Dieta del rebeco en el pirineo oriental: efectos del ganado doméstico y de los parásitos

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CERTIFIQUEN

Que **Arturo Leonel Gálvez Cerón** ha realizat sota la seva direcció el treball de recerca:

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A todas aquellas personas que sueñan y emprenden acciones por un mundo más justo y más armonioso.

CAPÍTULO 1

¿Qué sabemos sobre el solapamiento de dietas entre herbívoros salvajes y domésticos?

Los pastos: nuevos retos, nuevas oportunidades (2013) 161-169

Resumen

En el campo de la ecología trófica, un tema prioritario es estudiar cómo el solapamiento de dietas conduce a procesos de coexistencia o de competencia. Para conocer cuál es el estado de conocimiento actual sobre esta cuestión, realizamos una revisión bibliográfica en el buscador científico *Thomson Reuters Web of Knowledge* y en la bibliografía especializada no indexada escrita en castellano. La revisión se centró en trabajos sobre las familias Bovidae, Equidae y Cervidae. En concreto, evaluamos cómo la disponibilidad de recursos, el hecho de que las especies perteneciesen a una familia de ungulados concreta, y su naturaleza salvaje o doméstica, influyen sobre el solapamiento en la dieta. Para analizar la información usamos una selección de modelos basada en el criterio de Akaike. El solapamiento fue máximo cuando conviven bóvidos con equinos, y mínimo cuando lo hacen cérvidos con bóvidos. La disponibilidad de alimento tuvo poca importancia sobre el solapamiento, y el hecho de que las especies sean salvajes o domésticas no tuvo ninguna influencia. Para comprender en qué medida el solapamiento implica competencia, serán necesarios estudios que evalúen cómo los cambios en los hábitos alimenticios de especies en simpatría influyen sobre la aptitud biológica de las poblaciones (fitness).

Palabras clave: Competencia trófica, disponibilidad de recursos, pastoreo mixto.

What do we know about diet overlap between wild and domestic herbivores?

Abstract

A cornerstone in the field of trophic ecology is understanding whether diet overlap leads to coexistence or competence. In order to know the current understanding about this, we performed a literature review in both the *Thomson Reuters Web of Knowledge* and other specific journals written in Spanish. This review was focused on theBovidae, Equidae, and Cervidae families. Concretelly, our objective was to explored whether food availability, the co-occurrence of ungulates belonging to specific taxa and the fact of being wild or domestic influenced diet overlap. Model selection was based on the Akaike information criteria. Diet overlap peaked between bovidae and equidae members, but was low between bovids and equids. Food availability had few influence on diet overlap and the fact of being wild or domestic had no effect. Further research on the impact of dietary shifts due to species co-occurrence will be required to understand whether diet overlap results in competition for food.

Key words: Competition for food, food availability, mixed grazing.

Introducción

Un principio de la ecología de comunidades es minimizar la competencia entre las especies mediante el uso de recursos alimentarios distintos. El pastoreo mixto se basa en este principio, ya que pastorear con más de una especie animal permite optimizar el uso de las áreas de pastoreo minimizando el impacto sobre el medio (Walker, 1997). Aunque el pastoreo mixto reduce el impacto de los parásitos (Horak et al, 1999) y mejora la producción del ganado (Dickson et al, 1981; Connolly y Nolan, 1976), no todas las combinaciones son óptimas (Celaya et al, 2007). Por ejemplo el pastoreo con ovejas y vacas o cabras y ovejas, suele generar competencia (Nyangito et al, 2008). Un criterio habitual para detectar la competencia por el alimento es el estudio del solapamiento entre dietas (Beck y Peek, 2005), que se define como: la proporción de especies vegetales consumidas al mismo tiempo por dos especies animales que pastan en simpatría (Magurran, 1989). Se suele medir en forma de índices de similitud, siendo los más habituales los de Sorenson, Morisita-Horn (Magurran, 1989) y de Kulcyznski (Olsen y Hansen, 1977; Aldezabal y García-González, 2003). No obstante, solapamiento no suele implicar competencia si los recursos son abundantes (Gallina, 1993).

En ungulados salvajes, el estudio del solapamiento de dietas se usa como indicador de solapamiento de nicho ecológico entre especies (Acevedo y Cassinello, 2009). Además, se ha utilizado para evaluar el impacto de la ganadería sobre los ungulados salvajes o viceversa (Bhattacharya et al, 2012). A pesar de que existen muchos trabajos al respecto, aún se desconoce qué factores favorecen el solapamiento. En este trabajo, tras realizar una revisión bibliográfica de la literatura publicada desde los años 60, exploraremos qué influencia tienen la disponibilidad de recursos y la combinación de ungulados pertenecientes a distintas familias sobre el solapamiento entre las dietas.

Material y Métodos

Realizamos una revisión bibliográfica en la *Thomson Reuters Web of Knowledge* utilizando palabras clave: “*diet overlap*”, “*dietary overlap*”, “*feed overlap*”, “*diet competition*”, “*resource competition*”, “*mixed grazing*”, “*multispecies grazing*”, "*herbivores*", y "*domestic grazing*". El periodo de búsqueda fue desde 1960 al año 2012. Además se revisaron otras publicaciones especializadas pero no indexadas, en concreto los trabajos publicados en la revista PASTOS (periodo 1994 – 2011) y Actas de Reuniones Científicas de la Sociedad Española para el Estudio de los Pastos –SEEP- (1998 - 2012), revista Información Técnica Económica Agraria –ITEA- (2005 – 2012). En total, se revisaron 106 trabajos científicos sobre el tema, y se escogieron 82 que cumplían con las categorías buscadas. Los hábitats descritos por cada autor se reclasificaron en las ecorregiones propuestas por Olson et al (2001), cuya nomenclatura ha adoptado el Fondo Mundial para la Naturaleza (WWF, por sus siglas en inglés) para los programas de conservación en todo el mundo.

De cada publicación extrajimos la siguiente información, que se utilizó como variables explicativas: 1) la disponibilidad de recursos alimenticios durante el periodo de estudio (variable categórica: no limitante -NL- y limitante -L-), 2) las familias a las que pertenecían los herbívoros estudiados y 3) si los herbívoros eran domésticos o salvajes. Los trabajos fueron asignados a una de las categorías de recursos (NL, L), en función del criterio de los propios autores. Es decir, si los autores indicaban que el muestreo se había realizado en una zona o época en la que los recursos podían ser limitantes (p.ej., invierno en zona templada, y sequía en zona tropical), el trabajo se asignó a la categoría "L". Finalmente, la variable respuesta: "grado de solapamiento de la dieta", se codificó en tres categorías: Bajo, para coeficientes de solapamiento menores de 0.4; Medio: para valores mayores o iguales de 0,4 y menores de 0,6, y Alto: para los iguales o mayores de 0,6. Según Magurran (1989), un valor de coeficiente de solapamiento igual a 1 indica que las dietas se componen de las mismas especies vegetales, un valor de 0 indica lo contrario. Se contabilizó cada pareja de especies animales reportadas por los autores (n = 547), en sus distintas combinaciones: salvaje/doméstico, familias taxonómicas, recursos disponibles (limitados o no), hábitat, y grado de solapamiento de sus dietas (alto, medio o bajo).

Para estudiar cómo la disponibilidad de recursos, pertenencia a determinada familia taxonómica y su naturaleza doméstica o salvaje, influyó sobre el solapamiento de dietas, utilizamos modelos lineares generalizados (GLM) con distribución de errores

Poisson y función "log". Para este análisis, sólo consideramos aquellos trabajos que estudiaron el solapamiento de dieta entre combinaciones de especies de las familias Bovidae, Cervidae, y Equidae.

La selección de modelos estadísticos se realizó bajo una aproximación basada en la "Theoretic Information Approach" y el uso del criterio de Akaike (AIC). Además, calculamos el peso de Akaike (w_i), definido como la probabilidad de que un modelo sea el mejor entre los candidatos. Más información sobre este procedimiento puede encontrarse en Burnham y Anderson (2002).

Resultados y Discusión

Se encontraron 547 datos relacionados con el solapamiento de dietas entre herbívoros pertenecientes a diferentes familias, aunque sólo 381 se centraron en las familias Bovidae, Cervidae y Equidae. El 39% de los trabajos se han realizado sobre especies domésticas, el 22% con especies salvajes y un 39% en la interacción entre ambas. Los datos recogidos corresponden a trabajos realizados en el continente asiático (31,1%), europeo y africano (20,5% cada uno), australiano (3,8%) y suramericano, sólo Argentina, con el 5,9%. España aporta el 48,2% de la información publicada en Europa.

La selección de modelos indicó que el 20% de la variabilidad observada en el solapamiento de dietas se puede explicar por la disponibilidad de recursos (NL-L) y por el pastoreo en simpatría de especies de ungulados de familias determinadas ($w_{\text{familias + disponibilidad}}$ = 0.74, Tabla 1). Si los herbívoros son salvajes o domésticos, pareció no influenciar el solapamiento entre dietas. De forma general, el solapamiento fue superior cuando los recursos son abundantes ya que los animales tienden a seleccionar el alimento más nutritivo y palatable (Ego et al, 2003). Solapamientos medios se encontraron entre ovejas y cabras domésticas (Bartolomé et al, 1998) o salvajes (Martinez, 1988 y 2002). Sin embargo, el efecto de la disponibilidad de alimento sobre el solapamiento es pequeño (β = 0,03, ES = 0,07), con lo cual tenemos que ser precavidos a la hora de generalizar este resultado. De hecho, hay trabajos que describen que, cuando los recursos son escasos, el solapamiento de dietas suele ser alto entre bóvidos y cérvidos (García-González y Cuartas, 1992, Cuartas et al, 2000; Miranda et al, 2012) o entre bóvidos de diferentes especies (Xu et al, 2012). No obstante, e independientemente de si los recursos son o no abundantes, la selección de modelos indicó que el mayor solapamiento se produce cuando conviven especies de bovinos con equinos (β = 0.09, ES = 0.08, Z = 1.15). Una posible explicación es que ambos tienen un marcado hábito de pastoreo (Hofmann, 1993), principalmente de graminoides (Aldezábal et al, 2012), lo que justificaría un alto solapamiento durante todo el periodo de pastoreo. Al contrario, el menor solapamiento ocurrió cuando conviven especies de cérvidos y bóvidos (β = - 0.38, ES = 0.1, Z = -6.61). La familia Cervidae es mayoritariamente ramoneadora o intermedia, y ovinos y bovinos (Bovidae) pastadores, pudiéndose especializar cada una en una dieta distinta, p.ej. *Cervus elaphus* y *Bos taurus* (Pordomingo y Rucci, 2000). Las cabras, tanto salvajes como domésticas, generalmente se comportan como ramoneadoras, y ovejas y muflones, pastadores (García-González y Cuartas, 1989; Cuartas y García-González, 1992).

Solapamiento	Bovidae - Bovidae		Bovidae - Cervidae		Bovidae - Equidae		TOTAL	
	NL	L	NL	L	NL	L	NL	L
Bajo	11 (6,1)	14 (10,2)	26 (14,4)	10 (7,3)	4 (2,2)	3 (2,2)	41	27
Medio	18 (9,9)	22 (16,1)	10 (5,5)	7 (5,1)	5 (2,8)	5 (3,6)	33	34
Alto	68 (37,6)	37 (27,0)	6 (3,3)	8 (5,8)	33 (18,2)	31 (22,6)	107	76
Total	97 (53,6)	73 (53,3)	42 (23,2)	25 (18,2)	42 (23,2)	39 (28,5)	181	137

Bovidae – Bovidae, trabajos que estudian el solapamiento entre diferentes especies de bóvidos: Awan *et al*, 2006. *Mammalia.*70: 261-288; Bartolome *et al*, 1998. *J. Range Manage..* 51: 383-391; Breebaart *et al*, 2002. *Afr. J. Range & Forage Sc..*19: 13-20; Celaya *et al*, 2007. *Livestock Sc.* 106: 271-281; Celaya *et al*, 2008. *Animal.* 2: 1818-1831; Connolly y Nolan, 1976. *Anim. Prod..* 23: 63-71; Dailey *et al*, 1984. *J. Wildl. Manage..* 48: 799-806; Dawson y Ellis, 1996. *J. Arid. Environ..* 34: 491-506; de Iongh *et al*, 2011. *J. Trop. Ecol..* 27: 503-513; Dickson *et al*, 1981. *Anim. Prod..* 33: 265-272; Ego *et al*, 2003. *Afr. J. Ecol..* 41: 83-92; García-González y Cuartas, 1989. *Acta. Biol. Montana.* 9: 123-132; Horak *et al*, 1989. *Livest. Prod. Sci..* 61: 261-265; Karmiris y Nastis, 2010. *C. Eur. J. Biol.* 5: 729-737; La Morgia y Bassano, 2009. *Ecol. Res..* 24 : 1043-1050; Li *et al*, 2008. *J. Wildl. Manage..* 72: 944-948; Liu y Jiang, 2004. *J. Wildl. Manage..* 68: 241-246; Makhabu, 2005. *J. Trop. Ecol..* 21: 641-649; Mandaluniz *et al*, 1999. *Acta. Biol.* 9: 123-132; Martínez, 1988. *Arch. Zoot.* 37: 39-49; Martínez, 2002. *Acta. Theriol..* 47: 479-490; Namgail *et al*, 2004. *J. Zool..* 262: 57-63; Namgail *et al*, 2010. *J. Arid Environ..* 74: 1162-1169; Nyangito *et al*, 2008. *J. Human. Ecol.* 23: 115-123; Prins *et al*, 2006. *Afr. J. Ecol..* 44: 186-198; Quintana, 2003. *Mammalia.* 67: 33-40; Walker, 1994. *Sheep. Res. J.*52-64.

Bovidae – Cervidae, trabajos que estudiaron el solapamiento entre especies de bóvidos y cérvidos: Acevedo y Casinello. 2009. *Ann. Zool. Fenn..* 46: 39-50; Ahrestani *et al*, 2012. *J. Trop. Ecol..* 28: 385-394; Bertolino *et al*, 2009. *J. Zool..* 277: 63-69; Cuartas *et al*, 2000. *Acta. Theriol..* 45: 309-320; Ekblad *et al*, 1993. *Small. Rum. Res..* 11: 195-208; Elliott y Barret, 1986. *J. Range Manage..* 38: 546-550, Findholt *et al*, 2004. *N. Am. Wild. & Na. Res. Conf..* 69: 670-686; Gallina, 1993. *J .Range Manage..* 46: 487-492; García-González y Cuartas, 1992. *Mammalia.* 56: 195-202; Homolka y Heroldová, 2001. *Fol. Zool..* 50: 89-98; Ihl, y Klein, 2001. *J. Wildl. Manage..* 65: 964-972; Kingery *et al*, 1996. *J. Range Manage..* 49: 1-15; Larter y Nagy, 1997. *Rangifer.* 17: 13-17; Miranda *et al*, 2012. *Wildl. Res..* 39: 171-182; Pordomingo y Rucci, 2000. *J. Range Manage..* 53: 649-654, Thill y Martin, 1986. *J. Wildl. Manage..* 50: 707-713.

Bovidae-Equidae, trabajos que evaluaron el solapamiento entre especies de bóvidos y équidos: Aldezábal *et al*, 2012. *51ª RC SEEP*, 325-330; Krysl *et al*, 1984. *J. Range Manage..* 37: 72-76; Loiseau y Martinrosset, 1988. *Agronomie.* 8: 873-880; Mcinnis y Vrava, 1987. *J. Rang. Manage..* 40: 60-66; Menard *et al*, 2002. *J. Appl. Ecol..* 39: 120-133

Trabajos mixtos, aquellos que evaluaron más de una combinación anterior: Abaye *et al*, 1994. *J. Anim. Sci..* 72: 1013-1022; Aldezábal, 2001. CPNA, 317pp; Beck y Peek, 2005. *Rang. Ecol. & Manage..* 58: 135-147; Bhattacharya *et al*, 2012. *Proc. Zool. Soc..* 65: 11-21; Campos-Arceiz *et al*, 2004. *Ecol .Res..* 19:455—460; Chu Hong-Jun *et al*, 2008. *Acta. Zool. Sinica.* 54: 941-954; Heroldova, 1996. *For. Ecol. Manage..* 88: 139-142; Maccracken y Hansen, 1981. *J. Range Manage..* 34: 242-243; Kleynhans *et al*, 2011. *Oikos.* 120: 591-600; Mishra *et al*, 2004. *J. Appl. Ecol..* 41: 344-354; Mysterud, 2000. *Oecologia.* 124: 130-137; Olsen y Hansen, 1977. *J. Range Manage..* 30: 17-20; Osoro *et al*, 2005a. *XLV R.C. SEEP..* 45-71; Osoro *et al*, 2005b. *XLV R.C. SEEP.* 253-259; Puig *et al*, 2001. *J. Arid. Environ..* 47: 291-308; Ruben Vila *et al*, 2009. *J. Wildl. Manage..* 73: 368-373; Sietses *et al*, 2009. *Mamm. Biol.* 74: 381-393.

Tabla 1. Trabajos (n y %) que evalúan el solapamiento de dietas entre ungulados pertenecientes a las familias Bovidae, Cervidae y Equidae. Solapamiento Bajo (<0,4), medio (≤ 0.4 y < 0,6), alto (≥ 0,6).

Conclusiones

El estudio del solapamiento de dietas es un criterio comúnmente extendido para evaluar cuando las especies de herbívoros comparten los mismos recursos alimenticios. No obstante, alto solapamiento de dieta no implica competencia si los recursos son abundantes. Del mismo modo, la ausencia de solapamiento puede indicar que las especies se alimentan de forma diferente para minimizar la competencia. Desde un punto de vista ganadero, habrá que prestar especial atención cuando se realice pastoreo mixto con caballos y vacas, ya que sus dietas van a ser muy similares independientemente de la disponibilidad del alimento. En el caso de las interacciones entre herbívoros salvajes y domésticos, será necesario realizar trabajos a largo plazo para estudiar en qué grado modifican su dieta las especies salvajes en presencia de los domésticos y viceversa. Además, para concluir que el solapamiento entre dietas implica o no competencia, será necesario incluir medidas adicionales sobre el impacto de la convivencia entre especies sobre parámetros relacionados con la aptitud biológica (fitness) de las poblaciones salvajes.

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CAPÍTULO 2

Forage selection and diet overlap between Pyrenean chamois and livestock in eastern Catalan Pyrenees

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Abstract

In summer, the period of greatest primary production, Pyrenean chamois (*Rupicapra pyrenica*) shares territory with livestock (cattle, horse and sheep), causing interspecific interactions such as dietary overlap and spatial segregation. This study was conducted in two areas (Costabona and Fontalba) of the Freser-Setcases National Game Reserve (FSNGR), Eastern Pyrenees, Catalonia, Spain. The vegetation availability was determined by measuring the plant cover. The botanical composition of diets was determined by fecal microhistological analyses, in Summer and Autumn 2011-2012. The Kulzynski and Morisita-Horn similarity index was used to compare the diet of chamois and livestock. Spearman's rank order correlation coefficient was used to evaluate the correlation in diet composition between the pairs of animal studied. The Savage index was used to calculate foraging preferences of each animal species, for each plant species in each season. Availability of plant species in summer appears very similar in both study zones. Graminoids cover represents half of the vegetation, where *Festuca* sp. is clearly dominant followed by *Carex cariophyllea*. Forbs cover almost one third of the area and are dominated by *Trifolium alpinum*. The rest are woody species, the most common being *Calluna vulgaris*, *Juniperus communis* and the legume *Cytisus scoparius*. Over the whole sympatric period, a total of 42 taxa were identified in fecal samples. The diet of chamois is clearly different to livestock with a higher consumption of graminoids (*Calluna vulgaris* 25% and *Cytisus scoparius* 19%). The horse diet shows a higher consumption of graminoids (around 60%) and a lesser consume of woody species (around 7%). Cattle and sheep diets are similar, with a decreasing proportion of graminoids, forbs and woody species respectively. Chamois and sheep consume more woody species in Autumn than Summer and more forbs in Summer than Autumn. Horses consume more forbs in Autumn than Summer and cattle do not vary in any component. Both indices of similarity (SIK and C_{MH}) show the highest values in the comparisons between domestic species in both seasons and the lowest between chamois and the rest, mainly in Autumn. Our results suggest the chamois diets differ in composition and preference from livestock which graze in sympatrically. The diet of domestic species largely overlaps within them and is clearly dominated by grasses and forbs. However, the chamois diet that usually is considered a grass feeder in Summer, here appears as a browser with clear preferences for *Calluna vulgaris* and *Cytisus scoparius*. It seems obvious that the presence of livestock modifies the chamois´ diet.

Keywords: Vegetation availability, botanical composition, microhistological analysis, food preference, sympatry.

Introduction

Over the last decades, in the mountains of Europe, most of the large herbivores have increased in numbers as a direct result of conservation programmes, disappearance of predators, or some other human-induced changes (Loison et al, 2003). This growth affects specific areas of interest in ecology, such as plant-herbivore relationships or interactions between wild and domestic herbivores. When native ungulates coexist with alien species or free-ranging livestock, interspecific interactions can lead to lower density or even disappearance of one herbivore from its preferred habitats (Gordon & Illius, 1989; Forsyth & Hickling, 1998). Livestock, formed of several domestic species and a considerable number of animals, used to graze in alpine pastures during the optimum period of primary production, being a potential competitor for wild ungulates (Latham et al, 1999). Direct competition can trigger spatial segregation (e.g. Kie, 1996; Coe et al, 2001) or diet overlap between wild and domestic ruminants (e.g., Mysterud, 2000; Mussa et al, 2003).

The chamois (*Rupicapra pyrenaica* and *R. rupicapra*) is one of the most common large herbivores in European mountains, usually observed in Summer in open grasslands above the tree line. Summer is the key season for food intake and consequent body growth and the competition with livestock can have consequences on their development. The interspecific interactions between chamois and other herbivores have been studied in previous works. Diet overlap between livestock and chamois is surely the source of interaction most widely studied (e.g. Berdoucou, 1986; García-González et al, 1990; Rebollo et al, 1993; La Morgia & Bassano, 2009). Some works have shown that chamois, despite being considered an intermediate feeder, act as a grazer, feeding mostly on graminoid components, mainly when they co-exist with other wild intermediate feeders, such as red deer (*Cervus elaphus*), mouflon (*Ovis musinom*) or concentrate feeders, such as roe deer (*Capreolus capreolus*) (Bertolino et al, 2009; Ferretti et al, 2015). The highest diet overlap has been recorded between chamois and red deer despite the former prefering open habitats and the second forested habitats (Schröeder & Schröeder, 1984; Homolka & Heroldová, 2001). Some others have showed chamois entering forests in winter where it browses in woody plants (Hegg, 1961; Perle

& Hamr, 1985; García-González & Cuartas, 1996) and thus its diet changes from grazer to browser depending on the season.

The presence of livestock can force chamois to graze away from them, instead of forming mixed flocks, in safety areas where food quality is lower (Chirichella et al, 2013). Chamois often shows signs of intolerance, and the disturbance caused by interspecific encounters used to be pronounced (Forsyth & Clarke, 2001). One consequence of that disturbance is spatial segregation or space partitioning, where chamois are displaced by livestock; this behaviour having been recorded in several works (Berdoucou, 1986; García-González et al, 1990; Rebollo et al, 1993; Ruttimann et al, 2008). Segregation can also occur over time. The temporal partitioning of daily activities between chamois and other herbivores may contribute to their coexistence (Darmon et al, 2014). Sometimes, despite a large diet overlap, chamois segregation occurs in both axes, space and time (Darmon et al, 2012). A high dietary overlap between animal species can indicate competition by food resources, and chamois may have been forced to reduce their niche breath (Marchandeau, 1992; La Morgia & Bassano, 2009; Lovari et al, 2014). But in other cases, for instance chamois *vs* ibex (*Capra ibex*), both species feed on the same forage in seasons when plenty biomass is available without resource competition (Trutmann, 2009). In fact, understanding the mechanisms allowing coexistence between species, at least one dimension of their ecological niche (space, habitat resources or time) should be different (MacArthur & Lewins, 1967). One mechanism to promote segregation of wild herbivores is the avoidance for faeces of domestic animals, which is interpreted as a strategy to minimize endoparasites (Fankhauser, 2008). In fact, interactions between domestic animals and chamois can also occur through the transmission of diseases (Gaffuri et al, 2006). Usually infectious diseases have been found in wild ruminants due to transmission from livestock (Ferroglio et al, 2000; Belloy et al, 2003).

The chamois is a small ungulate species and coexistence with larger species, such red deer, cattle or horse can modify its diet, because often larger species prevail over smaller ones (Berger & Cunningham, 1998; Forsyth & Hickling, 1998; Ferretti et al, 2011). In this regard, it should be noted that small herbivores select feeds with high nutritive value and easily digestible (concentrate feed), eat more frequently, and have a greater passage rate compared with larger species (Van Soest, 1994). On the other hand, comparison between chamois and cattle showed a similar capacity to digest the diet, but cattle are most efficient in the synthesis of microbial protein because their flora maintenance requires less energy (Dalmau et al, 2006).

Chamois in sympatry with other large herbivores have also been studied in terms of nature conservation. When their feeding niches show extensive overlap during all seasons and population regulation by predators or by hunting are insufficient, then grazing can cause problems in conservation of valuable plant associations (Homolka & Heroldová, 2001; Kie & Lehmkuhl, 2001; Parkes & Forsyth, 2008).Interactions between chamois competitors and some abiotic factor, such as temperature increase due to climate change, have also been detected by Mason et al (2014).

The present study is focused on diet selection of chamois, cattle, horse and sheep in sympatry during Summer and Autumn, when all they coexist in alpine habitat, in order to contribute to better understanding interspecific relationships between different large herbivores profiles. A high diet overlap is expected because all of them are grazers or intermediate feeders (Hofmann, 1989). Previous works only has recorded changes of chamois feeding niche to a more grazer feeder when they are in sympatry with other intermediate feeders during the period of primary production. Here, we will test the hypothesis that feeding niche of chamois will changes to a more browser type when other large herbivores, such as domestic grazers coexist with them. This plasticity should contribute to sustainability of wildlife in mountain pasture ecosystem submitted to livestock extensive farming.

Material and methods

Study area

This study was conducted at the Freser-Setcases National Game Reserve (FSNGR), Eastern Pyrenees, Catalonia, Spain (42° 22′ N, 2° 09′ E, Figure 1). The FSNGR is a mountainous area of 20,200 ha where an alpine ecosystem predominates with an average altitude of 2,000 m.a.s.l. (ranging from 1,100 to 2,900 m.a.s.l. at Puigmal peak). Specifically, our work was carried out in two different areas separated by 20 Km of rough terrain: Costabona and Fontalba. The former is located in the northeast part of the FSNGR (42° 24′ N, 2° 20′ E, ranging from 1,093 to 2,429 m.a.s.l., area of 385,4 ha), whereas the later in the central part of the reserve (42° 22′ N, 2° 08′ E, ranging from 1,660 to 2,248 m.a.s.l., and an area of 823,7 ha). Both areas have similar features in terms of vegetation composition and structure belonging to the sub-humid alpine region. Annual mean temperature of 6 ºC (min = - 16.8, max = 39.2) and mean yearly accumulated rainfall (period 1999-2011) of 963 mm (min = 520.6, max = 1,324.8, data from Nuria meteorological station located at 1,971 m.a.s.l. in the core FSNGR, Servei Meteorològic de Catalunya< www.meteocat.com >).

Vegetation above 2,000 meters is mainly represented by a mosaic of alpine grasslands were graminoid species (e.g., *Festuca* and *Carex* genus) are dominant and *Trifolium alpinum* patches are abundant (for revision see Vigo, 1976; Vigo et al, 1987). Vegetation in the study lowest area consists of *Pinus uncinata* forests with a substrate of *Arctostaphylos uva-ursi*, *Calluna vulgaris*, and *Cytisus scoparius* (1,200 - 2,000 m.a.s.l.).

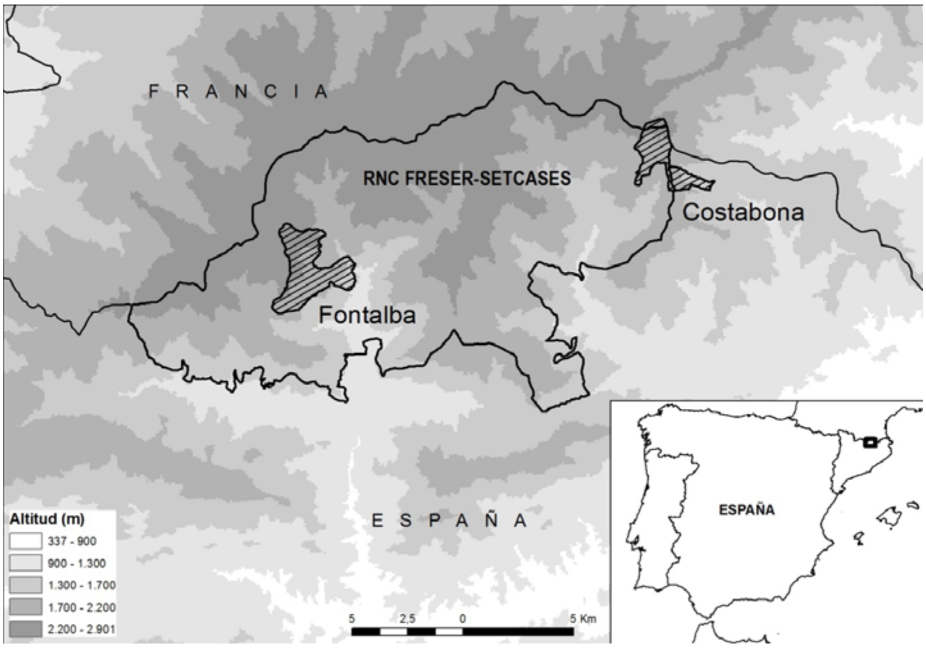

Figure 1. Location of Costabona and Fontalba zones, inside the Game National Reserve of Freser-Setcases, in the Pyrenees.

A population (average 2011-2012) of around 80 chamois from Costabona zone shares habitat with herds of approximately 600 cows, 300 sheep and 70 horses during the warm season (i.e., June to November). Another population of 150 chamois from Fontalba zone also shares habitat with 250 cows and 50 horses. No large predators are present but trophy hunting is allowed from August to February.

Sampling procedure

From June to November 2011-2012, 84 faecal samples chamois and livestock were collected by the following procedure: once a month, but often twice, each study area was visited by at least two observers. They walked two transects (one per location) of about 5 km each locating chamois groups and livestock herds by means of 10 x 42 binoculars and 20 - 60 x 65 spotting scopes. These transects cover the whole altitude range and the main vegetation communities of the study area. Once group size, composition and precise location of animals were recorded, observers collected fresh droppings at the exact place where animals were sighted and their surroundings. Fresh samples were chosen according to their colour and texture (Hibert et al, 2011). Faecal samples were collected in individually labelled plastic bags and transported to the laboratory. By mixing pellets from individual faecal groups we obtained population faecal samples that were transported to Universitat Autònoma de Barcelona facilities by keeping them in a cool box (4ºC). Following this procedure observers got between five to eight individual samples per sampling day, that constitutes a mixed sample. A total of 276 mixed samples chamois and livestock were obtained (108 for Fontalba and 168 for Costabona) from June to November 2011-2012.

