse species and a number of forb species contain considerable amounts of condensed tannins or other PSCs (Frutos *et al.* 2002). The amount of PSC-rich plants might increase with rangeland degradation as the less palatable species especially shrubs (Estell *et al.* 2012) are likely to become more predominant and palatability is a factor that is negatively correlated with PSCs (Muelley-Harvey 2006).

Shepherds by virtue of their experience and observations *in situ* have extensive knowledge about the flora of these natural vegetation and about the behaviour of the grazing animals (Meuret M 1991). Based on such local ecological knowledge, they design and implement daily grazing circuits and a seasonal pattern of rangeland utilization (Perevolotsky *et al.* 1998) . Local ecological knowledge (LEK) is defined as knowledge ‘‘integrally linked with the lives of people, always produced in dynamic interactions among humans and between humans and nature, and constantly changing’’ (Agrawal 1995). LEK is not a shared knowledge but is rather personal. It is the product of each farmer/shepherd competence especially in terms of observation combined with the “events” climatic, cultural, edaphic and geological, which he has witnessed. Moreover, LEK is not static but evolves with time and circumstances (Davis and Wagner 2003; Fazey *et al*.2 006). Since LEK is something quite personal and intimate, it does not have a standardised vocabulary but people use their own “language” to express and describe what they observe and as it is their main asset that

they have in earning their livelihood, it is therefore not something that they would likely readily share or disclose. In addition, the "values" of each person's LEK are not always comparable as the priorities and benchmarks used by each person would be different even within the same community.

Experiences and know-how of shepherds have over time become a part of the subconscious of the shepherd which is reflected in automatisms (Nonaka and Takeuchi, 1995) of shepherds with their animals on the rangelands (Bouche R., 2011). In the Mediterranean region, pastoral systems may be considered as a traditional way of farming and are of relevance with the need to protect environment and diversity (Ligda *et al.* 2011). Although to my knowledge there is almost no known work that has been published in the Mediterranean context on the relation between shepherd's practices and PSCs, nevertheless given that plants in the Mediterranean rangelands contains PSCs and since PSCs affect intake and livestock behaviour and performance, it may be presumed that experienced and skilled shepherds would have noticed such behaviour and would have consider it in their decision making. The shepherds may use specific terms to designate such interaction between the animals and the plants and these interactions are likely to vary over seasons and area as PSCs level and composition vary with species, season, soil type and plants parts, *cum* phenology.

6. The thesis: objectives and hypothesis

The scope of the present research work was to investigate how innovations may be brought in livestock production systems in the Mediterranean area.

The first part of the work aimed at investigating, in a stall-fed situation, how an indigenous by-product containing PSCs (carob pulp or dehydrated citrus pulp) could be incorporated in the diet of lambs. The hypothesis was that these by-products, although known to contain PSCs especially polyphenols, have the potential to substitute cereal to some extent without compromising performance, productivity and the well-being of the animal. The aim was to find out whether the inclusion of unusually high levels (i.e. 24% and 35%) in a diet would be feasible without causing heavy disruption in the system. This first study was carried out in Sicily on a stall-fed lamb growing farm whereby feeding is mainly cereal-based.

The second part of the work investigated, how PSCs ingestion at pasture may be increased by coaxing grazing animals to ingest a variety of plants including those considered as unpalatable or even toxic. However, the hypothesis here was that shepherds are knowledgeable of the behaviour of their animals towards PSCs containing plants in the rangelands and over time, they have accumulated experiences and shared knowledge from their predecessors and peers enabling them to make use of this feature to optimise the use of the natural vegetation of the rangelands. Collecting this knowledge of farmers (which we will refer to as "local ecological knowledge", LEK), will enable to understand whether animals ingest plants containing PSCs and if so how do the shepherds cope with it. Such knowledge was linked to the chemical composition of the plants to correlate shepherds practice with the plant characteristics. This second part of the study was carried out on the rangelands of the Middle atlas in Morocco in the Boulemane region whereby lambs are mainly grazed on the natural vegetation (steppes).

This approach was aimed at being holistic with investigation of PSCs role in small ruminant production under two contrasting system in the Mediterranean area. The materials and methods of these two parts of the thesis, the corresponding results and discussion are presented in the two following chapters, based on three scientific papers either accepted for publication or submitted. A general discussion and conclusion make the last chapter of the thesis.

First Article

Title: Carob pulp inclusion in lamb diet: effect on intake, performance, feeding behaviour and blood metabolites[1]

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Abstract

Carob (*Ceratonia siliqua*) is commonly found in the Mediterranean region and may be used as an alternative feed resource in livestock production. However, carob contains plant secondary compounds, such as polyphenols, which limit its use due to potential toxicity problems. This study aimed to investigate whether the substitution of barley by carob pulp at a relatively high level of up to 35% causes production-level reduction and has detrimental effects on animal welfare. Lamb performance parameters such as feed intake, liveweight and carcass weight were recorded and feeding behaviour was monitored. Blood metabolite and protein profiles were determined to detect signs of metabolic distress. The inclusion of carob pulp resulted in similar level of performance by animals in all the experimental diets. However, the feeding pattern was different with feed intake being significantly lower during the first 90 min post feed supply in the carob-fed lambs compared with the Control animals, although total daily intake was similar. In terms of welfare indicators, the inclusion of carob pulp in the lambs' diet reduced blood cholesterol while increasing both non-esterified fatty acid and urea levels. These indicate that the animals were probably under some form of metabolic stress but not at a level to cause concerns, as confirmed by the similar serum protein profile especially in terms of albumin to globulins ratio.

Additional keywords: carob, feeding behaviour, metabolic welfare, polyphenols, serum metabolites, serum protein profile.

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Livestock feeding systems rely heavily on cereals; locally available agro-industrial by-products can replace such conventional feedstuffs, often imported, reducing the cost of production even in terms of carbon footprint. Carob pulp contains substances that may negatively affect production levels and welfare; this study demonstrated that it can partially substitute barley in lamb diets, at relatively high level of inclusion, without harming the animals. Use of carob pulp could be a sustainable alternative feed resource easily to manage at farmer level.

Introduction

Carob (*Ceratonia siliqua*) is a leguminosae plant representing an important component of the Mediterranean vegetation (Battle and Tous 1997). The carob pod is exploited by the food industry: the carob gum, a common food thickener and stabiliser, is extracted from the seeds; the pulp, the carob pod by-product, is traditionally used as food and feed (www.feedipedia.org, verified 24 November 2014). Carob pulp presents a high sugar content [~44% of the dry matter (DM); Calixto and Cañellas 1982; Marakis *et al.* 1997], a low protein content (3–5% DM; Avallone *et al*. 1997; Marakis *et al.* 1997) and a variable amount of tannins (3–20% DM; Avallone *et al*. 1997; Priolo *et al.* 2000; Silanikove et al. 2006).

Carob pulp and pods can be potentially used as a source of energy for growing lambs and kids but the low protein content and the presence of tannins could limit their extensive use (Guessous et al. 1989; Karabulut *et al*. 2006; Silanikove et al. 2006). Obeidat *et al*. (2012) reported that the inclusion of carob pods up to 25% in lamb diets did not affect nutrient intake and digestibility in Awassi lambs whereas Priolo *et al*. (2000) showed that lambs given a diet containing 56% of carob pulp exhibited lower performance with the halving of the feed efficiency index compared with lambs fed with a conventional diet based on cereals.

Since the 1900s several tools for animal welfare measurement have been developed and discussed (Broom 1991). Metabolic distress is a situation arising from metabolic imbalances in the blood, which can lead to discomfort, impaired feed intake and eventually sickness, and is one of the means for measuring wellbeing in animals (Broom 1991; Ohl and Van der Staay 2012). Blood metabolite profile can assist in appreciation of the animals' welfare status, in particular in relation to their nutritional and health status. For example, it is well known that high blood level of non-esterified fatty acids (NEFA) is a consequence of body fat reserve mobilisation (Hatfield *et al.* 1999) and can be observed in starving animals, although other factors may also lead to a rise in blood NEFA. Also

the blood protein profile, which relates to the proportions of the various fractions of albumin and globulins, provides farmers with a welfare indicator for their animals. Alteration of serum protein profile may occur in the case of liver disorders, acute inflammation and other physiological disorders. Apaydin and Dede (2010) for instance observed this phenomenon in sheep affected by a protozoon disease. Protein profile may also vary with age (Piccione *et al*. 2014) and nutrition; however, the changes due to nutrition are often subtle and difficult to detect and interpret (Kaneko et al. 1997).

Most studies dealing with the use of non-conventional feeds focus on animal performance or product quality (Devendra 1988; Ben Salem *et al*. 2004; Vasta *et al*. 2008) but very few papers study their impact and implications on animal welfare. The feeding of non-conventional feeds rich in plant secondary compounds, such as carob, may cause malnutrition leading to increased vulnerability by animals to disease condition (Mahgoub *et al*. 2008). Tannin consumption can affect animal welfare as does many other plant secondary compounds (Durmic and Blache 2012). Tannins include a wide variety of chemicals, some of which may reduce feed intake (Waghorn 2008), cause astringency (Mueller Harvey 2006) and metabolic discomfort, indicated for instance by high blood urea concentration in sheep urine (Mahgoub *et al*. 2008). However, some tannins could lead to reduction of gastro-intestinal worms thus improving animal performance and welfare (Waghorn 2008).

The aim of this study was to assess the feasibility of partially substituting cereals by carob pulp in concentrate-based diets for growing lambs and to assess whether there are any metabolic disorders arising from intake of these diets. Additionally, the effect of carob-rich diets on the feed ingestion behaviour was also investigated.

Materials and methods

Animal management

Twenty-six Comisana male lambs of 90 days of age (±10 days) were selected. The animals were born on the same farm; during the pre-experimental stage, lambs had free access to their mother's milk (until 60 days) and to a ration composed of faba bean, wheat, barley and lucerne hay. At the age of 60 days all the animals were dewormed by injection of a broad spectrum antiparasitic drug of the avermectin family.

The animals were divided into three homogeneous groups, according to their weight [20.3 kg +/- 4.4 kg (s.e], and randomly assigned to three experimental diets. The Control group (eight animals) was fed a total mixed diet consisting of barley, lucerne hay, wheat bran and soya bean meal that were coarsely ground. Two groups received a mixed diet with the same ingredients as in the Control group but with the addition of different proportions of carob pulp (24% and 35%, on an as-fed basis, respectively for Ca24 and Ca35 groups; nine animals in each group). The diets were formulated in order to supply an equivalent crude protein (CP) allowance. The ingredients and chemical composition of the diets are shown in Table 1.

The animals were placed in individual boxes (1.2 m × 1.8 m) equipped with a feeding trough and a plastic bucket for drinking water. An adaptation period of 10 days was observed during which the pre-experimental diet was gradually replaced by the experimental one. The diets (Control, Ca24 and Ca35) were given on an *ad-libitum* basis from 9 a.m. to 6 p.m. throughout the 56 days of the experimental feeding trial. The daily intake was measured by weighing the refused feed at the end of the day.

The pattern of the individual feed intake was carried out on Days 20, 33, 47 and 53. On these days, the amount of feed left in the feeder was weighed at 1030 hours, 1200 hours, 1500 hours and 1800 hours. On the basis of the total individual dry matter intake (DMI) of the day, the proportions in the four periods of the day (Period 1 = 0900–1030 hours; Period 2 = 1030–1200 hours; Period 3 = 1200–1500 hours; Period 4 = 1500–1800 hours) were calculated and expressed as % of the total DMI.

The animals were weighed regularly on a weekly basis with an electronic weighing scale before feeding and slaughtered after 56 days of experimental feeding trial by captive bolt followed by exsanguination. The lambs were slaughtered in a public slaughterhouse 100 km away from the farm and 24-h lairage time was observed.

The trial was conducted at an experimental farm of the University of Catania (Italy). The experimental protocol used was approved by the University of Catania in which the animals were handled by specialised personnel following the European Union Guidelines (2010/63/EU Directive).

Feed and blood sampling

Fresh feed samples were collected four times during the trial (on Days 9, 30, 44 and 51, respectively), and stored at –30°C; analyses were done on a pooled sample for each diet.

Individual blood samples (10 mL) were collected, in the morning before feed allocation, from the external jugular vein using Vacutainer tubes (Terumo Corporation, Tokyo, Japan) with no additive. Trained professionals were assigned to carry out this operation to minimise stress. The blood samples were collected at Day –10 (i.e. before the commencement of the adaptation period) and Day 55 of the trial. The blood samples were allowed to clot at room temperature (20°C) and centrifuged at 2081*g* for 15 min to separate the serum. The serum samples were neither lipemic nor haemolysed and were dispensed into 2-mL capped centrifuge tubes and stored at –80°C before analyses, performed within 2 months. At the time of analysis, serum samples were thawed at 20°C for 30 min before assessing protein concentrations.

Laboratory analyses

Feed analyses

Feeds were analysed for DM, whereas the AOAC (1995) methods were used for the analyses of CP (method 984.13) and crude fat (CF; method 935.38) extracted with petroleum ether and neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) according to Van Soest *et al.* (1991); with sodium sulfite, without α amylase and expressed with residual ash method. Diet metabolisable energy (ME) was estimated by means of a commercial software (ASSIST.T Alimentazione, version 1.3.1, developed by CRPA spa, Italy, www.CRPA.it, accessed 24 November 2014).

For the analysis of total phenols in the feed, samples were first treated as described by Makkar *et al.* (1993) with minor modifications. Briefly, 200 mg of finely ground feeds was extracted with 5 mL of diethyl ether containing 1% acetic acid to remove pigments and the supernatant was discarded. For extraction of total phenolic compounds, 10 mL of 70% (v/v) acetone was added and samples were subjected to ultrasonic treatment for 30 min in a cold water bath. Samples were then extracted for 2 h using a rotating device and then centrifuged at 2500*g* for 10 min at 4°C. The supernatant was collected for subsequent analyses. The above extraction procedure was repeated and the supernatant collected. The residue from the acetone extraction was subjected to a further extraction using a modification of the method described by Silanikove *et al.* (2006). Briefly, 9 mL of citrate-phosphate buffer containing 0.5 mg/mL of urea (pH 4.7) was added to the residue and

samples were incubated at 90°C for 2 h. A clear supernatant was obtained by centrifugation at 2500*g* for 20 min at 20°C.

In all the above extracts, total phenols were determined using the Folin–Ciocalteau reagent. The concentration of total phenols in feeds was calculated as the sum of the concentration measured in each extract. The assays were calibrated using standard solutions of tannic acid and results were expressed as mg of TA equivalents/g of feed (on a DM basis).

Blood analyses

Two types of analysis were done on the blood serum, namely haematochemical and protein profile. The haematochemical profile included total protein, cholesterol, triglycerides, total bilirubin, iron, NEFA and urea; the protein profile determined albumin and globulins (α1, α2, β and γ globulins).

Total protein was measured by biuret method using an automated analyser (Knoelab20, Dasit, Helsinki, Finland). The protein standard was albumin (0.5 g/mL; Dasit, Milano, Italy). Electrophoresis was performed using a semi-automated AGE system (Helena Laboratories, Helena Biosciences, Gateshead, Tyne and Wear,UK) according to the manufacturer's procedure. For each serum sample 10 mL were applied to numbered sample wells containing agarose gel G previously prepared. Each gel could accommodate up to 24 samples. Films were electrophoresed for 28 min at 450 V. After electrophoresis, films were simultaneously fixed using an automated system (SAS2, Helena Biosciences), stained in blue stain acid solution (Coomassie Blue Brillant R250, Helena Biosciences) for 10 min, and then dried at 37°C. After distaining in acetic acid and drying completely for 15 min films were scanned on a densitometer (EZ-Scan, Helena Biosciences). Using the computer software Phoresis (Helena Biosciences), electrophoretic curves plus related quantitative specific protein concentrations for each sample were displayed. All samples were analysed by the same individual relative protein concentrations within each fraction were determined as the optical absorbance percentage, and absolute concentrations (g/100 mL) were calculated using the total serum protein concentration. The concentration of protein fractions were finally expressed as g/L.

Triglycerides and total cholesterol were assessed by means of a spectrophotometer (SEAC, Florence, Italy). Triglycerides and total cholesterol were determined after enzymatic hydrolysis by means of an enzymatic colourimetric test. Briefly, triglycerides were determined after enzymatic hydrolysis with lipoprotein lipase. The indicator was a coloured phenazone formed from hydrogen peroxide,

4-aminoantipyrine, and 4-chlorophenol under the catalytic influence of peroxidase. Total cholesterol was determined after enzymatic hydrolysis and oxidation. Hydrogen peroxide produced formed a red dyestuff by reacting with 4-aminoantipyrine in the presence of phenol and peroxidase. The colour intensity is directly proportional to the concentration of cholesterol. NEFA were measured enzymatically with a commercially available kit (Randox Laboratories, Crumlin, UK). Samples exhibited parallel displacement to the standard curve; the intra-assay coefficient of variation was less than 8%. Urea, total bilirubin and iron were determined with the use of commercial kits (Centronic GmbH-Am Kleinfeld 11 85456 Wartenburg Germany and finally measured using the UV Spectrophotometer (SEAC, Slim).

Statistical analyses

ANOVA was used to determine the effect of dietary treatment on performance indicators (feed intake, liveweight gain, feed efficiency and carcass yield) and serum haematochemical and protein composition. Regarding intake and feed efficiency data, the individual average value for the whole experimental period has been included in the database for ANOVA analysis. Data were analysed as a completely randomised design, with a model that included the diet as fixed effect. When the ANOVA was significant ($P < 0.05$), means were separated by pairwise comparison by means of the Tukey's method. Individual data of the feed intake pattern (expressed as % of the total DMI during the day) were analysed by including the fixed effects of animal (nested within the diet), diet (Control, Ca24 and Ca35), experimental day (20, 33, 47 and 53) and period of the day (Interval 1 = 0900–1030 hours; Interval 2 = 1030–1200 hours; Interval 3 = 1200–1500 hours; Interval 4 = 1500–1800 hours) and their interactions. The effect of the experimental day was not significant; therefore, factors in the model were reduced to only animal (nested within the diet), diet, period of the day and their interaction. Means were separated by Tukey's test pairwise comparison ($P < 0.05$).

Results

Dietary composition and lamb performance

The experimental diets were similar in terms of CP and NDF contents but differed in the proportion of the fibre fractions (Table 1). Total phenols content, as expected, was higher in the carob diets as compared with the Control due to the incorporation of carob pulp.

DMI was not significantly affected by the dietary treatment (Table 2). CP and NDF ingestion were not significantly affected by the experimental treatment. However, a different nutrient intake has been obtained for fibre fractions: the Ca35 group showed significantly ($P < 0.05$) lower hemicellulose intake compared with Control and Ca24 groups. Lambs in the Control group showed the lowest level of daily cellulose and ADL intake (Table 2). Total phenols consumption was also affected by the experimental diet, as expected, showing the lowest values in Control lambs compared with Ca24 and Ca35 ($P < 0.05$).

Regarding animals' performance, none of the parameters measured during the in vivo phase and at slaughtering were affected by the experimental diet (Table 2). This implies that no detrimental effect on animal performance indicators has been obtained even at the highest value of carob pulp inclusion.