Vegetation availability

The vegetation availability was determined from relative abundance of plant species by measuring the cover (Cummings & Smith, 2000) along 6 transects of 10 m length placed in five different altitudes (2000, 2100, 2200, 2300, 2400 and 2500 m.a.s.l.) of each area.

Faecal sampling processing

Once at the laboratory, faecal samples were stored frozen at -20ºC. Population samples from each sampling day were used for microhistological identification of epidermal fragments in faeces. For more than a half century (Crocker, 1959), this indirect assessment of diet composition has been by far the most common technique for assessing diet selection in both domestic (e.g. Bartolomé et al, 2011) and wild ruminants (e.g. Suter et al, 2008; La Morgia & Bassano, 2009). This technique allows collection of a representative sample of plant species ingested without interfering with animal behaviour (Bartolomé et al, 1998). However, some biases can occur due to differential digestibility of ingested plants and correction factors could be sometimes required (Holechek et al, 1982; Bartolomé et al, 1995). However, when making diet comparisons across seasons or years in the same study area, correction factors are not required.

The procedure employed in this work was adapted from Stewart (1967). Once samples were thawed, they were water washed to remove extraneous material and then ground in a mortar to separate the epidermal fragments. After that, 10 grams by sample were placed in a test tube with 5 ml of 65% concentrated HNO_3. The test tubes were then boiled in a water bath for 1 minute. After digestion in HNO_3, the samples were diluted with 200 ml of water. This suspension was then passed through 1.00 and 0.25 mm filters. The 0.25–1.00 mm fraction was spread on glass microscope slides in a 50% aqueous glycerine solution and cover-slips were fixed with DPX microhistological varnish. Two slides were prepared from each sample. Later, slides were examined under a microscope at 100–400x magnifications and plant fragments were recorded and counted until 200 units of leaf epidermis. An epidermis collection of 40 main plant species of the study area was made and used for fragment identification. Finally, plants were pooled into five groups: non legume woody sp (NLW), legume woody sp (hereafter LW), graminoids sp (G), legume forb sp (LF) and non legume forb sp (NLF).

Stocking rate

Stocking rate is the number of animals grazing in a determined space and time (García-González & Marinas, 2008). The animal unit month (AUM) concept is the most widely used way to determine the approximate amount of forage a 500 kg cow will eat in one month. All other animals were than converted to an "Animal Unit Equivalent" (AUE) of this cow: sheep = 0.20, horse = 1.25 and chamois = 0.15 (Pratt & Rasmussen, 2001).

Statistical analysis

In order to describe the diet of sympatric animals, the number of leaf epidermal fragments of each species was converted to percentages (as response variable) and subjected to arcsine (angular) transformation (Sokal & Rohlf, 1969) before statistical analysis. Species were grouped in three main groups: woody species, graminoids and forbs. Three factors that could influence the composition of epidermal fragments were considered: animal species (chamois, cattle and horse), zone (Costabona and Fontalba) and season (Summer and Autumn). A second analysis was done including sheep but in this case zone factor was not considered. Allocation of total sum of squares between factors was determined by analysis of variance (ANOVA) using StatView for Windows (SAS Institute, Inc.). Significant differences were determined by means of Fischer Protected LSD method (Fischer, 1949). According to Krebs (1999), there are more than two dozen measures of similarity available and much confusion exists about which measure to use. Similarity coefficients are mainly descriptive, not estimators of some statistical parameter. Here, relative abundance of diet species has been measured and three similarity coefficients have been calculated:

1.- The Kulczynski's similarity index (Kulczynski, 1927) has been employed in many works about diet overlapping between herbivores (e.g. García-González & Cuartas, 1992; Dikinson, 1994). Here, it has also been used to compare animal species diets:

$$S.I.K = \frac{2\sum c}{\sum(a+b)} + 10o$$

where *c* is the lesser percentage of a common plant species or taxon in the diet and ∑(a+b) is the sum of the percentages of all the species in the two diets.

2.- The Morisita-Horn index of similarity (Horn, 1966) was also calculated:

$$I_{M-H} = \frac{2\,\Sigma\left(an_i \times bn_j\right)}{(da + db)aN \times bN}$$

Where

an_i = number of individuals i in sample A

bn_j = number of individuals j in sample B

aN = total numbers of individuals in sample A

bN = total numbers of individuals in sample B

$da = \sum an^2_i / aN^2$

$db = \sum bn^2_j / bN^2$

This formula is appropriate when the original data are expressed as proportions rather than numbers of individuals and should be used when the original data are not numbers but biomass, cover, or productivity (Krebs, 1999).

3.- Spearman's rank order correlation coefficient (r_s) was used to evaluate the correlations in diet composition between the pairs of animals studied. It is employed when one do not wish to make the assumption of a linear relationship between species abundances in the two diets. But correlation coefficients may be undesirable measures of similarity because they are all strongly affected by sample size, especially when most of the abundances are zero in the samples (Krebs, 1999). Here, sample sizes were similar. The Savage index (Manly et al, 1993) was used to calculate foraging preferences of each animal species, for each plant species in each season. This index determines selectivity of a given resource by relating its use with its availability:

$$W = \frac{Oi}{ni}$$

Where, Oi, is the proportion of the sample of used resource units that are in category i and ni. the proportion of available resource units that are in category i.

The Savage index varies from zero (maximum rejection) to infinite (maximum preference), where 1 is the value defining the selection expected by chance. The statistical significance of these index was tested by comparing the Savage statistic with that corresponding to the critical value of freedom (Manly et al, 1993):

$$Savage\ statistic = \frac{(Wi - 1)^2}{se(Wi)^2}$$

The standard error of the index is:

$$se(Wi) = \sqrt{\frac{(1 - \pi)}{u_t + \pi i}}$$

Where u_t is the total number of used resource units sampled.

In order to evaluate differences between indices of selection of plant species, Savage index with the modification proposed by Kautz and Van Dyne (1978) was also calculated. This amended index allows obtaining preferential measures positive and negative, symmetrical with respect to zero, which allows an analysis of variance:

$$logWi = \frac{(Oi + 0.1)}{(\pi i + 0.1)}$$

To control the error produced by multiple comparisons in the Savage index, we used Bonferroni correction, to adjust the significance of the statistical test.

Results

Availability of plant species at the beginning of summer appear very similar in both study zones when Spearman rank correlation is applied (r_s = 0.765, P-value < 0,0001). Table 1 shows mean percentages of plant availability and diet composition for each animal. See Annexes minimums and maximums in the composition of the diet for each animal and each area (2011-212).

Graminoids cover represents half of the vegetation, where *Festuca* sp. is clearly dominant and is followed by *Carex cariophyllea*. Forbs cover almost one third of the area and are distributed half and half between legumes and non legumes. Legumes are dominated by *Trifolium alpinum* and any non legume species can be considered as dominant. The rest are woody species, where the most common are the dwarf shrubs (*Calluna vulgaris* and *Juniperus communis* ssp. *communis*) and the legume *Cytisus scoparius*. Over the whole sympatric period, a total of 42 taxa were identified in faecal samples. In general, the most available species are the most common in diet but it varies between animals and seasons. For a subsequent analysis of variance, plant species were grouped in three forage classes: woody species (including the dwarf shrubs), graminoids (Gramineae, Juncaceae and Cyperaceae families) and forbs.

Table 1. Mean percentages of plant cover (availability) and diet composition of each animal species in the Catalan Pyrenees during summer and autumn (2011-2012).

	Plant	**Chamois**		**Sheep**		**Cattle**		**Horse**	
	cover	**Sum.**	**Aut.**	**Sum.**	**Aut.**	**Sum.**	**Aut.**	**Sum.**	**Aut.**
Non-Leguminous Wood									
Calluna vulgaris	7.64	17.71	31.50	1.75	1.67	10.75	7.83	4.13	3.08
Juniperus communis	4.87	0.00	0.04	0.00	0.50	0.00	0.08	0.00	0.00
Pinus uncinata	1.49	0.88	0.79	2.92	3.75	1.88	5.38	1.92	1.04
Quercus sp.	0.04	0.08	2.63	0.00	1.17	0.00	0.00	0.00	0.00
Rhododendron ferrugineum	0.10	0.08	0.58	0.00	0.00	0.00	0.00	0.00	0.00
Rosa sp.	0.26	3.00	3.00	1.25	2.42	1.38	0.50	0.25	0.33
Rubus sp.	0.18	4.63	7.42	2.50	12.00	4.04	2.29	0.25	0.58
Subtotal	**14.6**	**26.46**	**46.71**	**8.42**	**21.50**	**18.04**	**16.08**	**6.54**	**5.04**
Leguminous Woody									
Cytisus scoparius	4.59	15.33	23.08	0.00	1.08	0.04	1.08	0.00	0.71
Subtotal	**4.59**	**15.33**	**23.08**	**0.00**	**1.08**	**0.04**	**1.08**	**0.00**	**0.71**
Graminoids									
Festuca sp.	33.07	20.54	14.25	36.75	46.17	39.29	39.63	48.96	45.17
Agrostis sp.	0.22	0.63	0.13	2.75	0.58	2.75	3.88	4.13	3.17
Avenula pratensis	1.57	1.42	1.46	2.08	2.42	2.58	3.67	2.21	1.79
Carex caryophyllea	11.03	0.25	0.42	0.33	1.42	0.63	0.42	1.50	1.04
Nardus stricta	3,03	0.00	0.33	0.00	0.00	0.00	0.00	0.13	0.00
Poa sp.	0.98	0.50	0.21	0.67	0.08	2.83	2.38	6.04	4.29
Subtotal	**50.285**	**23.92**	**16.92**	**42.75**	**50.83**	**48.08**	**50.00**	**63.04**	**55.63**
Non-Leguminous Forbs									
Antennaria dioica	0.68	0.33	0.04	0.08	0.00	0.17	0.00	0.08	0.17
Cerastium sp.	0.54	0.13	0.00	0.00	0.00	0.04	0.00	0.08	0.00
Cruciata glabra	1.04	1.08	1.71	0.50	0.00	1.29	0.58	0.13	0.17
Galium verum	0.59	0.21	0.00	0.17	0.33	0.58	0.17	0.29	0.29
Gentiana sp.	0.69	0.04	0.00	0.00	0.00	0.00	0.08	0.04	0.00
Helianthemum nummularium	0.08	0.25	0.13	0.00	0.42	0.54	0.04	0.00	0.08
Hieracium pilosella	1.9	2.25	0.88	1.92	2.17	0.83	0.42	1.29	3.58
Pedicularis pyrenaica	0.68	0.25	0.00	0.33	0.00	1.13	0.42	0.50	0.38
Plantago monosperma	2.06	4.50	0.88	5.08	4.33	3.38	4.04	3.71	5.67
Plantago media	0.28	2.25	0.08	0.83	1.50	0.54	0.17	0.42	1.29
Potentilla sp.	0.9	3.38	0.67	5.58	2.33	3.25	3.63	2.46	3.75
Ranunculus bulbosus	0.19	0.88	0.04	3.92	3.50	2.42	3.54	1.42	2.67
Sempervivum montanum	0.08	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Silene acaulis	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Taraxacum sp.	0.85	0.25	1.17	0.00	0.00	0.00	0.00	0.00	0.00
Thymus nervosus	0.96	0.46	0.13	0.42	0.00	0.38	0.08	0.75	0.75
Veronica sp.	0.07	1.63	0.33	1.83	1.42	1.46	1.00	0.88	0.79
Subtotal	**16.17**	**18.33**	**6.04**	**21.00**	**16.17**	**16.42**	**14.33**	**12.13**	**19.63**
Leguminous Forbs									
Anthyllis vulneraria	0,11	0,83	0.50	4.42	3.58	2.50	2.33	1.58	1.17
Astragalus sp.	0.24	0.00	0.29	0.17	0.17	0.88	0.83	1.08	0.92
Chamaespartio sagittalis	0.60	0.00	0.00	1.50	0.75	0.29	0.17	0.38	1.58
Hippocrepis comosa	0.75	0.13	0.00	1.67	0.08	0.38	0.00	0.08	0.00
Lotus corniculatus	1.18	1.42	0.71	1.75	0.42	0.96	1.38	0.92	0.67
Trifolium alpinum	9.75	8.08	5.25	10.42	2.58	8.21	8.88	8.71	8.00
Trifolium pratense	0.62	3.96	0.33	6.58	2.67	3.00	4.33	4.13	5.71
Trifolium repens	0.96	1.46	0.17	1.08	0.17	1.17	0.58	1.42	0.75
Vicia pyrenaica	0.12	0.08	0.00	0.25	0.00	0.04	0.00	0.00	0.21
Subtotal	**14.34**	**15.96**	**7.25**	**27.83**	**10.42**	**17.42**	**18.50**	**18.29**	**19.00**

All seasonal dietary overlap correlation coefficients between animal species are positive and highly significant (Table 2). Both index of similarity (SIK and C_{MH}) show the highest values in the comparisons between domestic species in both seasons and the lowest in the comparisons between chamois and the rest, mainly in Autumn.

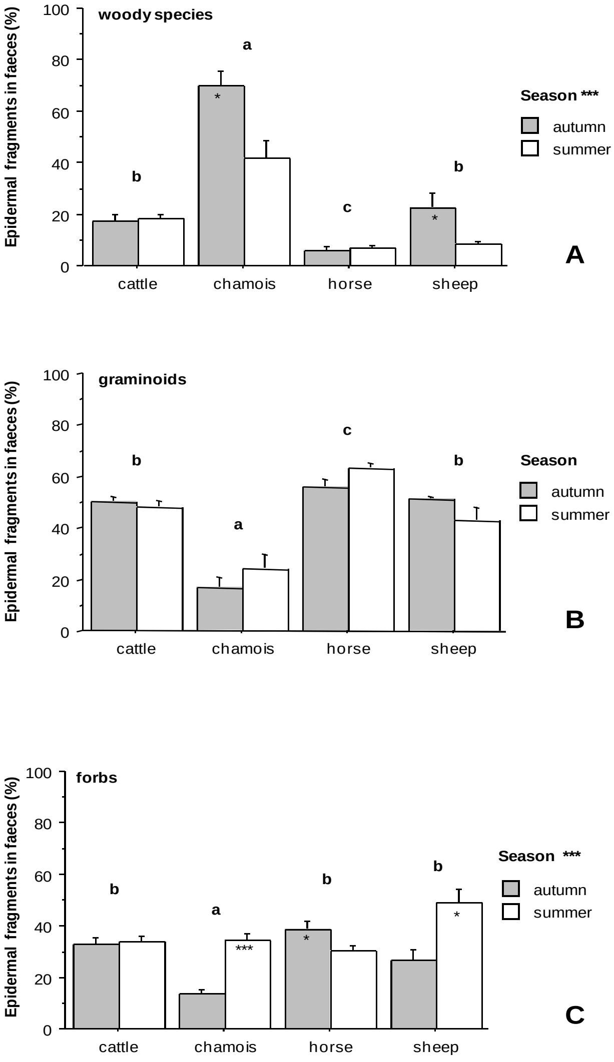

Figure 2. Comparison of diet components between sympatric animal species during Summer and Autumn in the Catalan Pyrenees. Lines above bars are standard errors. Different letters above bars indicate significant differences between animals ($p < 0.05$). Asterisks (*) next to season indicate significant differences between seasons and inside the bars indicates significant differences between seasons for each animal species (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Table 2. Dietary overlap among chamois, cattle, horse and sheep during the grazing seasons: Summer (June - August) and Autumn (September – November) in the Catalan Pyrenees. Data includes Kulczynski similarity index (SIK), Morisita-Horn index of similarity (C_{MH}) and Spearman's rank correlation coefficient (r_s). Significant relationships are denoted with asterisks (*p < 0.05, **p< 0.01, ***p < 0.001).

Pair of species	Season	SIK	C_{MH}	r_s
Chamois-Sheep	Summer	53.25	0.61	0.720***
	Autumn	27.83	0.33	0.533***
Chamois-Cattle	Summer	52.79	0.66	0.766***
	Autumn	40.25	0.44	0.513***
Chamois- Horse	Summer	44.54	0.57	0.618***
	Autumn	27.13	0.35	0.371*
Sheep-Cattle	Summer	65.25	0.87	0.888***
	Autumn	64.58	0.89	0.775***
Sheep-Horse	Summer	68.75	0.89	0.856***
	Autumn	64.17	0.88	0.721***
Cattle-Horse	Summer	69.21	0.90	0.880***
	Autumn	68.88	0.91	0.870***

There are 11 plant species that appeared as clearly avoided for all animal species. They are: *Carex cariophyllea*, *Cerastium* sp., *Gentiana* sp., *Juniperus communis*, *Nardus stricta*, *Quercus* sp., *Rhododendron ferrugineum*, *Sempervivum montanum*, *Silene acaulis*, *Taraxacum* sp. and *Vicia pyrenaica.* Tables 3 and 4 show data about animal preferences in Costabona and Fontalba zones respectively. The only species significantly preferred by all animals is the legume *Trifolium pratense*. *Anthyllis vulneraria* is also quite preferred, except by chamois in Fontalba. Another legume, *Astragalus* sp. is only preferred by cattle and horse. About woody species, *Cytisus scoparius* is exclusively preferred by chamois, which shows clearly preference for other woody species, such *Calluna vulgaris*, *Rosa* sp. and *Rubus* sp. Cattle and sheep shows also preference for these last two species and horse is the only animal that not shows preference for any woody species. Cattle and horse show preference for some graminoid species, such *Poa* sp. and *Agrostis* sp. and chamois avoided *Festuca* sp. A half of non-leguminous forbs appear significantly preferred by some animal species in one or both zones.

Stocking rate for the warm period (June to November) 2011-2012 was determined: 1.97 UAM/ha for Costabona (0.03 chamois, 1.55 cattle, 0.23 horse and 0.16 sheep), and 0.40 UAM/ha to Fontalba (0.03 chamois, 0.30 cattle and 0.70 horse).

Table 3. Savage preference index (Wi) of the average value for each plant species that were present in Costabona area, in late spring. Nf = not found.

Species	**Chamois**	**Cattle**	**Horse**	**Sheep**
Non-Leguminous Woody				
Calluna vulgaris	2.85*	1.612	0.13	0.25
Pinus uncinata	0.58	1.71	1.74	1.71
Rosa sp.	10.05*	2.46	2.51	7.39*
Rubus sp.	17.59*	7.39*	0.00	12.31*
Leguminous Woody				
Cytisus scoparius	3.03*	0.00	0.00	0.00
Graminoids				
Festuca sp.	0.77	1.19	1.65	1.13
Agrostis sp.	nf	nf	nf	nf
Avenula pratensis	0.80	1.83	1.07	1.05
Poa sp.	0.30	1.47	3.29*	0.29
Non-Leguminous Forbs				
Antennaria dioica	0.98	0.00	0.00	0.00
Cruciata glabra	1.52	2,99	0,00	0,75
Galium verum	0.00	0.57	0.00	0.00
Helianthemum nummularium	0.00	49.26*	0.00	0.00
Hieracium pilosella	0.90	0.44	1.35	1.76
Pedicularis pyrenaica	0.00	9.85*	5.02	4.93
Plantago monosperma	2.36	1.62	1.89	2.31
Plantago media	2.45	1.20	1.23	2.40
Potentilla sp.	3.29*	3.68*	2.35	5.06*
Ranunculus bulbosus	1.67	9.85*	5.02	13.14*
Thymus nervosus	0.45	0.44	0.45	0.44
Veronica sp.	nf	nf	nf	nf
Leguminous Forbs				
Anthyllis vulneraria	15.07*	24.63*	20.10*	44.33*
Astragalus sp.	0.00	2.40	1.23	0.00
Chamaespartium sagittale	0.00	0.00	0.90	2.64
Hippocrepis comosa	nf	nf	nf	nf
Lotus corniculatus	0.98	1.45	0.49	1.93
Trifolium alpinum	1.14	0.98	1.14	1.38
Trifolium pratense	5.66*	4.16*	5.66*	9.02*
Trifolium repens	1.03	1.00	1.03	0.67

Discussion

The four sympatric animals select fairly similar species but in different proportions when they are faced with similar plants components for choice. For species of the same trophic niche, the use of common limited resources would result in competitive interactions (de Boer & Prins, 1990). Species can avoid competition through resource partitioning (Hutchinson, 1959; MacArthur 1972; Schoener, 1974) although diet overlap exists.The dietary preferences could be due to differences in animal morphophysiology and foraging behaviour. In the Pyrenees, these differences between different kinds of livestock species facilitate the exploitation of pastures without excluding the presence of wild animals (García-González & Montserrat, 1986). Chamois, with lower body mass, is considered an intermediate feeder like sheep, at least since the late Pleistocene (Feranec et al, 2010) and cattle and horse, with higher body mass, are considered grazers (Hofmann, 1989), but in this study, chamois shows a diet composed mainly by woody species. This result seems to be in contrast with other studies where chamois is considered a grass feeder (Pérez-Barbería et al, 1997; Homolka & Heroldová, 2001), mostly during Summer (García-González & Cuartas, 1996). Bertolino et al (2009) also showed a predominance of graminoids when chamois grazes in sympatry with other wild ungulates, but their plasticity, that allows incorporating a relatively high proportion of woody species in diet mainly in Winter, is also well known (García-González & Cuartas, 1996; Häsler & Sen, 2012). Even where the chamois has been introduced as alien species, such in New Zealand forests, diet may consist almost entirely by woody plants (Yockney & Hickling, 2000). In fact, body mass and rumen types are currently considered poor predictors of diet composition (Redjadj et al, 2014).

It is assumed that when there is a high overlap of habitat use and diet, and resources are scarce, competition is the main interaction between herbivores (Belovski, 1986; Latham, 1999). In alpine grasslands, during the green up and maturity periods of vegetation, which occurs in Summer and Autumn, resources are often abundant (García-González et al, 1991). As for stocking rate, Aldezábal (1997) found values of 0.97 AUM/ha for cows, period July to September 1991, in Puerto Bajo Góriz (Ordesa y Monte Perdido National Park, Huesca, Spain). This value is higher for cows in Fontalba area (0.30 AUM/ha) but lower for Costabona area (1.55 AUM/ ha). The same author found values of 2.73 AUM/ha for mixed flocks of sheep and goats in September 1991 in that National Park, above the sheep value in Costabona area (0.16 AUM/ha). At high stocking rate, livestock requires the chamois to move to areas with lower quality forage (La Morgia & Bassano, 2009; Chirichella et al, 2013). Although one usually considers mixed-species stocking to refer to two or more of livestock species (Abaye, 1994), wildlife if present should be considered as part of the mix (Gallina, 1993; Anderson et al, 2012). Hopefully, here animal coexistence could lead to resource partitioning, which is common among large herbivores (e.g. Gordon & Illius, 1989; Homolka, 1996; Putman, 1996). Diet overlap among herbivores was well predicted by rumen type when measured over broad plant groups (Redjadj et al, 2014). In that sense, here chamois appears as a browser in comparison with domestic flocks, with highest amounts of woody species and lowest values of graminoids and forbs in their diet (Figure 2). Moreover, chamois shows some specific plant preferences and aversions over common species that do not occurs in domestic animals, such as *Calluna vulgaris* and *Cytisus scoparius* preference and *Festuca* sp. aversion (Tables 3 and 4). *Calluna vulgaris* consumption and preference is clearly higher in chamois than domestic animals. In fact, Ericaceous species have been recorded as an important diet component in mountain wild ungulates (Trutmann, 2009). Only *Trifolium pratense* could be susceptible to competence effects due its preference by all animal species. In addition, dietary overlap indicators are lower when comparing chamois with any other animal than when comparing domestic animals between them (Table 2). In the other size, horse shows clear grazer behaviour, with highest amounts of graminoids and lowest of woody species (Figure 2). Horses are hindgut fermenters, and their differences with ruminants provide support for the generality that morphologically more similar species will compete more than less morphologically similar species (Duncan & Forsyth, 2006). Cattle and sheep appear also as grazers using something more woody species and less graminoids than horses, but overlap indicators are high in all comparisons between the three species (Table 2). All three showed a clear indifference about *Festuca* sp., the most common resource (Table 3 and 4). That means competition could exist between them.

Other factor that can influence diet selection is plant phenology (Garel et al, 2011), which implies that the pattern of similarity between species can change according to the season (Homolka, 1993; Heroldová, 1996). In the case of chamois and sheep, it could explain the significant differences between Summer and Autumn diets, when forbs in Summer and woody species in Autumn are more grazed (Figure 2). In that sense, forbs availability could play an important role in diet selection. Taking into account that graminoids fraction do not varies between both seasons for any animal species and main woody species are present throughout the year, the appearance of palatable forbs (with many annual and geophytes species) could determine consume of ligneous species. It would be in agreement with the optimal foraging theory (Gross, 1986), when says that ruminants prefer food items with higher digestibility of organic matter, thus, forbs are preferred to grasses and some woody species, such as conifers. When chamois is the only large herbivore in alpine habitat selects grass-forb throughout the year, but its use increases when its quality is high, in Spring and Autumn, independently of quantity available (Pérez-Barbería et al, 1997). As a consequence of the high consume of forbs by domestic animals, mainly sheep, chamois may have been forced to reduce niche breadth and increasing percentages of woody species. Similar effect was observed by La Morgia & Bassano (2009) in the Western Italian Alps, where a reduction of highly digestible forbs in chamois diet was due to the presence of sheep during Summer.

Spatial segregation of the ecological niche is other factor that contributes to determine diet composition and allows the coexistence of similar species (Belovski, 1986; Putman, 1996; Latham et al, 1999). Free-ranging livestock can affect the spatial distribution of wild ungulates and modify their activity and diet (Kie et al, 1991; Kie, 1996; Coe et al, 2001; Mattiello et al, 2002; Brown et al, 2010). A marked spatial segregation was recorded between Pyrenean chamois and livestock in France and Spain (Berdoucou, 1986; García-González et al, 1990) and between Cantabrian chamois (*Rupicapra pyrenaica parva*) and livestock in Spain (Rebollo et al, 1993). Chamois usually show signs of intolerance when domestic sheep occupies their area (Ruttimann et al, 2008). The presence of domestic herds is a source of disturbance by chamois which responds moving upslope (Mason et al, 2014) or grazing next to refuge points, such are rocky areas or forest, where forage quality is lower (Hamr, 1988; Chirichella et al, 2013). Other reason of segregation can be its sensitivity to presence of faeces from other large herbivores on the grassland (Fankhauser et al, 2008). In addition, larger ungulate species sometimes prevail over smaller ones (Berger & Cunningham, 1998; Ferretti et al, 2011), especially when the coexistence of the two populations is quite recent (Forsyth & Hickling, 1998). Surely it is related with the fact that energy requirements are proportional to metabolic body weight, meaning that small animals require more feed per unit of body weight for maintenance than larger animals (Van Soest, 1994). In our case, the coexistence with cattle and horses, more larger and clearly grazers, could be determinant for the browser pattern of chamois, and the preference for *Cytisus scoparius* which is distributed next to the forest, could be a consequence of these pressures in the Catalan Pyrenees.

In summary, chamois plasticity or specialization towards ligneous species due the presence of domestic grazers moves their effect to these vegetation components. In the other side, the herbaceous components remains under the impact of three species of grazers that consume throughout the sympatric period almost the same diet, and their feeding niches show an extensive overlap that, in terms of conservation resources, are likely to be cumulative to an unknown extent.

Conclusions

In alpine habitats, our results suggest that the chamois diet differs in composition and preferences from those of the other three large domestic herbivores that grazing in sympatry (cattle, horse and sheep). Diet of domestic species largely overlaps within them and is clearly dominated by grasses and forbs. However, the chamois that usually is considered a grass feeder in Summer, here appears as a browser with clear preferences for common ligneous species, such *as Calluna vulgaris* and *Cytisus scoparius*. It seems obvious that the presence of livestock modifies chamois diet. Interaction between chamois and livestock suggest that resource partitioning is likely to occur because of direct competition, while among domestics species interaction would only provoke diet overlap, may be due to the abundance of forage resources. Resource partitioning between species may promote coexistence within animal communities by reducing trophic interference but these interactions may also lead to displacement of chamois from its preferred habitats. In addition, diet composition of small ruminants, in this case chamois and sheep, change according the season being more ligneous in Autumn than Summer, but it doesn’t occurs with large ruminants such as cattle.

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Anexo 1a. Seasonal diet composition of Chamois in Costabona (2011-2012). Values represent % fragments in faeces (Min-Max).

DIET CHAMOIS-C	SUMMER						AUTUMN					
	June		July		August		September		October		November	
Non-Leguminous Woody												
Calluna vulgaris	2.5	(0-5.0)	20.8	(19.0-23.0)	43.75	(43.0-44.5)	30.0	(28.0-32.0)	28.5	(25.5-31.5)	43.8	(43.5-44.0)
Pinus uncinata	1.0	(0-2.0)	2.0	(2.0-2.0)	0.0		0.0		0.0		4.75	(1.0-8.5)
Quercus sp.	0.0		0.0		0.0		0.0		1.25	(0-2.5)	4.75	(0-9.5)
Rosa sp.	3.0	(2.5-3.5)	2.5	(1.5-3.5)	0.5	(0-1.0)	0.0		2.75	(1.0-4.5)	0.25	(0-0.5)
Rubus sp.	5.0	(4.0-6.0)	4,25	(4.0-4.5)	0,5	(0-1.0)	3.0	(0-6.0)	5.5	(0.5-10.5)	1.5	(1.0-2.0)
Others	0.0		0.25	(0-0.5)	0.0		1.5	(0-3.0)	0.5	(0-1.0)	0.25	(0-0.5)
Total NLW	**11.5**		**30.3**		**44.8**		**34.5**		**38.5**		**55.3**	
Leguminous Woody												
Cytisus scoparius	5.5	(3.5-7.5)	15.8	(9.5-22.0)	18.25	(8.0-28.5)	51.5	(47.0-56.0)	12.5	(12.5-12.5)	17.5	(11.0-24.0)
Total LW	**5.5**		**15.8**		**18.3**		**51.5**		**12.5**		**17.5**	
Graminoids												
Festuca sp.	42.0	(34.0-50.0)	15.8	(13.5-18.0)	11.75	(9.5-14.0)	8.5	(6.0-11.0)	25.25	(18.0-32.5)	14.5	(8.0-21.0)
Agrostis sp.	2.75	(1.5-4.0)	0.25	(0-0.5)	0.25	(0-0.5)	0.0		0.75	(0-1.5)	0.0	
Avenula pratensis	1.75	(0-3.5)	2.0	(0-4.0)	0.25	(0-0.5)	0.0		4.0	(1.5-6.5)	0.5	(0-1.0)
Carex sp.	0.25	(0-0.5)	0.25	(0-0.5)	0.0		0.5	(0-1.0)	0.0		0.75	(0-1.5)
Nardus stricta	0.0		0.0		0.0		0.0		0.0		0.25	(0-0.5)
Poa sp.	1.75	(1.0-2.5)	0.0		0.25	(0-0.5)	0.0		0.5	(0-1.0)	0.0	
Others	1.75	(0-3.5)	0.25	(0-0.5)	0.0		0.0		0.0		0.25	(0-0.5)
Total G	**50.3**		**18.5**		**12.5**		**9.0**		**30.5**		**16.3**	
Non-Leguminous Forbs												
Antennaria dioica	0.5	(0.5-0.5)	0.0		0.25	(0-0.5)	0.0		0.0		0.0	
Cerastium sp.	0.0		0.5	(0-1.0)	0.0		0.0		0.0		0.0	
Cruciata glabra	0.75	(0-1.5)	1.25	(0-2.5)	1.25	(1.0-1.5)	1.0	(0-2.0)	2.5	(0-5.0)	0.0	
Galium verum	0.25	(0-0.5)	0.25	(0-0.5)	0.0		0.0		0.0		0.0	
Gentiana sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Helianthemum nummularium	0.0		0.0		0.25	(0-0.5)	0.0		0.0		0.25	(0-0.5)
Hieracium pilosella	0.25	(0-0.5)	2.25	(2.0-2.5)	1.0	(0-2.0)	0.5	(0-1.0)	1.0	(0.5-1.5)	0.25	(0-0.5)
Hippocrepis comosa	0.25	(0-0.5)	0.0		0.5	(0-1.0)	0.0		0.0		0.0	
Lotus corniculatus	0.75	(0-1.5)	1.25	(0.5-2.0)	1.0	(0-2.0)	0.0		0.25	(0-0.5)	0.75	(0.5-1.0)
Pedicularis pyrenaica	0.25	(0-0.5)	0.0		0.0		0.0		0.0		0.0	
Plantago monosperma	8.0	(7.0-9.0)	3.75	(2.5-5.0)	2.5	(1.0-4.0)	1.0	(0-2.0)	1.25	(0-2.5)	0.0	
Plantago media	1.0	(0-2.0)	1.0	(1.0-1.0)	0.25	(0-0.5)	0.0		0.0		0.0	
Potentilla sp.	4.0	(2.0-6.0)	4.5	(4.0-5.0)	2.0	(1.5-2.5)	0.5	(0-1.0)	0.5	(0-1.0)	0.25	(0-0.5)
Ranunculus bulbosus	2.0	(0-4.0)	0.0		0.0		0.0		0.25	(0-0.5)	0.0	
Sempervivum montanum	0.25	(0-0.5)	0.0		0.0		0.0		0.0		0.0	
Taraxacum sp.	0.0		0.5	(0-1.0)	0.0		0.0		1.75	(0-3.5)	0.0	
Thymus nervosus	0.25	(0-0.5)	1.0	(0.5-1.5)	0.0		0.0		0.0		0.0	
Veronica sp.	1.25	(1.0-1.5)	2.0	(0.5-3.5)	0.0		0.0		0.5	(0-1.0)	0.0	
Others	0.25	(0-0.5)	0.25	(0-0.5)	0.0		0.0		0.0		0.0	
Total NLF	**19.0**		**17.3**		**7.5**		**3.0**		**7.8**		**0.8**	
Leguminous Forbs												
Anthyllis vulneraria	0.5	(0-1.0)	1.5	(0-3.0)	2.5	(0-5.0)	0.0		1.25	(0-2.5)	0.5	(0-1.0)
Astragalus sp.	0.0		0.0		0.0		0.0		0.0		0.5	(0-1.0)
Chamaespartium sagittale	0.0		0.0		0.0		0.0		0.0		0.0	
Hippocrepis comosa	0.25	(0-0.5)	0.0		0.5	(0-1.0)	0.0		0.0		0.0	
Lotus corniculatus	0.75	(0-1.5)	1.25	(0.5-2.0)	1.0	(0-2.0)	0.0		0.25	(0-0.5)	0.75	(0.5-1.0)
Trifolium alpinum	7.5	(5.5-9.5)	8.25	(7.0-9.5)	9.0	(1.5-16.5)	0.5	(0-1.0)	8.75	(8.5-9.0)	8.5	(7.5-9.5)
Trifolium pratense	4.25	(3.5-5.0)	5.0	(4.5-5.5)	2.5	(1.0-4.0)	1.5	(0-3.0)	0.5	(0-1.0)	0.0	
Trifolium repens	0.5	(0-1.0)	1.75	(0-3.5)	1.5	(0-3.0)	0.0		0.0		0.0	
Vicia pyrenaica	0.0		0.5	(0-1.0)	0.0		0.0		0.0		0.0	
Total LF	**13.8**		**18.3**		**17.0**		**2.0**		**10.8**		**10.3**	

Anexo 1b. Seasonal diet composition of Chamois in Fontalba (2011-2012). Values represent % fragments in faeces (Min-Max).

DIET CHAMOIS-F	SUMMER						AUTUMN					
	June		July		August		September		October		November	
Non-Leguminous Woody												
Calluna vulgaris	1.5	(1.0-2.0)	21.8	(18.5-25.0)	16.0	(14.5-17.5)	32.3	(32.0-32.5)	19.0	(10.0-28.0)	35.5	(30.5-40.5)
Juniperus communis	0.0		0.0		0.0		0.0		0.0		0.25	(0-0.5)
Pinus uncinata	1.5	(0-3.0)	0.0		0.75	(0.5-1.0)	0.0		0.0		0.0	
Quercus sp.	0.0		0.0		0.5	(0-1.0)	1.0	(0-2.0)	2.5	(1.5-3.5)	6.25	(0-12.5)
Rhododendron ferrugineum	0.0		0.0		0.0		0.0		3.5	(3.0-4.0)	0.0	
Rosa sp.	3.25	(1.0-5.5)	3.5	(2.5-3.5)	5.25	(0.5-10.0)	3.5	(2.5-4.5)	10.0	(8.0-12.0)	1.5	(0-3.0)
Rubus sp.	3.25	(1.5-5.0)	6.5	(5.0-13.0)	8.25	(6.5-10.0)	7.0	(6.0-8.0)	24.5	(19.5-29.5)	3.0	(1.5-4.5)
Others	0.25	(0-0.5)	0.0		0.0		0.25	(0-0.5)	1.5	(1.0-2.0)	0.5	(0-1.0)
Total NLW	**9.8**		**31.8**		**30.8**		**44.0**		**61.0**		**47.0**	
Leguminous Woody												
Cytisus scoparius	17.0	(11.5-22.5)	19.0	(14.5-24.0)	16.5	(14.5-18.5)	16.5	(8.5-24.5)	22.5	(16.0-29.0)	18.0	(12.5-23.5)
Total LW	**17.0**		**19.0**		**16.5**		**16.5**		**22.5**		**18.0**	
Graminoids												
Festuca sp.	23.5	(20.0-27.0)	15.3	(13.0-18.0)	15.0	(6.0-24.0)	19.3	(17.5-21.0)	4.5	(4.0-5.0)	13.5	(10.0-17.0)
Agrostis sp.	0.25	(0-0.5)	0.25	(0-0.5)	0.0		0.0		0.0		0.0	
Avenula pratensis	2.5	(0-5.0)	1.25	(0-2.5)	0.75	(0-1.5)	2.25	(0.5-4.0)	0.0		2.0	(2.0-2.0)
Carex sp.	0.5	(0.5-0.5)	0.5	(0-1.0)	0.0		0.75	(0-1.5)	0.0		0.5	(0-1.0)
Nardus stricta	0.0		0.0		0.0		0.0		0.0		1.75	(0.5-3.0)
Poa sp.	0.5	(0-1.0)	0.0		0.5	(0-1.0)	0.0		0.0		0.75	(0-1.5)
Others	1.5	(0-3.0)	0.0		0.0		0.5	(0-1.0)	0.0		0.0	
Total G	**28.8**		**17.3**		**16.3**		**22.8**		**4.5**		**18.5**	
Non-Leguminous Forbs												
Antennaria dioica	0.75	(0-1.5)	0.5	(0-1.0)	0.0		0.0		0.0		0.25	(0-0.5)
Cerastium sp.	0.0		0.25	(0-0.5)	0.0		0.0		0.0		0.0	
Cruciata glabra	0.5	(0.5-0.5)	1.0	(0.5-1.5)	1.75	(1.0-2.5)	2.75	(1.5-4.0)	2.5	(1.0-4.0)	1.5	(1.0-2.0)
Galium verum	0.25	(0-0.5)	0.25	(0-0.5)	0.25	(0-0.5)	0.0		0.0		0.0	
Gentiana sp.	0.25	(0-0.5)	0.0		0.0		0.0		0.0		0.0	
Helianthemum nummularium	0.0		0.0		1.25	(0-2.5)	0.5	(0-1.0)	0.0		0.0	
Hieracium pilosella	5.75	(4.5-7.0)	3.5	(3.0-4.0)	0.75	(0.5-1.0)	1.25	(1.0-1.5)	2.0	(1.0-3.0)	0.25	(0-0.5)
Pedicularis pyrenaica	0.0		0,5	(0-1.0)	0.75	(0-1.5)	0.0		0.0		0.0	
Plantago monosperma	5.0	(2.5-7.5)	3.25	(3.0-3.5)	4.5	(3.0-6.0)	3.0	(3.0-3.0)	0.0		0.0	
Plantago media	2.0	(0.5-3.5)	2.25	(1.5-3.0)	7.0	(5.5-8.5)	0.5	(0-1.0)	0.0		0.0	
Potentilla sp.	3.25	(2.0-4.5)	3.5	(3.5-3.5)	3.0	(2.5-3.5)	1.5	(0.5-2.5)	0.0		1.25	(0-2.5)
Ranunculus bulbosus	2.75	(0-5.5)	0.5	(0-1.0)	0.0		0.0		0.0		0.0	
Sempervivum montanum	0.75	(0-1.5)	0.0		0.0		0.0		0.0		0.0	
Silene acaulis	0.0		0.0		0.0		0.0		0.0		0.0	
Taraxacum sp.	0.5	(0-1.0)	0.25	(0-0.5)	0.25	(0-0.5)	0.25	(0-0.5)	5.0	(3.0-7.0)	0.0	
Thymus nervosus	0.25	(0-0.5)	0.5	(0-1.0)	0.75	(0-1.5)	0.75	(0.5-1.0)	0.0		0.0	
Veronica sp.	0.75		0.75	(0-1.5)	5.0	(2.5-7.5)	1.0	(0-2.0)	0.0		0.5	(0-1.0)
Others	0.5	(0-1.0)	0.5	(0-1.0)	0.25	(0-0.5)	0.0		0.0		0.0	
Total NLF	**23.3**		**17.5**		**25.5**		**11.5**		**9.5**		**3.8**	
Leguminous Forbs												
Anthyllis vulneraria	0.5	(0-1.0)	0.0		0.0		0.25	(0-0.5)	0.0		1.0	(0-2.0)
Astragalus sp.	0.0		0.0		0.0		0.0		0.0		1.25	(0-2.5)
Chamaespartio sagittalis	0.0		0.0		0.0		0.0		0.0		0.0	
Hippocrepis comosa	0.0		0.0		0.0		0.0		0.0		0.0	
Lotus corniculatus	2.5	(1.0-4.0)	1.25	(1.0-1.5)	1.75	(1.5-2.0)	1.5	(1.0-2.0)	0.0		1.75	(0-3.5)
Trifolium alpinum	10.0	(7.0-13.0)	9.0	(7.0-11.0)	4.75	(4.0-5.5)	3.25	(1.0-5.5)	2.5	(0-5.0)	8.0	(4.5-11.5)
Trifolium pratense	5.5	(5.0-6.0)	3.5	(2.0-5.0)	3.0	(2.5-3.5)	0.0		0.0		0.0	
Trifolium repens	2.75	(2.0-3.5)	0.75	(0.5-1.0)	1.5	(0-3.0)	0.25	(0-0.5)	0.0		0.75	(0-1.5)
Vicia pyrenaica	0.0		0.0		0.0		0.0		0.0		0.0	
Total LF	**21.3**		**14.5**		**11.0**		**5.3**		**2.5**		**12.8**	

Anexo 2. Seasonal diet composition of Sheep in Costabona (2011-2012). Values represent % fragments in faeces (Min-Max).

DIET SHEEP-C	SUMMER						AUTUMN					
	June		July		August		September		October		November	
Non-Leguminous Woody												
Calluna vulgaris	0.5	(0.5-0.5)	1.5	(1.0-2.0)	3.25	(3.0-3.5)	2.0	(1.0-3.0)	3.0	(0-6.0)	0.0	
Juniperus communis	0.0		0.0		0.0		1.5	(1.0-2.0)	0.0		0.0	
Pinus uncinata	5.5	(4.0-7.0)	2.0	(2.0-2.0)	1.25	(0.5-2.0)	0.5	(0-1.0)	4.25	(3.5-5.0)	6.5	(4.0-9.0)
Quercus sp.	0.0		0.0		0.0		1.0	(0-2.0)	0.0		2.5	(2.0-3.0)
Rhododendron ferrugineum	0.0		0.0		0.0		0.0		0.0		0.0	
Rosa sp.	1.75	(1.5-2.0)	0.75	(0.5-1.0)	1.25	(0-2.5)	3.0	(2.0-4.0)	1.75	(0-3.5)	2.5	(2.0-3.0)
Rubus sp.	2.25	(1.0-3.5)	2.25	(1.0-3.5)	3.0	(2.5-3.5)	10.0	(10.0-10.0)	5.5	(3.0-11.0)	20.5	(20.0-21.0)
Others	0.0		0.0		0.0		0.0		0.0		0.0	
Total NLW	**10.0**		**6.5**		**8.8**		**18.0**		**14.5**		**32.0**	
Leguminous Woody												
Cytisus scoparius	0.0		0.0		0.0		1.5	(0-3.0)	0.75	(0.5-1.0)	1.0	(1.0-1.0)
Total LW	**0.0**		**0.0**		**0.0**		**1.5**		**0.8**		**1.0**	
Graminoids												
Festuca sp.	44.0	(42.0-46.0)	38.0	(34.0-42.5)	28.25	(28.0-28.5)	46.5	(45.0-48.0)	48.5	(45.0-52.0)	43.5	(42.0-45.0)
Agrostis sp.	2.5	(2.0-3.0)	2.75	(0.5-5.0)	3.0	(2.0-4.0)	0.0		1.75	(1.0-2.5)	0.0	
Avenula pratensis	3.0	(1.5-4.5)	1.5	(0.5-2.5)	1.75	(0-3.5)	3.5	(2.0-5.0)	1.25	(0-2.5)	2.5	(1.0-4.0)
Carex sp.	0.25	(0-0.5)	0.5	(0-1.0)	0.25	(0-0.5)	2.0	(1.0-3.0)	0.75	(0.5-1.0)	1.5	(1.0-2.0)
Nardus stricta	0.0		0.0		0.0		0.0		0.0		0.0	
Poa sp.	0.75	(0-1.5)	1.25	80.5-2.0)	0.0		0.0		0.25	(0-0.5)	0.0	
Others	0.0		0.25	(0-0.5)	0.25	(0-0.5)	0.0		0.0		0.5	(0-1.0)
Total G	**50.5**		**44.3**		**33.5**		**52.0**		**52.5**		**48.0**	
Non-Leguminous Forbs												
Antennaria dioica	0.0		0.0		0.25	(0-0.5)	0.0		0.0		0.0	
Cerastium sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Cruciata glabra	0.0		0.75	(0.5-1.0)	0.75	(0.5-1.0)	0.0		0.0		0.0	
Galium verum	0.25	(0-0.5)	0.25	(0-0.5)	0.0		0.5	(0-1.0)	0.5	(0-1.0)	0.0	
Gentiana sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Helianthemum nummularium	0.0		0.0		0.0		0.5	(0-1.0)	0.25	(0-0.5)	0.5	(0-1.0)
Hieracium pilosella	1.0	(0.5-1.5)	3.25	(1.0-5.5)	1.5	(1.0-2.0)	3.0	(3.0-3.0)	1.5	(1.5-1.5)	2.0	(1.0-3.0)
Pedicularis pyrenaica	0.0		0.0		1.0	(0-2.0)	0.0		0.0		0.0	
Plantago monosperma	4.0	(3.0-5.0)	4.5	(3.5-5.5)	6.75	(3.5-10.0)	4.0	(3.0-5.0)	6.0	(6.0-6.0)	3.0	(2.0-4.0)
Plantago media	0.75	(0-1.5)	1.0	(1.0-1.0)	0.75	(0.5-1.0)	0.0		3.0	(0.5-5.5)	1.5	(1.0-2.0)
Potentilla sp.	4.5	(3.5-5.5)	6.0	(4.0-8.0)	6.25	(5.0-7.5)	2.5	(2.0-3.0)	3.0	(1.5-4.5)	1.5	(0-3.0)
Ranunculus bulbosus	5.0	(3.0-7.0)	3.75	(2.0-5.5)	3.0	(2.5-3.5)	4.0	(2.0-6.0)	3.0	(2.5-3.5)	3.5	(3.0-4.0)
Sempervivum montanum	0.0		0.0		0.0		0.0		0.0		0.0	
Silene acaulis	0.0		0.0		0.0		0.0		0.0		0.0	
Taraxacum sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Thymus nervosus	0.25	(0-0.5)	0.5	(0-1.0)	0.5	(0-1.0)	0.0		0.0		0.0	
Veronica sp.	3.0	(2.0-4.0)	1.0	(0.5-1.5)	1.5	(0.5-2.5)	0.0		4.25	(2.5-6.0)	0.0	
Others	0.5	(0.5-0.5)	0.25	(0-0.5)	0.25	(0-0.5)	0.0		0.0		0.5	(0-1.0)
Total NLF	**19.3**		**21.3**		**22.5**		**14.5**		**21.5**		**12.5**	
Leguminous Forbs												
Anthyllis vulneraria	2.75	(2.0-3.5)	4.75	(4.5-5.0)	5.75	(5.0-6.5)	6.5	(6.0-7.0)	1.75	(0.5-3.0)	2.5	(1.0-4.0)
Astragalus sp.	0.0		0.25	(0-0.5)	0.25	(0-0.5)	0.5	(0-1.0)	0.0		0.0	
Chamaespartio sagittalis	0.5	(0.5-0.5)	1.75	(0-3.5)	2.25	(0-4.5)	1.0	(1.0-1.0)	0.75	(0-1.5)	0.5	(0-1.0)
Hippocrepis comosa	1.75	(0-3.5)	1.25	(0-2.5)	2.0	(0-4.0)	0.0		0.25	(0-0.5)	0.0	
Lotus corniculatus	1.0	(1.0-1.0)	1.0	(0-2.0)	3.25	(0.5-6.0)	0.0		1.25	(0-2.5)	0.0	
Trifolium alpinum	7.0	(6.5-7.5)	10.0	(9.5-10.5)	14.25	(14.0-14.5)	1.0	(0-2.0)	4.75	(4.5-5.0)	2.0	(1.0-3.0)
Trifolium pratense	6.25	(6.0-6.5)	7.0	(5.5-8.5)	6.5	(6.5-6.5)	5.0	(4.0-6.0)	1.5	(1.0-2.0)	1.5	(1.0-2.0)
Trifolium repens	1.0	(0.5-1.5)	2.0	(1.5-2.5)	0.25	(0-0.5)	0.0		0.5	(0-1.0)	0.0	
Veronica sp.	3.0	(2.0-4.0)	1.0	(0.5-1.5)	1.5	(0.5-2.5)	0.0		4.25	(2.5-6.0)	0.0	
Vicia pyrenaica	0.0		0.0		0.75	(0-1.5)	0.0		0.0		0.0	
Others	0.5	(0.5-0.5)	0.25	(0-0.5)	0.25	(0-0.5)	0.0		0.0		0.5	(0-1.0)
Total LF	**20.3**		**28.0**		**35.3**		**14.0**		**10.8**		**6.5**	

Anexo 3a. Seasonal diet composition of Cattle in Costabona (2011-2012). Values represent % fragments in faeces (Min-Max).

DIET CATTLE-C	SUMMER						AUTUMN					
	June		July		August		September		October		November	
Non-Leguminous Woody												
Calluna vulgaris	8.0	(1.0-15.0)	16.3	(10.0-23.0)	14.25	812.0-17.0)	6.0	(6.0-6.0)	7.5	(2.5-12.5)	1.5	(1.0-2.0)
Juniperus communis	0.0		0.0		0.0		0.0		0.0		0.0	
Pinus uncinata	1.75	(0.5-3.0)	2.5	(1.0-4.0)	4.75	(1.5-8.0)	5.0	(5.0-5.0)	3.5	(1.5-5.5)	14.0	(9.5-18.5)
Quercus sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Rhododendron ferrugineum	0.0		0.0		0.0		0.0		0.0		0.0	
Rosa sp.	0.25	(0-0.5)	0.5	(0-1.0)	0.0		0.0		0.25	(0-0.5)	0.75	(0-1.5)
Rubus sp.	2.75	(1.0-4.5)	2.0	(2.0-2.0)	0.25	(0-0.5)	0.0		2.0	(0.5-3.5)	2.25	(1.0-3.4)
Others	0.0		0.0		0.0		0.0		0.0		0.0	
Total NLW	**12.8**		**21.3**		**19.3**		**11.0**		**13.3**		**18.5**	
Leguminous Woody												
Cytisus scoparius	0.0		0.25	(0-0.5)	0.0		0.0		0.25	(0-0.5)	1.25	(0-2.5)
Total LW	**0.0**		**0.3**		**0.0**		**0.0**		**0.3**		**1.3**	
Graminoids												
Festuca sp.	39.25	(26.5-52.0)	35.3	(34.0-37.0)	41.75	(33.0-51.0)	40.0	(40.0-40.)	45.5	(42.5-49.0)	43.3	(35.5-51.0)
Agrostis sp.	3.75	(2.0-5.5)	1.75	(0.5-3.0)	3.75	(3.0-4.5)	2.5	(2.0-3.0)	3.75	(3.5-4.0)	6.0	(1.0-11.0)
Avenula pratensis	3.5	(3.0-4.0)	1.25	(0-2.5)	5.5	(4.5-6.5)	4.0	(4.0-4.0)	2.25	(2.0-2.5)	3.5	(3.5-3.5)
Carex sp.	0.25	(0-0.5)	0.5	(0-1.0)	0.0		0.5	(0-1.0)	0.75	(0-1.5)	0.5	(0-1.0)
Nardus stricta	0.0		0.0		0.0		0.0		0.0		0.0	
Poa sp.	2.0	(0.5-3.5)	1.5	(1.5-1.5)	4.25	(4.0-4.5)	2.0	(1.0-3.0)	3.0	(0-6.0)	3.5	(0.5-6.5)
Others	0.0		0.0		0.0		0.0		0.25	(0-0.5)	0.0	
Total G	**48.8**		**40.3**		**55.3**		**49**		**55.5**		**56.8**	
Non-Leguminous Forbs												
Antennaria dioica	0.5	(0-1.0)	0.0		0.0		0.0		0.0		0.0	
Cerastium sp.	0.0		0.25	(0-0.5)	0.0		0.0		0.0		0.0	
Cruciata glabra	1.5	(0.5-2.5)	2.5	(1.5-3.5)	1.5	(0-3.0)	1.0	(0-2.0)	0.0		0.0	
Galium verum	0.0		1.0	(0.5-1.5)	0.75	(0.5-1.0)	0.0		0.25	(0-0.5)	0.25	(0-0.5)
Gentiana sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Helianthemum nummularium	0.5	(0.5-0.5)	1.0	(0.5-1.5)	0.0		0.0		0.25	(0-0.5)	0.0	
Hieracium pilosella	1.25	(1.0-1.5)	0.25	(0-0.5)	0.0		0.0		0.0		1.0	(0-2.0)
Pedicularis pyrenaica	0.25	(0-0.5)	1.75	(1.5-2.0)	0.5	(0-1.0)	0.0		0.5	(0.5-0.5)	0.0	
Plantago monosperma	4.25	(3.5-5.0)	3.0	(2.0-4.0)	3.5	(3.5-3.5)	3.5	(3.0-4.0)	3.25	(3.0-3.5)	5.0	((3.0-7.0)
Plantago media	0.0		0.75	(0.5-1.0)	0.0		0.0		0.0		0.0	
Potentilla sp.	4.75	(4.5-5.0)	4.0	(2.0-6.0)	2.5	(1.5-3.5)	5.5	(5.0-6.0)	3.75	(3.5-4.0)	2.75	(2.0-3.5)
Ranunculus bulbosus	3.5	(1.5-5.5)	2.25	(0.5-4.0)	2.75	(0.5-5.0)	5.0	(4.0-6.0)	3.75	(0.5-7.0)	1.25	(1.0-1.5)
Sempervivum montanum	0.0		0.0		0.0		0.0		0.0		0.0	
Silene acaulis	0.0		0.0		0.0		0.0		0.0		0.0	
Taraxacum sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Thymus nervosus	0.0		0.75	(0-1.5)	0.25	(0-0.5)	0.0		0.0		0.25	(0-0.5)
Veronica sp.	0.5	(0.5-0.5)	2.75	(2.5-3.0)	0.25	(0-0.5)	1.0	(1.0-1.0)	1.25	(0-2.5)	0.5	(0-1.0)
Others	0.5	(0.5-0.5)	0.75	(0.5-1.0)	0.25	(0-0.5)	0.5	(0-1.0)	0.25	(0-0.5)	0.0	
Total NLF	**17.5**		**21.0**		**12.3**		**16.5**		**13.3**		**11.0**	
Leguminous Forbs												
Anthyllis vulneraria	4.5	(2.0-7.0)	3.0	(2.0-4.0)	0.5	(0-1.0)	0.0		2.0	(0-4.0)	2.25	(1.0-3.5)
Astragalus sp.	0.25	(0-0.5)	0.5	(0-1.0)	1.5	(1.0-2.0)	0.0		0.75	(0-1.5)	0.75	(0-1.5)
Chamaespartio sagittalis	0.0		0.25	(0-0.5)	0.0		0.0		0.0		0.0	
Hippocrepis comosa	0.25	(0-0.5)	0.5	(0-1.0)	0.75	(0.5-1.0)	0.0		0.0		0.0	
Lotus corniculatus	1.25	(0.5-2.0)	2.0	(1.5-2.5)	0.75	(0-1.5)	3.0	(3.0-3.0)	0.25	(0-0.5)	0.75	(0-1.5)
Trifolium alpinum	9.75	(9.0-10.5)	5.75	(5.5-6.0)	6.5	(4.5-8.5)	11.0	(10.-12.0)	9.5	(6.5-12.5)	8.0	(7.0-9.0)
Trifolium pratense	3.25	83.0-3.5)	3.5	(2.5-4.5)	2.25	(1.0-3.5)	8.5	(8.0-9.0)	4.0	(0-8.0)	0.5	(0-1.0)
Trifolium repens	1.75	(1.0-2.5)	1.75	(1.5-2.0)	1.0	(0-2.0)	1.0	(0-2.0)	1.25	(0.5-2.0)	0.25	(0-0.5)
Vicia pyrenaica	0.0		0.0		0.0		0.0		0.0		0.0	
Total LF	**21.0**		**17.3**		**13.3**		**23.5**		**17.8**		**12.5**	

Anexo 3b. Seasonal diet composition of Cattle in Fontalba (2011-2012). Values represent % fragments in faeces (Min-Max).