Pattern of feed intake

The distribution of the DMI throughout the day is shown in Fig. 1. This parameter was significantly affected by the period of the day ($P < 0.0005$) and by the diet × period interaction ($P < 0.0005$), while the effect of the diet was not significant ($P > 0.05$).

On average (data not shown), in the three experimental groups, lambs showed the highest percentage (35.6%) of the total daily DMI in the Interval 1, i.e. in the first 90 min after feed supply, and the lowest values (10.7%) in the second interval of observations (1030–1200 hours). In the third and fourth intervals, a significant increase in the proportion of the daily DMI was observed (respectively 22.0% and 31.8% for Intervals 3 and 4). All the average values of the four considered intervals were significantly different ($P < 0.05$).

Figure 1 shows a different DMI pattern in the Control lambs compared with those fed the carob-containing diets. Indeed, the proportion of the ingestion in the first 90 min following feeding was significantly ($P < 0.05$) lower in both Ca24 and Ca35 groups compared with the Control group.

In the other periods of the day the percentage of the total daily DMI was less affected by the experimental diet. Indeed, a different percentage of ingestion was observed exclusively in the interval 1200–1500 hours: Ca35 lambs ingested more DM as compared with Control lambs (24.3% vs 18.2%; $P < 0.05$).

In the last period of feed availability (1500–1800 hours), the Control group showed a significantly lower ($P < 0.05$) proportion of DMI compared with that observed in the first period of the day. On the contrary, in both Ca24 and Ca35 groups the percentage of DMI reached the same level as in the first period ($P > 0.05$). Overall, in our experimental conditions, the high levels of inclusion of carob in the diets offered to both Ca24 and Ca35 groups did not affect total DMI (Table 2) but affected feed ingestion behaviour (i.e. the distribution of the total DMI throughout the day), by lowering the rate of DM consumption in the period immediately following feeding.

Blood analyses

The values observed for the blood serum metabolites in all the experimental groups before the feeding trial (Day –10) and at the end of the trial (Day 55) are shown in Table 3. The consumption of the three experimental diets did not affect serum total protein and bilirubin but there was tendency in serum iron levels ($P = 0.091$), with the Control group showing the highest value. However, urea ($P < 0.001$), cholesterol ($P < 0.0005$), triglycerides ($P < 0.05$) and NEFA ($P = 0.0005$) were all significantly affected by the diets. Urea blood level was significantly higher ($P < 0.05$) in both carob groups compared with the Control, whereas the opposite pattern was observed for cholesterol, which showed significantly lower ($P < 0.05$) values in Ca24- and Ca35-fed animals in comparison with those supplied with the Control diet. Triglycerides values were also affected by the higher level of carob inclusion in the diet, showing Ca35 lambs significantly lower value ($P < 0.05$) compared with Control-fed animals, whereas in Ca24 lambs triglycerides were not different compared with both Control and Ca35 groups. NEFA blood content reached the highest values in Ca35 lambs, which showed significantly ($P < 0.05$) higher value compared with Control and Ca24 lambs.

Blood serum protein fractions are depicted in Table 4. Apart from the blood β and γ globulins, there was an effect of the diet on all the other parameters, though of variable amplitude. There was a marked effect of the diet on α1 and α2 globulins: α1 globulins were significantly lower in the Ca24 lambs, compared with Ca35 and Control ($P < 0.05$) although α2 globulins significantly ($P < 0.05$) differed between Ca24 and Control lambs. There was also a tendency ($P < 0.1$) in the ratio of albumin to globulins (A/G) proteins to rise with the inclusion of carob in the diets compared with the animals in the Control group. All these observations tend to indicate some effect of carob pulp ingestion in modifying the serum protein profile.

Discussion

Intake and performance

Carob pulp usually contains high level of total phenols with some of these being represented by tannins (Avallone *et al*. 1997). Such high level of tannins in the feeds could potentially reduce the nutritive value of feed, especially as tannins bind with the proteins making them unavailable to the animal (Kumar and Singh 1984; Provenza 1995), and negatively affect intake due to the astringency associated with tannins (Bate-Smith 1973). Surprisingly, in our experimental conditions, a positive correlation has been observed between DMI and total phenols intake (R2 = 0.53; P = 0.0005) and there was no effect of the diet on the various in vivo parameters (Table 2), despite the ~1.8–2.0 times higher ingestion of total phenolic compounds in carob groups. It is probable that the level of proteins and energy in the diets were more than sufficient such that the interaction of nutrients-toxins (tannins) was not detrimental in agreement with Freeland and Janzen (1974) and Priolo *et al*. (2000). Even if the diet effect was not significant, it is worthy to note that the carob groups showed a higher daily DM consumption and a lower feed efficiency than the Control group but with very similar carcass weights. This is in line with the findings of Priolo *et al*. (1998) who observed that the inclusion of 20% of carob increased feed intake, did not affect animals' growth and worsened feed efficiency. Similarly, Guessous *et al*. (1989) observed that at the rate of 200 g/kg of inclusion, carob pulp-fed lambs reached a gain comparable to the Control diet. The ability of animals eating carob to maintain similar performance to those not eating carob is an indication of the ability of the animal to cope with anti-nutritional factors in the diet, such as tannins. In this study, it has been observed that even at the level of 35%, carob pulp allowed to maintain a level of growth and carcass weight and yield comparable to animals fed on a conventional diet probably due to the high sugar content in carob, which offsets the detrimental effect of tannins by allowing a sufficient amount of energy.

The effect of carob secondary compounds on lamb feeding behaviour has been studied by measuring the proportion of the diet consumed during the day and by limiting the access to feed during the night, when small ruminants usually show a negligible eating activity (Avondo *et al*. 2013). Similar setup has been implemented by Villalba *et al.* (2006) in lambs fed diets containing tannins. As a consequence of this, as soon as the feed is given in the morning, the animals are stimulated to ingest. Under these conditions, the effect of carob pulp secondary compounds on feeding behaviour is expected to be highlighted. Indeed, in the present study, the inclusion of carob in the diet

appeared to affect the feeding behaviour of the animal. A clear pattern seems to be defined with a lower rate of ingestion immediately after feed administration being evident when carob is included in the diet. Thus, the animals in the Control group ate more than 40% of the whole daily intake in the first 90 min of feeding compared with only 33% in the carob groups. A similar behaviour has been observed in heifers supplemented with tannins extracted from the quebracho tree (Landau *et al*. 2000). It could be inferred that, at high level of inclusion, the astringency in carob pulp overcomes the palatability due to its sweetness, making the feed less appealing and inducing animals to differently modulate the daily feed consumption compared with the Control group. This is further confirmed by the recovery in intake observed in our study that occurred in the later monitoring periods of the day, in agreement with Provenza *et al.* (1992). It is also interesting to note that throughout the study, the overall pattern of feeding was unchanged as the date factor was not significantly different ($P > 0.05$) indicating that the animals were comfortable with that pattern. This finding is quite useful as it is generally regarded that to avoid reducing DMI it is advisable to follow a type of pattern with supply of tannins at specific times or in a particular sequence with other non-tanniferous feeds, mainly depending on the type of feeds used in the diets (Mote *et al*. 2007). In this study, carob-containing diets were supplied steadily and in a mixed diet, in order to avoid feed selection, resulting in no reduction in final intake. This is a valuable observation as it indicates that farmers, when using carob pulp at relatively high level, need not take demanding steps, like alternating two types of feeds, to maintain similar animal performance but should allow the animal sufficient time to consume the ration.

Blood metabolites

The metabolic response of the animals was a major concern in this study. Blood cholesterol level decreases with carob ingestion, in agreement with Silanikove *et al*. (2006) who found low levels of blood cholesterol in kids fed carob-containing diets. To our knowledge, it has been observed for the first time here in sheep. This effect could be related to the presence of tannins in carob, as the hypocholesterolemic effect of condensed tannins has been documented in humans (Chung *et al*. 1998; Bele *et al*. 2010). Few studies reported that in case of infections, blood cholesterol level goes down with amplitude depending on factors like breeds and physiological state (Wellde *et al*. 1989; Adamu *et al*. 2008). Urea concentration in plasma may increase due to overfeeding, resulting in higher level of protein intake and subsequent reflection in the level of urea excretion, or to

underfeeding, when animal mobilises the body reserves and tends to recycle the nitrogen and minimise its excretion in urine (Nozière *et al*. 2000). Caldeira *et al*. (2007) reported that animals with extreme body condition score have higher blood urea level compared with animals with an intermediate score. In this trial, blood urea increased when animals were fed the carob diets, which is contrary to the observations reported by Priolo *et al.* (2000) and Fernández *et al*. (2012) but complies with those reported by Silanikove *et al.* (2006) and Whitney *et al.* (2014). This issue is quite complex. Indeed, a possible relationship between carob tannins ingestion and blood urea level could be accounted for by the tannins effect in ruminal nitrogen metabolism. However, when two carob diets were supplied to kids, one with polyethylene glycol (PEG), which inhibits the effects of tannins in the rumen, and the other without, blood urea level in kids remains higher in both cases, when compared with kids fed the Control diet; unfortunately this issue was not elaborated further (Silanikove *et al.* 2006). In the absence of other parameters (e.g. faecal nitrogen, urinary nitrogen and hepatic function indicators), which are not available in this study, we can only hypothesise that carob could have altered the site of nitrogen metabolism, as suggested by Whitney *et al*. (2014) who studied the effect of redberry juniper, as a source of secondary compounds, in lamb diets.

In this study, the animals fed 35% level of carob had a significantly higher level of blood NEFA compared with the other groups. High levels of NEFA generally indicate a mobilisation of body fat reserves to meet metabolic needs. Ospina *et al*. (2010) and Huzzey *et al*. (2012) showed that animals under metabolic stress and having disease problems have elevated levels of blood NEFA. Lapierre *et al*. (2000) also found that higher blood NEFA corresponded to lower feed intake. NEFA may also increase in cases of physiological stress like inflammation (Bell 1995; Waldron *et al*. 2003; Pethick *et al*. 2005). Interestingly NEFA did not increase in animals fed the Ca24 diet, which may indicate that dietary carob pulp does not affect this metabolic parameter when it is included in the diet at levels up to 24%. In conclusion, in this study carob ingestion impaired both protein and energy metabolic parameters with a greater effect at the highest level of inclusion.

Protein electrophoresis

Serum protein electrophoresis is a tool used in the diagnosis of serum protein disorders (O'Connell *et al.* 2005). Plasma protein levels in terms of albumin and globulins (α1, α2 β and γ) reflect, with a good fidelity, changes associated with the body response to injury; α1 and α2 globulins are

considered as moderate indicators of health status in sheep (Cray *et al*. 2009). Albumin is one of the proteins in the plasma known as acute phase proteins (APP), whose levels change whenever the animal is subjected to external or internal challenges like trauma, inflammation and stress (Murata et al. 2004). Depending on whether the level increases or decreases, the various APP are termed as 'positive' or 'negative. In ruminants, APP are used as biomarkers of disease conditions (Ceciliani *et al.* 2012). For example, albumin is considered as a negative APP as it decreases in cases of health disorders (Ceciliani *et al.* 2012); thereby the application of the measurement of APP is a tool to indicate herd health (Cray *et al.* 2009). In this study it was found that the diet had some effects on the protein fractions with carob pulp-fed animals having higher albumin levels (P = 0.026) and a tendency for higher A/G (P = 0.06). Albumin level is a good indicator of nutritional status (Hoaglund *et al*. 1992; Hoffman *et al*. 2001). High A/G ratio may indicate low protein utilisation efficiency and liver dysfunction; conversely, low A/G ratio is due to some sort of mild infection (Farver 1997; Ndlovu *et al.* 2009) which, in the case of the present study, was not diagnosed. El-Sherif and Assad (2001) reported that ewes in lactation have higher A/G ratio than dry ones due to higher nutritional stress. Our results may indicate that the animals eating carob pulp had some nutritional stress compared with the Control ones; however, it may be inferred that they were able to cope with it as the performances were not affected by the diet.

Conclusions

This study showed that the inclusion of locally available carob pulp was pertinent in substituting barley in the diet of fattening lambs without causing metabolic distress. The measured indicators, some blood metabolites and serum protein profile, showed that carob inclusion did not heavily affect physiological welfare of the animals. This indicates that the animals were able to cope with a diet including up to 35% of carob pulp without hindrance to intake and performance. The animals adopted a different feeding behaviour, modulating the rate of ingestion throughout the day. The specific experimental conditions, supplying of mixed diets in order to avoid feed selection, could represent a feeding strategy that farmers could implement when dealing with feeds containing high level of carob pulp.

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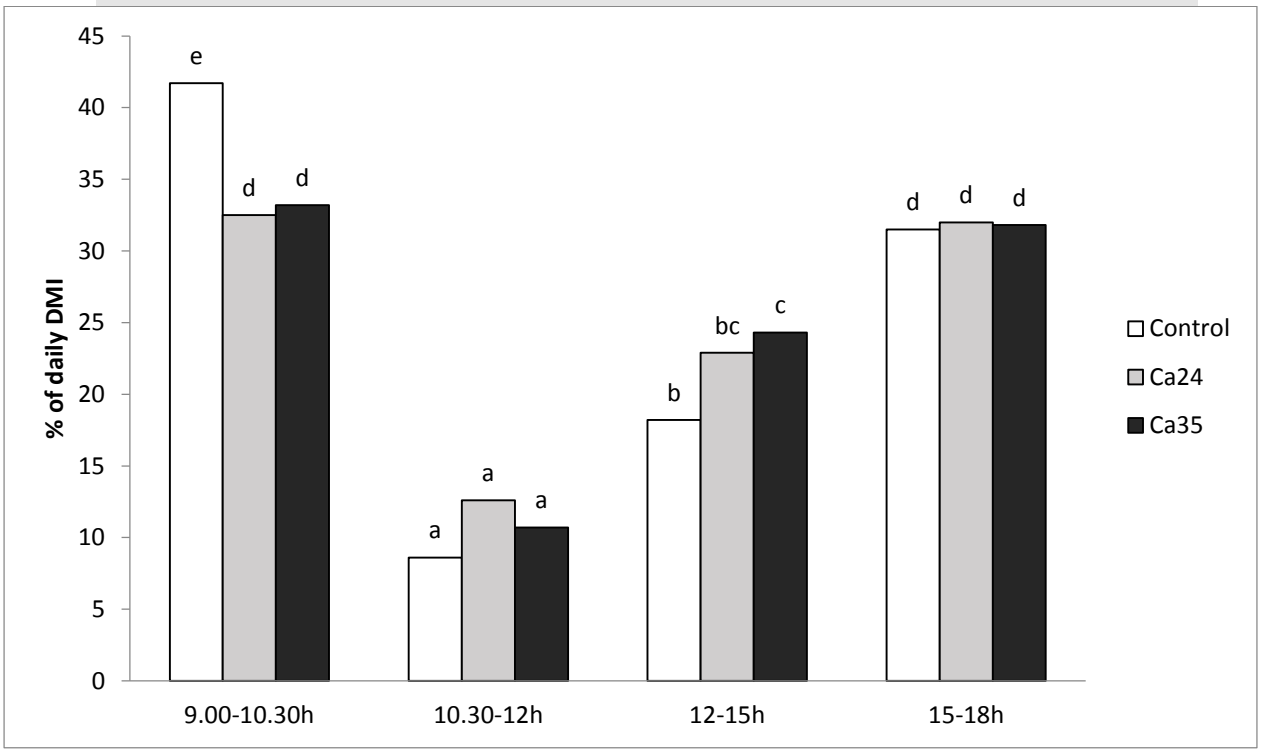
Fig. 1. The pattern of dry matter intake throughout the day in terms of percent of the total daily intake. a, b means with unlike superscripts differ ($P < 0.05$).



[a,b]means with unlike superscripts differ (P<0.05)

Table 1. Ingredients and chemical composition of the diets (Control, Ca24 and Ca35 groups)

Item	Diet		
	Control	Ca24	Ca35
Ingredients, % as fed			
Barley	60	33	23
Carob pulp	0	24	35
Dehydrated lucerne	20	20	17
Soya bean meal	9	13	16
Wheat bran	11	10	9
Chemical composition			
Dry matter, g/100 g fresh weight	88.9	88.2	87.8
Crude protein[A]	18.0	19.6	19.2
Neutral detergent fibre[A]	34.6	34.4	34.6
Acid detergent fibre[A]	13.7	18.0	22.7
Hemicellulose[A]	20.9	15.6	11.9
Cellulose[A]	5.4	7.9	11.3
Acid detergent lignin[A]	8.3	10.9	11.4
Ether extract[A]	2.0	3.3	2.2
Total phenols[B]	8.6	14.2	16.7
Metabolisable energy (MJ/kg)[C]	10.49	10.41	10.48

[A]Expressed as g/100 g of dry matter.

[B]Expressed as mg of tannic acid equivalents/g of dry matter.

[C]Expressed as fresh weight basis.

Table 2. Performance and nutrient intake of lambs fed on Control diet or two carob pulp-based diets (Ca24 and Ca35)

a, b Within a row, means with different letters differ ($P < 0.05$)

	Control	Carob 24 (Ca24)	Carob 35 (Ca35)	s.e.m.	*P*-value
		Performance			
No. of lambs	8	9	9		
Bodyweight Day 0 (kg)	19.3	18.7	18.6	0.73	0.923
Bodyweight Day 57 (kg)	29.6	30.0	28.3	1.08	0.797
Average daily gain (Day 0 to Day 56) (g/day)	181	198	170	8.8	0.418
Dry matter intake (g/day)	749	843	809	31	0.474
Feed efficiency (kg weight gain/kg dry matter intake)	0.24	0.23	0.21	6.86	0.126
Carcass yield (%)	43.8	42.3	43.8	0.32	0.111
Carcass weight (kg)	13.0	12.7	12.4	0.50	0.901
		Nutrient intake (g/day)			
Crude protein	135.1	165.4	155.6	6.2	0.133
Neutral detergent fibre	259.4	290.0	279.8	10.5	0.511
Hemicellulose	156.7b	131.7b	96.1a	6.7	0.0005
Cellulose	40.6a	66.3b	91.7c	4.9	0.0005
Acid detergent lignin	62.2a	92.1b	92.0b	4.2	0.001
Ether extracts	18.4a	22.0a	31.8b	1.3	0.0005
Total phenols (mg/day)[A]	6458a	11967b	13479b	731	0.0005

[A]Expressed as tannic acid equivalents.