DIET CATTLE-F	SUMMER						AUTUMN					
	June		July		August		September		October		November	
Non-Leguminous Woody												
Calluna vulgaris	3.5	(2.0-5.0)	10.8	(10.0-11.5)	11.75	(6.5-17.0)	18.3	(11.0-25.5)	9.0	(8.0-10.0)	4.75	(4.0-5.5)
Juniperus communis	0.0		0.0		0.0		0.0		0.5	(0-1.0)	0.0	
Pinus uncinata	0.5	(0.5-0.5)	0.25	(0-0.5)	1.5	(0.5-2.5)	1.5	(1.0-2.0)	0.0		8.25	(8.0-8.5)
Quercus sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Rhododendron ferrugineum	0.0		0.0		0.0		0.0		0.0		0.0	
Rosa sp.	3.75	(2.5-5.0)	3.75	(0-7.5)	0.0		0.75	(0.5-1.0)	0.0		1.25	(0-2.5)
Rubus sp.	8.75	(5.5-12.0)	8.5	(7.0-10.0)	2.0	(0-4.0)	5.25	(1.5-9.0)	0.5	(0-1.0)	3.75	(2.5-5.0)
Others	0.0		0.0		0.0		0.0		0.0		0.0	
Total NLW	**16.5**		**23.3**		**15.3**		**25.8**		**10.0**		**18.0**	
Leguminous Woody												
Cytisus scoparius	0.0		0.0		0.0		0.0		1.5	(1.0-2.0)	3.5	(0-7.0)
Total LW	**0.0**		**0.0**		**0.0**		**0.0**		**1.5**		**3.5**	
Graminoids												
Festuca sp.	47.5	(46.0-49.0)	31.8	(30.5-33.0)	40.25	(36.0-44.5)	35.5	(28.0-43.0)	33.5	(32.0-35.0)	40.0	(35.0-45.0)
Agrostis sp.	2.0	(2.0-2.0)	2.75	(2.5-3.0)	2.5	(1.0-4.0)	2.25	(1.5-3.0)	8.0	(7.0-9.0)	0.75	(0-1.5)
Avenula pratensis	0.5	(0-1.0)	2.5	(2.5-2.5)	2.25	(1.0-3.5)	4.75	(3.0-6.5)	7.5	(7.0-8.0)	0.0	
Carex sp.	0.75	(0.5-1.0)	1.75	(1.5-2.0)	0.5	(0.5-0.5)	0.25	(0-0.5)	0.0		0.5	(0-1.0)
Nardus stricta	0.0		0.0		0.0		0.0		0.0		0.0	
Poa sp.	3.25	(3.0-3.5)	1.25	(1.0-1.5)	4.75	(2.5-7.0)	2.0	(1.5-2.5)	2.5	(1.0-4.0)	1.25	(0-2.5)
Others	0.0		0.0		0.0		0.0		0.0		0.0	
Total G	**54.0**		**40.0**		**50.3**		**44.8**		**51.5**		**42.5**	
Non-Leguminous Forbs												
Antennaria dioica	0.0		0.25	(0-0.5)	0.25	(0-0.5)	0.0		0.0		0.0	
Cerastium sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Cruciata glabra	0.25	(0-0.5)	1.25	(1.0-1.5)	0.75	(0-1.5)	1.25	(0.5-2.0)	0.0		1.25	(0-2.5)
Galium verum	0.0		1.0	(0.5-1.5)	0.75	(0-1.5)	0.5	(0-1.0)	0.0		0.0	
Gentiana sp.	0.0		0.0		0.0		0.0		0.5	(0-1.0)	0.0	
Helianthemum nummularium	0.25	(0-0.5)	1.25	(1.0-1.5)	0.25	(0-0.5)	0.0		0.0		0.0	
Hieracium pilosella	2.5	(0.5-4.5)	0.5	(0.5-0.5)	0.5	(0-1.0)	0.5	(0-1.0)	0.0		1.0	(0-2.0)
Pedicularis pyrenaica	1.0	(0-2.0)	1.25	(0.5-2.0)	2.0	(1.5-2.5)	0.75	(0-1.5)	0.5	(0-1.0)	0.75	(0-1.5)
Plantago monosperma	1.75	(0.5-3.0)	2.75	(2.5-3.0)	5.0	(4.0-6.0)	3.25	(2.5-4.0)	5.5	(5.0-6.0)	3.75	(2.0-5.5)
Plantago media	0.25	(0-0.5)	1.5	(1.5-1.5)	0.75	(0-1.5)	0.5	(0-1.0)	0.0		0.5	(0-1.0)
Potentilla sp.	4.0	(4.0-4.0)	2.0	(2.0-2.0)	2.25	(2.0-2.5)	1.5	(0-3.0)	4.5	(4.0-5.0)	3.75	(2.5-5.0)
Ranunculus bulbosus	0.75	(0-1.5)	2.5	(1.5-3.5)	2.75	(2.0-3.5)	2.0	(0-4.0)	4.0	(4.0-4.0)	5.25	(2.0-8.5)
Sempervivum montanum	0.0		0.0		0.0		0.0		0.0		0.0	
Silene acaulis	0.0		0.0		0.0		0.0		0.0		0.0	
Taraxacum sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Thymus nervosus	0.0		0.0		1.25	(0-2.5)	0.25	(0-0.5)	0.0		0.0	
Veronica sp.	1.5	(0-3.0)	2.75	(2.0-3.5)	1.0	(0-2.0)	1.75	(1.5-2.0)	0.5	(0-1.0)	1.0	(0.5-1.5)
Others	0.0		0.75	(0.5-1.0)	0.25	(0-0.5)	0.0		0.0		0.25	(0-0.5)
Total NLF	**12.3**		**17.8**		**17.8**		**12.3**		**15.5**		**17.5**	
Leguminous Forbs												
Anthyllis vulneraria	1.75	(1.5-2.0)	3.0	(0.5-5.5)	2.25	(0.5-4.0)	4.25	(2.0-6.5)	3.5	(3.0-4.0)	2.0	(0-4.0)
Astragalus sp.	0.75	(0.5-1.0)	0.5	(0-1.0)	1.75	(0-3.5)	1.25	(0-2.5)	0.0		2.25	(1.0-3.5)
Chamaespartio sagittalis	0.75	(0-1.5)	0.5	(0-1.0)	0.25	(0-0.5)	1.0	(0-2.0)	0.0		0.0	
Hippocrepis comosa	0.0		0.75	(0.5-1.0)	0.0		0.0		0.0		0.0	
Lotus corniculatus	0.75	(0.5-1.0)	0.5	(0.5-0.5)	0.5	(0-1.0)	0.75	(0-1.5)	0.5	(0-1.0)	3.0	(1.5-4.5)
Trifolium alpinum	8.75	(8.0-9.5)	9.25	(8.0-10.5)	9.25	(9.0-9.5)	6.75	(4.5-9.0)	9.5	(9.0-10.0)	8.5	(7.5-9.5)
Trifolium pratense	3.5	(3.0-4.0)	3.0	(3.0-3.0)	2.5	(2.0-3.0)	2.75	(0-5.5)	7.5	(7.0-8.0)	2.75	(2.5-3.0)
Trifolium repens	1.0	(0-2.0)	1.25	(1.0-1.5)	0.25	(0-0.5)	0.5	(0.5-0.5)	0.5	(0-1.0)	0.0	
Vicia pyrenaica	0.0		0.25	(0-0.5)	0.0		0.0		0.0		0.0	
Others	0.0		0.75	(0.5-1.0)	0.25	(0-0.5)	0.0		0.0		0.25	(0-0.5)
Total LF	**17.3**		**19.0**		**16.8**		**17.3**		**21.5**		**18.5**	

Anexo 4a. Seasonal diet composition of Horse in Costabona (2011-2012. Values represent % fragments in faeces (Min-Max).

DIET HORSE-C	SUMMER						AUTUMN					
	June		July		August		September		October		November	
Non-Leguminous Woody												
Calluna vulgaris	0.25	(0-0.5)	1.5	(0.5-2.5)	1.25	(0.5-2.0)	1.0	(1.0-1.0)	6.25	(0.5-12.0)	0.5	(0-1.0)
Juniperus communis	0.0		0.0		0.0		0.0		0.0		0.0	
Pinus uncinata	3.5	(2.5-4.5)	3.0	(2.5-3.5)	2.5	(0-5.0)	0.0		2.75	(0.5-5.0)	3.25	(0.5-6.0)
Quercus sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Rhododendron ferrugineum	0.0		0.0		0.0		0.0		0.0		0.0	
Rosa sp.	0.5	(0-1.0)	0.25	(0-0.5)	0.5	(0.5-0.5)	0.0		0.0		0.0	
Rubus sp.	0.25	(0-0.5)	0.0		0.0		0.0		0.75	(0-1.5)	0.5	(0-1.0)
Others	0.0		0.0		0.0		0.0		0.0		0.0	
Total NLW	**4.5**		**4.8**		**4.3**		**1.0**		**9.8**		**6.5**	
Leguminous Woody												
Cytisus scoparius	0.0		0.0		0.0		0.0		0.0		2.25	(0-4.5)
Total LW	**0.0**		**0.0**		**0.0**		**0.0**		**0.0**		**2.3**	
Graminoids												
Festuca sp.	49.5	(45.5-53.5)	56.3	(56.0-56.5)	53.25	(43.0-64.0)	51.5	(47.0-56.0)	51.0	(50.0-52.0)	44.5	(28.5-60.5)
Agrostis sp.	3.25	(2.5-4.0)	2.5	(1.0-4.0)	4.5	(3.0-6.0)	8.0	(8.0-8.0)	1.75	(0-3.5)	0.25	(0-0.5)
Avenula pratensis	1.25	(0-2.5)	1.75	(0-3.5)	3.5	(1.0-6.0)	0.0		1.75	(1.5-2.0)	3.25	(0.5-6.0)
Carex sp.	1.75	(1.0-2.5)	0.5	(0-1.0)	0.5	(0-1.0)	0.0		0.5	(0-1.0)	0.25	(0-0.5)
Nardus stricta	0.75	(0-1.5)	0.0		0.0		0.0		0.0		0.0	
Poa sp.	4.0	(2.5-5.5)	6.25	(5.5-7.0)	5.5	(5.0-6.0)	4.0	(0-8.0)	3.5	(3.0-4.0)	6.5	(2.5-10.5)
Others	0.0		0.5	(0-1.0)	0.0		1.0	(0-2.0)	0.0		0.0	
Total G	**60.5**		**67.8**		**67.3**		**64.5**		**58.5**		**54.8**	
Non-Leguminous Forbs												
Antennaria dioica	0.0		0.0		0.0		0.0		0.5	(0.5-0.5)	0.0	
Cerastium sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Cruciata glabra	0.0		0.5	(0-1.0)	0.0		0.5	(0-1.0)	0.0		0.0	
Galium verum	0.0		0.0		0.0		0.0		0.0		0.0	
Gentiana sp.	0.0		0.25	(0-0.5)	0.0		0.0		0.0		0.0	
Helianthemum nummularium	0.0		0.0		0.0		0.0		0.0		0.0	
Hieracium pilosella	2.5	(1.0-4.0)	0.75	(0-1.5)	1.75	(0-3.5)	2.5	(2.0-3.0)	3.0	(1.5-4.5)	3.75	(2.0-5.5)
Pedicularis pyrenaica	1.0	(0-2.0)	0.25	(0-0.5)	0.5	(0-1.0)	0.0		0.75	(0.5-1.0)	0.25	(0-0.5)
Plantago monosperma	6.25	(4.5-8.0)	3.0	(2.0-4.0)	3.0	(2.0-4.0)	5.5	(2.0-9.0)	2.5	(2.5-2.5)	6.5	(3.5-9.5)
Plantago media	0.5	(0.5-0.5)	0.0		1.25	(0-2.5)	0.5	(0-1.0)	4.5	(0-9.0)	1.0	(0.5-1.5)
Potentilla sp.	1.5	(0.5-2.5)	2.5	(1.0-4.0)	3.0	(1.5-4.5)	2.5	(0-5.0)	1.75	(0.5-3.0)	5.0	(1.0-9.0)
Ranunculus bulbosus	1.5	(1.0-2.0)	1.0	(0.5-1.5)	1.75	(1.5-2.0)	4.0	(4.0-4.0)	1.5	(0.5-2.5)	2.0	(0.5-3.5)
Sempervivum montanum	0.0		0.0		0.0		0.0		0.0		0.0	
Silene acaulis	0.0		0.0		0.0		0.0		0.0		0.0	
Taraxacum sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Thymus nervosus	0.25	(0-0.5)	1.25	(0.5-2.0)	0.5	(0-1.0)	1.5	(0-3.0)	0.25	(0-0.5)	0.5	(0-1.0)
Veronica sp.	1.5	(0.5-2.5)	0.5	(0.5-0.5)	1.0	(1.0-1.0)	0.0		1.25	(1.0-1.5)	0.75	(0-1.5)
Others	0.5	(0-1.0)	0.0		0.0		0.0		0.0		0.0	
Total NLF	**15.5**		**10.0**		**12.8**		**17.0**		**16.0**		**19.8**	
Leguminous Forbs												
Anthyllis vulneraria	1.5	(0.5-2.5)	2.25	(0-4.5)	1.5	(1.0-2.0)	1.0	(0-2.0)	1.0	(0-2.0)	1.0	(0.5-1.5)
Astragalus sp.	0.25	(0-0.5)	0.5	(0-1.0)	0.75	(0-1.5)	0.0		1.75	(0-3.5)	0.75	(0-1.5)
Chamaespartio sagittalis	0.25	(0-0.5)	0.5	(0-1.0)	0.0		0.5	(0-1.0)	1.5	(0-3.0)	5.5	(4.0-7.0)
Hippocrepis comosa	0.25	(0-0.5)	0.0		0.0		0.0		0.0		0.0	
Lotus corniculatus	0.5	(0.5-0.5)	1.25	(0-2.5)	0.25	(0-0.5)	1.0	(0-2.0)	0.0		0.0	
Trifolium alpinum	10.75	(9.5-12.0)	7.5	(7.5-7.5)	7.5	(7.0-8.0)	10.0	(10.0-10.0)	5.5	(1.5-9.5)	6.25	(0.5-12.0)
Trifolium pratense	4.75	(2.0-7.5)	4.0	(3.5-4.5)	3.75	(0.5-7.0)	4.0	(1.0-7.0)	5.25	(5.0-5.5)	5.25	(2.5-8.0)
Trifolium repens	1.25	(0.5-2.0)	1.5	(0.5-2.5)	2.0	(2.0-2.0)	1.0	(0-2.0)	0.75	(0.5-1.0)	0.25	(0-0.5)
Vicia pyrenaica	0.0		0.0		0.0		0.0		0.0		0.0	
Total LF	**19.5**		**17.5**		**15.8**		**17.5**		**15.8**		**19.0**	

Anexo 4b. Seasonal diet composition of Horse in Fontalba (2011-2012). Values represent % fragments in faeces (Min-Max).

DIET HORSE-F	SUMMER						AUTUMN					
	June		July		August		September		October		November	
Non-Leguminous Woody												
Calluna vulgaris	4.0	(2.5-5.5)	7.0	(2.5-11.5)	10.75	(0.5-21.0)	1.0	(0-2.0)	9.0	(8.0-10.0)	0.75	(0-1.5)
Juniperus communis	0.0		0.0		0.0		0.0		0.0		0.0	
Pinus uncinata	0.5	(0-1.0)	0.5	(0-1.0)	1.5	(1.0-2.0)	0.0		0.0		0.25	(0-0.5)
Quercus sp.	0.0		0.0		0.0		0.0		0.0		0.0	
R. ferrugineum	0.0		0.0		0.0		0.0		0.0		0.0	
Rosa sp.	0.0		0.0		0.25	(0-0.5)	1.25	(0-2.5)	0.0		0.75	(0-1.5)
Rubus sp.	0.25	(0-0.5)	0.0		1.0	(0-2.0)	1.75	(1.5-2.0)	0.0		0.5	(0-1.0)
Others	0.0		0.0		0.0		0.0		0.0		0.0	
Total NLW	**4.8**		**7.5**		**13.5**		**4.0**		**9.0**		**2.3**	
Leguminous Woody												
Cytisus scoparius	0.0		0.0		0.0		0.0		0.5	(0-1.0)	1.5	(0-3.0)
Total LW	**0.0**		**0.0**		**0.0**		**0.0**		**0.5**		**1.5**	
Graminoids												
Festuca sp.	51.0	(50.5-51.5)	40.0	(38.5-41.5)	43.75	(37.0-50.5)	46.8	(40.0-53.5)	40.5	(38.0-43.0)	36.8	(23.5-50.0)
Agrostis sp.	2.5	(2.0-3.0)	4.75	(3.0-6.5)	7.25	(4.5-10.0)	4.5	(2.5-6.5)	1.5	(1.0-2.0)	3.0	(2.5-3.5)
Avenula pratensis	4.0	(1.0-7.0)	1.75	(0.5-3.0)	1.0	(0-2.0)	3.0	(1.5-4.5)	2.0	(0-4.0)	0.75	(0-1.5)
Carex sp.	2.0	(1.0-3.0)	1.5	(0.5-2.5)	2.75	(2.0-3.5)	2.25	(0.5-4.0)	0.5	(0-1.0)	2.75	(0-5.5)
Nardus stricta	0.0		0.0		0.0		0.0		0.0		0.0	
Poa sp.	8.75	(5.5-12.0)	7.5	(7.5-7.5)	4.25	(4.0-4.5)	6.5	(5.5-7.5)	2.0	(0-4.0)	3.25	(2.0-4.5)
Total G	**68.3**		**55.5**		**59.0**		**63.0**		**46.5**		**46.5**	
Non-Leguminous Forbs												
Antennaria dioica	0.25	(0-0.5)	0.0		0.25	(0-0.5)	0.0		0.0		0.5	(0-1.0)
Cerastium sp.	0.5	(0-1.0)	0.0		0.0		0.0		0.0		0.0	
Cruciata glabra	0.25	(0-0.5)	0.0		0.0		0.0		0.5	(0-1.0)	0.0	
Galium verum	0.0		0.5	(0-1.0)	1.25	(1.0-1.5)	0.25	(0-0.5)	0.0		1.5	(0-3.0)
Gentiana sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Helianthemum nummularium	0.0		0.0		0.0		0.0		0.5	(0-1.0)	0.0	
Hieracium pilosella	1.25	(0.5-2.0)	0.75	(0-1.5)	0.75	(0-1.5)	3.0	(3.0-3.0)	3.5	(3.0-4.0)	5.75	(4.0-7.5)
Pedicularis pyrenaica	0.5	(0-1.0)	0.75	(0.5-1.0)	0.0		0.75	(0-1.5)	0.5	(0-1.0)	0.0	
Plantago monosperma	3.5	(2.5-4.5)	3.75	(3.0-4.5)	2.75	(2.5-3.0)	4.0	(4.0-4.0)	7.0	(4.0-10.0)	8.5	(5.0-12.0)
Plantago media	0.5	(0.5-0.5)	0.25	(0-0.5)	0.0		0.25	(0-0.5)	0.5	(0-1.0)	1.0	(0.5-1.5)
Potentilla sp.	1.5	(1.0-2.0)	3.75	(3.5-4.0)	2.5	(1.5-3.5)	3.25	(2.0-4.5)	5.0	(5.0-5.0)	5.0	(4.0-6.0)
Ranunculus bulbosus	1.75	(1.5-2.0)	1.75	(1.5-2.0)	0.75	(0-1.5)	2.25	(2.0-2.5)	3.5	(2.0-5.0)	2.75	(1.5-4.0)
Sempervivum montanum	0.0		0.0		0.0		0.0		0.0		0.0	
Silene acaulis	0.0		0.0		0.0		0.0		0.0		0.0	
Taraxacum sp.	0.0		0.0		0.0		0.0		0.0		0.0	
Thymus nervosus	0.75	(0.5-1.0)	1.25	(0-2.5)	0.5	(0-1.0)	0.0		1.5	(0-3.0)	0.75	(0-1.5)
Veronica sp.	0.25	(0-0.5)	1.0	(1.0-1.0)	1.0	(1.0-1.0)	0.75	(0.5-1.0)	0.5	(0-1.0)	1.5	(0-3.0)
Others	0.0		0.0		0.0		0.25	(0-0.5)	0.0		0.0	
Total NLF	**11.0**		**13.8**		**9.8**		**14.8**		**23.0**		**27.3**	
Leguminous Forbs												
Anthyllis vulneraria	1.25	(0.5-2.0)	1.5	(1.0-2.0)	1.5	(1.5-1.5)	2.25	(1.5-3.0)	0.5	(0-1.0)	1.25	(0-2.5)
Astragalus sp.	1.0	(0-2.0)	2.25	(0-4.5)	1.75	(0-3.5)	1.25	(0-2.5)	0.0		1.75	(0.5-3.0)
Chamaespartio sagittalis	0.5	(0.5-0.5)	0.0		1.0	(0.5-1.5)	0.75	(0-1.5)	0.5	(0-1.0)	0.75	(0-1.5)
Hippocrepis comosa	0.25	(0-0.5)	0.0		0.0		0.0		0.0		0.0	
Lotus corniculatus	1.75	(0.5-3.0)	0.75	(0.5-1.0)	1.0	(0-2.0)	0.5	(0-1.0)	2.5	(1.0-4.0)	0.0	
Trifolium alpinum	9.75	(5.5-14.0)	10.3	(10.0-10.5)	6.5	(6.0-7.0)	9.25	(5.5-13.0)	9.5	(9.0-10.0)	7.5	(2.5-12.5)
Trifolium pratense	1.25	(0.5-2.0)	5.75	(0-11.5)	5.25	(4.5-6.0)	3.5	(1.0-6.0)	6.5	(5.0-8.0)	9.75	(4.5-15.0)
Trifolium repens	0.25	(0-0.5)	2.75	(2.0-3.5)	0.75	(0-1.5)	0.75	(0-1.5)	0.5	(0-1.0)	1.25	(0-2.5)
Vicia pyrenaica	0.0		0.0		0.0		0.0		1.0	(0-2.0)	0.25	(0-0.5)
Total LF	**16.0**		**23.3**		**17.8**		**18.3**		**21.0**		**22.5**	

CAPÍTULO 3

Diet composition and gastrointestinal parasitism of Pyrenean chamois

Abstract

According to Optimal Foraging Theory (OFT), optimal feeding implies to obtain the maximal benefit at the lowest cost including parasitic risk of infection. This challenge would be of particular relevance in herbivores, since main parasite groups (e.g., *Strongyles) are released trough the faeces that tend to be concentrated where animal grazes*. In this work we analyzed both gastrointestinal parasite loads and annual diet composition of Pyrenean chamois (*Rupicapra pyrenaica*) in order to explore OFT. Concretely we tested whether or not seasonal foraging behaviours are oriented to avoid parasite infection in Freser Setcases National Game Reserve (Eastern Pyrenees). From May 2009 to May 2012 we collected 76 population faecal samples from two localities (Costabona and Fontalba). Diet composition was determined by microhistological analysis of faeces and parasite load by coprological analysis. A partial least squares regression model was used to evaluate the effect of diet composition on parasitic load in two periods of contrasted food availability: Winter-Autumn and Spring-Summer. Diet analysis showed that woody species were the most consumed in winter, autumn and spring with a sharply decrease during summer. Forbs species remained low during the whole year except in summer when consume increased. Woody species were negatively related to parasitism while forbs were positively. The results suggest that cost of parasitism would be lower than the benefits from forbs intake in Pyrenean chamois inhabiting this extremely seasonal environment.

Keywords: Foraging Theory, diet composition, parasite load, tolerance.

Introduction

Natural selection may favour those feeding strategies that maximize the amount of energy gained per unit of time spent foraging (Stephens et al*,* 2007). Animals that acquire energy efficiently during foraging may increase their fitness by having more energy available for reproduction (Hennicke and Culik, 2005), antipredator vigilance (White and Berger, 2001) or immune response against pathogens (Beldomenico et al, 2008). But highly nutritious food may be less abundant or may be farther away than the less nutritious one and hence foraging for animals involves a trade-off between the food's energy content and the cost of obtaining it. According to Optimal Foraging Theory (OFT), optimal feeding implies to obtain the maximal benefit at the lowest cost (Stephens and Krebs, 1986), not only in terms of energy acquisition but also minimizing predation risk. However, sometimes highly energy intake is not the one that minimizes these additional costs (see Kie, 1999 for a review in ungulates).

One of the last contributions to OFT was the inclusion of the effects of parasitism on diet choice (Lozano, 1991). This new perspective is of particular relevance in herbivores, since dispersal forms of the main parasite groups (e.g., *S*trongyles) are released trough the faeces and tend to be concentrated where animal graze (Abaturov et al, 2008). Actually, faeces deposition improves pasture quality (Sasaki et al, 1998) creating patches of higher forage quality where herbivores tend to be aggregated (Fryxell, 1991). These vicious circles between dung concentration, forage quality, and parasites, forces to make foraging decisions involving trade-offs between the benefits of nutrient intake and the risk of infection (Hutchings et al, 1999).

Foraging behaviour to avoid parasitism would be even more challenged in those herbivore species that experience long periods of food shortage (e.g., mountain ungulates). In the seasonal mountain ecosystems, for example, phenological development of plants drives foraging behaviour of herbivores (Mysterud et al, 2011). Feeding habits match with yearly cycles of primary production (Parker et al, 2009), being herbivores forced to spend most part of their time grazing (hyperphagia) during the warm season in anticipation of winter (Barboza and Humme, 2006). What is more, voluntary intake of ruminants also depends on herbage characteristics (Decruyenaere et al, 2009), decreasing at low quality forages (are digested more slowly and remain in the rumen longer) but increasing with food quality (van Langevelde, 2008). Under such circumstances parasite encounter by means of feeding infective larvae released on to pastures would be highly probable in herbivores grazing in patches of high forage quality. This situation would be especially relevant in alpine ecosystems where highly nutritious pastures are available for herbivores during the short summer window (i.e., period without snow).

In this work, we will explore these constraints, between the accommodation of seasonal variations of food availability and the risk of parasite infection in the Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) inhabiting the highly seasonal habitats of the Pyrenees. This mountain ungulate browse when herbaceous availability is low but prefers feeding on graminoids and forbs (Aldezábal and García-González, 2004) coinciding with the increase of nutritive contents of pastures during the warm season (i.e., spring and summer, see Marinas et al, 2003). As a result, body mass recovering after winter in *Rupicapra* species depends greatly on Spring-Summer primary productivity (Garel et al, 2011). On the other hand, helminthofauna of this medium size mammal is highly diverse (Durand and Gautier, 1996), being prevalences of gastrointestinal infection close to 100% in most chamois populations (unpublished data). Taking the advantage of this exceptional host-parasite system, in this work we first described diet composition of a population of Pyrenean chamois from the Catalonian Pyrenees, Spain, and then explored whether or not their seasonal foraging behaviour maximizes acquisition of high quality food while minimizes parasitic infection as predicted by the OFT.

Material and Methods

Study area

This study was conducted at the Freser-Setcases National Game Reserve (FSNGR), Eastern Pyrenees, Cataluña, Spain (42° 22′ N, 2° 09′ E, Figure.1). The FSNGR is a mountainous area of 20, 200 ha where an alpine ecosystem predominates with an average altitude of 2000 m. a. s. l. (1200 - 2910 m. a. s. l. at Puigmal peak). Specifically, our work was carried out in two different areas separated by 20 Km of rough terrain: Costabona and Fontalba.

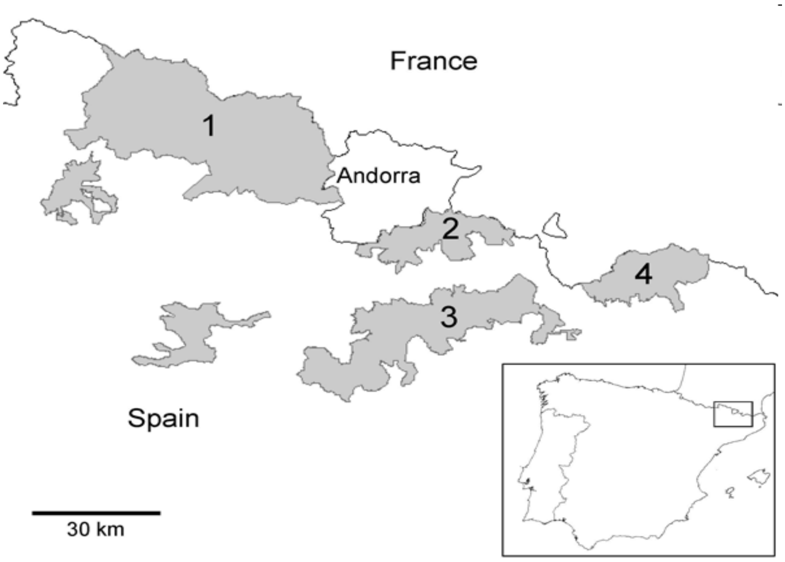

Figure 1. Study area in the Freser-Setcases National Game Reserve, Eastern Pyrenees Spain.

The former is located in the northeast part of the FSNGR (42° 24′ N, 2° 20′ E, ranging from 1093 to 2429 m. a. s. l.), whereas the later in the central part of the reserve (42° 22′ N, 2° 08′ E, ranging from 1660 to 2248 m. a. s. l.). Both areas have similar features in terms of vegetation composition and structure belonging to the sub-humid alpine region. Annual mean temperature of 6.05 ºC (min = - 16.8, max = 39.2) and mean yearly accumulated rainfall (period 1999-2011) of 963.38 mm (min = 520.6, max = 1,324.8, data from Nuria meteorological station located at 1971 m. a. s. l. in the core FSNGR, Servei Meteorològic de Catalunya < www.meteocat.com >).

Vegetation in the study lowest area consists of Pinus uncinata forests with a substrate of Arctostaphylos uva-ursi, Calluna vulgaris, and Cytisus scoparius (1200 - 2000 m.a.s.l.). Vegetation above 2,000 meters is mainly represented by a mosaic of alpine meadows were graminoid species (e.g., Festuca and Carex genus) are dominant and Trifolium alpinun patches are abundant (for revision see Vigo 1976, 1996, and Vigo and Ninot, 1987). Chamois from the FSNGR shares habitat with a small population of European muflons (Ovis musimon) and with herds of cows, sheep and horses during the warm season (i.e., May to October). No large predators are present but trophy hunting is allowed from August to February.

Sampling procedure

From 22nd May 2009 to 24th May 2012, 449 faecal samples were collected by the following procedure: once a month, but often twice, each study area was visited by at least two observers. They walked two transects (one per location) of about 5 km each locating chamois groups by means of 10 x 42 binoculars and 20 - 60 x 65 spotting scopes. These transects cover the whole altitude range and the main vegetation communities of the study area. Once group size, composition and precise location of chamois were recorded, observes collected fresh droppings at the exact place where animals were sighted and their surroundings. Fresh samples were chosen according to their colour and texture (Hibert et al, 2011), in order to avoid effects of possible chemical or physical agents (Lestie and Starkey, 1984) and to guarantee accurate gastrointestinal egg counts. Faecal samples were collected in individual labelled plastic bags and transported to the laboratory. By mixing pellets from individual faecal groups we obtained population faecal samples that were transported to UAB facilities by keeping them in a cold box (4ºC). Following this procedure observers got between five to eight individual samples per sampling day (a total of 197 for Costabona and 252 for Fontalba) that once mixed accounted for 76 population samples (39 for Fontalba and 37 for Costabona).

Faecal sampling processing

Once at the laboratory, samples were divided for both coprological and botanical diet composition analyses. For diet estimation, samples were stored frozen at -20ºC, whereas for parasite load estimation the samples were analysed within the following 12 hours. Gastrointestinal eggs count (GEC) was carried out in a pool of a minimum of 20 fresh droppings from each population sample. This sample size can be considered a reliable estimation of the parasitic status in an ungulate host population (Tayce et al, 2008). GEC technique is broadly used as a reliable indicator of parasite load in small ruminants; actually, it is the most common approach for the estimation of intensity of gastrointestinal strongyle infections (Rinaldi et al, 2009). Briefly, 2 g of faeces were emulsified in 20 ml of a zinc sulphate solution and GEC was determined microscopically by the modified McMaster method (MAFF, 1980). For each sample, all strongyle eggs in two chambers of McMaster slide are counted and then multiplied by 50 to determine the number of eggs per gram of faeces.

Diet composition estimation by microhistological analysis

Population samples from each sampling day (37 from Costabona and 39 from Fontalba) were used for microhistological identification of epidermal fragments in faeces. For more than a half century (Crocker, 1959), this indirect assessment of diet composition has been by far the most common technique for assessing diet selection in both domestic (Bartolomé et al, 2011) and wild ruminants (Suter et al, 2004; La Morgia and Bassano, 2009). This technique allows collection of a representative sample of plant species ingested without interfering with animal behaviour (Bartolomé et al, 1998). However, some biases can occur due to differential digestibility of ingested plants and correction factors could be sometimes required (Holechek et al, 1982; Bartolomé et al, 1995). However, when making diet comparisons across seasons or years in the same study area, correction factors are not required. The procedure employed in this work was adapted from (Stewart, 1967). Once samples were thawed, they were water washed to remove extraneous material and then ground in a mortar to separate the epidermal fragments. After that, 10 grams by sample were placed in a test tube with 5 ml of 65% concentrated HNO_3. The test tubes were then boiled in a water bath for 1 min. After digestion in HNO_3, the samples were diluted with 200 ml of water. This suspension was then passed through 1.00- and 0.25-mm filters. The 0.25–1.00mm fraction was spread on glass microscope slides in a 50% aqueous glycerine solution and cover-slips were fixed with DPX microhistological varnish. Two slides were prepared from each sample. Later, slides were examined under a microscope at ×100×400 magnifications and plant fragments were recorded and counted until 200 units of leaf epidermis. An epidermis collection of 40 main plant species of the study area was made and used for fragment identification. Finally, plants were pooled into five groups: non legume woody sp (NLW), legume woody sp (hereafter LW), gramineous sp (G), legume forb sp (LF) and non legume forb sp (NLF).