Table 3. Blood metabolic indicators before (Day –10) and at the end (Day 55) of the feeding experimental trial

a, b Within a row, means with unlike letters differ ($P < 0.05$)

	Control	Carob 24 (Ca24)	Carob 35 (Ca35)	s.e.m.	*P*-value
Total protein at Day –10 (g/100 mL)	5.89	5.93	6.14	0.09	0.520
Total protein at Day 55 (g/100 mL)	6.61	6.77	6.64	0.08	0.749
Urea at Day –10 (mmol/L)	6.65b	6.09ab	5.14a	0.26	0.043
Urea at 55 days[A] (mmol/L)	7.20a	9.40b	10.30b	0.37	0.001
Cholesterol at Day –10 (mg/100 mL)	44.25	56.56	67.60	4.87	0.156
Cholesterol at Day 55 (mg/100 mL)	46.13b	30.78a	27.56a	2.01	0.0005
Triglycerides at Day –10 (mg/100 mL)	17.63	23.22	25.44	1.41	0.066
Triglycerides at Day 55 (mg/100 mL)	24.88b	20.78ab	18.56a	1.00	0.030
Non-esterified fatty acids at Day –10 (mmol/L)	0.11	0.14	0.12	0.005	0.164
Non-esterified fatty acids at Day 55 (mmol/L)	0.18a	0.19a	0.46b	0.036	0.0005
Total bilirubin at Day –10 (µM/L)	0.28	0.31	0.32	0.03	0.876
Total bilirubin at Day 55 (µM/L)	0.33	0.37	0.47	0.04	0.333
Iron at Day –10 (µg/100 mL)	146.5	171.2	178.8	6.52	0.114
Iron at Day 55 (µg/100 mL)	238.6	181.0	199.2	10.9	0.091

[A]Statistical analysis includes the value at Day –10 as covariate; adjusted means are shown.

Table 4. Blood protein profile before (Day –10) and at the end (Day 55) of the feeding experimental trial

a, b Within a row, means with unlike letters differ ($P < 0.05$)

	Control (C)	Carob 24 (Ca24)	Carob 35 (Ca35)	s.e.m.	*P*-value
Albumin at Day –10 (g/100 mL)	3.36	3.04	3.16	0.07	0.159
Albumin at Day 55 (g/100 mL)	2.79a	3.30b	3.14ab	0.08	0.026
α1 globulins at Day –10 (g/100 mL)	0.24	0.32	0.33	0.18	0.069
α1 globulins at Day 55 (g/100 mL)	0.23ab	0.16a	0.30b	0.02	0.001
α2 globulins at Day –10 (g/100 mL)	0.75	0.78	0.84	0.02	0.266
α2 globulins at Day 55 (g/100 mL)	0.93b	0.81a	085ab	0.02	0.048
β globulins at Day –10 (g/100 mL)	0.40	0.43	0.45	0.02	0.680
β globulins at Day 55 (g/100 mL)	0.50	0.44	0.39	0.02	0.142
γ globulins at Day –10 (g/100 mL)	1.13	1.36	1.36	0.07	0.377
γ globulins at Day 55 (g/100 mL)	2.16	2.06	1.97	0.07	0.617
Ratio albumin/globulin, Day –10	1.38	1.07	1.16	0.07	0.156
Ratio albumin/globulin, Day 55	0.75	0.97	0.91	0.04	0.060

Second Article

TITLE: THE EFFECTS OF SUBSTITUTING BARLEY BY DEHYDRATED CITRUS PULP ON FEED INTAKE, PERFORMANCE, FEEDING BEHAVIOUR AND BLOOD METABOLIC INDICATORS IN LAMBS[1].

Target Journal: **Small Ruminant Research**

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Abstract

In the stall-fed system, we investigated the potential of replacing barley by dehydrated Citrus pulp (DCP) containing polyphenols. The animals were 90 days of age Comisana lambs assessed during fattening. Food intake pattern and blood metabolic profile were monitored. It was found that ingestion of a diet containing up 35% of DCP resulted in comparable performance, feed efficiency and carcass weight and yield as compared to animals ingesting a cereal based diet (control). The daily feed consumption pattern was also not affected by the inclusion of citrus pulp in the diet. . In terms of blood metabolic profile, DCP ingesting animals had similar levels. Pertaining, to the blood protein profile, DCP addition had minor effects, there was a significant incraese in the albumin and

the albumin to globulin (AG) ratio; in the case of DCP inclusion in lambs diet, the serum albumin and the AG ratio were higher being at 1.07 in the animals ingesting 35% DCP compared to only 0.72 in the control; but the values were not a a level to cause metabolic distress.

Key words: Dehydrated Citrus Pulp, feeding behaviour, metabolic welfare, polyphenols, serum metabolites, serum protein profile

Introduction

Ruminants have an intrinsic capability to convert several agro-industrial by-products into valuable product, namely meat and milk, allowing a waste to become a feed resource. Citrus pulp is an agro-industrial by products derived from extraction of juices from fresh fruits. Citrus are widespread in the Mediterranean region which produces a fifth of world citrus production and Sicily is the main producer in Italy, accounting to around 90% of the country total production (USDA-FAS, 2011). In livestock production cost of feeding is in most cases the main expenditure incurred by breeders; with increasing concerns about the high dependence on cereals, and their highly volatile prices, the potential use of this relatively cheap and locally available by products as a cereal substitute is highly attractive. There is some considerable amount of literature on the use of citrus by products in ruminants feeding (Bampidis and Robinson 2006, Arthington *et al.* 2002, Wadhwa & Bakshi 2013). However, due to wide variation in citrus species and varieties, agronomic systems of production as well as processing conditions which affect the by-products nutritional composition (Kale and Adsule 1995; Bampidis and Robinson 2006; Wadhwa and Bakshi 2013) there is no universally recognised formula for its inclusion in animal rations but only guidelines.

Citrus pulp are known to be a good source of dietary fibre (Welch and Smith, 1971) and soluble sugars (Wadhwa and Bakshi 2013)and to be rich in plant secondary compounds such as phenolic compounds (Balasundram *et al.* 2006)and essential oils (e.g. limonene) (Tao *et al* 2009, Robinson, 1999). The inclusion of citrus pulp in ruminant feed formulation may not only substitute to some extent traditional feed resources like cereals but may also confer advantages such as improved animal health status (Calloway *et al*. 2011[a,]) or reduced nematodes eggs hatching suggesting a

tendency to shed fewer eggs to the environment (Nordi *et al*. 2014) or improved meat shelf life (Gravador *et al.* 2014; Inserra *et al.* 2014) or confer disadvantages such as hindered performance and metabolic discomfort (Durmic 2012) . The present study aims at investigating whether the inclusion of locally available dried citrus pulp in a total mixed ration, as a substitute for barley, affects lamb productivity and metabolic welfare as assessed by blood metabolites level and blood protein profile.

Materials and Methods

Twenty-six male lambs of 90 (± 10) days of age of milk breed (Comisana) were selected. The pre-experimental period treatment is described in Inserra *et al.* (2014).The animals were weighed, divided into three homogeneous groups and housed in individual pens. Each group was allocated to an experimental diet (table 1). The control group (Control, n = 8) was fed a total mixed diet mainly consisting of barley and lucerne hay, coarsely ground in order to avoid feed selection. Two groups received a mixed diet with the same ingredients as in Control group with the addition of different proportions of dehydrated citrus pulp (DCP), respectively 24% in Cp24 group (n = 9) and 35% in Cp35 group (n = 9).

The respective diets were given on an *ad-libitum* basis from 9am to 6pm and this system was maintained throughout the duration of the feeding trial, which lasted for 56 days. An adaptation period of 10 days was observed during which the pre-experimental diet was gradually replaced by the experimental ones. Every day, a fixed amount of feed was offered in the morning and replenished regularly throughout the day as and when required such that the animals were, at no time during the day, left without feed. The daily intake was measured by weighing the refused feed which was then discarded. Water was always available.

The animals were weighed regularly on a weekly basis with an electronic weighing scale before feeding. The animals were slaughtered after 56 days of feeding trial by captive bolt followed by exsanguinations. The experimental protocol was approved by the University of Catania; the animals were handled by specialized personnel following the European Union Guidelines (2010/63/EU Directive).

Feed and Blood Sampling

Fresh feed samples (i.e. the mixed diets for Control, Cp24 and Cp35 groups) were collected four times during the trial (on day 9, 30, 44 and 51 respectively) for subsequent laboratory analysis, carried out on a pooled sample. The feeds were stored at -30°C until analyses.

Individual blood samples (10 mL) were collected, prior to feed allocation, from the external jugular vein using Vacutainer tubes (Terumo Corporation, Tokyo, Japan) with no additive. Trained professionals were assigned to carry out this operation to minimise stress. The blood samples were collected on day -10and 55 of the feed trial. The blood samples were allowed to clot at room temperature (20°C) and centrifuged at 2081g for 15 minutes at 4°C to separate the serum. The serum samples obtained were neither lipemic nor haemolysed and were dispensed into plastic tubes and stored at -80°C for up to 2 months prior to analysis. At the time of analysis serum samples were thawed at 20°C for 30 minutes before assessing protein concentrations.

Laboratory analysis

Feed Analysis

Feeds were analysed for dry matter (DM); the AOAC (1995) methods were used for the analyses of crude protein (CP; method 984.13) and crude fat (CF; method 935.38) extracted with petroleum ether. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al.* (1991)with sodium sulphite, without alpha amylase and expressed with residual ash method. The metabolisable energy (ME) was estimated by the use of "ASSIST.T Alimentazione" software , version 1.3.1 developed by CRPA spa, Italy (www.CRPA.it).

For the analysis of total phenols in the feed, samples were first treated as described by Makkar *et al*. (1993) with minor modifications. As described by Makkar *et al.,* 1993, total phenolic compounds were extracted from the feeds using aqueous acetone (70% v/v), analysed by means of the Folin-Ciocalteau reagent and expressed as tannic acid equivalents

Blood analysis

Haemochemical and electrophoresis of blood serum were selected as indicators of metabolic welfare. The parameters measured in the haemochemical part were total protein, albumin, cholesterol, urea, bilirubin, NEFA and triglycerides, and in the electrophoresis part the parameters

were the alpha1, alpha 2, beta and gamma proteins. Total protein was measured by biuret method using an automated analyser (Knoelab20, Dasit, Helsinki, Finland). Electrophoresis was performed using a semi-automated AGE system (Helena Labs, Helena Biosciences, UK) according to the manufacturer procedure.

For each serum sample 10 mL were applied to numbered sample wells containing AG previously prepared. Each gel could accommodate up to 24 samples. Films were electrophoresed for 28 minutes at 450 V. After electrophoresis, films were simultaneously fixed using an automated system (SAS2, Helena Biosciences), stained in blue stain acid solution (Coomassie Blue Brillant R250, Helena Biosciences) for 10 minutes, and then dried at 37°C. After destaining in acetic acid and drying completely for 15 minutes films were scanned on a densitometer (EZ-Scan, Helena Biosciences). Using the computer software Phoresis (Helena Biosciences), electrophoretic curves plus related quantitative specific protein concentrations for each sample were displayed. All samples were analysed by the same individual (I.V.). Relative protein concentrations within each fraction were determined as the optical absorbance percentage, and absolute concentrations (g/dL) were calculated using the total serum protein concentration. Values obtained were multiplied by 10 in order to express protein fraction concentration in g/L (SI).

Triglycerides and total cholesterol were assessed by means of a spectrophotometer (SEAC, Florence, Italy). Triglycerides and total cholesterol were determined after enzymatic hydrolysis by means of an enzymatic colorimetric test. Briefly, triglycerides were determined after enzymatic hydrolysis with lipoprotein lipase. The indicator was a coloured phenazone formed from hydrogen peroxide, 4-aminoantipyrine, and 4-chlorophenol under the catalytic influence of peroxidase. Total cholesterol was determined after enzymatic hydrolysis and oxidation. Hydrogen peroxide produced formed a red dyestuff by reacting with 4-aminoantipyrine in the presence of phenol and peroxidase. The colour intensity is directly proportional to the concentration of cholesterol. NEFA were measured enzymatically with a commercially available kit (Randox Laboratories, Crumlin, UK). Samples exhibited parallel displacement to the standard curve; the intra-assay coefficient of variation was less than 8%.

Urea, total bilirubin and iron were determined with the use of commercial kits (Centronic GmbH-Germany) and finally measured using the UV Spectrophotometer (SEAC, Slim, Florence, Italy).

Statistical Analysis

Analysis of variance (ANOVA) was used to determine the dietary effect on performance indicators, namely intake, liveweight gain, feed efficiency, live and carcass weights and on serum heamatochemical and serum protein composition. Data were analysed as a completely randomised design, with a model that included the diet as treatment effects. When the ANOVA was significant ($P < 0.05$), means were separated by pairwise comparison.

Individual data of the feed intake pattern in terms of DMI during the day were analysed by including the fixed effects of diet (Control, Cp24 and Cp35), experimental day (20, 33, 47 and 53) and period of the day (interval 1 = 09.00-12.00; interval 2 = 12.00-15.00; interval 3 = 15.00-18.00) and their interactions. Animals, nested within the group, were included as random factor. The effect of the experimental day was not significant, so a simplified model considering only diet, period of the day and their interaction was used pairwise comparisons allowed to compare the mean values ($p<0.05$). Data were analysed by using Minitab software (version 16.0).

Results

- Diets composition and lambs performance

The various proportions of ingredients and the chemical composition of the Control, Cp24 and Cp35 diets are displayed in table 1. The effects of the diets on in vivo and post mortem performances, presented in table 2, was not significant for the most part of the parameters. Statistical differences were indeed observed in nutrients intake. The total fibre content of the various diets was very close but, as expected, differed in terms of composition mainly due to the substitution of barley by DCP in the Cp24 and Cp35 diets. As a consequence, the intake of the fibre fractions was significantly affected by the experimental diet. In particular, ADF and ADL fractions intake was highly affected by DCP inclusion ($P= 0.044$ and $P=0.016$ respectively for ADF and ADL) with the Cp35 group ingesting significantly higher amount as compared to Control fed lambs($P<0.05$); on the other side, the highest intake of the hemicellulose has been observed in the control group when compared to both Cp24 and Cp35 groups. According to the different polyphenols content in the three diets (table 1), lambs total phenols intake (table 2) was significantly affected by the diet ($P<0.0005$), showing citrus pulp-fed lambs significantly ($P<0.05$) higher values as compared to control-fed ones.

- Feed intake pattern

The daily distribution of the DMI is shown in figure 1. It was significantly affected by the period of the day ($p<0.0005$) and the interaction diet * period ($p<0.0005$), while the effect of the diet was not significant ($p>0.05$).On the whole, considering all the three experimental groups data, the lambs ingested the higher quantity of feed immediately after its supplying, i.e. in period 1 (9.00 – 12.00 h). They showed a decrease in intake consumption in period 2 (12.00 – 15.00 h) followed by a recovery in the last period of feed availability (15.00 – 18.00). The percentages of the total daily DM intake were equal to 48%, 21% and 31% for period 1, 2 and 3 respectively; all these values were significantly different ($P<0.05$).Fig. 1 put into evidence the different dry matter intake pattern among the experimental groups in the three intervals of the day. The proportion of the ingestion in the first 3 hrs following feed distribution was not statistically different among the three groups; however a lower proportion of DMI in Cp35 group compared to Control group (45 compared to 51%) could be pointed out. In the interval 12.00 – 15.00 h the Cp35 lambs ingested more DM as compared to Control lambs (25% *vs* 18%, $P<0.05$) while Cp24 lambs showed no different dry matter intake percentages as compared to Control and Cp35 groups. In the last period of feed availability (15.00 – 18.00 h) no significant differences in the proportion of DMI among groups have been detected. However, it is worthy to note that Cp35 lambs showed similar percentage of intake in period 2 and 3 (25 and 31%, $P>0.05$), differently to what observed in the other two groups. Overall, in our experimental conditions, citrus pulp inclusion in the experimental diets offered to lambs did not affect total DMI (Table 2) but, when added at the highest level, differently modulated the rate of feed consumption.

- Blood Metabolites

The results on the blood serum metabolites in all the animals before and at the end of the experimental trial are shown in table 3.

There was no effect ($P>0.05$) of the diets on all the indicators measured after 55 days of study. It can be summarised that, in our experimental conditions, the ingestion of citrus pulp did not affect the serum metabolic indicators.

- Blood Protein electrophoresis

The blood protein profile results are showed in table 4. Many indicators were found to be significantly different among the groups at the beginning of the experimental trial (i.e. 10 days before onset of trial) despite the randomisation of animals which came from the same flock and were subjected to same nutrition and husbandry practices. At the end of the experimental trial, for most of these parameters an effect of the experimental diet has been observed. Considering that indicators measured are relatively quickly changed as a response to nutritional and health status and that the covariate statistical procedure has been used, when necessary, the results of statistical analysis are reliable. At the end of the experimental feeding trial, i.e. at day 55, the albumin content and the ratio albumin to globulin (AG ratio) was significantly ($P=0.001$ and $P=0.002$ respectively) affected by the diet supplied to lambs. Indeed, for both the parameters, the Cp35-fed lambs showed significantly higher values compared to Control fed lambs ($P<0.05$) while Cp24 lambs maintained average values that were not different from the other two groups. Among the globulins fractions, a significant ($P=0.046$) decreasing trend due to citrus pulp inclusion in the diet has been observed for α2 globulins, which were significantly lower in the case of Cp35-fed lambs compared to the control ones.

Discussion

- Intake and animal performance

In this study substitution of barley by DCP, to up to 35%, did not affect intake and all performance indicators providing an equivalent output as exhibited by the similar carcass weight and yield. Literature shows similar results for calves (Ahooei *et al.* 2011) and sheep (Gilaverte *et al.* 2011; Morales *et al.* 2010; Sartori *et al.* 2004); whilst an improvement has been observed in kids (Bueno *et al.* 2002) and in dairy cows (Miron *et al.* 2002).

Our results tends to indicate that, in our experimental conditions, the organic matter derived from DCP was equivalently degraded as compared to barley. Despite the different ingestion in terms of fibre fractions, in particular the much higher ADL ingested, and polyphenols there were no detrimental effects on intake and even on feed conversion efficiency in DCP ingesting animals. Thus it can be inferred that inclusion of DCP in this study did not cause nutritional inadequacies. These observations tend to agree with findings by Ben Ghedalia *et al.* (1989) and Madrid *et al.* (1997) who attributed high digestibility to cell walls from citrus by products which, to some extent, justify the

similar carcass weight in the DCP ingesting animals when compared to those eating the control diet. Nevertheless, the advantage that inclusion of DCP confers in terms of improved feed digestibility due to improved rumen microflora activity (Ben Ghedalia *et al.* 1989; Moss, 1994) was limited in the present trial as the latter is much more evident in a straw based diet, which was not the case of the present trial.

This study seems to confirm that the restriction of feeding to only daytime (from 9.00 to 18.00 hr) does not adversely affect the animals in terms of meeting their nutritional requirements, as already observed in a similar research focusing on carob pulp inclusion in lambs diet (Gobindram *et al.* 2014). According to (Gill, 2011), this could be explained by the fact that sheep are diurnal animals predominantly eating during daytime. Another interesting aspect was that intake was not abated despite negative palatability issues (Battacharya *et al.* 1973) usually associated with DCP. However, it could be hypothesised that the differences observed in the pattern of ingestion among the three groups all along the day could be due to a negative effect of the highest level of citrus pulp inclusion on diet palatability. On the other hand, the shift produced in the feed consumption in the Cp35 group made DM intake a little bit more constant during the day.

DCP has the potential of having toxic effects in ruminants (Saunders *et al.* 2000; Rihani 1991) but, by virtue of the highly variable amount and types of substances which may cause such toxicity, it is very difficult to pinpoint the causing agent. Polyphenols is one of the group of secondary compounds that are present in citrus by-products (Balasundram *et al.* 2006). In our study we focused on determination of polyphenols content to try to surmise any observable differences between the various diets; as expected, the polyphenols ingestion was strongly affected by the substitution of barley with dried citrus pulp. Frutos *et al.* (2004) suggested that there is a threshold level at which polyphenols adverse levels overcome positive effects and thence result in toxic symptoms and that threshold is variable depending on a range of factors due partly to the complexity of the polyphenols that can be found in feeds. It can be hypothesized that the nature and the amount of ingested polyphenols, even by Cp35-fed lambs, maybe not at a level for toxic symptoms to be observed, as reflected in the assessed parameters, or else that its effects have been countered by other substances present in the diet. Moreover, a possible effect of essential oils could be excluded. Indeed, the limonene content of the DCP used in the present study was found to only around 48

mg/kg DM (Diani 2014, unpublished) which is at least 100000 times less than what could be expected normally in citrus pulp (Amparo *et al.* 2002).

- Blood Metabolic profile

Blood metabolic profile can be considered as one of the means that can be used to detect welfare discrepancies (Broom, 1991; Ohl *et al.* 2012). In this study, it was observed that in all of the measured metabolic parameters there was no significant difference between the diets. This is a promising result as it showed that ingestion of DCP up to 35% level did not cause any significant metabolic discomfort when compared to a traditional finishing diet based on barley, despite the higher levels of ingestion of polyphenols in the DCP diets. To our knowledge, this is the first paper in which the effect of high DCP level in the diet on ruminant metabolic parameters has been evaluated. Indeed, similar results have been observed in calves (Ahooei *et al.* 2011) and in dairy cows (Belibasakis and Tsirgogianni 1996) but, in those studies, DCP percentages in the experimental diets were 12% and 20% respectively. Citrus pulp can contain also other secondary compounds, such as saponins and steroids (Mathur 2011) that could have interfere with the polyphenols limiting their effect in agreement with Provenza *et al.* (2003) who reported on the antagonistic, synergistic and complementary relationship that exist between various PSCs and nutrients. It could have been expected that DCP diets would have provided a more energy than the Control diet as DCP is often considered as an energy supplement in ruminant ration (Kim *et al.*, 2007); however, this was not the case in the present study (Table 2).The composition of DCP may indeed vary a lot mainly depending on the processing conditions thereby leading to variable composition of nutrients and secondary compounds both qualitatively and in quantity (Kale and Adsule 1995). In conclusion, it can be inferred that in our experimental conditions the higher polyphenols intake and the different fibre fractions ingestion and the potential presence of other PSCs due to citrus pulp addition to the diet did not affect protein and carbohydrates utilization by the experimental lambs.

- Blood Protein electrophoresis

Protein is the most abundant component in blood plasma and its levels and its profile can assist in the diagnosis of immune system disruption (Eckersall 2008). In particular, the levels of a group of serum proteins called as APP (Acute Phase proteins) are correlated with infection, trauma and inflammation in animals (Eckersall 2008). These APPs are termed as positive or negative APPs

depending whether they increase or decrease in case of distress (Ceciliani *et al.* 2012). For example, albumin decreases with inflammation and is therefore a negative APP (Ceciliani *et al.* 2012). It has even been suggested (Anderson *et al.* 2002) that almost all diseases affect blood proteins and they suggested the use of proteomics to monitor changes in blood proteins as a mean to diagnose disease conditions. In this study, the experimental diets caused an increase in the albumin content and in the albumin to globulins (AG) ratio in DCP groups, probably linked to the higher polyphenol ingestion. Indeed, the regression analysis between total phenols intake and these two blood protein indicators gave interesting results (R^2=33.7%, P=0.002 and R^2=26.1%, P=0.007, respectively for albumin and AG ratio). Several authors have reported that AG ratio can be used as an indicator of metabolic disorders (Alberghina *et al.* 2010, Waziri *et al.* 2010) but seasonal variations have also been observed in sheep and goats (Piccione *et al.* 2011). Usually high AG ratio may be linked to impaired protein utilisation efficiency and liver dysfunction, while low AG ratio may indicate some sort of mild infection (Farver 1997, Ndlovu *et al.* 2009). The values of the AG ratio recorded in this study were quite far from those reported in Valle de Belice sheep, ranging from 1.2 to 1.7 (Piccione *et al.* 2011). However, the breed, age and sex effect may provide justification, to some extent, for such variation. In the absence of any other published reference values, it is quite pertinent to consider the values of a group of healthy animals, i.e. the control group in our case, as benchmark rather than confronting against published reference values obtained in different experimental conditions. Considering that no symptoms of any pathology in the animals during the course of the trial have been observed, such significantly different level of albumin and AG ratio can be attributable to the inclusion of DCP which was the only variable factor among the three groups of animals. However, the reason why this occurs is relatively obscure as there is little knowledge on the composition of the diets in terms of secondary compounds, other than total polyphenols. DCP are known to have antioxidant properties (Middleton and Kandaswami 1994; Fattouch *et al.* 2007) and have the inherent capacity to reduce pathological bacteria like E.coli O157:H7 (Calloway *et al.* 2011[b]) and salmonella (Calloway *et al.* 2011[c]). It may be inferred here that somehow the inclusion of DCP affected the protein profile but not to a level to shown any apparent signs of distress.

Conclusions

This study showed that dehydrated citrus pulp may be confidently used up to 35%, on as fed basis, as a substitute for barley in diets fed to fattening lambs. This can potentially lead to a significant reduction in feeding cost and provide a value addition to a local by product whilst avoiding any metabolic distress to the animals. Moreover, complementary studies have demonstrated an improved meat shelf life in lambs fed citrus pulp diets. The highly variable nature of citrus pulp requires detailed analyses on nutrient composition and, above all, on secondary compounds in order to fully understand the metabolic reasons of the experimental results. Further studies could be advisable on this issue.

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Table 1: Ingredients and chemical composition of the diets (Control, Cp24 and Cp35 groups)

Item	Diet		
	Control	Cp24	Cp35
Ingredients, % as fed			
Barley	60	35	23
Citrus pulp	0	24	35
Dehydrated lucerne	20	19	20
Soya bean meal	9	12	13
Wheat bran	11	10	9
DM, g/100 g freshweight	88.9	89.4	90.6
CP[1]	18.0	18.5	17.8
NDF[1]	34.6	31.8	33.1
ADF[1]	13.7	16.0	18.0
Hemicellulose[1]	20.9	15.8	15.1
Cellulose[1]	5.4	5.9	6.5
ADL[1]	8.3	10.0	11.5
Ether extract[1]	2.2	1.6	2.2
Total phenols[2]	4.0	6.7	7.9
Metabolisable Energy (MJ/kg)[3]	10.49	10.52	10.51

[1] expressed as g/100g of DM

[2] expressed as mg of tannic acid (TA) equivalents / g of DM

[3] expressed as freshweight basis

Table 2 - Performance and intake of lambs fed on Control (C) diet or two citrus pulp based diet (Cp24 and Cp35).

	Control (C)	Citrus24 (Cp24)	Citrus 35 (Cp35)	SEM	P value
Performance					
No. of Lambs	8	9	9		
BW day 0, kg	19.3	19.2	18.2	0.653	0.763
BW day 57,kg	29.6	29.6	28.5	1.04	0.876
ADG (day 0 to day 57), g/day	181	181.3	179.2	8.37	0.994
DMI (g/d)	749.2	767.0	756.4	30.2	0.886
Feed efficiency (kg wt gain/kg DMI)	0.24	0.25	0.25	0.011	0.000
Carcass yield (%)	43.8	44.0	43.7	0.416	0.953
Carcass weight, kg	13.0	13.1	12.49	0.530	0.882
Nutrient Intake					
Crudeprotein[1]	135.1	141.8	134.2	5.29	0.824
NDF[1]	259.4	243.6	250.3	9.71	0.824
ADF[1]	102.8[a]	122.5[ab]	136.4[b]	5.58	0.044
Hemicellulose[1]	156.6[b]	121.1[a]	113.9[a]	5.97	0.005
Cellulose[1]	40.6	45.5	49.2	1.93	0.199
ADL[1]	62.2[a]	77.01[ab]	87.21[b]	3.69	0.016
Ether Extracts[1]	16.7[b]	11.96[a]	17.04[b]	0.761	0.005
Total Phenols[2]	2996[a]	5156[b]	5950 [b]	315	0.0005

[a,b] Within a row, means with unlike superscripts differ (P<0.05)

[1] expressed as g/d

[2] expressed as mg tannic acid equivalents/d

Table 3: Levels of blood metabolic indicators before the feeding experimental trial (day -10) and at the end of the study (day 55)

	Control (C)	Citrus 24 (Cp24)	Citrus 35 (Cp35)	SEM	P value
Tot. protein at d -10 (g/100ml)	5.89[b]	4.33[a]	6.48[b]	0.224	0.0005
Tot. protein at d 55 (g/100ml)*	6.64	6.95	6.87	0.0976	0.482
Urea at d -10 (mmol/l)	6.65	5.24	5.73	0.275	0.120
Urea at 55 d (mmol/l)	7.12	8.28	7.96	0.247	0.159
Cholesterol at d -10 (mg/100ml)	44.25	40.22	53.80	2.81	0.110
Cholesterol at d 55 (mg/100ml)	46.13	42.56	43.00	1.73	0.691
Triglycerides at d -10 (mg/100ml)	17.63[a]	15.78[a]	24.30[b]	1.15	0.002
Triglycerides at d 55(mg/100 ml)*	25.83	22.24	20.62	1.17	0.209
NEFA at d -10 (mmol/l)	0.11	0.14	0.14	0.0089	0.422
NEFA at d 55 (mmol/l)	0.18	0.32	0.23	0.0334	0.247
Total Bilirubin at d-10 (µM/l)	0.28	0.22	0.35	0.033	0.246
Total Bilirubin at d 55 (µM/l)	0.33	0.33	0.45	0.039	0.347
Iron at d -10 (µg/100ml)	146.5[a]	165.7[ab]	172.8[b]	4.27	0.031
Iron at d 55 (µg/100ml)*	232.0	188.6	225.0	10.7	0.222

[a,b] Within a row, means with unlike superscripts differ (P<0.05).

*Statistical analysis includes the value at d-10 as covariate; adjusted means are showed.

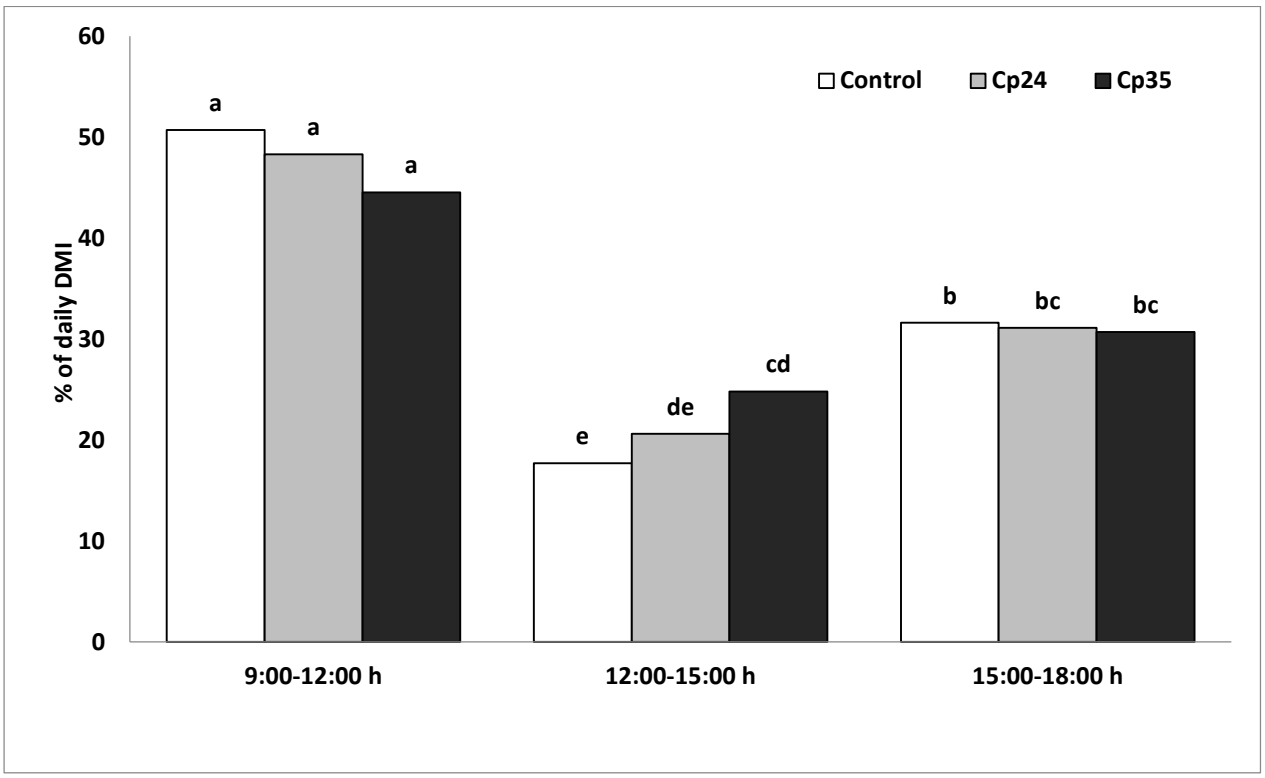
Table 4: Blood protein profile of animals in the study, before the feeding experimental trial (d -10) and at the end of the study (d 55).

	Control (C)	Citrus 24 (Cp24)	Citrus 35 (Cp35)	SEM	P value
Albumin at d-10 (g/100ml)	3.36[b]	2.21[a]	3.06[b]	0.115	0.0005
Albumin at d 55 (g/100ml)[*]	2.76[a]	3.23[ab]	3.43[b]	0.0791	0.002
α1 globulins at d-10 (g/100ml)	0.24[a]	0.26[a]	0.46[b]	0.0329	0.003
α1 globulins at d 55(g/100ml)[*]	0.23[a]	0.33[b]	0.19[a]	0.0178	0.003
α2 globulins at d-10 (g/100ml)	0.75	0.67	0.81	0.0468	0.488
α2 globulins at d 55 (g/100ml)	0.93[b]	0.89[ab]	0.83[a]	0.0185	0.046
β globulins at d-10 (g/100ml)	0.40[a]	0.34[a]	0.83[b]	0.0572	0.0005
β globulins at d 55 (g/100ml)[*]	0.51	0.51	0.40	0.0236	0.391
γ globulins at d-10 (g/100ml)	1.13[ab]	0.86[a]	1.32[b]	0.0622	0.003
γ globulins at d 55 (g/100ml)[*]	2.15	2.24	1.84	0.105	0.417
Ratio Albumin/Globulin, d-10	1.38[b]	1.07[a]	0.94[a]	0.0567	0.003
Ratio Albumin/Globulin, d 55[*]	0.72[a]	0.86[ab]	1.07[b]	0.0454	0.025

[a,b] Within a row, means with unlike superscripts differ (P<0.05).

[*]Statistical analysis includes the value at d-10 as covariate; adjusted means are showed.

Figure 1: The pattern of DMI throughout the day in terms of % of the total daily intake.



[a,b] means with unlike superscripts differ (P<0.05)

Third Article

Title: Shepherd's local ecological knowledge: Characterisation, strategic importance and perspectives in a traditionally pastoral area of Morocco.[1][2]

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Abstract

Local ecological knowledge (LEK) of shepherds and analysed the possible relationship between the grazing management implemented and the PSCs content of rangeland plants. Three sites were investigated in the Middle Atlas of Morocco, around the towns of Boulemane, Guigou and El Ksabi. Thirty-five farmers were encountered to describe local farming systems, and eleven shepherds were selected, which were subsequently interviewed *in situ* to collect their LEK about plant species, seasonality, grazing strategies and animal ingestion behaviour.In parallel, based on the interviews, samples of plants ingested were collected and the chemical composition in terms of nutrients and PSCs i.e. total phenols and tannins. It was found that each site could be associated to a type of grazing system and that the rangelands were classified based on the most predominant plant. The amount of LEK of the shepherds increased with experience and involvement in decision making. Only a handful of plants were considered as important in the opinion of shepherds and plants were classified as a function of their role in the daily intake (e.g. fodder, apetizer, …). Polyphenols were relatively low in most of the plants assayed ranging from 0.7 %DM in *Festuca* sp. to 4.0%DM in *Quercus rotundifolia*. Shepherds identified the plants which differed in circumstantial palatability and designed the grazing circuits to stimulate the intake of a diversity of plants. Due to the government concern about rangeland over-utilization and the increasing reliance on conserved feed, there is a risk for the pastoral systems to become less dependant on rangelands resources and for the LEK of shepherds to be lost over generations

[1] Preliminary findings were presented on 24th June 2004 at Clermon Ferrand in the FAO-CIHEAM Joint Meeting and published in proceedings.

Key words: Local Ecological Knowledge, Plant Secondary compounds, Rangelands, Shepherds, Polyphenols, Tannins, Morocco

Introduction

In the Mediterranean region, rangelands are widely used as a source of grazed fodder, especially in farming systems producing meat (Jouven *et al*. 2010). Small ruminants are the species most herded. There are varying degree of sheep and goat keeping depending on the region, tradition and resources (Iniguez 2011) but there are indication of increasing importance of goats and sheep substituting cattle, e.g. in Kenya (Degen 2007); Solomon et al. (1991) reported that goats and sheep are more popular in drought prone regions as they have a better recovery rate. Cattle keeping requires more forages and reliable water supply whilst sheep and goats are less demanding (Degen 2007). Moreover, for many populations of pastoralists, small ruminants are their sole source of income and their small format makes them easier to sell; they can also be easily slaughtered to feed a family (Squires 2009; Pfister *et al*. 1983).

North Africa has extensive rangelands (about 130 million hectares: Ben M'hamed 1990) which were traditionally exploited for small ruminant rearing in pastoral systems (Sihdamed *et al*. 1996). Small ruminant production in arid areas is mainly for meat production (Iniguez 2011). Over the past decades, tremendous changes have occurred in such pastoral systems due to social, climatic and economic factors: subsidies for the purchase of feeds, diversification of farming activities and sources of income, new generations working in urban areas (Bourbouze 2000). Such changes have resulted in increased reliance of farming systems on purchased inputs especially feeds and drugs and to a decrease in the relative importance given to the livestock keeping activity. This is true for a majority of the drylands of Africa, as previously reported by Sidahmed (1992) who observed a shift from extensive livestock production in the Near East and North African region to systems which are heavily dependent on imported and subsidized feed grain. Taking the example of Morocco: in January 2013, the custom duties have been removed on animal feeds and concentrates leading to a decrease of 13% in the price (Source: Ministry of agriculture of Morocco, http://www.agriculture.gov.ma/). Coupled with the various incentives given to pure bred animals

by the state, accessibility to these production inputs has increased (source: ANOC, http://www.anoc.ma/).