Statistical analysis

In order to describe the diet of chamois, we first analyzed whether percentage of plant consumed (as response variable) depended on plant groups (i.e., categorical explanatory variable with five categories: LW, NLW, G, LF and NLF) and seasons using generalized lineal models (GLM). Plant consume showed a clear non-linear yearly pattern (Figures 2-6), thus monthly trends of consume were analyzed using a set of specific generalized additive mixed models (GAMM) (Wood, 2006), in which percentage of plant consume (response variable) was explained by the effect of month of sampling (fixed explanatory factor) and population as random factor (i.e, Costabona and Fontalba). In all cases, only plants representing more than 3% of chamois diet were included in our additive modelling. Finally, the effect of diet composition on parasitic load was evaluated by a Partial Least Squares Regression approach (PLSR). This statistical tool is an extension of multiple regression analysis where associations are established with factors (e.g., combinations of dependent variables extracted from predictor variables that maximize the explained variance in the dependent variable). PLSr allows coping with multicollinearity better than generalized linear models (Geladi and Kowalski, 1986).

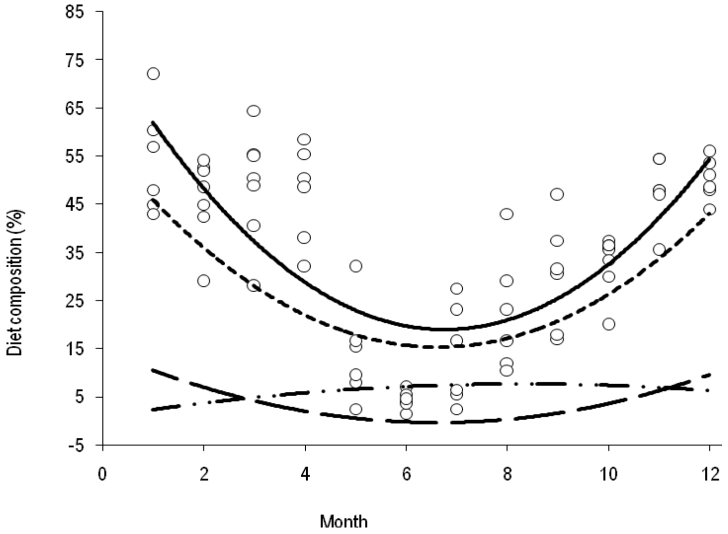

Figure 2. Monthly trends of non legume woody species (NLW) consume in Pyrenean chamois from FSNGR, northeast Spain. Solid line = NLW, short dashed line = *Calluna vulgaris*, long dashed line = *Quercus* sp, dash-dot-dotted-line = *Rubus* sp.

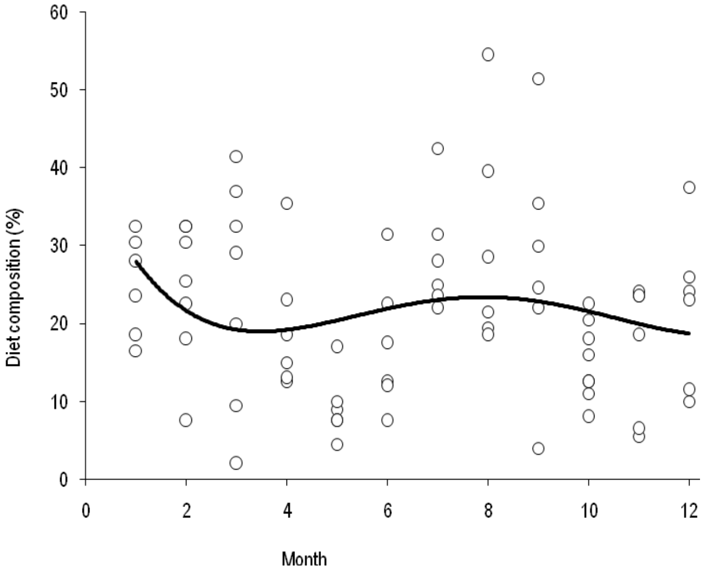

Figure 3. Monthly trends of legume woody species (LW) consume (*Cytisus scoparius*) in Pyrenean chamois.

Partial least squares regression is probably the least restrictive of the various multivariate extensions of the multiple linear regression models. This flexibility allows it to be used in situations where the use of traditional multivariate methods is severely limited, such as when there are fewer observations than predictor variables. Furthermore, partial least squares regression can be used as an exploratory analysis tool to select suitable predictor variables and to identify outliers before classical linear regression. This technique has been used in chemistry, economics, medicine, psychology, and pharmaceutical sciences where predictive linear modelling with a large number of predictors is necessary. A revision of its use in the field of ecology can be read at Carrascal et al*,* 2009. The relative contribution of each variable to the derived factors was calculated by means of the square predictor weights. In our case, the response variable was gastrointestinal parasitic load (eggs/grams of faeces) whereas diet composition (composed by LW, NLW, G, LF and NLF) was the explanatory one. Due to our moderate sample size, samples for the PLSR analyses were pooled into two year periods related to plant phenology: Spring-Summer as the warm period when vegetation growth is high and Autumn-Winter as the cold period when graminoids and both legume and non legume forbs begin to wither.

Generalized additive models (GAMM) were implemented in the "mgcv" library version 1.7-18 (Wood, 2011), and partial least square regression (PLSr) in the "plspm" version 0.2-2 (Sánchez and Trinchera, 2010) of the R statistical package version 2. 15. 1 (R Development Core Team, 2012).

Results

Diet composition

A total of 36 species were identified in faecal samples along the four years of study and only 12 appeared in a greater amount than 1% of the total number of fragments. These 12 species represent 91.4% of the diet composition (Table1A and 1B). Almost half of the diet was composed by NLW (40.2%), being: *Calluna vulgaris* (26.9% of the total of fragments), *Quercus* spp (3.8%) and *Rubus* spp (5.9%) the most consumed plants. The G resulted in the second most important component of diet (21.9%) with predominance of *Festuca* spp (18.6%). *Citysus scoparius,* the only LW, was also a main component of chamois diet with a presence over 20%. Both LF and NLF represented less than 10%. *Hieracium pilosella, Cruciata glabra* and *Plantago monosperma* were the most common plants among NLF, while *Trifolium alpinum* and *Trifolium pratense* dominated LF. The most part of the observed variability on yearly plant consume, was explained by the interaction between plant type and seasons (F = 14.953; p-value < 0.001 and R^2 = 60.6%, Table 2). The NLW resulted the most important group in Autumn (39.8%), Winter (49.9%) and Spring (37.3%), but suddenly declined in Summer (13.4%, Table 2, Fig. 2). The LW, however, showed very stable values being always around 20% of the diet in all seasons (Table 2, Fig. 3). On the other hand, G peaked in spring (29.5%) and summer (22.9%), especially in May (53.5%), and remained low during cold seasons (17.3% in Winter and 16.9% in Autumn, Table 2, Fig. 4). Yearly NLF consumes were around 10%, but increased in Summer (18.2%) reaching its highest value in July (21.1%, Table 2, Fig. 5). Similar trends, but at lower consume rate, were observed for LF (Fig. 6).

Table 1A. Annual diet composition consumed of Pyrenean chamois in FSNGR, northeast Spain. Values represent percentage of fragment apparition (Min-Max) of both non legume (NLW), legume woody (LW) species and graminoids G

	WINTER						SPRING						SUMMER						AUTUMN						
	December		January		February		March		April		May		June		July		August		September		October		November		Total
NLW																									
Calluna vulgaris	40.2	(35.5-45.5)	41.3	(32.0-64.5)	34.6	(22.0-46.0)	32.9	(12.5-53.0)	39.5	(33.5-53.0)	10.6	(0-29.5)	1.7	(0-6.0)	11.9	(2.5-25.0)	21.4	(8.5-43.0)	26.2	(9.5-44.0)	22.6	(10.0-33.5)	39.6	(33.0-47.0)	26.9
Juniperus communis	0.1	(0-0.5)	0.3	(0-1.0)	1.1	(0-3.5)	0.3	(0-1.5)	0.4	(0-2.0)	0.3	(0-1.5)	0.0		0.0		0.0		0.1	(0-0.5)	0.0		0.4	(0-1.5)	0.2
Pinus uncinata	0.4	(0-1.5)	0.4	(0-1.0)	3.0	(0-10.0)	11.9	(2.0-31.5)	4.5	(1.0-15.5)	2.2	(0-8.5)	1.2	(0-3.0)	0.3	(0-2.0)	0.3	(0-1.0)	0.1	(0-0.5)	0.2	(0-1.5)	0.8	(0-2.0)	2.1
Quercus spp.	9.4	(3.0-13.0)	11.1	(5.5-18.0)	7.0	(0-13.5)	3.3	(0-10.5)	2.6	(0-13.0)	0.0		0.1	(0-0.5)	0.0		0.5	(0-1.0)	1.1	(0-3.5)	3.4	(0-7.5)	6.8	(0-12.5)	3.8
R. ferrugineum	0.0		0.0		0.0		0.1	(0-0.5)	0.0		0.0		0.3	(0-1.0)	0.1	(0-0.5)	0.0		0.0		0.6	(0-3.5)	0.0		0.1
Rosa spp.	0.0		0.0		0.2	(0-1.5)	0.6	(0-2.5)	0.2	(0-1.0)	1.0	(0-2.5)	1.0	(0-2.5)	1.2	(0-3.0)	0.1	(0-0.5)	2.4	(0-8.0)	4.4	(0-10.0)	0.0		0.9
Rubus spp.	3.3	(0-6.5)	2.6	(0.5-6.5)	3.5	(0-15.0)	6.1	(0-18.5)	7.8	(0-28.0)	3.1	(1.5-6.0)	5.9	(1.5-14.0)	3.8	(0.5-5.5)	7.3	(0-18.5)	8.7	(0-17.0)	15.5	(5.0-27.0)	3.3	(1.0-8.0)	5.9
Others	0.1	(0-0.5)	0.1	(0-0.5)	0.3	(0-2.0)	0.0		0.0		0.0		0.2	(0-0.5)	0.1	(0-0.5)	0.0		0.4	(0-1.5)	1.9	(0-8.0)	0.4	(0-1.0)	0.3
Total	**53.5**		**55.8**		**49.7**		**55.1**		**54.9**		**17.1**		**10.3**		**17.4**		**29.7**		**38.9**		**48.7**		**51.2**		**40.2**
LW																									
Cytisus scoparius	22.0	(10.0-37.5)	24.9	(16.5-32.5)	24.1	(7.5-32.5)	24.5	(2.0-41.5)	19.6	(12.5-35.5)	9.3	(4.5-17.0)	17.3	(7.5-31.5)	28.8	(22.0-42.5)	30.3	(18.5-54.5)	27.9	(4.0-51.5)	15.1	(8.0-22.5)	16.9	(5.5-24.0)	**21.7**
G																									
Festuca spp.	12.2	(7.0-18.0)	14.5	(11.0-22.0)	15.6	(7.5-23.5)	12.8	(4.5-21.0)	16.9	(14.5-24.5)	47.6	(34.0-60.5)	31.1	(17.5-50.0)	12.5	(6.0-16.5)	15.8	(9.5-24.0)	15.0	(7.5-27.5)	15.1	(4.5-22.5)	14.8	(8.0-25.0)	18.6
Agrostis spp.	0.0		0.0		0.0		0.8	(0-2.0)	3.2	(0.5-6.0)	1.4	(0-8.5)	0.7	(0-1.5)	0.3	(0-0.5)	0.0		0.1	(0-0.5)	0.0		0.4	(0-2.5)	0.6
Avenula pratensis	0.4	(0-1.5)	0.2	(0-0.5)	0.8	(0-3.0)	0.5	(0-2.5)	0.2	(0-0.5)	1.0	(0-5.0)	2.2	(0-5.0)	0.4	(0-1.5)	0.6	(0-1.5)	1.3	(0-5.5)	1.1	(0-6.5)	0.8	(0-2.0)	0.8
Carex spp.	0.6	(0-2.5)	0.3	(0-1.0)	0.5	(0-2.5)	0.0		0.3	(0-1.0)	0.8	(0-1.5)	0.3	(0-0.5)	0.4	(0-1.0)	0.3	(0-1.0)	0.6	(0-1.5)	0.3	(0-2.0)	0.8	(0-1.5)	0.4
Nardus stricta	1.5	(0-5.5)	1.4	(0-4.5)	0.6	(0-2.5)	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.9	(0-4.0)	0.4
Poa spp.	0.5	(0-1.5)	0.0		0.5	(0-1.0)	0.7	(0-2.0)	2.1	(0-8.5)	2.3	(0-6.0)	1.3	(0-3.5)	0.6	(0-1.0)	0.4	(0-1.0)	0.0		0.0		0.8	(0-2.5)	0.8
Others	0.0		0.2	(0-0.5)	0.1	(0-0.5)	0.0		0.3	(0-1.0)	0.4	(0-1.5)	1.8	(0-3.5)	0.1	(0-0.5)	0.2	(0-1.0)	0.3	(0-1.0)	0.0		0.6	(0-2.5)	0.3
Total	**15.2**		**16.5**		**18.1**		**14.8**		**22.9**		**53.5**		**37.4**		**14.3**		**17.3**		**17.3**		**16.4**		**18.9**		**21.9**

Table 1 B. Annual diet composition consumed of Pyrenean chamois in FSNGR, northeast Spain. Values represent percentage of fragment apparition (Min-Max) of both legume (L) and non legume forbs (NLF) species.

	WINTER						SPRING						SUMMER						AUTUMN						
	December		January		February		March		April		May		June		July		August		September		October		November		Total
NLF																									
Antennaria dioica	0.0		0.0		0.0		0.1	(0-0.5)	0.0		0.6	(0-2.0)	0.6	(0-1.5)	0.9	(0-1.5)	0.3	(0-1.0)	0.1	(0-0.05)	0.0		0.0		0.2
Cerastium spp.	0.0		0.0		0.0		0.0		0.0		0.0		0.3	(0-1.0)	0.3	(0-1.0)	0.0		0.2	(0-1.0)	0.4	(0-3.5)	0.0		0.1
Cruciata glabra	0.3	(0-1.0)	0.3	(0-2.0)	1.4	(0-9.5)	0.9	(0-5.5)	0.0		4.6	(1.0-15.0)	1.6	(0-5.5)	2.1	(0.5-4.0)	2.3	(1.0-4.0)	1.7	(0.5-2.5)	5.3	(0-10.5)	0.4	(0-2.0)	1.7
Gentiana spp.	0.0		0.0		0.0		0.0		0.0		0.0		0.1	(0-0.5)	0.0		0.0		0.0		0.0		0.0		0.1
Helianthemum nummularium	0.0		0.0		0.0		0.0		0.0		0.0		0.2	(0-0.5)	0.1	(0-0.5)	0.1	(0-0.5)	0.7	(0-4.0)	0.0		0.1	(0-0.5)	0.1
Hieracium pilosella	2.1	(0-4.5)	0.6	(0-1.0)	1.1	(0-5.5)	0.3	(0-1.0)	0.7	(0-3.0)	1.6	(0-3.5)	6.1	(0-10.5)	4.7	(0.5-17.0)	3.7	(1.0-9.0)	1.3	(0-4.0)	1.9	(0-4.0)	2.9	(0.5-11.0)	2.2
Pedicularis pyrenaica	0.0		0.0		0.0		0.0		0.0		0.2	(0-1.0)	0.0		0.0		0.0		0.0		0.0		0.0		0.0
Plantago monosperma	0.3	(0-0.5)	0.1	(0-0.5)	0.1	(0-0.5)	0.1	(0-1.0)	0.3	(0-1.0)	2.4	(0.5-7.5)	5.6	(1.5-9.0)	3.3	(1.5-5.0)	2.2	(0-6.0)	2.0	(0-5.0)	0.0		1.2	(0-5.0)	1.5
Plantago media	0.0		0.0		0.0		0.4	(0-2.0)	0.3	(0-1.0)	0.8	(0-2.5)	0.9	(0-2.5)	1.8	(0.5-1.5)	1.1	(0-5.5)	0.3	(0-1.0)	0.0		0.0		0.5
Potentilla spp.	0.0		0.0		0.4	(0-3.0)	0.0		0.1	(0-0.5)	3.1	(0.5-6.5)	2.7	(0-3.5)	5.3	(3.0-8.5)	2.5	(0-6.5)	0.6	(0-2.0)	0.0		0.2	(0-1.0)	1.2
Sempervivum montanum	0.0		0.0		0.0		0.0		0.0		0.0		0.3	(0-1.5)	0.0		0.0		0.0		0.0		0.0		0.1
Silene acaulis	0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.0		0.4	(0-3.0)	0.0		0.1
Taraxacum spp.	0.0		0.0		0.1	(0-1.0)	0.3	(0-2.0)	0.1	(0-0.5)	0.3	(0-1.5)	0.5	(0-1.0)	0.5	(0-1.0)	0.5	(0-2.0)	0.7	(0-2.5)	2.8	(0-6.5)	0.0		0.5
Thymus nervosus	0.0		0.0		0.0		0.0		0.0		0.0		0.3	(0-0.5)	0.3	(0-1.0)	0.0		0.1	(0-0.5)	0.0		0.0		0.1
Veronica spp.	0.0		0.0		0.0		0.0		0.0		0.1	(0-0.5)	1.2	(0.5-1.5)	2.0	(0-8.5)	0.8	(0-2.5)	0.3	(0-1.5)	0.3	(0-2.0)	0.0		0.4
Others	0.5	(0-1.5)	0.1	(0-0.5)	0.1	(0-0.5)	0.1	(0-0.5)	0.2	(0-1.0)	0.3	(0-1.0)	0.2	(0-0.5)	0.0		0.0		0.1	(0-0.5)	0.0		0.8	(0-4.0)	0.2
Total	**3.1**		**1.1**		**3.2**		**2.1**		**1.6**		**13.8**		**20.4**		**21.1**		**13.3**		**7.9**		**11.0**		**5.5**		**8.7**
LF.																									
Hippocrepis comosa	0.0		0.0		0.0		0.0		0.0		0.1	(0-0.5)	0.3	(0-1.0)	0.1	(0-0.5)	0.0		0.0		0.0		0.0		0.1
Lotus corniculatus	1.2	(0-2.0)	0.2	(0-1.0)	0.6	(0-2.0)	0.6	(0-2.5)	1.0	(0.0-4.5)	0.3	(0-2.0)	1.3	(0-4.0)	0.8	(0-1.5)	0.5	(0-1.5)	0.6	(0-2.0)	0.0		0.3	(0-0.5)	0.6
Trifolium alpinum	4.8	(0-7.5)	1.6	(0-4.0)	4.2	(0-12.0)	2.6	(0-16.0)	0.0		2.3	(0-6.0)	6.3	(3.0-10.5)	7.1	(5.5-8.5)	4.2	(1.5-6.5)	5.8	(0.5-24.5)	7.3	(2.5-10.0)	7.3	(4.5-9.5)	4.4
Trifolium pratense	0.0		0.0		0.0		0.0		0.0		2.9	(1.0-7.0)	4.3	(1.5-10.0)	5.1	(2.0-8.5)	3.0	(0.5-7.0)	1.1	(0-2.0)	0.4	(0-3.5)	0.0		1.4
Trifolium repens	0.0		0.0		0.0		0.2	(0-1.0)	0.0		0.8	(0-2.5)	2.2	(1.0-3.5)	5.2	(0.5-8.0)	1.6	(0-3.0)	0.4	(0-1.5)	0.8	(0-2.0)	0.0		0.9
Trifolium total	4.8	(0-7.5)	1.6	(0-4.0)	4.2	(0-12.0)	2.8	(0-16.0)	0.0		5.9	(0-7.0)	12.8	(1.0-10.5)	17.3	(0.5-8.5)	8.8	(0-7.0)	7.3	(0-24.5)	8.4	(0-10.0)	7.3	(0-9.5)	6.8
Vicia pyrenaica	0.3	(0-2.0)	0.0		0.0		0.1	(0-0.5)	0.0		0.0		0.3	(0-1.0)	0.3	(0.0-1.0)	0.3	(0-1.5)	0.0		0.4	(0-3.0)	0.0		0.1
Total	**6.3**		**1.8**		**4.9**		**3.5**		**1.0**		**6.3**		**14.7**		**18.5**		**9.5**		**7.9**		**8.8**		**7.5**		**7.6**

Table. 2. Mean percentages (±SE) and minimum and maximum values of plant groups: non legume woody spp (NLW), legume woody spp (LW), gramineous spp (G), legume forb spp (LF) and non legume forb spp (NLF), consumed by Pyrenean chamois in FSNGR, northeast Spain.

	WINTER	SPRING	SUMMER	AUTUMN
NWL	49.9 ± 2.8 (0-29.0)	37.3 ± 4.4 (2.5-64.5)	13.4 ± 2.7 (1.5-3.0)	39.8 ± 2.2 (17.0-56.0)
WL	24.5 ± 2.1 (7.5-37.5)	18.1 ± 2.7 (2.0-41.5)	25.4 ± 2.7 (7.5-54.5)	20.0 ± 2.1 (4.0-51.5)
G	17.3 ± 1.4 (8.5-23.5)	29.5 ± 4.3 (5.5-63.5)	22.9 ± 3.1 (8.0-46.5)	16.9 ± 1.4 (4.5-34.0)
LF	3.4 ± 0.9 (0.0-12.0)	3.5 ± 1.0 (0.0-16.0)	13.9 ± 1.2 (5.5-6.0)	7.5 ± 0.9 (0.0-25.5)
NLF	2.2 ± 0.8 (0.0-10.5)	5.6 ± 1.3 (0.0-18.5)	18.2 ± 1.3 (6.0-7.0)	7.1 ± 1.1 (0.5-20.5)
	F=115.2 , p-value < 0.01	F value = 22.21, p-value < 0.01	F value = 5.1, p-value < 0.01	F value = 64.6, p-value < 0.01

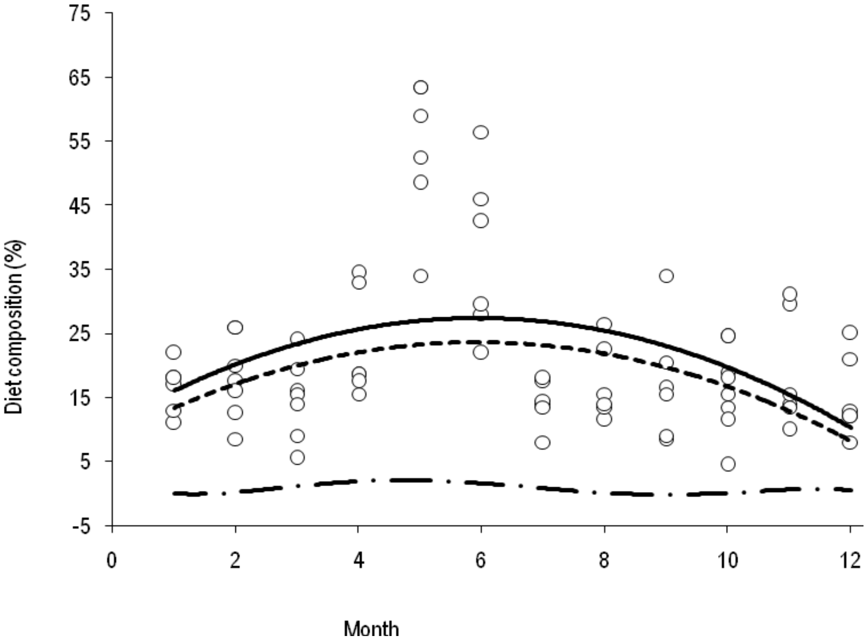

Figure 4. Montly trends of graminoids species (G) consume in Pyrenean chamois. Solid line = G, short dashed line = Festuca sp, dash-dotted-line = *Poa* sp.

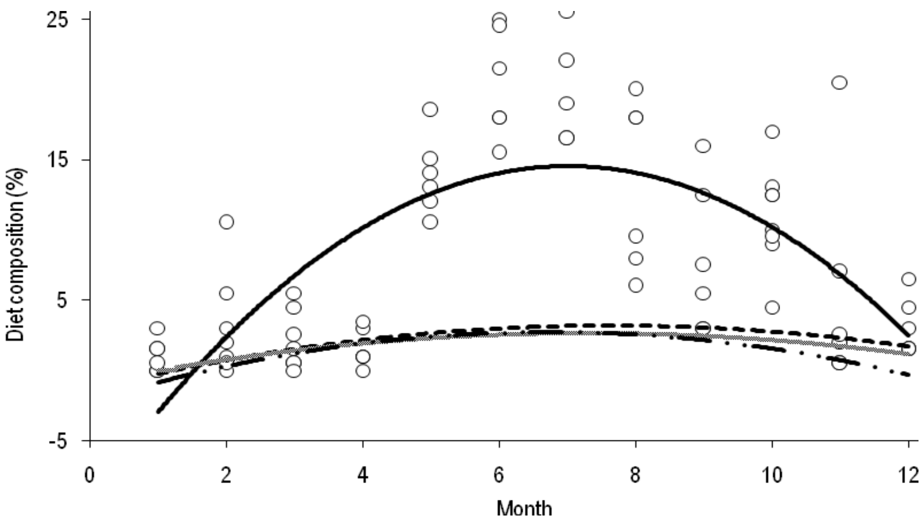

Figure 5. Monthly trends of non legume forb species (NLF) consume in Pyrenean chamois. Solid line = NLF, short dashed line = *Hieracium pilosella*, dash-dot-dotted-line = *Plantago monosperma* sp, grey line = *Cruciata glabra.*

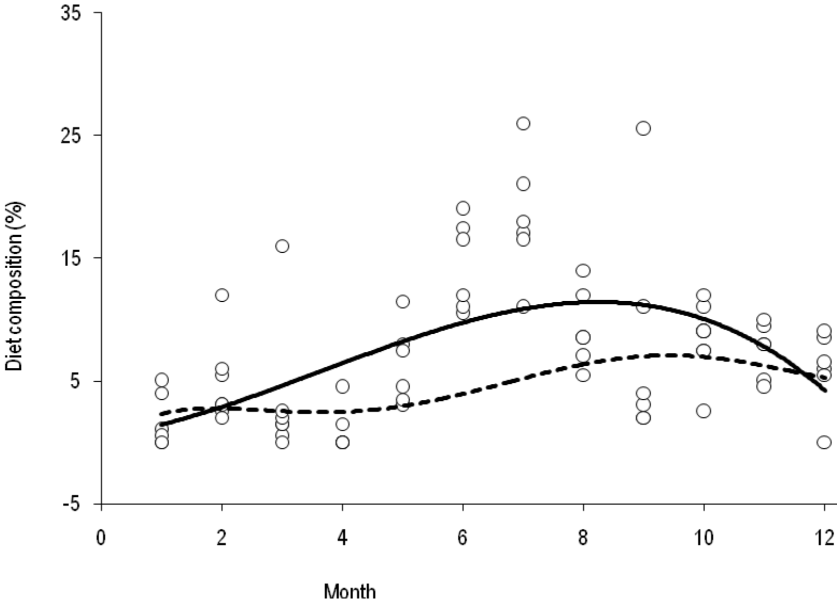

Figure 6. Monthly trends of legume forb species (LF) consume in Pyrenean chamois. Solid line = LF, dashed line represents both *Trifolium alpinum* and *Trifolium pratense* species.

Diet and gastrointestinal parasite load

PLSr analyses provided a component based on diet composition explaining between 22.6 to 44.9% of variance in Autumn-Winter and Spring-Summer gastrointestinal parasitic load of chamois respectively. Contribution of each plant to diet composition component varied among seasons (Table 3). The F were further the most important plant group in the cold period explaining more than the half of diet composition. In a lesser extent, NLW and LW were the other determinant plant types. Concretely, higher parasitic loads were associated to higher NLF, LF and G consumes while lower loads occurred in diets showing higher proportions of NLW and LW (Table 3, Fig. 7). In the same line, during the warm season (Spring-Summer), F were again the most important plant group characterizing diet composition but just slightly higher than NLW. In the cold period, NLW consume was the only negatively related group to parasitic burden whereas NLF and LF consume was associated with high parasitic loads. Curiously, LW species were slightly negatively related to parasite status in Winter-Autumn but positively in Spring-Summer.

Table. 3. Predictors weights of the Partial Least Square Regression (PLSr) analyses explaining the parasitic load effect of gastrointestinal parasites on alpine chamois in Winter-Autumn period (September, October, November, December and January) and in Spring-Summer period (March, April, May, June, July and August) by different plant types of diet composition: Non legume woody spp, Legume woody spp, Graminoids spp, Non legume forbs spp and Legume forbs spp. Pedictor weights represent the contribution of each plant type variable to the X component: Diet composition. Variables explaining more than 10% of the total variance are shown in bold type.

Predictor variables	Weights	% Variance explained
Winter-Autumn		
Non legume forbs spp	0.71	50.41
Non legume woody sp	- 0.59	28.81
Legume woody sp	-0.35	12.25
Legume forbs spp	0.15	2.25
Graminoids spp	0.06	0.36
Spring-Summer		
Non legume forbs spp	0.61	37.21
Non legume woody spp	- 0.59	34.81
Legume forbs spp	0.49	24.01
Legume woody spp	0.15	2.25
Graminoids spp	0.12	1.44

Discussion

As expected, patterns of plant consume in chamois matched with plant phenology. In general, Woody plants (*Cytisus scoparius*, and NLW) were more than the half of chamois annual diet. However, NLW sharply decreased in late spring and remained at low percentage of apparition until middle Autumn. At the same time ingested NLW declined, LF and NLF consume doubled, especially in May-June coinciding with their greater nutritive values (Marinas et al, 2003). La Morgia and Bassano (2009) found that chamois feed on browses only during Autumn and Winter as a result of low availability and quality of herbaceous plants. Our results are consistent with this foraging pattern, indicating a greater consumption and selection of high nutritious forbs when available. Moreover, chamois groups (as in other northern ungulate species, see Mysterud et al, 2001) migrate during summer above the forest limit (direct observations), probably to select resources in an optimal phenological state. At this places (over 2000 m. a. s. l.) woody species are scarcest and only *Cytisus scoparius*, are present. In fact, *Cytisus* remains green during the whole year (it has photosynthetic stems), probably being an important source of nitrogen. As Aldezábal and García-González (2004) pointed, chamois prefers legume to non legume plants due to its elevated nutritional value and digestibility (Marinas et al, 2003). On the other hand, G percentages would remain stable throughout the year due to its high fiber content. These results corroborate the feeding strategy of Chamois, that would prefer herbaceous plants when available (La Morgia and Bassano, 2009), even in forested habitats (Cuartas et al, 1996). Therefore chamois ingest G and LW regularly throughout the year in order to increase fiber and nitrogen intakes. When forbs availability is low during Autumn and Winter due to plant phenology (Marinas et al, 2003) or snow cover (Aldezábal and García-Gonzalez, 2004), chamois adapts to new conditions by browsing woody species.