In Northern Africa, the changes observed in pastoral systems since three decades have changed the herding practices and affected the rangeland. Chiche (2004) stated that there has been a shift in the paradigm of pastoral farmers from the importance of equilibrating the rangelands resources to that of increased profitability with the consequence of increased degradation of pastoral resources. Resorting to purchased feed has enabled to artificially increase livestock density far beyond rangeland's carrying capacity. In 1991, Lynn already argued that there were three heads of cattle on rangelands of Tunisia, Algeria and Morocco where only one could thrive. Overgrazing coupled with increased temperature and rarefication of rainfall in the last decade have led to the reduction of palatable plant species and to the proliferation of less desired species if not mere desertification (Teague *et al.,* 2013). In many instances, the proliferation of less palatable species may be correlated to an increased concentration of Plant Secondary Compounds (PSCs) in the sward (Provenza 1996). Tannins are a group of PSCs which has been widely studied. Tannins are known to have a significant influence on animal feeding behaviour (Muelley-Harvey 2006). Thus, in areas where rangelands are degraded by over-utilization, one would expect to find more PSC-rich plant species, altered feeding behaviour of the grazing flocks and/or specific herding techniques aimed at encouraging the animals to consume such species. Such herding techniques are usually based on a deep knowledge about the ecology of plants, animals and plant-animal interactions, which the shepherd acquires with practice and local observations

The objective of the present study was to collect the local ecological knowledge (LEK) of shepherds in a pastoral area of Morocco, and to analyse the strategies implemented to feed the flock on less palatable plants (possibly containing high amounts of PSCs) which have become abundant in over-grazed areas. Based on the studies carried out in France by Michel Meuret (Meuret *et al.*, 2014; Meuret *et al.,* 1997), we hypothesised that: 1) shepherds characterize feeding units (rangeland vegetation, plant parts) in relation to animal feeding behaviour; 2) shepherds have such LEK that enables them to implement management practices aimed at stimulating the consumption of less palatable species, e.g. organizing the timing (within a grazing circuit and the whole year) of the utilization of the diversity of forage resources.

The study was conducted in the province of Boulemane, Morocco where there is a significant herding community. Thirty-five farmers were interviewed about their production system, whereby eleven shepherds were selected for a comprehensive interview about their herding practices and LEK of rangelands and animal behaviour. The study area was split into three zones namely: Boulemane, El Ksabi and Guigou according to the type of vegetation and the characteristics of the feeding system of the flock.

Materials and Methods

1. The area of Study

Morocco has more than 33 million hectares of rangelands (Mahyou H. 2012) and the region of Middle Atlas in Morocco is considered as one of the four sheep producing regions of Morocco (Boulanouar *et al.* 1995) accounting for about 17% of the country sheep population. The province of Boulemane was selected because it has a significant pastoral-based livestock sector (particularly sheep) and it is well known by the local extension services (ANOC, i.e. Association Nationale des Eleveurs Ovins et Caprins). Together with the province of Ifrane, it is the only place in Morocco where it snows in winter. The province of Boulemane has substantial livestock rearing activity and is one of the poorest regions of the Kingdom of Morocco (Haut commissariat du plan 2013., URL: www.hcp.ma). ANOC is the only non-governmental organization that directly works with sheep farmers and is locally well integrated, thus it was chosen as the contact point in the region. According to ANOC the activity of farming in the area has changed over time with an increasingly number of farmers resorting to supplementation with concentrate due to impoverishment of rangelands in terms of biomass and increasing frequency of droughts.

Within the province of Boulemane, we investigated three small agricultural regions, around the cities of Boulemane, El Ksabi and Guigou. Boulemane is at an altitude of around 1800m and is the regional capital of the province. Local rangelands in Boulemane are dominated by either Stipa sp or sagebrush, and appear more or less degraded, probably both by overgrazing and drought. The degradation is visible in terms of reduced biomass growth and increased “patchiness” but authors are not conclusive on that (Davis 2005). Due to a lack of reliable assessment of the extent of the degradation process, most projects about land degradation are based on expert opinions (Mahyou

2012). El Ksabi also known as “Ksabi Moulouya”(alt. around 1600m) is a relatively remote area halfway between the city of Missour and Midelt with many similarities common to an “Oasis” type of farming , using relatively arid halfa-dominated rangelands. In such system, water is a key component and it has several types of farming, namely orchards, cereals fields and livestock which are all integrated into each other (Dollé V. 1990) and rangelands called as “agdals” are key in livestock rearing. Guigou for its part, supports “Bour” type of farming with large cultivated areas (cereals and onion cultures, mainly) and is surrounded by low hills covered by cedar and pine forests. The “Bour” is an Arabic word used to depict systems of cereal cultures without irrigation, which are very common in the Atlantic part of Morocco (Bechchari *et al.* 1995). In Boulemane sheep keeping is in most cases the sole economic activity of the farmer, whilst in El Ksabi and Guigou, farmers are also involved in olive and cereals cultivation respectively (Pers. Comm. Office of the Caid of El Ksabi Moulouya) and so sheep keeping can be considered as an annexed activity. The three areas of the study were only delimitated after following field observations with focus on types of rangelands and foremost the availability of experienced shepherds who are willing to accept our intrusion. These three localities were contrasting with each other as in a radius of less than 50km three rather distinct types of management were observed. In all the three sites, the rangelands were relatively degraded with patches of bare soil and “dwarfed” plants, to the extent that it was often difficult to distinguish between the species even for a trained observer. Moreover, parched land was not uncommon and watercourses were rare.

2. Choice of pastoral systems and shepherds

Two rounds of interviews were carried out. In the first one, the aim was to identify the various types of pastoral systems in the area and to select the shepherds that would be interviewed in the second round. The choice of farmers and shepherds interviewed was governed by two main criteria namely the use of rangelands as being an integral part of the rearing activity and the propensity to communicate and share their views and opinions.

Three types of stakeholders were identified during these interviews: farmers, farmer-shepherds and shepherds. Farmers are the owners of the flock and hire shepherds for conducting the animals on the rangeland. A shepherd is only responsible for the feeding of the animal on the rangelands and

is not generally involved in the decision making. A farmer-shepherd is responsible for the whole management of the flock, and makes all the decisions.

In the first round of interviews, thirty-five farmers were encountered. A set of relatively short and technical questions were asked, involving a discussion about the technical aspects of the rearing activity (breeding calendar, flock size, history of activity, breed and rangelands utilisation). The aim was to establish a trustful relationship with the stakeholders in the area by explaining in unambiguous terms the goals of the study and secondly describe and identify the various types of rearing activity in the region. The local customs were followed scrupulously so as to get "accepted" by the farming community and these involved encountering the village chief and "caïd" first. These interviews were carried out individually or in groups, in most cases during a gathering (lunch, meeting) at the farmers' home and in the presence of a local representative (member of farmers association or village council). A PhD student (A. Boughalmi) from the Institut Agronomique et Vétérinaire Hassan II (IAV) was involved as an interpreter. From this first encounter, shepherds for the collection of LEK were selected.

3. Shepherd's interviews

Eleven shepherds were identified from the first round of interviews and encountered, with the aim of collecting local ecological knowledge (LEK) and feeding management practices. The survey was carried out by in situ encounters while accompanying the flock on the rangelands and conversation with the shepherd was based on a series of "question sheets" each one covering a specific topic to ensure that the relevant information was collected. The conversations were digitally recorded to ensure an accurate transcription of shepherd's sayings. The interview method used is described by Kauffman (1996) and was adapted for pastoral studies by Meuret *et al.* (1997). Each "question sheet" comprised a central question: sheet 1- shepherd's experience, 2-use of rangelands, 3- grazing behaviour and 4-"forage" species present on the rangelands. Each "question sheet" also contained a series of potential "booster" questions whose aim was to provide the interviewer with alternative requests so as to minimise ambiguities and ascertain that the interviewer and the interviewee understood clearly each other. The use of such booster questions was circumstantial, and the questions themselves were adapted to avoid antagonism, clichés responses and foremost to ensure the reliability of the information collected. Interviews' duration varied from 3 hrs to a whole day as

utmost care was taken to minimise disruptions to the habit of the shepherd in his duty. This chosen setup has been adopted in several similar studies (Debit 2005; Meuret 2006) and the underlying principle is reverse from the traditional role whereby information flows from the technician to the operator (farmer or shepherd). Making the shepherd feel that his sayings are valued is the key of the selected approach. The interview was intentionally not linear to achieve as closely as possible a casual conversation rather than a formal interrogation. The question sheets allowed thus the interviewer to keep track of the "progress" of the encounter. Interviews were carried out in the language of the shepherd (mainly Berberian) with the assistance of the interpreter. In Boulemane and Guigou, most of the interviewees spoke a local variant of Moroccan Berberian language while in El Ksabi, Moroccan dialect was spoken most. The recordings were transcribed later according to the topics. Feeding practices on both daily and seasonal scale were recorded.

4. Photos sequence

In all the interviews a "photo" sequence was included, in most cases at the end of the encounter. A series of pictures of plant species expected to be found in the local rangelands were downloaded from botanic resources of the web in particular www.cabi.org database and other personal collections. There were several pictures for a species in particular one showing the whole plant; close-up pictures of flowers and fruits (if applicable) were also annexed. In addition, a number of pictures were taken in situ. All pictures were shown on a Lcd screen or printed scaled to the size of an A4 paper. The shepherd's opinion was recorded in terms of cognizance, fodder importance, seasonality, animal preferences and grazing behaviour. The sequence and order in which the pictures were shown was contextual varying from one farmer to another as they were shown according to the importance attributed to the species during the course of the interview (the pictures of the most important or most cited species were shown first). The shepherd was asked first whether he identified the plant, then what was its importance in terms of abundance and as a feed resource for his flock. His opinion was also requested on the seasonality, phenology and distribution on the rangelands.

5. Plant sampling and analysis

The plant species considered as being important from the viewpoints of the shepherds interviewed were collected in situ on the rangelands visited. In all, four rangelands were sampled near Boulemane, two near Guigou and two near El Ksabi. The plant part collected was the part considered as ingested and whenever possible seeds and flowers were collected separately. The plant samples were placed in plastic bags and kept in refrigerated container, then transferred to the plant laboratory at the IAV (Institut Agronomique et Véterinaire Hassan II) in Rabat where they were dried at 40°C over a period of 48 hrs until constant weight was reached (around 95% Dry weight) and then grounded to 1mm size. These samples were subsequently sent to CIRAD, Montpellier for proximate analysis for and analysed for dry matter (DM); the AOAC (1995) methods were used for the analyses of crude protein (CP; method 984.13) and crude fat (CF; method 935.38) extracted with petroleum ether. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest *et al.* (1991) with sodium sulphite, without alpha amylase and expressed with residual ash method. For the analysis of total phenols in the feed, samples were first treated as described by Makkar et al. (1993) with minor modifications. As described by Makkar *et al.,* 1993, total phenolic compounds were extracted from the feeds using aqueous acetone (70% v/v), analysed by means of the Folin-Ciocalteau reagent and expressed as tannic acid equivalents

Results

1. Characteristics of the shepherds interviewed and of the associated feeding systems

The characteristics of the shepherds interviewed are reported in table 1. The age of the shepherds was rather difficult to guess because of the ageing due to the outdoor activity. We estimated that it varied from 30 to more than 60 yrs.; when younger shepherds were seen, they were not in charge of a herd but rather assisted the shepherd in maintaining the herd within a given perimeter. All the shepherds interviewed had started the activity in their childhood and were males. A few female shepherds were also noted in the area; they were mainly responsible for small herds of goats that belonged to them and they carried their task along with the children, which implied staying close to the house. It was difficult to talk with them due to constraints related to local customs and

traditions. The hired shepherds were in one way or another family related although not always part of the nuclear family. In all cases, the shepherds were part of the community and the salary was very variable consisting of payment in money, in kind (cigarettes, shoes or clothes) and/or fringe benefits like housing and meals. In some cases, the payment was correlated to the performance of the herd, such that for every four kids, one was given to the shepherd who may or may not mix it to the herd under his responsibility or even sell it. Another interesting observation was that the herd size under the supervision of a shepherd did not seem to be correlated to the site, type of herdmanship or experience of the shepherd. The average size of 250 heads seems to be the size, which is considered as manageable by one person in the area. In Boulemane, based on the data reported in this table, the shepherds were relatively older with longer experience and who have worked in less different places. Moreover, they all had sheep in the flock whilst in the other site only two farmers, namely F and H had animals in the flock and they were relatively younger and have changed farms more often.

In the three sites, the animal production systems were all pasture-based but there were some variations among them (Table 2). Boulemane is the site where the pastoral system was predominant with transhumance to other rangelands or woods in winter (steadily becoming disused replaced by in-barn supplementation) and targeted supplementation at the shed. Guigou is what may be considered as a typical agro-pastoral system whereby animal keeping is supported by cereal cultures and supplementation is well established. Finally, El Ksabi bears similarities to an "oasis" type of system whereby the animals are in rangelands almost all year round but the rangelands sites change between summer and winter and orchard undergrowth is determinant for animal feeding.

All the rangelands used were communal and each farmer had a defined area of rangeland on which he could bring his herd for grazing. The flocks were grazed on the rangelands for a duration of 5 months to the whole year, the period being shortest in El Ksabi and longest in Guigou. In fact, the rangelands in El Ksabi are located in a warmer area and the land is more arid with poorer natural vegetation both in terms of density and diversity whereas in Guigou, farmers have access to woods and to significant expanses of cereals stubbles which enables them to reduce the grazing pressure and extend the duration of use of the surrounding rangelands. In general, the rangelands were grazed from spring to autumn. In all three sites, grazing circuits varied in onset and duration depending on the climate and vegetation. The distance covered by the flock increased from spring

to summer. The total distance covered could double from 3 km in spring to an estimated more than 6km in summer, due to both the reduced vegetation available and the drying up of water points. In Guigou, the length of the grazing circuit varied less, because water is more easily available and the farms visited were all strategically located close to cereals fields. The direction and amplitude of the flock movement also varied depending on the location of the farm. A shepherd whose farm is located in the hills would generally move downhill towards the steppes and then uphill to return home; the circuit would go the other way round for a farm located on the steppes. Thus, all shepherds enabled their animals to have access to at least two types of rangelands and so two types of meals during the course of a day.

2. Rangeland description based on shepherds' sayings

Generally, rangelands were characterised by shepherds either by the most prominent fodder species "Aalf" or by the topography. In Boulemane and El Ksabi, rangelands were generally called foothills "Asfal al jabbal" or hillside "haafa" or steppes "alemou" or rangelands "marahi" . Therefore based on the topography, rangelands are called by above mentioned names and associated with the plants. Based on the prominent fodder plants, they were named "halfa" (stipa) or "shiha" (sagebrush). In the rare cases where the two plants could be found together, the rangeland was considered as "halfa", probably because stipa is more visible than sagebush. In Guigou two types of rangelands were identified namely "saffs" (woods) and "Idmren" (foothills). "Saffs" refer to the woods of "karoush" (Holm Oak) which are located on the hills surrounding the town of Guigou whilst "idmren" refers to the foothills and plains where "casdir" i.e. *Genista quadriflora* and "agarbaz" i.e. *Nonaea mucronata*, are the most prominent forage plants. Therefore, a rangelands can be called a "alemou" of "halfa" describing both the topography and the main species or only by the species or topography.

The same principle of naming a portion of rangeland by its mains forage plant was applied also at patch scale. The main patches were "harmal" i.e. *Peganum harmala* or "azoukini" i.e *Thymus sp* (thym) While the types of rangelands were areas sharing common topographic features and clear borders (hills, ridges or water), the patches were defined strictly by their vegetation. These patches were included in the choice of the grazing itinerary depending on the season and phenology. For instance, "harmal" patches were included in the circuit generally when the plants are at a wilting

stage whilst “azoukini” patches were included in spring, when they are at a flowering stage. The distribution and relative importance in terms of biomass was very variable depending on the rangelands and thereby their variable level of importance in the decision making process of shepherds. Guigou was the site whereby such “patches” were less visible and subsequently the vegetation of the various rangelands was rather uniform. In Boulemane, the patches were found to be located in topographical niches with concentration of “shook” i.e. *Adonis aestivalis* or “haarein” (Roquette)near water sources and flowering species (mainly crucifers and brassica) on hilltops. Such situation may be due to the fact these species are located such that they are not easily accessible and therefore more protected against grazing. In El Ksabi, patches of “azir” and “azoukini” were found more frequently on ridges and nearby homesteads.

3. Management Practices implemented by the shepherds to optimize rangeland utilisation

The LEK of the shepherd about the characteristics and dynamics of the vegetation and the feeding behaviour of the animals was used to decide on the itinerary and pace of the flock. The objective for the shepherds was to optimise the use of the natural forage resources available, and to adapt to the changing conditions over the seasons and years. Shepherds managed grazing: at a yearly scale, in order to take the best advantage of the local diversity of forage resources; at a daily scale in order to include a variety of plants (with different palatability) in the grazing circuit and thus enable the animals to manage a sufficient intake..

We found that the dominant “halfa” rangeland is grazed at different times, depending on the surrounding vegetation. In Boulemane, “halfa” rangeland is grazed later in the season because there is a large availability of crucifers which are very palatable but last for only a few weeks in spring and so the animals are grazed on those first. “Haarein” and “harmal” were the most common patches found in Boulemane in particular in sagebrush steppes while “azoukini”, “harmal” and “azir” were most common in El Ksabi. In El Ksabi, the availability of flowering species is very short (restricted only to a few weeks in early spring) in contrast to Boulemane or Guigou where “haarein” and “talzazat”, respectively, are available for weeks especially in beginning spring. Thus, the shepherds in El Ksabi have to tap on the “halfa” rangeland earlier compared to those in the other sites .

Whenever possible, the shepherds designed their grazing circuit so as to bring diversity to the animal diet and at the same time manage the foraging resources to last as long as possible. This was

particularly visible in Boulemane where a diversity of rangeland types and patches were available, and the flocks transhumed to the woods of Guigou during the winter. Shepherds A brought their herd on halfa followed by “shiha” steppes or vice versa depending mainly on the location of the shed. Farmers who dealt with a more diverse range environment implemented more complex grazing circuits. For instance, farmer C would bring the herd in spring on a patch of mixed “tawarra” and “shiha” followed by “halfa” ; in summer, he would include “harmal” and “azoukini” in the circuit due mainly to a reduced availability of “shiha” in particular. In El Ksabi, the rangelands used were more uniform, which restricted the duration of the grazing season i.e. the animals grazing were restricted to a handful of species and therefore, they used up these resources at a faster pace compared to Boulemane and therefore such as “shiha” gets grazed up earlier. That is why, shepherd J can use “halfa” and “shiha” in the grazing circuit only in spring and later one he makes the animal grazed on “halfa” and “azir”. In Guigou, the availability of “casdir” and “karoush” almost all year round and the extensive use of supplementation and cereals stubbles reduced the need to resort to such strategic grazing. For example, shepherd G has access to stubbles of barley as from the month of May and therefore he includes the latter in his grazing circuit till the end of summer and so he feels no need to manage the various species according to season but just makes the animals graze on the “idmren” and the “saffs” are used mainly during the winter months.

The availability of feeding alternatives to rangeland vegetation results in an occupation of the rangeland more focussed on maintaining a presence so as to keep the rights on land and water than on feeding the flock on rangeland plants. Each shepherd has a period allocated to him to bring his flocks for watering at communal water points on the rangelands and this is what primarily influences the grazing circuit in Guigou. In addition, in Guigou the shepherds met were mostly salaried men and they just followed the day to day instructions given by the owner. Availability of alternative source of fodder is the main reason explaining why at Guigou shepherds are able to include grazing of rangelands in their animal management for the longest compared to the other sites. Thus, the role of a type of rangeland or patch depended on the type and diversity of the associated forage resources (rangelands, supplementation or stubbles).