PLSr analyses showed that higher consumes of NLW were negatively related to higher parasitic burdens. Some of the species (*Quercus* sp, *Pinus* sp, *Rubus* sp, *Rosa* sp) have to be browsed reducing the risk of infection (Hutchings et al, 2001). However, *Calluna vulgaris*, the most ingested NLW, *Junniperus communis* and *Rhododendrum ferrugineum* are small shrubs with heights between 20-50 cm. On the other hand, some ungulates have been described to use plants for self medication purposes (e.g., see Torres-Acosta et al, 2008 in goats, and Lisonbee et al, 2009 or Juhnke et al*,* 2012 in sheep), since plant extracts contains secondary compounds (tannins) showing antiparasitic properties (Hutchings et al, 2003; Hoste et al, 2005). The high concentration of tannins found in NLW and in LW species (e.g., *Juniperus* genus, *Calluna vulgaris*, *Quercus* genus) (Frutos et al, 2004) would justify their apparition in chamois diet. Controversially, both LF and NLF were positively associated to parasite load during the whole year due to parasites mainly concentrate in swards and tussocks, especially in Summer (Hutchings et al, 2003). Curiously, LW species were slightly negatively related to parasite status in Winter-Autumn but positively in Spring-Summer. Probably chamois acquires parasites mainly during Summer and Spring period, whereas in Winter-Autumn chamois infection would be reduced due to intake of plant species with higher tannin concentrations (LW and NLW). Nevertheless, when chamois consume the highest nutritious plants forbs (Marinas et al, 2003), would face a trade-off between risk of parasitism and nutrient intake. In fact, chamois doubled the ingestion of both LF and NLF when availability was higher but infective larvae of gastrointestinal parasites are abundant on herbaceous plants (Hutchings et al, 2003). According to OFT, chamois decision is based in the lower cost of parasitism compared to the benefit from the nutrient intake. However, and extra nutrient intake could enhance immunological system allowing chamois coping with parasites (resistance). Nevertheless, this behaviour would have meant a negative correlation between LF and NLF with parasitism. Hence, chamois behaviour can only be explained as chamois assuming the costs of parasitism and feeding with greater nutritive plants in order to minimize the harm caused by pathogens (tolerance).

To conclude, risk of parasite infection may be ignored by Pyrenean chamois at different temporal scales as diet seems more related to plant phenology. Tolerance strategy must be taken into account in OFT and complement foraging behaviours analyses. Studies concerning tolerance are just starting to be developed but further research is needed to throw some light on the relation between nutrition and parasitism.

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CAPÍTULO 3

Gastrointestinal nematodes and dietary fibre: Two factors to consider when using FN for wildlife nutrition monitoring

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Abstract

Faecal nitrogen (FN) – the combination of metabolic nitrogen and residual food nitrogen – has been used as a proxy for diet quality in wild and domestic ruminants for over half a century. However, a common misconception in some of these studies is that FN is a direct proxy for dietary N, in spite of experimental evidence that links FN to general diet digestibility. Additionally, gastrointestinal nematodes (GIN) can alter N metabolism and increase FN by various mechanisms. To clarify the role of dietary N, fibre and GIN as a factor in FN excretion, 10 naturally parasitized sheep were fed two different isocaloric diets (LPF: low-protein, low-fibre; HPF: high-protein, high-fibre). One month after these diets began, a single anthelmintic treatment was applied to remove GIN, after which the sheep were kept on the same diet for an additional 2 weeks. Throughout the experiment, individual faecal samples were obtained to estimate both FN and GIN intensity (using faecal egg counts, FEC). In addition, two blood samples were taken before and after deworming to measure serum total protein concentrations (TP) as a proxy for protein absorption. In spite of the difference in dietary protein, FN was higher on an LPF diet, supporting the overall digestibility concept. The influence of GIN on FN was later revealed by the anthelmintic treatment, which led to a decrease of FEC and FN in both dietary groups. Serum total protein showed as light but non-significant increase in both groups after the anthelmintic treatment. Our study supports not only the concept that FN is a proxy for diet digestibility, and not directly for dietary N, but also that gastrointestinal nematodes limit its use as a proxy for diet quality in ruminants, especially under high parasite loads (e.g., 1000 faecal eggs per gram of faeces). Such limitations should be considered before using FN for wildlife nutrition monitoring. Some recommendations are given to avoid misinterpretations.

Keywords: Acid detergent fibre, faecal nitrogen, diet quality, gastrointestinal nematodes.

Introduction

For over half a century, faecal indices have been used as a proxy for food quality in ruminants. One of the best known is faecal nitrogen (FN), an index that was initially proposed as a proxy for metabolic N in rats in 1934 (Schneider, 1934), and 1 year later accepted as an excellent indicator of the nutritional status of a wide variety of mammals (Schneider, 1935).

The use of FN as a proxy for metabolic nitrogen is based on the following formula:

$$FN = MFN + FRFN$$

Where FN is the combination of metabolic (MFN) and food residue nitrogen (FRFN) excreted in faeces (Schneider, 1935). In many animals, there is a correlation between dietary N and FN, a fact that has favoured its wide use as a proxy for diet crude protein in livestock since the 1940s (Raymond, 1948). Even while the influence of secondary plant compounds, a common constraint on this relationship, is well understood (Leslie et al, 2008), one cannot always assume that dietary protein is directly reflected in the faeces. However, in herbivores, FRFN represents only a minor fraction of FN; the major fraction of FN consists of MFN (Mason and Frederiksen, 1979; Van Soest, 1994; Schwarm et al, 2009; Steuer et al, 2014). As a concentration, FN will be higher if there are less in digestible substances in the diet (e.g., indigestible fibre components) and more substrates on which intestinal microbes can grow (e.g., digestible fibre components and other carbohydrates). Because FRFN is mostly associated with indigestible fibre (Van Soest, 1994), FN will decrease if FRFN increases due to the increased faecal fibre concentration and hence reduced overall digestibility (Schwarm et al, 2009).

Therefore, FN in herbivores should be considered a proxy for diet quality linked to overall diet digestibility (Clauss et al, 2013), which again is often, but not necessarily, higher in diets of higher N. Use of FN as a proxy for digestibility was experimentally validated in domestic and wild ruminants (Lancaster, 1949; Robbins et al, 1987a; Lukas et al, 2005; Wang et al, 2009) and horses (Mésochina et al, 1998). In diets with secondary plant compounds such as tannins, higher FN values occur because tannins make dietary N partly indigestible (Robbins et al, 1987b; Núñez-Hernández et al, 1992; Schlecht and Susenbeth, 2006). Thus, in the absence of secondary plant compounds, it should be possible to achieve a decrease in FN with a diet rich in N if that diet is at the same time less digestible due to high fibre content.

Despite the considerations mentioned above, monitoring FN in wild ruminants is common practice (Leslie et al, 2008). The duration of environmental exposure of faeces in the wild does not seem to affect FN (Kamler et al, 2003) and droppings are easily detected during field surveys even several days after excretion. FN was widely incorporated in to wild ruminant studies in the early 1970s (Robbins et al, 1975), becoming the most commonly employed faecal indicator of food quality in wildlife in the1980s. In fact, since 1984 at least one article using FN as a diet proxy has been published annually, especially in studies of feeding ecology (e.g. Beier, 1987; Leslie and Starkey, 1985), population ecology (Choquenot,1991; Albon and Langvatn, 1992), or even in long-term monitoring programmes (Blanchard et al, 2003; Hamel et al, 2009) of cervids and bovids.

Gastrointestinal nematodes (GIN), on the other hand, are ubiquitous parasites of herbivores that cause cell damage, cell excretion (Holmes, 1993), reduction in the activity of digestive enzymes (Ritchie et al, 1966; Jones, 1983) and amino acid malabsorption (Poppi et al, 1986; Brown et al, 1991) in the infected hosts. Thereby, GIN are recognized sources of metabolic N in ruminants (Kimambo et al, 1988; MacRae, 1993; Haile et al, 2004). There is no easy way to distinguish microbial (i.e., physiological) and truly endogenous N (such as gut epithelium, enzymes), or nematode tissue in the metabolic N fraction in herbivores (Schwarm et al, 2009). Despite that limitation, no work has evaluated the effect of GIN parasite load on the interpretation of FN in ruminants.

In this work, we conducted an experiment to evaluate the influence of dietary fibre and GIN on FN in sheep (*Ovis aries*). Our objectives were three-fold: [1] to perform a systematic review on the use of FN as a proxy for diet quality in wildlife, expanding the work of Leslie et al. (2008) to the present; [2] to assess the difference in FN in two tannin-free diets, one being high in protein but also high in lignocellulose, the other being low in protein and low in lignocellulose; and [3] to evaluate the effects of deworming on FN measurements.

The experimental results presented in this work underline the importance of considering both gastrointestinal parasite load and dietary fibre content when assessing diet quality of herbivores by means of FN.

Materials and methods

Systematic review on the use of FN as a proxy for diet quality in wildlife

We performed a bibliographic search using specific key words (e.g., "Diet quality indices" OR "Faecal indices" OR "Faecal metabolic nitrogen" OR "Non-dietary faecal nitrogen" OR "Faecal crude protein"), following the recommendations outlined by the Cochrane Handbook for Systematic Reviews (http://www.cochrane.org/). After finding that FN was published together with other faecal indices we performed a second search using other faecal indices such as "Faecal crude protein", "Faecal neutral detergent fibre", "Faecal acid detergent fibre", "Faecal lignin", "Faecal phosphorous" and "Faecal 2,6-diaminopimelic acid", as well as some recent diet-quality indices such as "Faecal chlorophyll" and "Faecal carbon isotopes. In each case, we also performed a search using the American spelling 'fecal' instead of faecal. Only works focused on wildlife species were considered (Table1).

Experimental study

This experimental study was performed in the installations of the Veterinary School of the Autonomous University of Barcelona (UAB) from 7 April to 3 June 2011. All animal care activities and study procedures complied with the guidelines of the Good Experimental Practices of the Ethical and Animal Welfare Committee of the UAB.

Animals and diets

The experiment was carried out on ten 2–3-year-old female sheep (Manchega × Ripollesa crossbreeds) from a local flock. Once the presence of spontaneous GIN infection had been confirmed by coprological examination, selected animals were transported to the Veterinary School and randomly assigned to three experimental groups.

A randomized block design was used (Pinheiro and Bates, 2000). For the first 6 days, sheep were fed an adaptation diet (500g barley + 250g alfalfa + straw ad libitum) with low protein levels designed to prevent Clostridium – related digestive upset. We analysed the ingredients – barley, alfalfa hay and straw – to ensure that all the diets contained the protein levels that had been assigned to each group (Table2). Nutrient analyses were performed following guidelines of the Association of Official Analytical Chemist (2000): Dry matter (DM) (AOAC934-01), ash (AOAC942-05), crude protein (CP) analysed as nitrogen (N) using the DUMAS combustion method (kit Leco® TruSpecN, AOAC977-02), gross energy (GE) in an adiabatic bomb calorimeter C 4000. Crude fibre (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using the ANKOM method (220 Fibre Analyzer ANKOM).

Table1. Systematic review on the use of FN as a proxy for diet quality invertebrates. The first column indicates the taxonomic group in which the study was carried out. The numbers in the column 'Source' correspond to the numbered list of references shown in the electronic supplementary Material S1.*no species differentiation.

Taxonomic group	Source
Artiodactyla	
Bovidae	
Buffalo *(Syncerus caffer)*	[15,26,51,52,121,122,153,169,170]
Hartebeest *(Alcelaphus buselaphus)*	[15,48]
Eland *(Taurotragus oryx)*	[15,48]
Thomson'sgazelle *(Eudorcas thomsonii)*	[15,48]
Grant's gazelle *(Nanger granti)*	[15]
Duiker *(Sylvicapra grimmia)*	[48]
Dik-dik *(Madoqua sp.)*	[15]
Klipspringer *(Oreotragus oreotragus)*	[15]
Impala *(Aepyceros melampus)*	[15,22,51,79,80,107]
Bison *(Bisonbison)*	[55,96]
Kudu *(Tragelaphus strepsiceros)*	[51,52,78]
Springbok *(Antidorcas marsupialis)*	[46,161,165]
Dorcas gazelle *(Gazella dorcas)*	[54]
Mongolian gazelle *(Procapra gutturosa)*	[91]
Blackbuck *(Antelope cervicapra)*	[89]
Blue wildebeest *(Connochaetestaurinus)*	[51,52,79,161]
Blue duiker *(Philantomba montícola)*	[76]
Muskox *(Ovibos moschatus)*	[27,28]
Japanese serow *(Capricornis crispus)*	[21]
Bighorn sheep *(Ovis canadensis)*	[2,3,6,14,33,88,155]
Desert bighorn sheep *(Ovis canadensis nelsoni)*	[95]
Mountain goat *(Oreamnos americanus)*	[14,154]
Pyrenean chamois *(Rupicapra pyrenaica)*	[11,167,172]
Nyala *(Tragelaphus angasii)*	[107]
Roan *antelope (Hippotragus equines)*	[111]
Sable *antelope (Hippotragus niger)*	[160,162,170]
Waterbuck *(Kobus ellipsiprymnus)*	[15,63]
Sheep *(Ovis aries)*	[4,8,29,48,99,100,125,148,164]
Cattle*/steer (Bos taurus or Bos indicus)*	[4,8,29,48,64,65,70,83,102,103,117,118,121,130,139,164]
Goat *(Capra aegagrus)*	[70]
Cervidae	
Red deer *(Cervus elaphus)*	[16,31,35,36,40,43,50,55,61,62,67,68,85,86,87]
Sika deer *(Cervus nippon)*	[5,7,34,90,142,145,147]
Fallow deer *(Dama dama)*	[25,128,129,136]
Roosevelt elk *(Cervus canadensis)*	[13]
Sambar *(Cervus unicolor)*	[143]
Mule deer *(Odocoileus hemionus)*	[9,38,44,69,93,138]
White-tailed deer *(Odocoileus virginianus)*	[10,17,35,37,41,42,45,47,49,56,58–60,71–73,75,104,106,127]
Black-tailed deer *(Odocoileus h. columbianus)*	[13,66,87,92]
Reindeer *(Rangifer tarandus)*	[81]
Moose *(Alces alces)*	[41,150]
Roe deer *(Capreolus capreolus)*	[20,40,61,112,124,152,154]
Hippopotamidae	
Hippopotamus *(Hippopotamus amphibious)*	[30]
Antilocapridae	
Pronghorn *(Antilocapra americana)*	[24]
Musk deer *(Moschus moschiferus)*	[53]
Giraffidae	
Giraffe *(Giraffa camelopardalis)*	[51]
Proboscidea	
Elephantidae	
African elephant *(Loxodonta africana)*	[77]
Perissodactyla	
Rhinocerotidae	
Rhinoceros *(Rhinoceros unicornis)*	[82]
Equidae	
Asiatic *wild ass (Equus hemionus)*	[54]
Zebra *(Equus zebra)*	[51,79,170]
Horse *(Equus ferus)*	[97]
Camelidae	
Camel *(Camelus dromedarius)*	[1]
Llama *(Lama glama)*	[151]
Suidae	
Warthog *(Phacochoerus africanus)*	[107]
Wild boar *(Sus scrofa)*	[163]
Pig *(Sus domesticus)*	[123]

Table 1. continued

Taxonomic *group*	Source
Diprotodontia	
Macropodidae	
Grey kangaroo *(Macropus giganteus)*	[101]
Wallaroo*(Macropus robustus)*	[108]
Red kangaroo *(Macropus rufus)*	[140]
Petauridae	
Sugar glider *(Petaurus breviceps)*	[74]
Northern Brown bandicoot *(Isoodon macrourus)*	[157]
Phascolartidae	
Koala *(Phascolarctos cinereus)*	[19,110]
Pseudocheiridae	
Greater glider *(Petauroides volans)*	[115]
Possum *(Trichosurusvulpecula)*	[114,115,173]
Rodentia	
Cricetidae	
Muskrat (Ondatra zibethicus)	[126]
Geomyidae	
Pocket gopher *(Thomomys bottae)*	[133]
Muridae	
Rat *(Rattus rattus)*	[98,130]
Caviidae	
Guinea pig *(Cavia porcellus)*	[134]
Chinchillidae	
PlainsViscacha *(Lagomostomus maximus)*	[84]
Erethizontidae	
Porcupine *(Erethizon dorsatum)*	[113,116]
Lagomorpha	
Leporidae	
Hare *(Lepus europaeus)*	[144]
Carnivora	
Musteloidea	
Mink *(Neovison vison)*	[119]
Procyonidae	
Raccoon *(Procyon lotor)*	[166]
Ursidae	
Blackbear *(Ursus americanus)*	[105]
Brown bear *(Ursus arctos)*	[168,171]
Primates	
Cercopithecidae	
Leaf monkey *(Trachypithecus obscurus)*	[19]
Birds	
Sturnidae	
European starling *(Sturnus vulgaris)*	[39]
Phasianidae	
Red grouse *(Lagopus lagopus scotica*	[18]
Actinopterygii	
Salmonidae	
Rainbow trout *(Oncorhynchus mykiss)*	[141]
Ictaluridae	
Channe lcatfish *(Ictalurus punctatus)*	[131]
Chichilidae	
Hybridtilapia *(Oreochromis mossambicus x O. niloticus)*	[131]
Pomacentridae	
Reef-dwellingj ewel damselfish *(Plectroglyphldodon lacrymatus)*	[146]
Sparidae	
Blackporgy *(Acanthopagrus schlegeli)*	[158]
Sauropsida	
Trionychidae	
Chinesesoft-*shelled turtle (Pelodiscus sinensis)*	[130]
General	
Review	[32,57,125,132]
Wildlife*	[23,147]
Wildlife+Livestock*	[94,109,135,137,156]

Subsequently, on day 6 the sheep were randomly assigned to one of two different diets at an n of 5 animals per diet: low-protein, low-fibre (LPF;1000 g barley + 1100 g straw per animal and day); and high-protein, high-fibre (HPF; 1600 g alfalfa + 1100 g straw). Neither barley, alfalfa nor straw are rich in secondary plant compounds (such as tannins) and hence there is no effect on gastrointestinal nematode control. The mean amount of straw ingested was approximately 1 kg per animal and day in LPF, and 0.5 kg per day in HPF diets, which resulted in a difference in nutrient content of the ingested diets in CP, ash, CF and ADF (and the not analysed soluble carbohydrates, Table 2). To guarantee the complete consumption of all the provided food, each daily portion was divided into two different rations. Before adding new food, straw fallen on the floor was completely removed. Before the feeding of the assigned diets was initiated, a *t*-test (for FN and total serum protein) and a Mann–Whitney test for faecal egg count were used to check that the FN (t = 0.06, P = 0.95), the TP (t = 0.36, P = 0.72) and the FEC (U = 15, P = 0.67) did not differ between groups (Table3). In addition, on day 6 we performed a coproculture, maintaining a wet faecal mixture collected from individuals from each group at 20°C for a minimum of 15 days (Pereira Lima and Delgado, 1961), to confirm that LPF (64% of larvae belonged to *Ostertagia* and 36% to other *Trichostrongylus* species, most of which were *Nematodirus*) and HPF animals (62% of larvae belonged to *Ostertagia* and 38% to other *Trichostrongylus* species) were infected with the same genus of nematodes.

Table2. Chemical composition of the diet fed to sheep. DM = dry matter, CP = crude protein, CF = crude fibre, NDF = neutral detergent fibre, ADF = acid detergent fibre, GE = gross energy. Diet nutrient content as summing complete consumption of 1kg barley and 1kg straw for LP, and 1.6 kg alfalfa and 0.5 kg straw for HP. Data are expressed in %.

	$DM_{as\ fed}$	CP	Ash	CF	NDF	ADF	GEKcal/100g
Nutrient content on dry basis							
Barley	90.43	11.05	2.59	5.35	15.89	5.54	461.35
Alfalfa	90.03	19.62	10.66	30.96	39.73	31.12	442.97
Straw	92.38	5.21	8.48	43.74	77.94	48.55	449.25
LP diet	91.41	8.10	5.57	24.75	47.25	27.27	455.24
HP diet	90.59	16.12	10.13	34.06	49.01	35.35	444.49

Experimental design

Faecal samples were collected directly from the rectum of each sheep on experimental days 0 (arrival at the experimental facility), 6 (start of diet), 19, 26, 33, 34, 35, 36, 37, 38, 39, 40, 46, 47, 48, 49, 50 and 51. Even though GIN egg output shows no circadian pattern (Rinaldi et al, 2009), samples were collected in the morning. On day 33, all sheep were dewormed with a subcutaneous injection of ivermectin (Ivomec®) with a dosage of 200 µg/kg body-weight. Blood samples were collected on days 19, 33, 46 and 51.

Laboratory analysis

Quantitative and qualitative coprological analyses were performed according to MAFF (1980). In brief, we performed a flotation in 33% zinc sulphate solution, and a faecal egg count (FEC) of the GIN eggs was performed with the modified McMaster method. For each 3-g faecal sample, all strongyle-like eggs observed in the two chambers of a McMaster slide were counted and then multiplied by 50 to determine the number of eggs per gram of faeces. FEC techniques remain the most common and reliable approach for indirectly estimating GIN infection intensity (Cringoli et al, 2004; Villanúa et al, 2006; Rinaldi et al, 2009).

A sample of individual faecal samples was frozen for FN determination. These frozen faeces were thawed and oven-dried at 60◦C to a constant weight (24h) and subsequently ground with a laboratory mill with 1 mm pitch (Cyclotec 1093, FOSS Tecator, Höganäs, Sweden). Subsamples were used in duplicate to determine DM, N and ADF concentrations as described above. Although to our knowledge, faecal ADF concentrations have not been established as markers of digestibility, we assume higher levels to reflect a lower diet digestibility in this study. Blood samples were collected from the jugular vein and serum was stored at −20◦C until biochemical analyses were performed. Serum was analysed with an OLYMPUS AU400® (Olympus, Tokyo, Japan) automated chemistry analyser to obtain serum total protein concentration (TP) based on the Biuret reaction (Gornall et al, 1949). Due to its relationship to body reserves, TP can be used for monitoring body condition in wild ungulates (Serrano et al, 2008).

Statistical analysis

Systematic review

We used linear models to explore the trend in the number of publications on FN since 1981, when publications on FN became common.

FN, faecal ADF and FEC in LPF and HPF diets

For describing FN, faecal ADF and FEC patterns in sheep on different diets, we used generalized mixed models (GLMM) including FN, faecal ADF or FEC as response variables and diet treatment, experimental days (ED) and their interaction as explanatory variables. To test for an effect of FEC on FN, we performed a randomized block design where the response variable was the FN measured from day 19 (13 days after diet assignment) until the administration of the anthelmintic treatment (day 33). In the fixed part of the model the response variables were the single effects of both FEC (as a proxy for GIN load) and the diet treatment (i.e., LPF and HPF diets) and their interactions. In addition, to control for the potential effect of diet digestibility on FN estimates, faecal ADF was included as a covariate in the fixed part of our mixed models. Finally, given that we took repeated measurements from the same individuals, we included individual sheep as a blocking factor in the random part of the GLMM. We chose the optimal random structure (e.g., the random intercept, results not shown) in the statistical modelling as recommended by Zuur et al (2009).

Changes in FN and TP due to anthelmintic treatment

In this analysis, we tested whether or not the suppression of gastrointestinal nematodes influenced FN excretion and TP concentration in sheep on the two different diets by comparing FN measurements from before (days 6–34 for FN comparisons and on days 19–33 for TP analysis) and after (days 40–50 for FN and 46–51 for TP analysis) the anthelmintic treatment. In the fixed part of this randomized block design, we included the single effects of drug administration, diet treatment and their interaction.

Table 3. Percentage of faecal nitrogen (FN); gastrointestinal parasite faecal egg counts (FEC); serum total protein concentrations (TP); low-protein (LPF), and high-protein (HP) diets. Adaptation: time until beginning of diet (6 days). Treatment: time that sheep were on their specific diets (33 days). Dewormed: time from the anthelmintic application onwards.

	FN %			ADF %			FEC (egg/ g				TP (g/L)		
	Mean	Min	Max	Mean	Min	Max	Mean	Median	Min	Max	Mean	Min	Max
LPF Adaptation	1.68	1.51	1.93	37.2	29.88	40.54	229.8	100	49	700	67.02	56	74.9
Treatment	2.41	1.99	2.91	28.76	19.9	36.61	1390	1125	200	3050	66.94	51.3	79.5
Dewormed	1.95	1.12	2.59	27.94	13.06	34.45	3.33	0	0	50	71.59	62.8	81.2
HPF Adaptation	1.68	1.51	1.86	35.01	23.98	42.53	240	300	5	50	68.7	61.9	81.9
Treatment	1.97	1.68	2.26	40.45	29.94	47.86	275	175	100	600	70.07	53.8	79.6
Dewormed	1.88	1.61	2.31	40.34	34.51	47.5	136.7	100	0	450	78.31	70.7	88.7

As in the previous analysis, faecal ADF contents were also included as a covariate in the fixed part of our mixed models for FN. Following the same rationale, individual sheep were included as a blocking factor in the random part of the GLMM. Furthermore, for both mixed models we tested whether or not the inclusion of the initial value of FN (measured during the adaptation period before assignment of the diets, to characterize the digestive efficiency of individual sheep) as a covariate reduced the residual error. To avoid underestimating the variance components (Pinheiro and Bates, 2000), we used restricted maximum likelihood to fit both statistical models.

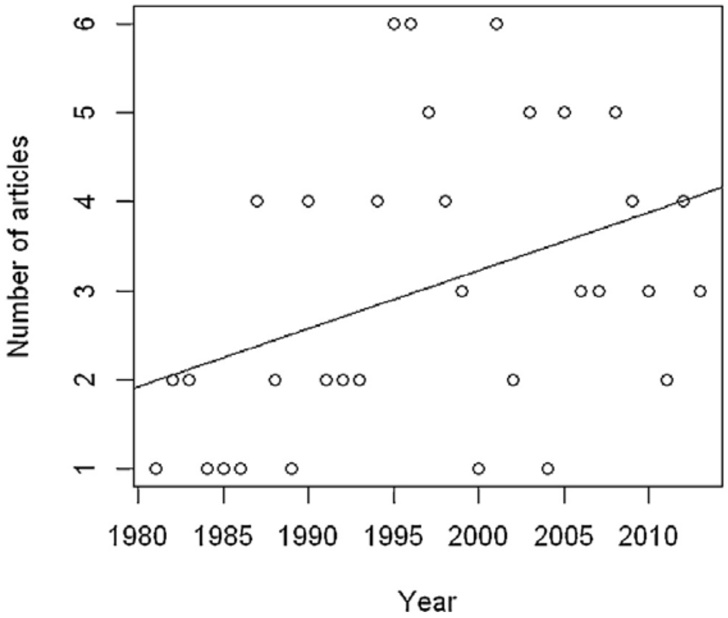

Figure. 1. Trend in the number of articles published from 1980 to 2013 that use faecal nitrogen as a proxy for diet quality in vertebrates. Though the first record appeared in the 1940s, only works published from 1980 to 2013 were considered in this analysis.

Before model interpretation, we also checked for the lack of a residual pattern and for normality using graphical procedures. Thus, for the first statistical modeling procedures FEC was log-transformed to reduce the residual pattern. The residual error associated with our mixed models was not reduced by the inclusion of the initial values of FN as a covariate, either in the model exploring the relationships between FEC and FN (*L*-ratio test = 2.16, P = 0.14, after comparing the model with and without initial FN values) or in the model for exploring the effects of parasite suppression on FN (*L*-ratio test = 2.41, P = 0.12). Thus, the initial FN was not taken into consideration in our statistical models. Mixed models were fitted using the 'nlme' package (Pinheiro et al, 2012) from R 3. 1. 1 (R Development Core Team, 2014).

Results

Use of FN for diet quality assessment in wildlife

Table 1 shows the results of a thorough search of the literature contained in Thomson Reuters Web of Knowledge (covering the period 1905–2013). Since the 1980s, an average of three articles per year were published (min = 1, max = 6), using FN as a proxy for diet quality in a broad range of vertebrate species. On year later, the number of articles using FN invertebrates increased every year (β = 0.06, SE = 0.02, P = 0.02, Fig.1). FN has been used to assess the diet quality of fish, reptiles, birds and mammals, and in particular in artiodactyls.

FN, faecal ADF and FEC in the different diets

Our mixed model revealed that FN ($F_{1,38}$ = 6.05, P = 0.01, for the ED × Diet treatment interaction), ADF ($F_{1,38}$ = 10.30, P = 0.002; for the ED × Diet treatment) and FEC ($F_{1,38}$ = 5.06, P = 0.03; for the ED × Diet treatment) before anthelmintic treatment (day 33) depended on the diet assigned. In fact, from day 19 onwards, FN in the animals on a LPF diet began to exceed that of their counterparts fed a HPF diet (Figs. 2A and 3A for ED 19, mean LPF = 2.17, min = 1.77, max = 2.47, mean HPF = 1.86, min = 1.6, max = 2.07). However, significant differences only occurred beginning on ED 28 (t = 2.56; P = 0.036, mean LPF = 2.41, min = 1.99, max = 2.77 vs. mean HPF = 1.97, min = 1.71, max = 2.26). The concentration of faecal ADF decreased over time in the LPF diet (βLogFEC × LPF = −0.6, SE = 0.18, t-value = −3.21, P = 0.002) but increased in HPF (Fig.2). In fact, ADF was lower in the LPF diet than in the HPF diet from day 19 onwards (mean LPF = 30.41, min = 28.95, max = 32.20 vs. mean HPF = 36.92, min = 29.94, max = 43.78) but statistical differences only occurred from ED 28 on (t = 4.55; P = 0.003, mean LPF = 28.17, min = 23.51, max = 32.91 vs. mean HPF = 41.91, min = 36.65, max = 47.86). Likewise, FEC was also higher in the LPF diet on ED 28 (w = 23; P = 0.036, median LPF = 1070, min = 200, max = 2300, vs. median HPF = 280.4, min = 100, max = 500).

FEC was correlated with FN (β = 0.28, SE = 0.12, t-value = 2.33, P = 0.02), especially in the LPF diet (Table 2, Fig. 2A and B).The influence of FEC on FN was 0.44 units higher in the LPF diet than in the HPF diet ($\beta_{\text{LogFEC×HPF}}$ = −0.44, SE = 0.21, t-value = −2.09, P = 0.043, Figs. 2 and 3). In fact, animals on the LPF diet excreted on average 1.2 times more FN than animals on the HPF diet. The FEC effect on FN excretion was no longer significant when FN was corrected for faecal ADF ($\beta_{\text{LogFEC×HPF}}$ = −0.32, SE = 0.2, t-value = −1.58, P = 0.12). In these analyses, 40% of the observed variability in the relationship between FEC, faecal ADF, diet group and FN was due to inter-individual variation.