The spatial distribution of the rangeland types and patches and the location of the shed were determinant in the choices made by shepherds. As already mentioned, the patches generally coincide with topographical and hydrological variations in the rangeland. Usually, the objective of

the shepherd was to exploit resources further from the starting point as early as possible and to "finish" with the resources close to the shed. In this scope, the use of supplementation with concentrates was strategically introduced at either the beginning or the end of the day depending on the season and the aim of the shepherd. Supplementing at the beginning of the day enabled the shepherd to move the flock at a faster pace in the morning, while the animals used to being supplemented at end of day and tended to move faster in the afternoon. The pace of the herd depended on the distance of the grazing site from the house and sometimes on the watering timing and location which may vary depending on sites, extent of drying of water points and allocated time frame for watering. The shepherd would time his pace to meet the water point within a specific period.

Another strategy used by shepherds to take the best advantage of the rangeland environment was to adapt the proportion of goats in the flock. Goats are generally kept under the care of children and women, but can be included in the sheep flock. The goats advance at a faster pace and are included at a relatively higher proportion if the distance to be covered is longer. Pertaining to goat keeping, from the farms visited, a trend was visible among the sites with a much higher number and proportion of goats in El Ksabi compared to Boulemane (table 2). When asked about the motivations for choosing the proportion of goats, the farmers argued that goats are poorly adapted to cold but very well adapted to heat and eat shrubs rather than grass. Favourable climatic and vegetation conditions were met in El Ksaby, which could explain the higher proportion of goats in the flocks (up to 50%). Conversely, Boulemane is reputedly very cold in winter, and displays open rangeland, which could explain the lower proportions of goats in the flocks (<20%).

4. Shepherds' knowledge of rangeland plants

Based on the photo sequence session during the interview, the information gathered about shepherds' cognizance and evaluation of rangeland plants is summarised in table 3. Although there were several pictures of a given species like for Stipa ("halfa") for instance, recognition of at last one picture was considered as positive.

Based on the photo sequence, it was found that despite the biodiversity of the various types of rangelands in the area of study, shepherds considered only a limited number of species (the most abundant) as being relevant for grazing. For instance in similar type of rangelands, Acherkouk *et al.*

(2011) reported more than 25 species whilst in the present study Shepherds recognised 7 to 15 species only. The number of species recognized was not correlated to the biodiversity of the rangelands visited but rather to the observational skills and experience of the shepherd interviewed. The information given was subsequently verified in situ whenever it was feasible so as to corroborate about the presence of the species in the rangelands visited.

The relative preferences of sheep between these plants varied according to shepherds encountered and sites: in Boulemane and Guigou, the most preferred plants were those bearing flowers such as "haarein" and "tinegmit" while in El Ksabi, where the biodiversity of the rangelands was poorer, the preferred plant was "halfa". However, shepherds explained that preferences changed with the season due mainly to plant availability. For example, in summer, "Harmal" which is generally disregarded by the sheep is grazed probably due to the scarcity of other species and also because when wilted it is less "smelly" which is a peculiarity of that species and could be the cause of the avoidance observed in sheep. Interestingly, some species which are quoted in the literature (Berkat *et al.* 2004) as edible forage species for the area, such as *Dactylis glomerata, Hieracium pseudopilosella , Medicago suffruticosa* and *Globularia alypum* were not observed in the rangelands not cited by the shepherds.

5. Physical and chemical characteristics of the plants ingested by sheep

Most of the plants ingested in the rangelands contain polyphenols (0.7 to 4.0%) among which tannins which vary from 0.06 % in the leaves of *Genista quadriflose* and *Thymus ciliatus* to 1.0 % in *Quercus rotunfolia*. In terms of chemical composition, there was a relatively high variability in the parameters measured with crude protein varying from as low as 5.6% in *Ranunculus sp* to up to 21% in Eruca vesicaria. The in vitro digestibility was moderate with the lowest (34%) being in Thymus ciliatus.

From the above table, the plants may be grouped into 4 categories namely basal diets, flowering plants, bush and aromatics. The first category is considered as the basal diet as providing the greatest biomass ingestion and is made up of mainly grasses ("aalf") especially *Stipa Sp*, *Poa sp* and *Festuca sp.* and *Artemisia herba-alba* , hereby the dry matter and digestibility is determining. In this group, the total phenols and tannins level was relatively low but the digestibility was variable and

low as 28% for “shiha” (sagebrush). In the second group, there were mainly crucifers, on one hand the both the polyphenols and tannins contents although still relatively low were the highest of all groups attaining 1.9% and 0.8% respectively in “lafoudfoulous” (Buttercup) i.e. Ranunculus sp. but on the other hand the digestibility were the highest attaining 82% in “Haarein” (Roquette), i.e. *Eruca vesicaria*. The third group is limited to one species namely “karoush” i.e. Quercus rotundifolia(Holm oak) and it has the highest polyphenols level at 4%DM and a relatively good in vitro digestibility at 69% DM. Finally the aromatics i.e. “azoukini” (thym) i.e. *Thymus ciliatus* and “azir” (rosemary) i.e. *Rosmarinus officinalis* had surprisingly low levels of total phenols and tannins at around 0.7% DM and 0.1%DM respectively.

In Boulemane, the main species recognized by shepherds were Stipa sp. “halfa”, Thymus ciliatus “azoukini”, Poa sp. “tawarra”, *Santolina rosmarinifolia* “shiha”, Eruca vesicaria ”haarein”, Rosmarinus officinalis “azir”, Ranunculus sp “lafoudfoulous” and Adonis aestivalis “shook”. In El Ksabi, shepherds identified *Stipa sp.* “halfa” as the main forage species and *Peganum harmala* “harmal”, Artemisia herba-alba “shiha” and *Thymus ciliatus* “azoukini” as the other significant forages found on the rangelands. In Guigou, *Quercus rotundifolia* “karoush” was considered by shepherds as the main source of forage in winter; the other species considered as important were *Poa Sp.* “tawarra”, *Thymelea sp.* “talzazat”, *Scorzonera pygmaea* “tinegmit”, *Genista quadriflora* “casdir” and *Nonaea mucronata* “agarbaz”.

Rather than species, shepherds talked about “plants” and their “forage value” but not in terms of the nutritional composition but rather on the behaviour of animals towards them. Thus they called plants as “appetiser” (“metchi”), or “repulsive” (“Makroof”) or simply fodder (“Matetcha”). For instance “halfa” would be a “Tiramt” (fodder or meal) and “tawarra” a “mustasat” i.e. an appetising and “harmal” a “Makroof” literally meaning to be avoided. . They also use generic terms like “Idjejiguen” which literally means flowers but the term is also indiscriminately use to indicate one type of flowering plants like “haarein” or “shiha”. Thus, a plant may have different names depending on the phenologcial stage or different plants may have the same name due to their appearance or function

Supplementation was frequent and usually consisted in concentrate, mainly barley, beet pulp and commercial feed distributed in the shed in troughs or on the soil at the shed. In Guigou mainly, cereal stubbles (mainly barley) could also be grazed. Although Lucerne hay is used almost exclusively for cattle, it was nevertheless used only in case of emergency and sparingly and this occurred only at El Ksabi. Supplementation frequency and quantity was highly variable, being almost automatic at Guigou but used more sparingly in Boulemane and El Ksabi. In Boulemane and El ksabi, supplementation is used strategically being given to rams, pregnant ewes and lambs earmarked to be slaughtered in the coming weeks. In addition, from the interviews, farmers reported that shed supplementation is used to "correct" whatever was grazed during the day and depends heavily on availability and above all on support schemes given by the state through organisations such as ANOC. Supplementation is given mainly in summer and winter when the use rangelands is minimal and in most cases, provided at night. It is also worthwhile to note that there is almost no beddings and almost no measures is taken to avoid bullying resulting in uneven ingestion of supplements across the herd.

Discussion

The objective of our study was to collect the local ecological knowledge (LEK) of shepherds in a pastoral area of Morocco, and to analyse the strategies implemented to feed the flock on less palatable plants (possibly containing high amounts of PSCs). Based on 35 interviews of local farmers, we found that although pastoral farming systems in the area were mainly devoted to meat sheep production with flocks of about 250 animals, they differed in terms of herd composition (goat/sheep ratio) and feeding management (type and amount of supplementation, grazing circuits, time spend on the rangeland) depending on the range of feeding resources available and the local climatic conditions. Based on 11 interviews of local shepherds, we found that the latter categorize rangelands and patches based on the most abundant plant considered as a forage resource. Such plants are further categorized depending on their function, with a given local name possibly covering different species. The laboratory analysis of plant samples confirmed that the plants of the rangeland contained PSCs, in variable amounts. The plants also widely differed in terms of nutritive value.

1. Shepherds organized their daily grazing circuits as a function of the place and timing of access to water, and the seasonal availability of the forage resource. Within a grazing circuit, they implemented strategies (grazing order of different vegetation types, grazing before/after watering, supplementation before/after grazing) in order to take the best advantage of the diversity of the rangeland vegetation, and especially stimulate the ingestion of the less palatable plants. The knowledge of plant species of the rangeland was highly variable among shepherds, with more experienced shepherds (usually fully responsible for the flock) recognizing more species.

2. Methodology and reliability

   One important potential drawback of studies that as this one is the difficulty to screen the information collected so as to get reliable facts (Linstädter *et al*. 2013). Farmers are generally suspicious especially towards officials and strangers and so, adequate time was devoted prior to the start of the study per se to follow their proper local etiquettes and customs so as to achieve a better integration and acceptance within the community. The prime rule was to avoid being associated or confused to any local or national authority or body and specific emphasis was laid on the solely scientific motivations of the work. The fact that the objective of the encounter was to gather information about plants but not on sensible information pertaining to herd size, selling price etc. contributed in making the encounters cordial. The information we were interested in was often considered benign and even ludicrous by the local farming community. Recording such information both on writing and on a voice recorder, gave a sense of importance to the sayings of the farmers, a situation to which they are not accustomed. These situations ultimately created an atmosphere which was conducive for collection of "confidences" and reliable observations from the part of the interviewees despite the relatively heavy nature of the interviews because of the language barrier and the complexity of the subject matter. The fact that the interviews were open and non-linear made the exercise less cumbersome for the farmer/shepherd and at the same time thanks to the question sheets, the completeness of sought after information was ensured. Also, the method of a semi-structured interviews, has been extensively used in similar studies (Meuret *et al*. 2006 and Meuret *et al*. 2014) and so was deemed as being well adapted to meet the set objectives.

The language and translations can lead to misinterpretations. In our study, the language of the shepherds interviewed included many local terms, difficult to translate. To avoid confusions, we chose to keep to the local names to characterize rangeland and rangeland plants. Though, such local names might have a slightly different meaning depending on the area and the experience of the person interviewed. For instance, a drought condition ("jafaf") is one such term whereby its meaning is variable. In Guigou not having rain for a few weeks would be considered a drought whilst in Boulemane the absence of rain need to be over a months for them to call it a "jafaf".

Apart from strictly linguistic aspects, confusion might have occurred in our study between similar rangeland plants, although we did our best to avoid it. During the interviews, less experienced shepherds might have confused plants developing close physical resemblance, such as "halfa" and "smar" i.e. *Juniperus maritimus*; found close to water, which experienced shepherds call "false halfa". In the field, it was very difficult even for trained botanists to identify some species that were brought from the rangelands as they were stunted and often devoid of flowers and seeds which would have facilitated their identification and so only plant samples that have confirmed as such by a botanist were kept for analysis and for example two species were named as "shiha" i.e. *Artemisia herba-alba* and *Santolina rosmarinifolia*.

3. The LEK collected in the study is relevant, and similar to what can be found in other Mediterranean areas (France)

The study area was found to have a thriving and well established herding community with well codified and structured rules for rangelands use as per communal/clan customs. All the farmers and shepherds were in one way or another resuming a family or clan tradition of herdmanship based on the grazing of the natural vegetation of the rangelands. The area is well known for its pastoral system (Berkat *et al*. 2004) and there are three categories of rangelands based on topography and climate. Rangeland feed resources were categorised by shepherds according to their functions and effects on feeding behaviour of animals. This knowledge contributes to the LEK and enables the shepherd to establish a sound ground on which he can base himself to design his grazing circuits and schedule the exploitation of rangelands resources over the seasons. In the course of the various interactions that occurred with shepherds and farmers,

some subtle differences were noted not only on the nature of the LEK but also on its importance in the decision-making. These differences were more pronounced between sites as schematically it can be inferred that LEK was given more importance in Boulemane and less in Guigou and this coincides with the level of reliance on non-rangeland resources namely concentrate feeding and cereal stubbles which were more important in Guigou. Moreover, it can be noted that the grazing circuit and rangelands resources management were relatively more complex and elaborated in the Boulemane area and this coincides with the age and experience of the shepherds encountered.

All shepherds classified rangelands based on criteria, which vary with seasons, topography, biomass, nutritional quality, water availability and distance from camp. This situation is analogous to what have been described in studies done elsewhere as in Mongolia for instance (Fernandez-Gimenez 2000). The shepherds investigated, attributed to a forage plant, patch or type of rangeland a certain function, which depended on the diversity and spatio-temporal distribution of the forage resources available. Such functional typology served as a tool in achieving the double objective of ensuring a sufficient animal intake at a daily scale, and securing sufficient feed resources to feed the flock during the seasons and years. In no way, can the shepherds extend the area on which they can bring their herd due to the strict rules guiding the use of communal lands (called “urf”), in which the various types of rangelands fall. Therefore, in case of unsufficient ingestion or inadequate weight gain, shepherds resorted to supplementation of concentrate feeds namely barley, beet pulp or sometimes even pelleted feeds. At a daily scale, the association of complementary feed resources described by the farmers matched quite well the “menu” model described by Meuret *et al.* (1997) based upon herding practices in southern France: in most cases, especially in Boulemane and El ksabi, “halfa” was considered as the “main course” or contextually as a “reserve” whilst the “haraein”, “casdir” and “agarbaz” for instance (flowering plants, mainly crucifers) were considered as “appetisers” or “rewards” and the aromatic plants (like “azir” or “azoukini”) as “boosters” since their taste and nutritive value differ greatly from “halfa”.

4. The function attributed by shepherds to a plant can be correlated with its physical and chemical characteristics

The function of the plants in animal intake can be correlated to their physical and chemical properties (Baumont *et al*. 1996). In the region we investigated, for example, ”halfa” has a big leaf to stem ratio and is generally at a comfortable height for grazing which should lead to a high bite mass (not measured in our study) , especially compared to other plant species present in the rangelands. Thus, “halfa” is a plant which may be ingested quickly and in high amounts. Such plants enable the animals to ensure a sufficient intake over the day (Agreil *et al*. 2006). This might explain why “halfa” plays such a central role for the shepherds. Moreover, “halfa” has a relatively low level of tannins (0.3%) which may be an indication that it has low astringency (Muelley-Harvey 2006). “Harmal” is a plant whose status changes from “makroof” (repulsive) to “aalf” (forage) from spring to summer or when moving from Boulemane to El Ksabi whilst “halfa” has a permanent status of “aalf”. “Harmal” is a very pungent species which has a characteristic strong smell specially when at the flowering stage (as encountered in the course of the study) and can be found in patches across the various steppes of Boulemane and El Ksabi. Plant scents especially those from the flowers are due to volatile compounds (Knudsen *et al.* 1993) which are PSCs. Many volatile compounds emitted by plants have a role against herbivory (Paré *et al.* 1999) and some authors have even defined the concept of “smell good but taste bad” (Yang *et al.* 2013) in relation to some flowers’ scent. This may be applicable to “harmal” which at flowering stage and green, has a very strong smell but when wilted the smell is heavily attenuated and the plant is ingested by sheep. A similar situation was reported by Papachristou TG and Nastis AS (1993) who showed that *Vicia fenuifolia* and *Vicia cracca,* two Mediterranean wildly occurring species of vetch, although they had a protein content up to 17% in spring were not grazed until their senesce in summer when the phenolic content was lower. A decrease in tannin content due to the process of wilting was also reported for sainfoin i.e. *Onobrychis viciifolia* by Girard *et al.* (2013). Although it was not possible to carry out PSCs assays across the seasons in the present study, it may be inferred that the status shift from “repulsive” to “fodder” in the sayings of shepherds is due to a reduction in the levels of PSCs among which polyphenols. Polyphenol content of “harmal” (sampled in late spring) was around 3.6% of DM which was the highest of all the species assayed from the rangelands of Boulemane and El Ksabi. Keeping in mind that “harmal” had a relatively good protein content (12.9% DM), low level of lignin (ADL of

5.6% DM) and an in vitro digestibility of 75% of DM, lead to the supposition that the reason behind the avoidance by animal was not nutritional but rather the "Som" (toxic) in shepherds'terms.

In Guigou "karoush" and "tinegmit" were the most important species, with contrasting physical and chemical characteristics. "Karoush" is either a bush or tree and has a CP content of 14.2% DM while "tinegmit" is creeping type and contains only 8.7% CP. However, according to the sayings of shepherds, a normal diet especially in spring will constitute almost exclusively of these two species i.e young leaves of "karoush" and leaves and flowers of "tinegmit", leading to the inference that these two by virtue of their characteristics i.e. big bites high biomass in the former and small bites but higher rate in the latter, complement each other in the viewpoint of the herd. "karoush" was the species which had the highest polyphenol levels (4 % DM) of all the species sampled in the study, but only a quarter of it was condensed tannins which agrees with previous findings by Custódio *et al.* (2015) about *Quercus suber*. It is interesting to note that "Karoush" is ingested rather in the form of very young leaves and "tinegmit" in the form of leaves and flowers, which are the plant parts with usually the highest concentrations of PSCs (Mole and Waterman 1987; Mole *et al*. 1988; Harborne 1997).

For *Rosmarinus officinalis* i.e. "Azir", many authors reported the polyphenols levels, for example, Genena *et al.,* 2007 reported 14.20 g of TAE/100 g of extract and Akrout *et al.,*2012 reported 85.6 EAG/L. For "azoukini", i.e. Thymus Ciliatus, 0.7 mg of GAE per g DM was reported by Guesmi *et al.,* 2014 in *Thymus hirtus* and 175.53 mg Gallic Acid Equivalents by Achour *et al.,* 2012 in Thymus capitus. For both plants, there is a very wide variation of values depending on species, locations and growth conditions and thereby the difficulty to benchmark the findings of the present study. Nevertheless, although "azir" has significant nutritional value with a CP of 18.2 %DM, that of "azoukini" was among the lowest of the species analysed, the CP being only 5.6%. and the in vitro DM digestibility being only 34%. However, these two plants are determinant according to shepherds in Boulemane as they are highly appreciated by the herd when found and the fondness increases just after having been watered. Moreover, it may be suspected that these aromatic plants have some beneficial health effects which account why the shepherds in all sites reported almost inexistent use of veterinary drugs and little health issues with their herd. The link between PSCs

and livestock health is well known in particular tannins was reviewed by Min *et al.* 2003, saponins by Francis *et al* 2002, to name just two examples. It is known that ruminants especially sheep which is a highly gregarious animal have the self ability to learn to attenuate effects of PSCs (Villalba *et al.* 2010), modulate intake of PSCs or even self-medicate (Villalba *et al.* 2014). So, it is probable that despite the low apparent nutritional level, the plants such as "azoukini" is ingested either for hedonism especially to break the monotony of the diet as suggested by Provenza *et al.* 1995 or have an ability to balancing their requirements to some extent (Faverdin P. 1999) or for improvement of health as already mentioned. All these makes that why each and every species especially in the context of rangelands should not be viewed only on the aspect of forage value but both on its physical ,chemical composition and circumstantial palatability (Meuret *et al.,* 1997) in agreement with O'Reagain PJ (2011).

5. Shepherds implement strategies to increase the diversity of plants ingested

The shepherds interviewed kept moving the flock to a different type of vegetation although they could have stayed in the same site and covered less distance. The explanation they gave for this strategy was the need for the animals to eat a "bit of everything". Although animals spend much time eating what is most palatable and nutritive, they usually sample the diversity of edible items (Provenza FD 1995 and Provenza FD 1996). In addition, the ingestion of an unpalatable plant may be improved if the shepherd finds a way to improve its "circumstantial palatability" as described by Meuret *et al.* (1997). This situation was witnessed in Boulemane with shepherds who grazed "shiha" (*Santolina rosmariniflora*) just after watering the animals as this – in tier own words -compensated the salty taste of the plants. The objective of shepherds to make the animals ingest at least two or three different plants is consistent with the chemical analysis that showed contrasting chemical profiles between plants. For example, a typical sheep diet at Boulemane would associate "shiha" with 17% CP but low digestibility (23%) with "halfa" which has lower CP 12% but higher digestibility (49% DM) and "azoukini" which has low CP (5.6%) but lower condensed tannins (0.1% DM). Similarly, the complementarity between "karoush" and "tawarra" in Guigou could be accounted for by the contrasting polyphenols level which is more than 4 times higher for "Karoush".

6. Issues related with the perpetuation of shepherding and the associated LEK

The LEK of shepherds, as measured in our study, varied widely depending on their previous experience and social position.

There was a wide gap in the LEK among shepherds not only due to experience but also due to involvement and social position in the farming activity. A salaried shepherd would normally only follow the day-to-day instructions of the owner. Thus, since he is not associated to decision making, he has merely the role of a guardian ("Assa"), while a farmer-shepherd would implement his own decision system. All the shepherds met were experienced ones, being in the activity since childhood and it was expected that they would be relatively knowledgeable on the issues at hand. However, the changes due to modernisation (availability of water tanks, forage chippers and access to subsidised feeds) coupled with the increasing erratic climatic conditions, have led many shepherds to feel that their knowledge is no longer pertinent. Besides, it is easier to supplement with concentrates rather than to design complex grazing circuits. The fact that shepherd knowledge has been rendered increasingly redundant, i.e. less and less determining for being hired, makes that owners don't hesitate to give their flock to a novice and eventually disrupt the transmission of LEK from one generation to another. Our results suggest that the extent of knowledge of the rangeland vegetation and animal behaviour is linked to the commitment and the extent to which the shepherd is involved in decision making. For example, in Boulemane one shepherd who was a salaried man and relatively young could only cite and recognise a maximum of 7 species during the interview and photo session whilst another shepherd from the same site but older and who was also an owner, recognised all the species in the photo session and cited over 22 species present in the rangelands. Farmers did complain about the difficulty to find competent shepherd especially due to competition from recently implanted nature reserves for breeding of wildlife, which gives attractive packages with good salary against whom owners can't fight. All this adds up and contributes to the decline of traditional pastoral systems and of transhumance.

LEK is something quite personal, the "personalised intuitive expert system" being a resultant of personality and nature and working environment (Nuthall 2012). . This is why, LEK collected from one farmer is often not applicable to another. However, in our study, the "rules and system" were similar for all the shepherds interviewed (with one exception namely shepherd C) particularly

relating to the rules and customs for rangelands use, climatic and edaphic conditions and market conditions. LEK is transmitted within a farm/clan through family members but not shared with others. Davis (2005) reported that although she found that generally LEK is quite accurate, it is not considered in policy making decisions in Morocco. Such one sided decision-making was witnessed in Guigou and Ifrane whereby expanses of rangelands were fenced and grazing prohibition implemented for a few years in the objective of restoring the degraded vegetation, but accompanied by compensation to shepherds through ANOC (Pers. Comm. ANOC). Such a situation is not novel as in other parts of the world pastoralist are considered as the "problem" not part of the solution (Reed *et al.* 2007) i.e. policy makers view the pastoralist as the cause of the problem, in this case land degradation and therefore decision are taken at their level without feedbacks from grassroots. However although participatory approach is becoming increasingly adopted in rangelands management around the world (Cooke and Kothari 2001), in Morocco this is still at a budding stage.

7. Rationality for Rangelands use

In the course of the encounters with the shepherds, there was a contrast on the way rangelands resources were regarded upon and used. The rationality behind grazing management adopted by Boulemane was to make the natural vegetation last for as long as possible, ideally until the first snow and thereby the animals' needs were secondary. That is, the aim of bringing the animals on the rangelands was not primarily to ensure that the herd had ingested up to satiety or requirement but rather to take advantage of a free resource for as long as possible. To achieve this objective, the farmers and shepherds in our study used mobility (grazing circuits and sometimes transhumance) and herd composition (goat/sheep ratio), which are typical adaptations in pasture-based systems (e.g. Butt 2010; Dwyer and Istomin 2008).

In Guigou, the situation is different as rangelands resource is considered "secondary" because of the high availability of other forage resources especially in terms of woodlands, cereals stubbles and vegetables fields leftovers (mainly onion, crucifers and potatoes). In fact, most rangelands are grazed sporadically or grazed in winter by transhumant flocks from other areas (mainly Boulemane). These observations support the idea that the issue of overgrazing in this part of Morocco or at least the contribution of shepherds to it is very variable from one site to another, and in general probably

overestimated (as reported by Davis 2005) . In fact, among the shepherds interviewed, none complained about a degradation in natural vegetation or overgrazing- but almost all expressed their concern about water availability. In all sites, rangelands seldom satisfy the needs of the flock except for some weeks in spring resulting in the shepherd designing elaborate grazing circuits, tapping resources which are more difficult to access like on ridges and hill tops or resorting increasingly to supplementation. Moreover, shepherds reported that that as long as they can recall, both the number of herds grazing on the rangelands and herd size has remained constant although the herd may have increased but the excess number of heads are not brought to the rangelands but rather stall-fed or transferred to other localities.

Of all the eleven shepherds interviewed, all were content to be able to continue earning their livelihood in this way. Very few of the shepherds openly expressed a desire to increase production, flock size or productivity but in fact they were very happy with their present level of performance and seldom expressed what could be interpreted as a concern about issues such as dwindling biodiversity. In fact, their ability to have survived so far, makes them rather confident and very little preoccupied about the future of their activities although they remain adamant about the next generation to take over their role. During the encounters, a strong sense of confidence in their own capacity to cope to whatever would be thrown to them exuded especially from the farmers. Although the shepherds met were hand selected for their experience and above all their eagerness to accept our intrusion in their daily activity, a relatively reliable insight on the preoccupations and practices of shepherds in the region was collected as the shepherds met were among the most experienced ones and also some were office bearers of farmers association or local councils and this make why such inferences can be made.

Conclusion

The present study though not completely novel in its conception nevertheless is original in two aspects: 1° it is the first time that such a study is carried out in eastern Morocco and 2° it is the first time that LEK and PSCs are linked together. The LEK collected, bore some similarities in the three sites under investigation but also differed in other aspects. Despite the relatively small area of the study (radius of around 25km) and the limited number of farmers and shepherds interviewed, some

interesting information was obtained. The criteria for rangelands classification and the rationality guiding herd conduct in the rangelands were described for each site. Although only one type of PSCs was investigated, i.e. polyphenols, the levels found in conjunction with the chemical composition, have enabled to explain to some extent why the shepherds chose to graze their animals on a particular type of vegetation and in a particular sequence or time. It may be speculated that other PSCs are present in the plants of the rangelands of this part of the Middle Atlas, namely flavonoids, essential oils and saponins, which are known to influence grazing behaviour. It is quite interesting to note how shepherds and farmers decisions differed from sites to sites. Their adaptive management based on years of experience and field observations, has enabled them to maintain their activities over the years. A concern is whether the present relatively traditional system of animal herding based on specific LEK to make best use of natural rangelands will be resilient enough to be able to sustain forthcoming challenges especially due to climatic changes and rangelands biodiversity.

Table 1: Characteristics of the 11 shepherds interviewed an of the associated flocks

Farmers	Boulemane Site					Guigou Site			El Ksabi Site		
	A	B	C	D	E	F	G	H	I	J	K
Status	Owner-shepherd	Owner	Owner-shepherd	Shepherd	shepherd	Shepherd	Shepherd	Owner-shepherd	Shepherd	Owner-shepherd	Shepherd
Relation with owner	Brother	-	-	cousin	Same clan	Son	-		-	-	Same clan
Age (approx.)	50+	46	65	60+	40	35	43	40+	33	30+	40+
Experience (years)	35+	30+	45+	45+	15	20+	30	20	16	15+	25+
Previous farms worked	3	-	1	-	3	-	4	2	6	1	8
Flock size	280	300	250	265	230	400	180	275	250	125	250
Animals owned by the shepherd in the flock	0	300	250	30	20	150	-	5	-	-	-
Animals owned by the shepherd not in the flock	50	300	-	50	20	50	35	-	100	-	50

Table 2: Major characteristics of the sheep farming systems depending on the area of study.

Site	Boulemane	Guigou	El Ksabi
Production System	Pastoral with transhumance	Agro-Pastoral ("Bour" type)	Pastoral ("Oasis" Type)
Calendar			
Lambing	Generally in winter	Sep-Feb	Sep-Mar
Flock size	Around 250 hds per shepherd	150-400	125-250
Flock composition	<20% goat	<35% goats	<50% goats
Sheep breed	Exclusively Timahdite	75% Timahdite, 25% Beni Guil	50% Timahdite, 50% Beni guil
Period in Rangelands	6-8 months	All year round	5-6 months
Rangelands used	Sagebrush and Halfa	Sagebrush, Woods, stubbles	Halfa and sagebrush
Grazing Circuit:			
in spring	Sagebrush-halfa-sagebrush	Quercus (Oak)	Sagebrush Sagebrush-Halfa
in summer	Halfa-Sagebrush-Halfa	Quercus- cereal stubbles	Halfa + orchard and olive undergrowth
in winter	Barn	Woods-barn	Transhumance to Midelt

Table 3: Number of shepherds who recognised each plant species in the list and cited the species as an important forage resource.

Species	No. of shepherds that identify				No. of shepherds that considered it as important forage				Period grazed
	Boulemane (n=5)	**Guigou (n=3)**	**El Ksabi (n=3)**	**Total 11**	**Boulemane (n=5)**	**Guigou (n=3)**	**El Ksabi (n=3)**	**Total 11**	
Artemisia herba-alba	**5**	**3**	**3**	**11**	**5**	**0**	**1**	**6**	**spring**
Astragalus sp.	1	0	0	1	0	0	0	0	-
Dactylis glomerata	0	0	0	0	0	0	0	0	-
Eruca vesicaria	5	5	2	10	5	0	0	5	Spring
Festuca Sp.	4	2	0	6	2	0	0	2	
Genista quadriflora									Summer
Globularia alypum	0	0	0	0	0	0	0	0	-
Hieracium pseudopilosella	0	0	0	0	0	0	0	0	-
Medicago suffruticosa	0	0	0	0	0	0	0	0	-
Nonaea mucronata	3	2	3	8	0	0	1	1	Spring, summer
Peganum harmala	5	3	3	11	1	0	2	2	Summer
Poa sp.	5	3	3	11	5	0	2	7	Spring
Quercus rotundifolia	5	3	3	11	0	3	0	3	Summer, winter
Ranunculus sp	3	1	1	5	1	0	0	1	Spring, summer
Rosmarinus officinalis	5	2	3	10	3	0	0	3	All year round
Santolina rosmarinifolia	5	5	3	11	5	0	3	8	Spring , summer
Scorzonera pygmaea	1	3	1	5	0	3	0	3	summer
Stipa sp.	5	3	3	11	5	0	3	8	All year round
Thymelea sp.	1	3	0	4	0	1	0	1	All year round
Thymus ciliatus	5	2	3	10	5	0	3	8	Spring, summer

Table 4: Chemical composition of main fodder species collected from the rangelands in the study area.

				Chemical Analysis						
Species	Local name	Parts Ingested	% DM	Crude Protein (%DM)	NDF	ADF	ADL	Total polyphenols (%DM)	Condensed Tannins (%DM)	% DM *in vitro* digestibility
Grasses "aalf"										
Artemisia herba-alba	Shiha	leaves	37	16.3	51.1	7.4	4.0	0.7	0.1	23
Festuca Sp.	Azkoun	Blades	55	17.2	39.4	25.3	7.9	0.7	0.2	65
Poa sp.	Tawarra	Blades + flower	63	15.0	43.5	26.0	5.8	0.9	0.2	66
Santolina rosmarinifolia	*Shiha*	*Leaves + spikes*	*37*	*16.6*	*26.3*	*17.5*	*4.2*	*0.7*	*0.1*	*93*
Scorzonera pygmaea	Tinegmit	Leaves + flowers	47	8.7	36.4	27.9	8.3	0.7	0.1	67
Stipa sp.	Hhalfa	Blades	71	12.4	60.3	29.2	4.3	1.0	0.3	49
Flowering plants										
Adonis aestivalis	Shook	With spines	18	14.1	27.7	21.4	12.6	1.6	0.6	84
Eruca vesicaria	Haarein	With flowers	39	21.3	32.9	19.7	5.2	1.1	0.4	83
Genista quadriflora	Casdir	Leaves + twigs	54	11.5	33.4	19.8	6.2	0.7	0.1	80
Noaea mucronata	Agarbaz	Leaves	67	6.4	57.5	38.3	12.4	1.0	0.1	47
Peganum harmala	Harmal	Wilted leaves + seeds	40	12.9	37.0	21.1	5.6	3.6	0.9	75
Ranunculus sp	Lafoudfoulous	Leaves + flowers	22	5.6	34.0	22.4	10.7	1.9	0.8	70
Thymelea sp.	Talzazat	Leaves	53	16.2	30.1	18.4	5.7	0.7	0.1	86
Bush										
Quercus Rotundifolia	Karoush	Leaves + twigs	38	14.2	41.1	21.8	7.5	4.0	1.0	69
Aromatics										
Rosmarinus officinalis	Azir	Leaves	66	18.2	46.0	27.0	4.4	1.1	0.1	69
Thymus ciliatus	Azoukini	Blades + flower	43	5.6	73.9	43.5	5.7	0.7	0.1	34

General Discussion

1. Scope of study (Objectives and Hypothesis)

The PhD project has been developed within the AgTraIn call on "Plant secondary compounds in small ruminant feeding: an alternative to drugs for improving animal metabolic state and product quality in low input farming systems".

The scope of the study was defined by two keywords namely small ruminant livestock systems and local alternative feed resources, in the context of the Mediterranean area. The main preoccupation is to bring innovations in two type of livestock system, namely stall-fed and pastoral. In the former, the challenge was to make use of indigenous local resources in particular agro-industrial by products whose use is limited by their content in PSCs. In the latter case, the challenge was to optimise use of the biodiversity in the rangelands for livestock nutrition and in order to do so, the experience and knowledge of local shepherds was tapped so as to understand how they cope with unpalatable and toxic plants in their day to day management of their herd. These two apparently unrelated systems have two things in common: 1) they are both in the Mediterranean area and therefore are subjected to similar realities in terms of climate, traditions and markets; 2) they both need to innovate to become more sustainable. Sustainability for these systems implies adaptation to the challenges of increased volatility in both inputs and market and foremost become "green" i.e. reduce their environment impact especially in terms of methane emission, carbon foot print and at the same time ensure the livelihood of the stakeholders. However, the two types of farming systems studied also have some intrinsic differences in that in the stall fed system, feeding is the main expenditure with heavy reliance on cereals while in the pastoral system, animals graze on the rangeland but performance cum. output level is heavily correlated to the quality and quantity of the natural resources they ingest and foremost on the competence of the farmer or more specifically the shepherd to optimise these resources over time.

In order to be able to have a more comprehensive picture of the central role of PSCs in livestock nutrition, the choice was made to have two case studies in two different countries. The choice of systems and location was motivated by a number of factors. One was to be within the guidelines as laid in the Agtrain philosophy which are:

. i) To obtain tangible deliverables that can be used in improving sustainability in the agricultural sector and, ii) to develop "*joint research with leading research organisations in Africa*". Given that the current PhD study was to be carried out under the supervision of the University of Catania (Institution n.1, Italy) and SupAgro Montpellier (Institution n. 2, France) the north Mediterranean site was chosen locally in Sicily, where sheep breeding is traditionally widespread. For the south Mediterranean site Morocco was selected for a number of reasons. Firstly, in Morocco there is a very wide variety of sheep production systems in which plants rich in PSCs substances are suspected to be involved and few studies have been done in this field in this area. Secondly, the Institut Agronomique et Vétérinaire Hassan II (IAV) in Rabat is a reputable institution with whom both the University of Catania and SupAgro Montpellier have substantial collaboration on a variety of subjects, including livestock, and therefore on the supervising and logistics aspects there were sound ground to ensure the smooth running of the proposed study. Such a study was set up as it also contrasts with the type of works carried out in the initial stage of the doctoral study, which involved monitoring an experiment and laboratory analysis. In the field study in Morocco, different methods and corresponding skills were developed , in direct line with the one of the objective of AgTraIN which is to foster student to acquire complementary skills to achieve greater versatility.

Another choice that was made during the course of the PhD was to focus on only one type of PSCs. Based on the state of the knowledge and competencies available, polyphenols and in particular tannins were chosen and assayed whenever possible.

2. What are the scientific findings that were learnt from this doctoral study?

A-Experimental trial on the use of agro-industry by-products

Two types of locally available polyphenols-rich by-products were evaluated, namely carob pulp and citrus pulp. From the first experiment, it was found that inclusion of carob pulp which contains polyphenols and tannins in lieu of barley up to level of 35% of the total diet, was feasible in the fattening of Comisana lambs. Three experimental groups were compared: a control group, fed with a traditional barley-based concentrate, and the two carob groups, fed with a concentrate in which carob pulp replaced 24% and 35% of barley. Three types of parameters were evaluated, namely: in vivo performance and carcass yield, feeding behaviour and metabolic welfare as assessed by blood metabolite levels. In all of them, the levels were comparable to the animals that were fed a non-

tannin containing diet indicating that carob pulp can be considered as an alternative that can be implemented in a lamb fattening diet without any drastic changes in the usual operating practice of farmers. A similar profile of results were obtained with the inclusion of dehydrated citrus pulp (DCP) leading a similar inferences as those drawn in the case of carob pulp. However, if the results are analysed in depth, some tangible effects of carob pulp ingestion and citrus pulp ingestion were noted and some of them, in particular those regarding metabolic welfare, have been reported for the first time in the present study.