Effects of anthelmintic treatment on FN excretion and serum TP

Ivermectin treatment resulted in a clear reduction in GIN load in the LPF (W = 6, $P < 0.001$) and HPF (W = 150, $P < 0.01$) diets (Table3, Figs. 2B and 3B). In contrast, faecal ADF levels were not affected by deworming in either the LPF (t = 1.73603, P = 0.08, Table 3, Fig. 2A) or HPF (t = 0.238, P = 0.81, Fig. 3A) diet. On the other hand, mixed modeling showed that the anthelmintic application resulted in a reduction in FN ($F_{1,79}$ = 7.02, P = 0.009, for the Anthelmintic × Diet treatment interaction). This interaction was also significant when FN was corrected for faecal ADF ($F_{1,78}$ = 8.83, P = 0.003). Averaged overall animals, FN was 1.1 times higher before deworming than after. However, FN diminished more in the LPF diet (1.23 times, Table 3) than the HPF diet (1.04 times, Table 3) after the anthelmintic treatment. The individual sheep effect on the variability in the observed pattern was 14.2%.

Finally, although no significant differences were found between the groups, the highest TP in serum was observed in the HPF diet (Table3). Both groups increased their TP to the same degree after the anthelmintic treatment (F2, 28 = 20.22, P = 0.0001).

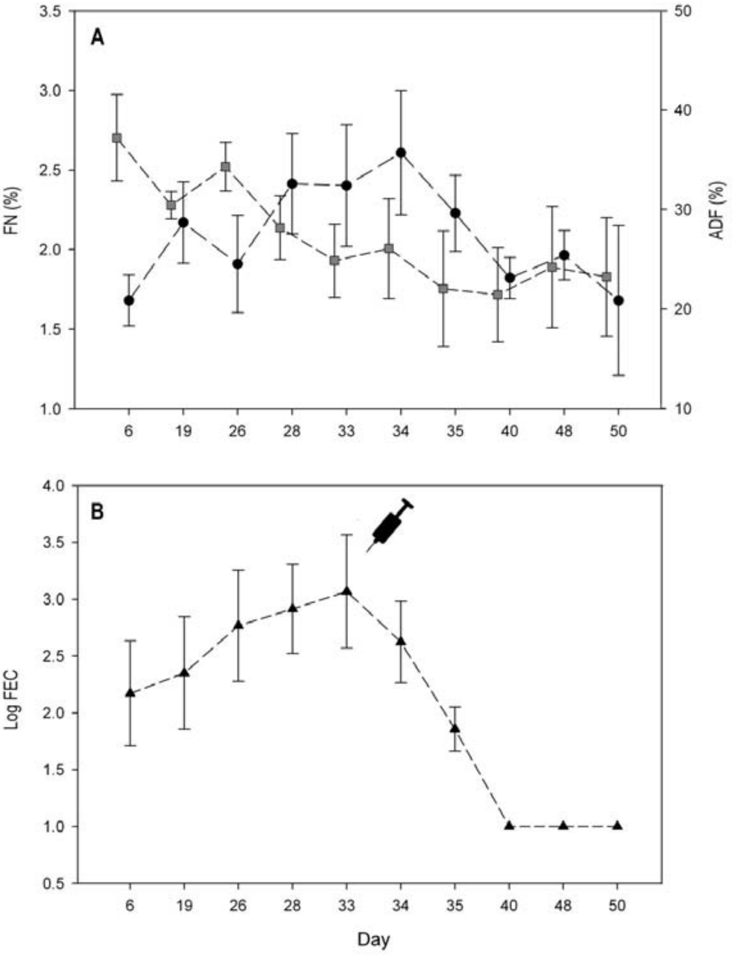

Figure 2. The plot 2A shows the mean percentage of faecal nitrogen (black circles) and acid detergent fibre (grey squares) in five sheep maintained on a low-protein, low-fibre diet. Figure 2B shows changes in log-transformed gastrointestinal faecal egg counts over time in the same group of sheep. The syringe icon indicates that ewes were given an anthelmintic treatment (ivermectin) on day 33. X axes have been shifted slightly to avoid overlap. Bars represent the standard deviation of the mean.

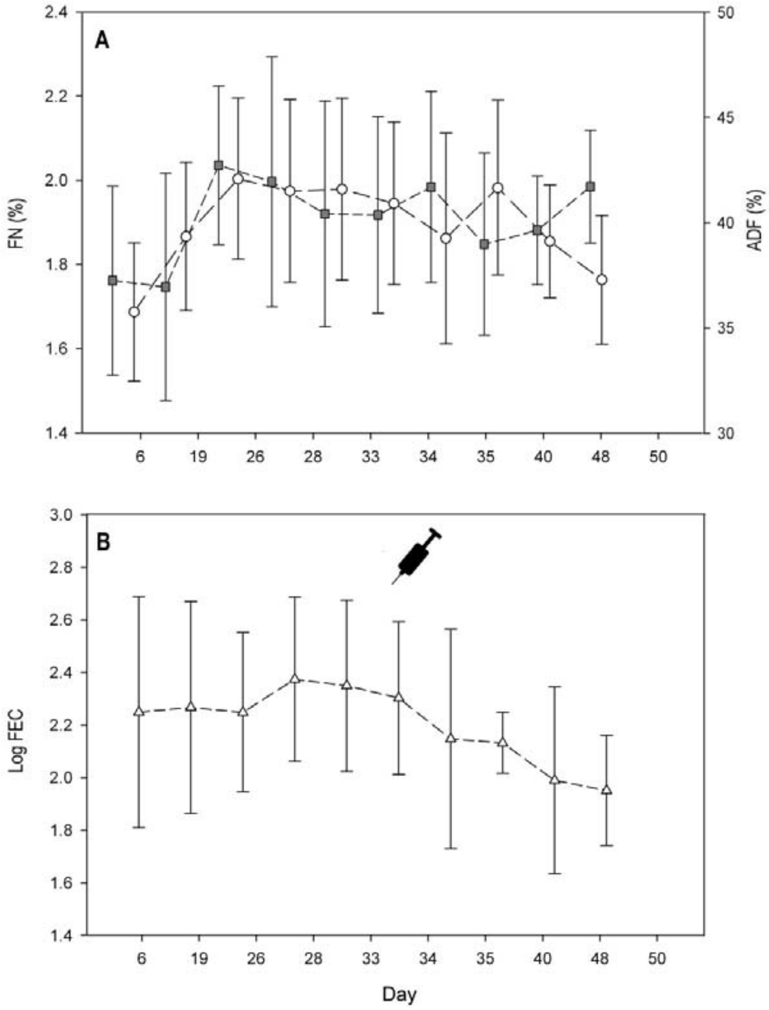

Figure 3. The plot 3A shows the mean percentage of faecal nitrogen (open circles) and acid detergent fibre (grey squares) in five sheep maintained on a high-protein, high-fibre diet. Figure 3B shows changes in log-transformed gastrointestinal faecal egg counts over time in the same group of sheep. The syringe icon indicates that ewes were given an anthelmintic treatment (ivermectin) on day 33. X axes have been shifted slightly to avoid overlap. Bars represent the standard deviation of the mean.

Discussion

Faecal nitrogen (FN) is still widely used as a proxy for diet quality in a broad range of vertebrate species, especially in the assessment of habitat quality in free-ranging herbivores. Our results support this practice, but provide important evidence that the widespread simplistic interpretation of a direct link between dietary protein content and

FN should be reconsidered. The most frequent caution in using FN as a proxy for dietary N is related to the influence of secondary plant compounds, which may lead to an increase in FN at similar dietary N levels (reviewed by Leslie et al, 2008). This approach may erroneously assume a direct link between dietary N and FN, ignoring modifications by secondary plant compounds. However, in the absence of such metabolites, the low-protein diet (LPF) in our study (at a protein content of about 8% DM) led to higher FN values than the high-protein diet (HPF) (which had twice the protein content at about 16% DM).

This effect is mostly explained by the higher content of lignocelluloses (ADF) in the HPF diet and the higher content of easily digestible carbohydrates, mainly starch, in the LPF diet. The LPF diet used in our study was clearly artificial due to the inclusion of grain, and will have no equivalent in the natural environment of grazing ruminants, for which a decrease in dietary N is usually linked to an increase in fibre and a decrease in digestibility. Nevertheless, it serves to illustrate that FN does not always directly reflect dietary N, but rather is the result of two different biological effects: diets higher in N are usually (in the absence of secondary plant compounds) more digestible and more digestible diets lead to higher FN in herbivores. Gastrointestinal helminthes also influenced FN excretion. Endoparasites are recognized sources of protein losses in ruminants (Parkins and Holmes, 1989), but their potential impact on FN estimates has usually been ignored by wildlife ecologists. In the first part of our manipulation, the increased FN and FEC in the group on the LPF diet was best explained by the high digestibility of this diet, and the corresponding lower proportion of diluting fibre in the faeces. However, the influence of helminthes on FN was later demonstrated by the decrease in FN once parasites had been removed by anthelmintic treatment. Despite the fact that sheep on the LPF diet had a higher FN excretion, they also had numerically lower concentrations of serum TP, which highlights the absence of an association between FN and nutritional condition in cases of severe parasitism. Once dewormed, sheep probably lost less nitrogen due to protein catabolism and specific immune response, as suggested by the increase in TP.

The decrease in FN excretion after the anthelmintic treatment (even when dietary fibre was corrected for) was probably due to the reduction of damage in the digestive tract caused by gastrointestinal nematodes (Fox, 1997) leading to the increase in metabolic nitrogen losses (Parkins and Holmes, 1989). In addition, the increased efficiency of digestive enzymes in the absence of parasites (Jones, 1983) may also explain the decrease in FN as a result of a decrease in residual dietary N. The loss of proteins in the gastrointestinal tract due to parasites is associated with the loss of plasma and erythrocytes, exfoliated epithelial cells and mucus in the gastrointestinal tract (Parkins and Holmes, 1989). In infections associated with gastrointestinal haemorrhage, such as *Haemonchus contortus*, *Oesophagostomun* spp. or *Trichurus colubriformis*, a massive loss of erythrocytes can be partially absorbed in the ileum (Rowe et al, 1982) and the small intestine (Poppi et al, 1981). In fact, small FN increases due to blood losses in ewes experimentally infected with these parasite species had been reported in the late 1970s (Sykes and Coop, 1977; Symons et al, 1981). These FN increases due to endogenous protein losses would increase in the case of infections by nematodes infecting both the abomasums and the small intestine (Haile et al, 2004), as in the current study.

Implications for population monitoring

Long-term assessment of FN has become common (Blanchard et al, 2003; Hamel et al, 2009; Gálvez-Cerón et al, 2013) in programmes of wild ungulate population monitoring. FN should be used with the understanding that it is a proxy for digestibility but not directly related to diet protein content. Researchers working in feeding ecology should take gastrointestinal parasite load into account when assessing diet quality by means of FN. Other faecal indicators of diet quality should also be incorporated into programmes as a means of avoiding the effects of GINs on FN (see Christianson and Creel, 2009). Further research is still needed to explore how these results might be applied to other ungulate species with other types of foraging strategies (e.g., browsers such as roe deer) and diets with higher levels of tannins, which act as parasiticides (Hoste et al, 2006) and limit protein absorption.

Supporting information

Literature published since 1905 on the use of faecal nitrogen as a proxy for diet quality in wildlife and livestock.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind. 2014.11.020. See page 121.

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Appendix A. A systematic review on the use of faecal nitrogen (FN) as a proxy for diet quality in wildlife and domestic animals.

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CAPÍTULO 4

Predicting seasonal and spatial variations in diet quality of Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) using near infrared reflectance spectroscopy

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Abstract

Monitoring nutritional status of wildlife is key to understand the population response to changes in food availability. Several direct and indirect methods have been proposed for such purposes, but faecal nitrogen (FN) is by far the most commonly used indirect non-invasive indicator of free-ranging ruminant diet quality. In this work, two methods were used to estimate the FN content in 291 pellet samples of Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) collected during a two year-round period (May 2009 to May 2011) in two chamois populations from the Catalan Pyrenees, Spain: the Dumas LECO analyzer and near infrared reflectance spectroscopy (NIRS). In order to optimize further FN estimations we performed three different NIRS calibrations: 1) general, across year, season and populations, 2) population-specific and 3) season-specific, i.e., different periods of vegetation growth. Faecal nitrogen ranged from 1.66 to 2.85% (dry matter basis) and was higher in the main vegetation growing season than in Winter. Concerning NIRS calibration, our study confirmed that FN of Pyrenean chamois can be accurately determined by NIRS, since the general calibration was accurate enough to predict FN. It is concluded that NIRS represents a non destructive, emission-free and low-cost analytical technique which may reduce the use of conventional laborious methods for estimating FN in long-term wildlife monitoring.

Keywords: Foraging ecology, faecal nitrogen, NIRS, Southern chamois.

Introduction

Monitoring nutritional interactions between an animal populations and its environment is one of the main challenges for wildlife researchers. Several approaches (e.g., based on body condition assessment) have been suggested for assessing such relationships in free-ranging populations of ungulates, but most of them require animal capture (López-Olvera et al, 2006; Pérez et al, 2003), or even shooting (Serrano et al, 2008, 2011). These invasive methods are inappropriate for continuous monitoring over time, especially for protected populations or outside the game season. In fact invasive methods Faecal indicators of diet quality, particularly faecal nitrogen (FN), have been the default technique to assess foraging ecology and life history traits of wild ungulates since the early 1970s (Robbins et al, 1975) up to now (Navarro-González et al, 2011, but see Leslie et al, 2008, for a comprehensive reading, and Table 1 for a summary on the uses of FN in wild ruminant research).

FN is a combination of both metabolic nitrogen and food residue nitrogen excreted in faeces (Schneider, 1935). In ruminants, there is a close relationship between dietary nitrogen and the percentage of nitrogen in faeces (Orksov, 1982), which has led to the use of FN as a proxy for diet quality (particularly crude protein) in livestock since the 40s (Raymond, 1948). This is particularly true for grazer species, since herbaceous vegetation has little content of secondary compounds (Leslie et al, 2008). In fact, monitoring FN is a simple and useful method for assessing diet quality, since FN is stable in faeces under field conditions at least for a week and up to a month, depending on environmental conditions (Kamler & Homolka, 2005; Leite & Stuth, 1994). Moreover wild ruminant droppings are easily detected during field surveys even several days after being shed. However, FN determination using traditional techniques, namely the combustion method, are expensive (especially to analyze large sample sets from long-term monitoring), and involves the destruction of the sample. Thus, any methodological improvement for estimating FN would provide an inestimable benefit for wildlife research.

Near infrared reflectance spectroscopy (NIRS) could overcome these constraints since it provides quick, non-destructive and quantitative analyses of a range of organic constituents, including FN (Dixon and Coates, 2009; Foley et al, 1998). The NIRS has been used with success to predict diet quality through faecal analysis in domestic ruminants (Leite & Stuth, 1995; Li et al, 2007; Lyons et al, 1992, 1995) and several free-ranging *Cervidae* species such as white-tailed deer *Odocoileus virginianus (*Showers et al, 2006), red deer *Cervus elaphus*, and roe deer *Capreolus capreolus (*Kamler et al, 2004). However, although there have been some attempts to multispecies calibration for FN in ungulates (Tolleson et al, 2004), FN reference values should be estimated within species, seasons or habitats (Leslie et al, 2008).

The Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) is the subspecies of the genus *Rupicapra* inhabiting the Pyrenees. This mountain ungulate has developed several physiological and behavioural strategies for managing the strong seasonal variations in food availability of mountain ecosystems (Parker et al, 2009). One of them is a marked seasonal altitudinal migratory behaviour, probably to optimize the exploitation of food resources (Crampe et al, 2007). In Summer and early Autumn this *Caprinae* species generally remains above 1,800 meters, grazing in the alpine meadows, and moves to lower forested areas coinciding with the first snowfalls of the Winter (García-González & Cuartas 1996, Aldezábal & García-González, 2004).

We analysed seasonal and spatial variations in diet quality (estimated by means of FN) in two populations of Pyrenean chamois inhabiting an Alpine ecosystem situated in the northeast of Spain. Our objectives were: [1] to explore seasonal variations in diet quality for two populations of Chamois and [2] to evaluate three different FN calibrations: general, population-, and season-specific. Despite the marked seasonal and spatial variations of FN in the study area, in the present article we will see how a single NIRS equation is robust enough for predicting FN in Pyrenean chamois.

Table 1. Main uses of Faecal Nitrogen for assessing different ecological aspects concerning: diet quality, nutritional status and life history traits in wild ruminant research.

Uses	Family	Species	Source
Diet quality and Nutritional Status	Bovidae	Hartebeest (*Alcelaphus buselaphus*)	1
		Waterbuck (*Kobus ellipsiprymnus*)	1
		Eland (*Taurotragus oryx*)	1
		Thomson's gazelle (*Eudorcas thomsonii*)	1
		Grant's gazelle (*Nanger granti*)	1
		Dik-dik (*Madoqua sp*)	1
		Klipspringer (*Oreotragus oreotragus*)	1
		Springbok (*Antidorcas marsupialis*)	4, 5
		Blue wildebeest (*Connochaetes taurinus*)	5, 6
		Impala (*Aepyceros melampus)*	1, 6
		Dorcas Gazelle (*Gazella dorcas*)	7
		Blackbuck (*Antelope cervicapra*)	8
		Buffalo (*Syncerus caffer*)	1, 12
		Kudu (*Tragelaphus strepsiceros*)	13
		Bighorn Sheep (*Ovis canadensis*)	19, 20, 67, 40
		Roan Antelope (*Hippotragus equines*)	24
		Pyrenean Chamois (*Rupicapra pyrenaica*)	23
		Sable Antelope (*Hippotragus niger*)	25
	Cervidae	Red deer (*Cervus elaphus*)	28, 29, 39, 44
		Sika deer (*Cervus nippon*)	30, 31, 32, 33, 35, 37
		Fallow deer (*Dama dama*)	36
		Black-tailed deer (*Odocoileus hemionus*)	38, 66
		Elk (*Cervus elaphus*)	38, 41,42, 43
		Sambar (*Cervus unicolor*)	45
		Mule deer (*Odocoileus hemionus*)	47, 48, 49
		White-tailed deer (*Odocoileus virginianus*)	52, 54, 55, 56, 57, 58, 59, 63, 64, 65
		Moose (*Alces alces*)	60
		Roe deer (*Capreolus capreolus*)	29, 68, 69, 70, 71
	Equidae	Zebra (*Equus zebra*)	6
	Equidae	Zebra (*Equus zebra*)	2
	Giraffidae	Giraffe (*Giraffa camelopardalis*)	2
Life history traits	Bovidae	Kudu (*Tragelaphus strepsiceros*)	1, 3
		Blue wildebeest (*Connochaetes taurinus*)	2, 3
		Bison (*Bison bison*)	9, 10
		Buffalo (*Syncerus caffer*)	2, 3, 11
		Muskox (*Ovibos moschatus*)	14, 15
		Impala (*Aepyceros melampus)*	2
		Japanese Serow *(Capricornis crispus)*	16
		Bighorn Sheep (*Ovis canadensis*)	17, 18, 21
		Mountain Goat (*Oreamnos americanus*)	22
		Waterbuk (*Kobus ellipsiprymnus*)	26
		Elk (*Cervus elaphus nelsoni*)	9
		Red deer (Cervus elaphus)	27
		Sika deer (Cervus nippon)	33, 34
		Mule deer (Odocoileus hemionus)	46, 50, 51
		White-tailed deer (Odocoileus virginianus)	53, 61, 62

See supplemental material S1 for the full list of references used in this table.

Material and methods

Study area

The study area is located in the Freser-Setcases National Game Reserve (FSNGR), Eastern Pyrenees, Spain (42º 21'N, 2º 09' E). This mountainous area of 20,200 ha is mostly composed of an alpine ecosystem ranging from 1,800 to 2,910 m.a.s.l. in the Puigmal peak). The FSNGR is a sub-humid alpine region, with annual mean temperatures of 6.05 ºC (min = - 16.8, max = 39.2) and mean yearly accumulated rainfall (period 1999-2011) of 963.38 mm (min = 520.6, max = 1324.8, data from the Nuria station at 1,971 m.a.s.l. in the core FSNGR, Servei Meteorològic de Catalunya < www.meteocat.com >). The vegetation of the study area consists of alpine meadows dominated by graminoid species (e.g., Festuca and Carex genus), which represent over 50% of the plant cover, and scattered Pinus uncinata forest patches with a substrate of small woody **groundcover** shrubs of Arctostaphylos uva-ursi, Calluna vulgaris, and Juniperus communis (for revision see Vigo et al, 2006).

Pyrenean chamois populations

We studied two populations of Pyrenean chamois from two different areas within the FSNGR, called Costabona and Fontalba. Costabona is located in the northeast part of the FSNGR (42º 24' N, 2º 20' E, ranging from 1093 to 2429 m.a.s.l.), whereas Fontalba is located in the central part of the reserve (42º 22' N, 2º 08' E, study area ranging from 1660 to 2248 m.a.s.l.). Both have similar landscape features in terms of vegetation composition. However, they are separated by 20 km of rough terrain. Maximum dispersal distance from wintering areas recorded by female chamois is about 9.2 km (Crampe et al, 2007), and from 26 individuals marked with colour plastic collars in both areas (unpublished data), none has been seen in the other area in the last three years (2009-2011). Therefore, the two populations can be considered independent for the purpose of this study.

Sampling procedure

From 14 May 2009 to 6 May 2011, 291 faecal samples were collected in 59 sampling sessions. Each study area was visited at least once a month (range 1–3 sampling days by location). Chamois groups were located using 10 x 42 binoculars and 20 - 60 x 65 spotting scopes until defecations occurred. Once size and composition of group was recorded, fresh droppings without signs of dung beetle activity were collected in individual plastic bags, recording coordinates and altitude at the precise location of the chamois groups. Fresh samples were chosen according to their colour and texture (Hibert et al, 2011). A group of droppings collected in one bag is considered a faecal sample, even if they belonged to different individuals. Samples were transported refrigerated at 4ºC and stored frozen at -20ºC until FN determination.

Faecal Nitrogen determination

Faecal samples were thawed and dried in an oven at 80°C for 48 hours. Subsequently, samples were ground in a Cyclotec mill of 0.5 mm screen (FOSS Tecator, Höganäs, Sweden). FN on a dry matter basis (DM) was determined by the Dumas dry combustion method using a LECO analyser (LECO Corporation, St Joseph, Michigan, USA). Descriptive statistics of FN values can be found in Table 2.

Table 2. Monthly mean values and associated standard errors of faecal nitrogen percentage (FN) from 291 faecal samples of Pyrenean chamois (*Rupicapra p. pyrenaica*) sampled in Fontalba and Costabona, National Game Reserve of Freser–Setcases (NGRFS), Catalan Pyrenees, Spain.

Month	Costabona	Fontalba	Total
January	2.11 ± 0.05	1.66 ± 0.05	1.92 ± 0.06
February	2.02 ± 0.05	1.68 ± 0.02	1.79 ± 0.03
March	2.17 ± 0.04	1.66 ± 0.03	2.03 ± 0.06
April	2.50 ± 0.16	1.97 ± 0.08	2.12 ± 0.08
May	2.85 ± 0.07	2.36 ± 0.04	2.46 ± 0.05
June	2.79 ± 0.10	2.81 ± 0.13	2.79 ± 0.09
July	2.70 ± 0.13	2.80 ± 0.14	2.79 ± 0.09
August	2.40 ± 0.13	2.48 ± 0.05	2.47 ± 2.53
September	2.19 ± 0.02	2.31 ± 0.06	2.61 ± 0.03
October	1.93 ± 0.06	2.13 ± 0.05	2.07 ± 0.04
November	2.11 ± 0.05	1.90 ± 0.06	1.99 ± 0.04
December	2.06 ± 0.03	1.91 ± 0.06	2.00 ± 0.03

Statistical analysis for exploring seasonal patterns of FN

Since the relationship between date of sampling and FN showed a non-linear pattern (see Fig. 1), a set of additive mixed models was performed (GAMM), in which FN was explained by the main effects of sampling month (as a continuous variable), population (discrete variable with two categories: Costabona and Fontalba), and their two-way interactions. Samples collected in the same day were not considered independent; therefore, sampling day was included in the statistical models as a random factor (i.e., random intercept). Then, we performed a model selection based on the information-theoretic approach and the Akaike's Information Criterion corrected for small sample sizes (AICc).

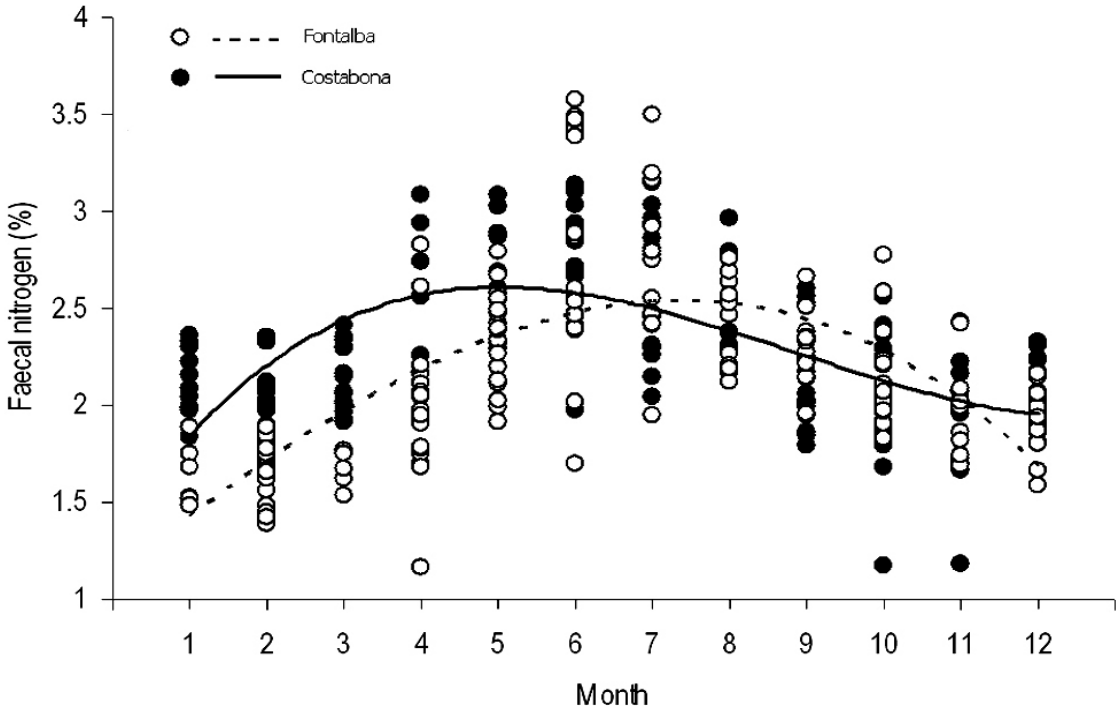

Figure 1. Monthly variations of faecal nitrogen percentage in two populations of Pyrenean chamois (*Rupicapra p. pyrenaica*) from the Catalan Pyrenees, northern Spain: Costabona and Fontalba. Costabona is represented by solid line and black circles whereas Fontalba by a broken line and white circles.

Subsequently, we estimated the Akaike weight ($w_{i,}$), defined as the relative probability that a given model is the best model among those being compared. Once the best model was selected (for a review see Johnson and Omland 2004, but Burnham and Anderson 2002 as reference book) we confirmed the general assumptions of GAMM (normality and the absence of residual pattern in the data) following Wood (2006) and Zuur et al (2009) recommendations. Statistical analyses were performed using "mgcv" package version 1.7-12 (Wood, 2006) of the statistical software R version 2.14. 2 (R Development Core Team 2012). Subsequently, we estimated the Akaike weight ($w_{i,}$), defined as the relative probability that a given model is the best model among those being compared. Once the best model was selected (for a review see Johnson and Omland 2004, but Burnham and Anderson 2002 as reference book) we confirmed the general assumptions of GAMM (normality and the absence of residual pattern in the data) following Wood (2006) and Zuur et al (2009) recommendations. Statistical analyses were performed using "mgcv" package version 1.7-12 (Wood, 2006) of the statistical software R version 2.14. 2 (R Development Core Team 2012).

NIRS analysis

FN estimation using NIRS was calibrated and validated using a subset of 192 samples (66% of the total sample size) representative of the study period (years 2009 and 2010) and sampling locations. Table S3 (supplemental material S3) shows the characteristics of the sample set used for calibration and validation. Samples in the validation set were not used in the calibration set or vice versa and FN content variability in both calibration and validation sets was similar.

Three different calibrations were carried out: [1] a single general calibration (G) including samples from both populations collected all year long, [2] a population-specific calibration for each study site Costabona (C) and Fontalba (F), including all year round samples, and [3] a seasonal calibration combining samples from both populations but divided by period of vegetation growth: Spring-Summer (S-S) when primary productivity is maximal and Autumn-Winter (A-W), coinciding with the decrease of vegetation growth and presence of snow (Gómez, 2008).

Briefly, after determining FN content by Dumas elementary analysis, ground faecal samples were packed in a ring-cup sample cell. Samples were then scanned from 1,100 to 2,500 nm using a NIRSystems 5000 scanning monochromator (FOSS, Hillerød, Denmark). Reflectance was recorded at 2 nm intervals as log (1/R), where R represented reflected energy, resulting in 692 data points for each sample. The analysis was carried out in duplicate and all measurements were performed by the same operator.

Table 3. Model selection based on an additive mixed modelling for exploring the seasonal and spatial variations of faecal nitrogen percentage (FN) determined in 291 faecal samples of Pyrenean chamois from Fontalba (n = 173) and Costabona (n = 118) in the NGRFS, Catalan Pyrenees, Spain. K = effective number of parameters in the additive modelling, AICc = Akaike Information Criterion corrected for small sample sizes, ΔAICc = difference of AICc with respect to the best model, w_i = Akaike weight, Mo = null model only with the constant term. In bold the best model for explaining the observed FN variability

Biological models	**K**	**AICc**	**Δ_i**	**w_i**
Month + Population	6	107.06	0	0.98
Month * Population	7	120.55	13.50	> 0.01
Month	5	121.06	14.00	> 0.01
Mo	3	335.88	228.83	> 0.01
Population	4	336.90	229.85	> 0.01

A WinISI III (v. 1.6) software program was employed for spectra data analysis and developing chemometric models. Prior to calibration, log 1/R spectra were corrected for the effects of scatter using the standard normal variate (SNV) and detrend (DT) method and by multiplicative scatter correction (MSC) to reduce the effects of particle size. The calibrations were performed by modified partial least square (MPLS) regression using first, second and third derivatives of the spectra. In brief results of the calibration were checked by observing "t" and global "H" (GH) outliers being those extreme values with t > 2.5 and GH > 10 were not considered for calibration. Finally, the number of outlier elimination passes was 2. Calibrations by MPLS regression were performed using different pre-treatments of spectral data to remove or reduce disturbing effects not related to the chemical absorption of light. To validate NIRS FN estimation, the performance of the model was determined by the following statistics: standard error of calibration (SEC), standard error of prediction (SEP), coefficient of determination (R^2), linear correlation coefficient between reference methods and estimated by prediction models (r^2), the ratio of performance to deviation (RPD, defined as the ratio of standard deviation for the validation samples to SEP), and the range error ratio (RER, defined as the ratio of the range in the reference data from the validation set, to the SEP, for a detailed description see Williams and Sobering, 1996).

Results and discussion

Our model selection procedure showed that chamois from both Costabona and Fontalba showed a similar seasonal pattern of FN ($wi_{\text{Month of sampling + Population}} = 0.98$, Table S2, Fig. 1). The month of sampling explained most of the observed FN variability (54%), in agreement with the foraging strategy of this mountain ungulate (e.g., browsing when alpine pastures are covered by snow and grazing highly nutritious herbaceous plants as soon as the snow starts to melt, see García-González & Cuartas, 1996). In fact, the maximum values of FN occurred in Spring and Summer in both populations (i.e., May to September, mean = 2.54, min = 2.19, max = 2.85, Table 2), probably coinciding with the increase in nitrogen experienced by plants during the early stages of growth (Mattson, 1980). On the contrary, minimum FN values occurred from October to March (mean = 1.97, min = 1.66, max = 2.13), probably due to a loss of the nutritive value of the main abundant grasses and forbs species of this kind of grasslands (Marinas et al, 2003). Mean yearly values of FN for Costabona were slightly higher than in Fontalba (t-value = 2.04, p-value = 0.04), being the FN in the earlier in average 1.05 times higher than in the former (mean FN = 2.26, SE = 0.03 in Costabona, vs, mean FN = 2.16, SE = 0.036 in Fontalba). Differences of FN between both populations seemed to be greater in late Winter and early Spring (i.e., January to April) than in the rest of the seasons (see Table 2 and Fig. 1). However, this difference between populations is not supported by our statistical modelling (i.e., the model including the interaction between FN and population was at 13.5 points from the best model, (Table S2). Variability due to differences on FN estimates between samples was 37.5% (i.e., random effect).

Regarding FN prediction by NIRS, the results obtained in the development of both calibration and validation equations are summarized in Table 4 (but also see Fig. S3). The optimal spectral pre-treatment were the second and the third derivative treatment, combined with SNV and MSC. Prediction equations for FN content were satisfactory in all cases, probably due to an easy identification by NIRS of C-N and N-H bonds contained in the sample. Although all calibrations (i.e., G, F, C, and S-S, A-W) were excellent in terms of accuracy, population-specific calibrations (F-C) showed the higher coefficients of determination (0.99 in both cases, Table 3). Both SEP and SECV values were very low in comparison to other studies on wild ungulates (Kamler et al, 2004, obtained SECV values of 0.147 and 0.12 for red deer and roe deer, respectively), which guaranties accurate FN predictions. For all models, the mean bias (mean difference between chemical and NIRS values) was near zero and the slopes of the equations did not differ significantly from one, indicating that values of FN obtained by the two methods were closely correlated in all pools of data sets.

Table 4. Calibration and validation statistics for determination of nitrogen content in Pyrenean chamois - *Rupicapra p. pyrenaica* - faecal samples by NIRS.

	Math [a] treatment	Scatter [b] correction	R^2	SEC	SECV	SD/SECV	r^2	SEP	Bias	Slope	RPD	RER
			Calibration set				Validation Set					
Costabona (C)	3,4,4,1	SNV	0.99	0.03	0.06	6.89	0.98	0.07	-0.01	0.93	6.09	30.34
Fontalba (F)	3,4,4,1	SNV	0.99	0.05	0.08	6.17	0.97	0.11	-0.01	0.96	6.17	31.73
Spring-Summer (S-S)	2,4,4,1	MSC	0.98	0.06	0.08	6.02	0.97	0.11	-0.03	0.96	5.78	29.16
Autumn-Winter (A-W)	3,4,4,1	MSC	0.97	0.04	0.06	4.58	0.95	0.066	0.003	0.88	4.31	16.61
Global (G)	2,4,4,1	MSC	0.97	0.08	0.08	5.48	0.97	0.11	0.02	0.97	5.45	30.60

[a] Values correspond to designations as follows: derivative order (i.e., 1 means first derivative), gap (number of data points over which derivation was computed), first smoothing (number of data points for spectral smoothing), and second smoothing (data points for second spectral smoothing); [b] Standard Normal Variate (SNV) and Multiple Scatter Correction (MSC) transformations. Key: R^2 = coefficient of determination for calibration, SEC = standard error of calibration, SECV = standard error of cross validation, SD = standard deviation, r^2 = coefficient of determination for validation, SEP = standard error of prediction, RPD = standard deviation of reference data and RER = range error ratio.

The accuracy of the calibration in terms of RPD and the RER statistics confirms the high precision of the equations developed, with values over the minimum recommended for prediction (i.e., RER over 10 and RPD over 3 according to Williams and Sobering, 1996). Regarding coefficients of determination, our results were similar or even greater than those reported for other wild ungulate species (e.g., red deer and roe deer, see Kamler et al, 2004) and even for domestic ruminants (e.g. Alonso et al, 2005). On the other hand, a single general calibration (G) would exhibit enough precision for estimating FN in the FSNGR even outside the study area, however further calibrations using samples beyond the study area would be required before making predictions of diet quality in chamois showing a different range of FN values.

In conclusion, NIRS is a viable technology for predicting FN content in free-ranging Pyrenean chamois. Moreover, NIRS is non-destructive (samples can be re-utilized, for example, to determine botanical composition of the diet); it is emission-free since it does not require the use of chemical products; and its less-time consuming once calibrations are in place, and have a low cost of analysis. All of these reasons should encourage researchers to use NIRS for quantitative analyses of a range of organic constituents with applications in wildlife research.

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S1. Model selection based on an additive mixed modelling for exploring the seasonal and spatial variations of faecal nitrogen percentage.

Table S1. Model selection based on an additive mixed modelling for exploring the seasonal and spatial variations of faecal nitrogen percentage (FN) determined in 291 faecal samples of Pyrenean chamois from Fontalba (n = 173) and Costabona (n = 118) in the NGRFS, Catalan Pyrenees, Spain. K = effective number of parameters in the additive modelling, AICc = Akaike Information Criterion corrected for small sample sizes, ΔAICc = difference of AICc with respect to the best model, wi = Akaike weight, Mo = null model only with the constant term. In bold the best model for explaining the observed FN variability

Biological models	**K**	**AICc**	**Δ_i**	**w_i**
Month + Population	6	107.06	0	0.98
Month * Population	7	120.55	13.50	> 0.01
Month	5	121.06	14.00	> 0.01
Mo	3	335.88	228.83	> 0.01
Population	4	336.90	229.85	> 0.01

S2. Full list of references used in Table 1.

The following reference list summarizes bibliographic records found in the Thomson Reuters Web of Knowledge (http://www.accesowok.fecyt.es/) between the years 1899 and 2011. The following key words: Diet quality indices, Faecal indices, Faecal nitrogen, Faecal metabolic nitrogen and Non-dietary faecal nitrogen have been used. The numbers match with those appearing in Table 1 in the main body of the manuscript.

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Table S2. Descriptive statistics of samples for both calibration and validation NIRS equations for estimating FN in Pyrenean chamois (*Rupicapra p pyrenaica*) faecal samples from Costabona and Fontalba, two populations from the National Game Reserve of Freser – Setcases, Catalan Pyrenees, Spain.

Kind of calibration	Calibration set				Validation set			
	N	Range	Mean	SD	n	Range	Mean	SD
Costabona (C)	59	1.69 - 3.45	2.37	0.40	20	1.90 - 3.10	2.44	0.35
Fontalba (F)	85	1.53 - 4.10	2.28	0.50	28	1.16 - 3.73	2.29	0.62
Spring-Summer (S-S)	71	1.68 - 4.10	2.58	0.50	24	1.70 - 3.73	2.59	0.54
Autumn-Winter (A-W)	73	1.53 - 2.51	2.04	0.27	24	1.81 - 2.45	2.13	0.16
General (G)	144	1.53 - 4.10	2.34	0.46	48	1.62 - 3.49	2.27	0.51

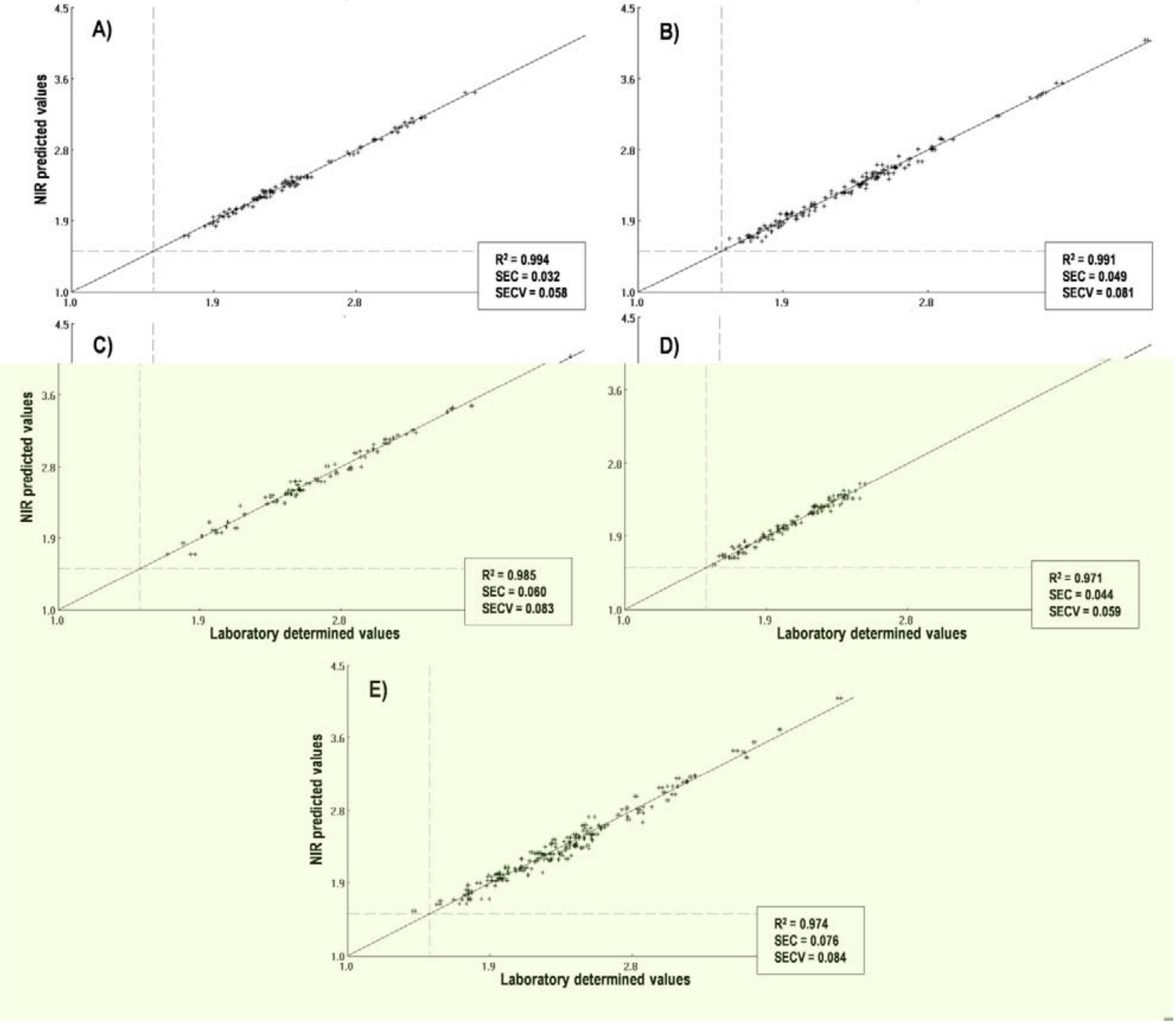

Figure S2. Regression relationships between faecal nitrogen estimated by LECO ("Laboratory determined values", see Material and Methods section) and near infrared reflectance predicted values. (A) Equations for Costabona, (B) for Fontalba, (C) for Spring – Summer, (D) for Autumn – Winter and (E) General calibration including all year round samples from both populations.

DISCUSIÓN GENERAL

El estudio de la dieta de herbívoros silvestres y todas sus derivaciones ha motivado la curiosidad de muchos científicos en el mundo entero, más cuando conviven en simpatría con animales domésticos (p.e. García-González et al, 1990; La Morgia y Bassano, 2009). La gestión de áreas de conservación cada vez mayores, donde puedan convivir varias especies de herbívoros, conlleva cada vez un mayor número de elementos de análisis. Al tradicional estudio de la disponibilidad de recursos alimentarios, la composición y el solapamiento de la dieta, en este trabajo se añadió el análisis de otros elementos influyentes, como la presencia de parásitos gastrointestinales en la relación pasto-herbívoro. Además se valoró el uso de técnicas de detección de indicadores de la calidad de la dieta, como el nitrógeno fecal (Nf) a través de la esprectroscopía de reflectancia en el infrarrojo cercano (NIRS, por sus siglas en inglés), el cual se ha revelado muy útil en procesos de monitoreo a largo plazo (long-term), como es el caso del estudio del rebeco en la Reserva Nacional de Caza Freser-Setcases, en los Pirineos Orientales, donde se realizó el presente estudio.

La comparación de dietas de rebeco y ganado doméstico en dos zonas (Costabona y Fontalba) con una disponibilidad vegetal similar al inicio del verano, permitió detectar diferencias atribuibles a la especie animal. En general, tanto el rebeco como el ganado doméstico (vacas, caballos y ovejas) seleccionan especies similares pero en diferentes proporciones. Se trata de herbívoros que comparten el mismo nicho trófico y si los los recursos alimentarios fueran limitados se esperarían interacciones competitivas (de Boer y Prins, 1990; Lovari y Ferreti, 2013). Sin embargo, entre el rebeco y el ganado doméstico se evitaría la competencia mediante la repartición de recursos, como se ha observado en otros estudios (Hutchinson, 1959; MacArthur, 1972; Schoener, 1974). Las diferencias en cuanto a preferencias alimentarias se deberían a diferencias en la morfofisiología de los animales (Hofmann, 1989; Clauss et al, 2010), las cuales obligan a caballos, vacas y ovejas a alimentarse principalmente de herbáceas (especies pastadores) mientras que el rebeco podría adaptarse tanto a una dieta herbácea como a una leñosa. En este trabajo, el rebeco, durante la época favorable, muestra una dieta compuesta principalmente por leñosas, en contraste con otros estudios donde se le considera principalmente pastador (Pérez-Barbería et al, 1997; Homolka y Heroldová, 2001), sobre todo en verano (García-González y Cuartas, 1996), carácter que se acentuaría cuando pasta en simpatría con otros ungulados silvestres (Bertolino et al, 2009). En nuestro caso, la clara preferencia por leñosas indicaría que los recursos se reparten entre especies salvajes y domésticas, evitando así la competencia. De hecho, es bien conocido que el rebeco tiene la capacidad de incorporar una proporción relativamente alta de especies leñosas en su dieta, sobre todo en invierno (García-González y Cuartas, 1996; Häsler y Sen, 2012). Incluso, cuando el rebeco se ha introducido como especie exótica, como en los bosques de Nueva Zelanda, la dieta puede consistir casi en su totalidad por plantas leñosas (Yockney y Hickling, 2000). El rebeco aparece aquí, entonces, como ramoneador con preferencia sobre arbustos como la ericácea *Calluna vulgaris* y la leguminosa *Cytisus scoparius*. El consumo de ericaceas se ha registrado como un componente importante en la dieta de ungulados silvestres de montaña (Trutmann, 2009), quizás relacionado con el efecto antiparasitario de los taninos que poseen (Moreno-Gonzalo et al, 2014; Fernández-Olaya y San Miguel-Ayanz, 2007; Osoro et al, 2009; Celaya et al, 2010; Landau et al, 2010).

De acuerdo con la hipótesis del reparto de recursos, los indicadores de solapamiento de la dieta más bajos se obtuvieron al comparar el rebeco con cualquiera de los animales domésticos, y los más altos en las comparaciones de las especies domésticas entre sí. El caballo mostró un claro comportamiento pastador, con las cantidades más altas de gramíneas y las más bajas de leñosas. Las vacas y las ovejas también aparecieron como pastadoras, aunque utilizando algo más de leñosas y menos gramíneas que el caballo. El mayor índice de solapamiento se dio entre los equinos y los vacunos, probablemente debido a su mayor tamaño, y en consonancia con otros estudios realizados en distintos ambientes (Menard et al, 2002; Osoro et al, 2005; Aldezabal et al, 2012), incluso entre vacunos domésticos y equinos salvajes (Krys et al, 1984).

Un factor que puede influir en la selección de la dieta es la fenología de la planta (Garel et al, 2011), lo que implica que el índice de similitud entre las especies puede variar de acuerdo a la estación (Homolka, 1993; Heroldová, 1996). En el caso del rebeco y las ovejas, podría explicar las diferencias significativas entre las dietas de verano y otoño, siendo las herbáceas más consumidas en verano, y las leñosas en otoño. En este sentido, la disponibilidad de las herbáceas podría desempeñar un papel importante en la selección de la dieta. Teniendo en cuenta que la fracción de gramonoides no varía entre las dos estaciones, en ninguna especie animal, y que las principales especies leñosas están presentes durante todo el año, la aparición de las herbáceas, en general más palatables, podría determinar el consumo de las especies leñosas. Esto estaría de acuerdo con la teoría del forrajeo óptimo (*Optimal Foraging Theory*) (Sthepens y Krebs, 1986; Gross, 1986), que dice que los herbívoros prefieren alimentos con mayor digestibilidad de la materia orgánica; por lo tanto, las herbáceas son preferidas a las gramíneas y a algunas leñosas. De este modo, cuando el rebeco es el único herbívoro en el hábitat alpino, selecciona gramíneas y herbáceas durante todo el año, y su consumo se incrementa en primavera-verano, cuando su calidad es alta (Pérez-Barbería, 1997). Como consecuencia del elevado consumo de herbáceas por los animales domésticos, sobre todo por ovejas, los rebecos se verían obligados a aumentar su consumo de leñosas. Este efecto también se ha observado en los Alpes italianos occidentales, donde la reducción de las herbáceas altamente digestibles en la dieta del rebeco se debió a la presencia de ovejas durante el verano (La Morgia y Bassano, 2009).

La segregación espacial del nicho ecológico es otro factor determinante de la composición de la dieta y posibilita la coexistencia de herbívoros (Belovski, 1986; Putman, 1996; Latham et al, 1999). El pastoreo libre puede afectar la distribución de los ungulados silvestres y modificar su actividad y la dieta (Kie et al, 1991; Kie, 1996; Coe et al, 2001, Marttielo et al, 2002; Brown et al, 2010). Una segregación espacial marcada entre el rebeco y el ganado doméstico se ha registrado en Francia y España (Berdoucou, 1986; García-González et al, 1990), y entre el rebeco cantábrico (*Rupicapra p. parva*) y el ganado doméstico en España. Además, los rebecos suelen mostrar algún grado de intolerancia cuando las ovejas ocupan su zona (Ruttimann et al, 2008). La presencia de rebaños domésticos es una fuente de perturbación para los rebecos, que responden moviéndose por la pendiente (Mason et al, 2014) o pastando en áreas rocosas o boscosas, donde la calidad del forraje es menor (Hamr, 1988; Chirichella et al, 2013). Otra razón de la segregación puede ser la presencia de heces de otros herbívoros en la zona de pastoreo (Frankhauser et al, 2008). En nuestro caso, la coexistencia del rebeco con vacas, caballos y ovejas, todos claramente pastadores, podría ser determinante para el patrón ramoneador del rebeco, y la preferencia por *Cytisus scoparius*, que se distribuye junto al bosque, podría ser una consecuencia de estas presiones en el Pirineo oriental catalán.

El análisis microhistológico de las heces a lo largo del año mostró que, en general, las plantas leñosas, principalmente *Calluna vulgaris*, *Juniperus communis*, y *Cytisus scoparius* representaron más de la mitad de la dieta anual del rebeco. Sin embargo, el consumo de las leñosas no leguminosas disminuyó hasta mediados del otoño, al tiempo que se duplicó el consumo de las herbáceas leguminosas y no leguminosas, especialmente en mayo-junio, coincidiendo con sus mayores valores nutritivos (Marinas et al, 2003). En los Alpes occidentales italianos, la dieta del rebeco contenía leñosas solamente en otoño e invierno, como consecuencia de la baja disponibilidad de las herbáceas (La Morgia y Bassano, 2009). Nuestros resultados coinciden en parte con este patrón de alimentación, con un mayor consumo de herbáceas nutritivas cuando están disponibles. El hecho que el *Cytisus scoparius* permanezca verde todo el año debido a sus tallos con actividad fotosintética, justifica su presencia en la dieta del rebeco durante todo el año, pues en general prefiere plantas leguminosas a las no leguminosas (Aldezábal y García-González, 2004) debido a su elevado valor nutricional y digestibilidad (Marinas et al, 2003). Los rebecos consumen sobretodo graminoides y *C. scoparius* regularmente todo el año, con el fin de aumentar la ingesta de fibra y nitrógeno. Cuando la disponibilidad de herbáceas es baja, como en el otoño e invierno, debido a la fenología de las plantas (Marinas et al, 2003), o a la capa de nieve (Aldezábal y García-González, 2004), o a la presencia de ganado doméstico (Berdoucou, 1986; García-González et al, 1990; La Morgia y Bassano, 2009), el rebeco se adapta a las nuevas condiciones, aumentando el ramoneo de especies leñosas.

Los análisis de los modelos de regresión de cuadrados mínimos parciales (PLSR) mostraron que los consumos altos de leñosas no leguminosas se relacionan negativamente con cargas parasitarias altas de nematodos gastrointestinales. Para algunos ungulados, se ha descrito el uso de plantas con fines de automedicación, por ejemplo en cabras (Torres-Acosta et al, 2008; Moreno-Gonzalo et al, 2014) y ovejas (Lisonbee et al, 2009; Juhnke et al, 2012). La alta concentración de taninos que se encuentra en algunas especies leñosas, como *Calluna vulgaris*, *Juniperus sp.* y *Quercus sp.*(Frutos et al, 2004), podría justificar su aparición en la dieta anual del rebeco. Las herbáceas, tanto leguminosas como no leguminosas, se asociaron positivamente con la carga parasitaria durante todo el año, debido a que los parásitos se concentran principalmente en las herbáceas y graminoides, sobre todo en la época estivante (Hutchings et al, 2003). *Cytisus scoparius*, como única leñosa leguminosa en la dieta, resultó negativamente relacionada con la carga parasitaria en invierno-otoño, pero positivamente en primavera-verano. El rebeco adquiere parásitos principalmente en el periodo primavera-verano, mientras que la infestación disminuiría en otoño-invierno debido a la ingestión de plantas con mayores concentraciones de taninos. Cuando el rebeco consume gran cantidad de plantas herbáceas (Marinas et al, 2003), se enfrenta al dilema entre el riesgo del parasitismo y la ingestión de nutrientes. De hecho, el rebeco duplicó el consumo de herbáceas leguminosas y no leguminosas cuando la disponibilidad fue mayor, y las larvas infectivas de los parásitos gastrointestinales abundan en estas plantas (Hutchings et al, 2003). Según la teoría del forrajeo óptimo, la decisión del rebeco se basaría en asumir el riesgo de parasitismo, a la vez que obtiene el mayor beneficio de la ingestión de nutrientes. Además de la ingestión extra de nutrientes, podría mejorar el sistema inmunológico, permitiéndole hacer frente a los parásitos (resistencia). Por consiguiente, el comportamiento del rebeco asumiría los costos del parasitismo, a la vez que se alimentaría con cantidades mayores de plantas nutritivas que contribuirían a minimizar el daño causado por los patógenos (tolerancia).

En procesos de monitoreo a largo plazo de la fauna silvestre, como en el caso del rebeco en la Reserva Nacional de Caza de Freser-Setcases, se suele determinar el contenido de nitrógeno fecal en heces, como indicador comúnmente utilizado para determinar la calidad de la dieta (Blanchard et al, 2003; Hamel et al, 2009; Gálvez-Cerón et al, 2013). En este estudio, esta determinación se realizó mediante espectroscopía de reflectancia en el infrarrojo cercano (NIRS). Los resultados mostraron la utilidad de esta técnica. Sin embargo, la interpretación simplista generalizada de una relación directa entre el contenido de proteína en la dieta y el N-fecal debe ser reconsiderada. Un aspecto a tener en cuenta es la presencia de metabolitos secundarios, que puede producir un aumento del N-fecal en niveles similares a los del N de la dieta (revisión de Leslie et at, 2008). Sin embargo, en ausencia de dichos metabolitos secundarios, una dieta baja en proteína, como en el caso de la experiencia con ovejas, (p.e. 8% en base a materia seca), llevó a valores más altos de N-fecal que la dieta alta en proteína. Este efecto se explicaría principalmente por el mayor contenido de lignocelulosa (fibra ácido detergente) en la dieta alta en proteína, y un mayor contenido de carbohidratos de fácil digestión, principalmente almidones, en la dieta baja en proteína. La dieta baja en proteína utilizada en nuestro estudio fue claramente artificial, debido a la inclusión de grano, y no tendría ningún equivalente en el medio natural de rumiantes en pastoreo, donde una disminución en el N de la dieta suele estar vinculado con un aumento de la fibra y una disminución de la digestibilidad. Sin embargo, sirve para ilustrar que el N-fecal no siempre refleja directamente el N de la dieta, sino más bien es el resultado de dos efectos biológicos diferentes: las dietas altas en N son generalmente más digeribles, en ausencia de metabolitos secundarios, y las dietas más digeribles conducen a un mayor Nf en los herbívoros.

Los endoparásitos se reconocen como una fuente de pérdida de proteína en rumiantes (Parkins y Holmes, 1989), pero su impacto en la medición del N-fecal ha sido ignorado por los investigadores de la vida silvestre. En la primera parte de nuestra investigación, el aumento del N-fecal y del número de huevos fecales en el grupo de la dieta baja en proteína se explica mejor por la alta digestibilidad de la dieta y la menor proporción de fibra en las heces. Sin embargo, la influencia de los helmintos en el N-fecal se demostró más tarde por la disminución del N-fecal cuando los parásitos se eliminaron debido al tratamiento antihelmíntico. A pesar que las ovejas de la dieta baja en proteína tuvieron una excreción de N-fecal superior, también tenían concentraciones inferiores de proteína sérica total, lo que pone de relieve la ausencia de una asociación entre el N-fecal y el estado nutricional en los casos de parasitismo grave. Una vez desparasitadas, las ovejas probablemente perdieron menos N debido al catabolismo de las proteínas y la respuesta inmune específica, como sugiere el aumento de la proteína sérica total.

La disminución en la excreción del N-fecal después del tratamiento antihelmíntico (incluso cuando la fibra se corrigió) se debió probablemente a la reducción de los daños causados por nematodos gastrointestinales en el tracto digestivo (Fox, 1997), que conduce al aumento de las pérdidas de N metabólico (Parkins y Holmes, 1989). Además, el aumento de la eficiencia de las enzimas digestivas en ausencia de parásitos (Jones, 1983), también puede explicar la disminución del N-fecal, como resultado de una disminución del N residual de la dieta. La pérdida de proteínas en el tracto gastrointestinal debido a parásitos se asocia con la pérdida de plasma y de eritrocitos, células epiteliales exfoliadas y moco (Parkins y Holmes, 1989). En infecciones asociadas con hemorragia gastrointestinal, p.e. *Haemonchus contortus*, *Oesophagostomum* spp. o *Trichurus colubriformis,* una pérdida masiva de eritrocitos puede ser absorbida por el ileon (Rowe et al, 1982) y el intestino delgado (Poppi et al, 1981). Se han detectado pequeños incrementos del N-fecal debido a la pérdida de sangre en ovejas infectadas experimentalmente con estas especies de parásitos (Sykes y Coop, 1977); Symons et al, 1981). Estos aumentos en el N-fecal, debido a las pérdidas de proteína endógena, aumentarían en el caso de infecciones por nematodos que infectan el abomaso y el intestino delgado (Haile et al, 2004), como en el estudio actual.

El seguimiento de la calidad de la dieta del rebeco durante dos años (mayo de 2009 a mayo de 2011), a partir del N-fecal determinado por NIRS, mostró que los rebecos siguen un patrón estacional similar de N-fecal en dos zonas distintas (Costabona y Fontalba) con vegetación similar. El periodo de muestreo explica la mayor parte de la variabilidad del N-fecal observado, de acuerdo con la estrategia de búsqueda de alimento por parte de los ungulados de montaña con dominio del ramoneo cuando las herbáceas están cubiertas de nieve, y del pastoreo cuando la nieve empieza a derretir (García-González y Cuartas, 1996). Los valores máximos de N-fecal se produjeron en primavera-verano, coincidiendo con el aumento de N de las plantas en crecimiento (Mattson, 1980) y los mínimos en otoño-invierno, probablemente debido a la pérdida del valor nutritivo de los pastos (Marinas et al, 2003).

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CONLUSIONES

1. El el Pirineo oriental estival del rebeco difiere en su composición y preferencias de vacas, caballos y ovinos que pastan en simpatría debido al consumo de *Calluna vulgaris* y *Cytisus scoparius*.

2. Las dietas de las especies domésticas se solapan en gran medida entre sí, y están claramente dominada por gramíneas y herbáceas.

3. La presencia del ganado doméstico parece modificar la dieta del rebeco. La interacción entre éste y la ganadería sugiere una repartición de los recursos, mientras que la interacción entre los domésticos provocaría solapamiento de dietas, que posiblemente no derivaría en competencia debido a la abundancia de recursos forrajeros.

4. Rebecos y ovejas varian su dieta en función de la estación, siendo más leñosa en otoño que en verano, mientras que vacas y caballos, muestran una dieta similar en ambas estaciones.

5. El rebeco parece compensar las consecuencias de la infección por parásitos gastrointestinales con un incremento del consumo de herbáceas ricas en proteina. Además, el N-fecal debería considerarse como un indicador de la digestibilidad más que un estimador del contenido de proteína de la dieta, ya que este parámetro se ve influido por la carga parasitaria gastrointestinal.

6. La espectroscopía de reflectancia en el infrarrojo cercano (NIRS) es una técnica válida para predecir el contenido de N-fecal en el rebeco pirenaico, que resulta más barata que los análisis por vía húmeda, no contaminante y que permite reutilizar las muestras para otros análisis (p.ej., microhistológico).

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