The set up of the trial allows the comparison of the two by products, which is an approach that have not been tackled in the articles published (Gravador et al., 2014; Gobindram et al., 2014; Inserra et al., 2014) and submitted (Lanza et al., 2014; Gravador et al., 2014), to be made as the experimental conditions in both cases were the same. This is pertinent as it shed some light on some aspects not discussed yet in the articles and may potentially give some insights on the complementary or antagonistic nature of these two by products. Some of the observations and results obtained confirmed what were to be expected according to the literature but some were less expected and even counter-current with respect with what was already known..

On the expected side, it was found that both carob and citrus pulp (DCP) contained polyphenols. The polyphenols content, expressed as mg tannic acid equivalents/mg DM, ranged between 14.2 and 16.7 in the two carob diets and between 6.7 and 7.9 in the two citrus diets. However, comparisons of these two set of values is not possible because the method of polyphenol assay used in the case of carob pulp and citrus pulp was not the same. Two different methods was used because the nature of polyphenols that could be expected to be found in these two by-products is different. Hagerman et al. (1989) and Singleton et al. (1999) both recommended to adapt the method of assays on the sample to be analysed. Since, the primary aim of the trial was not to compare carob pulp with citrus pulp but to confront each feed resources against a purely cereal diet, the need to be precise was more important than uniformity which in any case was not scientifically justified. Furthermore, the two by-products, although evaluated on the same experimental conditions, were nevertheless independent from each other in their follow up. So, it would be useless to compare

the two trials from the polyphenols content perspective but other factors may be considered such as nutrient composition, energy level, intake behaviour, welfare indicators to name a few.

In both by-products, the performance indicators both in vivo and at slaughter were unaffected compared to the control diet. This is quite unexpected as our analysis found that both feeds contained significantly higher levels of polyphenols with respect to the cereal based diet and given that there are ample literature that demonstrate a positive or negative effect of ingestion in high quantity of these feeds. Many authors have documented the effects both positive and negative of these two alternative feeds resources in animal diet: Silanikove *et al.* (2004), Marakis *et al*. (1996) and Heuzé *et al*. (2013) have all reported on carob pulp while Heuzé *et al*. (2014), Belibasakis et al. (1996), Arthington *et al*. (2002), Bampidis & Robinson *et al*.(2006) are some of the authors who have reported on citrus pulp. In both cases, the effects are mainly attributed to the PSCs content of these feeds namely tannins for carob pulp and flavonoids and essential oils for DCP. At high levels of ingestion which was the case in the present study, both carob pulp and DCP are likely to have reduce palatability and causes anti-nutritive effects (www.feedipedia.org) but none of these occurs. Polyphenolic compounds like tannins (Frutos *et al.* 2004) and flavonoids (Oskoueian et al. 2013) are known to offset ruminal digestion at high levels but such a situation was not apparent. With the fact that all the diets had similar protein and energy levels, there is likely to be other factors in the feeds that may explain the above. Apart from PSCs, the diets were different in fibre and marginally in fats. In a concentrate based diet as in the present trial, the role of fibre is important as such diets generally contains high level of fermentable carbohydrates and highly digestible protein and lower levels of fibre if compared to a forage based diet. Fibre is important for ruminal function (Mirzaei-Aghsaghali *et al.* 2011). All the diets had similar total fibre but the proportion of the various fibre fractions differed and this may hint to the fact that these fibre especially ADL and cellulose have offset the binding activity of PSCs in the carob and DCP diets by binding with them (Padayachee *et al*., 2012a, b,; Renard et al. 2001). These fibres especially lignin would not have contribute much to growth of the animals as they have low digestibility and therefore, it may be probable that by blocking some PSCs molecules, less proteins and carbohydrates get bound and thereby the growth of animals was not affected. Another potential explanation may simply be that the animals have over the period of more than 50 days of exposure to the same diet, developed some defensive mechanism and get accustomed to the diet as inferred by Waghorn *et al*. (2003).

Regarding the ingestion behaviour issue, in both by-product based diets and in the control diet, around 50% of the total daily intake was done in the first three hrs of feeding. This tends to infer that, probably, there were no marked differences in both palatability and "toxic" effect of polyphenols in both type of diets. This is somehow, contrary to what could have been expected. Indeed, according to literature, differently from citrus-fed lambs, the carob-fed animals were ingesting tannins, which are not present in citrus pulp ; in the light of the above, some differential feeding behaviour would have been in the normality. Tannins ingestion generally abate intake rate (Landau et al., 2000) with recovery attained later in the day (Provenza *et al.* 1992). The results showed in the carob pulp study agree with these previous studies, showing an effect of carob pulp inclusion in lowering the intake in the first 90 minutes immediately following feed supply, followed by a recovery in the later 90 minutes. A fully comparison between carob pulp and citrus pulp studies is not possible because of the slightly different time interval set up in the two trials which do not allow the comparison in the first 3 hours immediately following supply.

The metabolic welfare parameters is where the contrast between the two by products were the highest. In the case of carob pulp, most of the blood metabolites level were affected by the carob ingestion whilst in DCP diets the levels were comparable to that of the control group. This situation is highly probably linked to the presence of tannins. Though the hypo-cholesterolemic effect of tannins could have been expected (Silanikove et al. 2006), the effect on NEFA and urea level is more complex and this have already been elaborated upon in the carob paper. The absence of any apparent effects on the blood metabolites parameters in DCP fed lambs is comparable to that reported by Gawad et al. (2013) in bulls.

However, the most interesting difference between the carob pulp and the citrus pulp diets were on the blood protein profile data. Indeed, a statistically higher ratio of albumin to globulin (A/G) in animals ingesting 35% level of DCP was shown, while in the carob experiment only a tendency for higher AG ratio has been observed as a consequence of carob pulp inclusion in the diet. Simultaneously, the albumin level was significantly higher, compared with the control fed lambs, in both carob pulp and citrus pulp ingesting animals. Considering that albumin is an acute phase protein (APP) which is negatively correlated to health, i.e. it lowers with infection (Murata *et al.* 2004), and that AG ratio increases in case of low protein utilisation efficiency and liver dysfunction (Eckersall PD 2008)) or nutritional stress (El-Sherif and Assad, 2001), it may be inferred that AG ratio

increasing trend observed in both carob pulp- and citrus pulp-fed lambs is not due to an adverse health condition but rather to a metabolic impairment. However, the comparison between the two experimental trials allows to hypothesise that when animals were ingesting citrus pulp the A/G ratio was much higher affected as compared to carob pulp ingestion. Thereby, there is ground to infer that instead of tannins (usually contained only in carob pulp), there are other factors (fibre composition and/or content) or other substances, probably flavonoids, which by impairing ruminal digestion may have also affected hepatic metabolism, which is reflected in the A/G ratio. Carob pulp diets had a higher level of fibre, particularly cellulose, compared to the citrus pulp diets. The light difference in diet composition, coupled with the different fibre fractions intakes, may probably be accounted by a slightly more efficient ruminal digestion in the carob containing diets. In addition, citrus pulp diets could have some other compounds, especially flavonoids, polyphenols generally found in citrus pulp (Levaj et al., 2009) but highly variable in carob pulp (Sebai et al., 2013), which are known to reduce rumen degradability (Oskoueian et al., 2013). However, this avenue cannot be explored further as flavonoids were not quantified in the present study.

Finally it is worth to mention that the same experimental groups observed in the two studies above described and discussed were also used to evaluate the effect of carob and citrus pulp inclusion on some aspects of meat quality. Gravador et al. (2014, submitted) studied the effect of carob pulp inclusion on meat quality and observed an increase in meat beneficial n-3 fatty acids and a decrease of the n-6/n-3 polyunsaturated fatty acids (PUFA) ratio due to a higher intake of linoleic and linolenic acids and a favourable effect on ruminal fatty acids biohydrogenation, compared to the control diet. Carob pulp dietary supply, moreover, did not modify the oxidative stability of meat after storage (aerobic and refrigerated conditions for six days). Similarly, the use of dietary dried citrus pulp in feeds resulted in an improved meat fatty acid profile, due to an increase in the levels of beneficial PUFA (Lanza et al. 2014, submitted). Even in this case the results were attributed to the increased the intake of essential polyunsaturated fatty acids and to the effect on ruminal fatty acid biohydrogenation. Differently to what obtained with carob pulp based diets, the inclusion of citrus pulp in lamb diet improved oxidative stability of lipids (Inserra et al. 2014) and proteins (Gravador et al. 2014) in stored meat. These last results have been attributed to the citrus pulp antioxidant phenolic compounds.

Pertaining to the carob pulp evaluation, a free-choice group was also set up with 10 animals each having the same ingredients than the animals in carob pulp trial but instead of a total mixed ration (TMR), the five individual ingredients were given in a cafeteria-system, i.e. each feed separately in small boxed box and the animal was left to eat freely whichever ingredient. The intake behaviour was monitored by observation based on Altman method (Altman, 1974) and both frequency of ingestion of each ingredient and quantity was recorded . The data has been compiled and would be analysed with the prospect of producing a scientific article for publication. The aim was to evaluate how the animals modulate their intake behaviour to cope with the carob pulp and appreciate the "individuality" effect in the formulation of the diet for each animal to meet its requirements. Besides these specific behavioural aspects, the feed intake pattern and in vivo and post mortem parameters were also measured such as in the other TMR experiments. This study shall enable to correlate the intake of carob pulp with respect to these performance indicators.

B . Field Survey in Morocco on pastoral system

Following the on-farm stall-fed experimental trial carried out in Sicily, a field survey was set up in collaboration with IAV Hassan II with pastoral based sheep farmers in the Middle Atlas region of Morocco. Understanding how tannins containing plants in natural rangelands affect sheep ingestion behaviour and evaluate how shepherds and farmers are cognizant of these species and how they include this feature if ever, in their decision making process particularly in designing grazing circuit and seasonality. The methodology, findings and issues encountered have been discussed in the paper enclosed.

To our knowledge, it was the first time that a study was carried out in Morocco about local ecological knowledge (LEK) of shepherds in terms of plants consumed in the rangelands. The study had three components : 1) a semi-structured interview with farmers relying on the natural . It was found that in a relatively small area, there were several types of management practices that were adopted by stakeholders involved in sheep fattening based on rangelands grazing. Despite the relatively high variability in the types and topography of the rangelands studied, some common features came out.

It was noted, that in all cases, despite the biodiversity of the rangelands, only a few species were attributed importance in terms of forage and decisions are taken with respect to these species only. Strategies used by shepherds are analogous to what have been reported in similar studies by farmers elsewhere especially in Europe. For example, in the south of France (Meuret *et al.* 2014)b where they described how shepherds "let" the herd to graze or not on flowering plants depending on the distance to cover. In Spain (Oteros-Rozas *et al*. 2013) where shepherds can makes forecast on rain based on and knowledge about animal behaviours such as general nervousness, nervous feeding, and excessive stillness, depending on the area, season, breed and thereby modify their grazing circuit accordingly or in Greece (Kizos *et al.* 2013) whereby shepherds use the strategy of burning astivi (*Sarcopoterium spinosum*) to clear unpalatable species .

Small ruminants grazing in rangelands whereby there is a mixture species, combines both herbaceaous and browse species and ingest both green and dry plant parts (Meuret M 1997). Factors such as phenology affect leaves availability for instance and so with grazing, the size of bites declines compensated to some extent by increase in bite rate (Spalinger and Hobbs 1992; Shipley *et al.* 1999). Morever, the types and availability of other neighbouring species may also influence intake (Bryant et al. 1991). Plant secondary compounds generally cause aversion like tannins in blackbrush (*Coleogyne ramosissima*) or terpenes in big sagebrush (*Artemisia tridentata*) (Provenza FD 1995). But the animals in rangelands have feeding strategies that make them able to ingest more of a particular "toxin" i.e. PSCs that they would have been expected (Launchbaugh *et al.* 2001; Provenza *et al.* 2003). Shepherds also have strategies that make them tap the various resources available to them on the rangelands like the "menu" system reported by Meuret (1997). In the present study, the plants in the rangelands had relatively low levels of PSCs compared to those that were present in the trial with Carob pulp and dehydrated citrus pulp(DCP) although the method of assay used were not the same whereby the difficulty to make comparisons. Although the concentration of total phenols are higher in carob pulp, i.e. 16 mg/ g DM i.e. (1.6% DM) compared to the range of 0.7 to 4.0% DM in the species sampled in the rangelands. However, keeping in mind that the dry matter of the carob or DCP was more than 85% but that of the plants in the rangelands

was very variable ranging from 16 to 70%. This implies that the amount of PSCs ingested s likely to considerably higher in the stall-fed animals when compared against those grazing.

The species that have been identified as being "key" ones either by their preference or abhorrence by the herd were found to have relatively low levels of tannins leading to the suspicion that other PSCs may be having greater incidence on grazing behaviour and shepherd decisions. Finally, it was clear that as already suggested by a number of authors, the pure pastoral way of sheep rearing is in decline and one of the main cause is the access to input especially concentrate and too high variability in the rangeland resources due to increased frequency of extreme climatic conditions particularly drought. This situation has resulted in rendering LEK of shepherds and other experts in the activity, less determining. The lesser dependence on rangelands is a source of potential redundancy of experienced shepherds and may eventually lead to a loss of the indigenous knowledge and knowhow as no longer needed thereby the pertinence of the study that have been carried out.

3. Value of the results

From the various studies that have been carried out during the course of this doctoral study, some valuable information was gathered. The main ones were:

i. Carob pulp and dehydrated citrus pulp (DCP) can potentially be included in lieu of barley, at a level of up to 35% of the ration DM with comparable performance and productivity level in comisana sheep.
ii. Carob pulp and DCP contains polyphenols and this only mildly affect metabolic welfare of animals.
iii. Plants in the rangelands of the Middle Atlas of Morocco contains PSCs, notably polyphenols and tannins and are ingested as forage by grazing sheep.
iv. Shepherds are knowledgeable of the biodiversity in the rangelands that they grazed their animals on and have strategies to optimise their use which are comparable to practices done by shepherds elsewhere, in particular in the Mediterranean.

v. Despite the biodiversity, only a few species are considered as important not because of their nutritional properties but because of the effects they have in relation to the behaviour of animals on the rangelands. This is the basis for classification of rangelands resources.

vi. These behaviours may be correlated to some extent to the PSCs level in the plants

vii. The LEK of shepherds vary between sites , age and "importance" in the decision taking of the herd management

viii. The importance of shepherds knowledge is diminishing due to increased reliance on feeding other than that of the rangelands.

ix. Perception on degradation and future of perspectives of rangelands resources are relatively positive from shepherd's viewpoint.

Considering the above list, much of these findings are quite new from previous knowledge. Although carob pulp and DCP have been amply investigated in relation to ruminant feeding and performance (www.feedipedia.org), there are little work that has been done in evaluating the welfare issues that their use entail. Thereby, they either aimed at unravelling the mechanisms by which the "toxic" effects of PSCs or determine the optimal system to counteract the "anti-nutritive" effects. The novelty of the present work lies in the fact that the concern was mainly to correlate PSCs with welfare and to rise and dealing wih the "toxic" effects of PSCs in livestock production,

In the rangelands, the variety of PSCs to which the animal is exposed and ingested is much greater compared to the stall-fed animals which is limited to the five ingredients of the ration and thereby the challenges in these two situations are different. The stall-fed animals are in a nutritional context where the high availability of nutrients probably enhanced the detoxification processes but at rangelands, the issue is more complex. Depending on situations, animals may either "eat the best and leave the rest" or "mix the best first, then eat the rest" (Villalba *et al.* 2004) adapting to the composition of the plants available to them. Trying to extrapolate what have been observed in the present doctoral study, it can be hypothesised that:

a) At pasture a higher consumption of PSC-rich plants could be triggered in a favourable nutritional context (obtained either at pasture by associating complementary feed resources, or by strategic and specific supplementation);

b)In stall, especially for short durations such as fattening periods, the negative side-effects of incorporating high amounts of PSC-rich by-products could be controlled with feeding strategies inspired from those observed on the rangeland (= combining species with complementary features, providing water and nutrients in a given order during the day).

4. Issues

Taking into account all the studies that have been done in the present doctoral thesis, the common denominator is the pursuance of the objective of investigating means that can be used to improve sheep farming system efficiency in the Mediterranean region by promoting a better utilisation of local resources. At the same time, in lines with the trend of "clean ethical and green" (CEG) (Martin et al. 2004; Bickell et al. 2010) concept of livestock production, the means investigated should ensure animal welfare, be environmentally sound and be ethical allowing a level of livelihood of the farmers.

The issue of plant bioactive substances, especially the PSCs, is one of the main factors affecting livestock nutrition either in stall-fed or pastoral based system and a considerable amount of literature is available on the bearing that PSCs have in modulating feeding behaviour, performance and animal health in diverse types of sheep rearing system all around the Mediterranean. There are both positive and negative aspects of PSCs and therefore, any means would entail a balancing exercise of these aspects. Considering only the realities of the Mediterranean system of sheep production, there are many common features especially a very long tradition of pastoral system, which is dwindling, or getting "diluted" in many areas as found in the survey carried out in Morocco. Meuret (1997,) described how shepherds are cognizant of the ecological and biological making up of their landscape and animal behaviour; how they ingeniously integrate these observations in designing an ingenious and yet relatively simple system to better tap resources available to them. The experimental trial showed how it is feasible to achieve good performance by incorporation of polyphenols rich agro-industry byproducts, in partial substitution of cereals in lambs fattening diets formulated in such a way that animals cannot avoid unpalatable feed, and at the same time

maintaining welfare. A similar parallel can be drawn in the rangelands of Morocco, whereby the animals were coaxed to ingest diverse types of vegetation modulated with seasons and sites, and no notable health and performance issues were reported by shepherds. Shepherds decision was not based on nutritive value of the plants but rather on complementarity of the species in terms of their availability and on animal behaviour.

Rangelands across the Mediterranean, although very diverse in terms of the species found, are nevertheless subjected to similar types of pressures especially in terms of climate change and human activity. Many authors have reported on the uniqueness of the Mediterranean livestock products and have stressed out the importance of livestock system, particularly exploitation of natural grasslands and rangelands by shepherds and other pastoralist putting into perspective their influence in the maintenance of human activity in remote and rural area and in maintenance of the natural environment avoiding their degradation (Ligda et al., 2013). There are several studies that have brought in light the role of livestock production in particular in maintenance of natural environment and landscape and these are the strengths on which policy makers should consider instead of the solely economic aspects (Belibasaki, 2012; Boyazoglu and Morahnd-Fehr 2001; Casabianca 2011; Casabianca and Matassino 2006; da Gama, 2006; Sossidou *et al.* 2004; de Rancourt *et al.* 2006). For example, herders activity prevent encroachment of natural rangelands on hills, for instance by shrubs, which can increase propensity for fire outbreak (Casasus *et al.* 2007). This is one typical example of how the activity of grazing animals on grazelands can be valorised. The other issue arising from this thesis project is that the agro-industrial by-products finds a new perspective and a new reason for their use in livestock feeding based on the potential beneficial effects of PSCs on animal health and welfare and product quality. The old vision of animal as a means to recycle problematic and even polluting biomasses is an extremely hot topic, considering that the new European Union research and innovation programme, Horizon 2020, gives to the “waste resources” a huge importance.

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