What is on the menu today? Creating a microwear reference collection through a controlled-food trial to study feeding management systems of ancient agropastoral societies

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Abstract

The study of ancient herd-feeding systems is essential to investigate livestock management and the interactions of humans with domestic animals and past environments. This topic for historic periods has recently been investigated through dental microwear analyses. This approach, however, must be used with caution, as it is based on comparisons of established microwear patterns of modern wild animals. Here we present an experimental reference collection of dental microwear for domestic sheep (Ovis aries), created by a controlled-food trial to fill this methodological gap. Fifty sheep were split into five groups of ten, fed with four different types of vegetation potentially used by agropastoral societies (alfalfa, ray-grass, forage, and barley), and administrated following different techniques of processing (wet, dried and fresh). After being fed with a specific and controlled diet, the animals were slaughtered and the microwear patterns on the enamel surface of the lower molars were analysed via standard light stereomicroscopy. The differences found in our experiment between the different diets and processed plants have allowed us to characterize each dietary group and feeding management system. This information is extremely important to correctly interpret the archaeological record.

Keywords

Archaeozoology; Ancient livestock strategies; Diet; Ovis aries; Tooth wear patterns; Phytoliths

1. Introduction

In the last decades, research focused on ancient livestock strategies have increased considerably. This is due to its importance to study various aspects of past societies, such as economy, social relations or environment, among others (Delgado et al., 1999; Gandini and Villa, 2003; Floyd et al., 2003; Mawdsley et al., 1995; Steinfeld and Mack, 1995). In archaeozoology, this is vital in understanding the relationship between humans, domestic animals, and the environment, and this relationship in relation to the social and economic organization of past communities.

One of the main lines of research that concerns archaeozoology is how and where domestic animals were fed, as well as the food management systems used by ancient societies. Traditionally, this information was obtained from the written texts of Classical authors like Cato the Censor, Varus, or Columella. These ancient authors, however, wrote about theorical and optimal models of livestock management, not providing reliable descriptions of every-day food management systems. Recently, the dental microwear technique has been proved as a

useful tool to help us better understand ancient husbandry (Gallego et al., 2017; Mainland, 1998a, 2006; Rivals et al., 2011; Rieau, 2012, 2014; Vanpoucke et al., 2009 among others), since it allows us to know the last meals of each individual, which is also known as "Last Supper phenomenon" (Grine, 1986). This principle is based on the fact that the enamel surface is worn due to attrition (tooth-on-tooth contact) and abrasion (tooth-to-food contact) during mastication based on the composition of what animals ate (Dahlberg and Kinzey, 1962; Fortelius and Solounias, 2000).

The application of this technique relies on previous knowledge of the kind of diet that produces a particular pattern on the enamel. Archaeozoologists, biologists and palaeontologists have started to create dental microwear reference collections to strengthen the interpretation of the micro-traces observed in the palaeontological and archaeological records. Some of these reference collections, however, are based on specimens from museum collections and slaughterhouses (Rieau, 2014; Solounias and Semprebon, 2002; Ward and Mainland, 1999). Others were obtained from animals in which only a controlled experiment of their natural grazing diet was possible to perform (Mainland, 1998b, 2000, 2001, Mainland, 2003a,b). Finally, some researchers conducted controlled-food trials on domestic animals (Hoffman et al., 2015; Merceron et al., 2017; Winkler et al., 2019) but with the intent to answer biological and paleontological questions related to the formation of tooth microwear and the agents responsible of it.

Here we present the first dental microwear reference collection created from a controlled-food trial in order to specifically study the feeding systems of ancient agropastoral societies. The composition of the nourishment, the environment where the herd grazed, and the selection of every single individual used on this trial were designed and decided before the start of the experiment and were under our supervision during its undertaking. We created five diets that simulated different potential feed-management systems of ancient agropastoral societies. The first two groups were designed to reproduce an intensive feeding system characterized by feeding the herd in a barn with wet ensilaged fodder. The third group recreates a mixed system, allowing the sheep free access to fresh plants from the countryside during the day and wet ensilaged fodder later in a barn. The fourth group replicates the utilization of a mixture of dried cereal hay, with its grains (barley products) fed to a stalled herd. While the last group characterizes a flock that fed on an over-grazed meadow in which the grass is scarce, and animals eat closer to the ground with a logical increase of soil ingestion.

The experiment is focused on one domestic mammal, sheep (Ovis aries) for the following reasons. First, to avoid micro-traces generated by biomechanical factors such as the different chewing between species (Gailer and Kaiser, 2014). And second, because this taxon is the most represented animal in most Holocene Mediterranean sites (Saña, 2013; Colominas et al., 2017; Helmer and Gurichon, 2017; De Cupere et al., 2017).

This study allows us to understand how the different plant resources ingested by sheep affected dental microwear. The different microwear patterns observed from each diet generate models to compare with the patterns observed in the archaeological record. These models make it possible to infer the distinct livestock strategies used by past societies to

nourish this species and therefore, to better understand the organization of ancient agropastoral societies and their interactions with the environment and husbandry systems.

2. Material and methods

2.1. Experimental conditions

The controlled-food experiment was carried out at the facilities of the Farm Service and Experimental Fields from Veterinary Faculty of the Autonomous University of Barcelona (Bellaterra, Cerdanyola del Vallès, Catalonia). There, a flock of sheep is kept for experimental studies on extensive livestock farming conditions for more than forty years. The flock is fed in the main barn with a mixture of plant-sources and in the fields and forests that surround the research centre.

Fifty domestic ewes (Ovis aries) ranging in age from one to nine years were used for this controlled-trial. They belong to Ripollesa, Manchega, and Lacaune breeds all raised and keep at the Farm Service (see Table 1 of the supplementary materials for detailed data of each individual). The ewes were distributed into five different feeding groups (Table 1). The first group was fed with wet ensilaged alfalfa (Medico sativa) (ALF group). The second group was fed with wet ensilaged ray-grass (Lolium hybridum Hauuskn) (RG group). The third group was fed in a semi-extensive livestock system (FOR group). This system was comprised of fodder with a mixture of monocotyledonous and dicotyledonous plants when the animals were in the barn, combined with free forage during the day (see section 3.2 for detailed description of plants consumed by the herd). The fourth group was fed with a mixture of seeds and straw of dry barley (BAR group). While the last group was fed using wet ensilaged alfalfa mixed with soil to simulate animals feeding on plant parts close to the ground or on vegetation covered with heavy loads of dust or grit (described throughout as the 'dusty alfalfa diet') (DA group) (Table 1).

Table 1. Summary of the five diets simulated in this trial: plant and plant parts, technique, and period when each group was fed with the specific diet.

Diet group	Diet	Part of the plant	Technique	Feeding period
ALF	Alfalfa	All	Wet	27/3/2017 to 3/4/2017
RG	Ray-grass	All	Wet	25/4/2017 to 2/5/2017
FOR	Forage	All	Fresh	1/5/2017 to 8/5/2017
BAR	Barley	Stalk, grain	Dry	8/5/2017 to 15/5/2017
DA	Alfalfa + soil	All	Wet	17/7/2017 to 24/7/2017

Food was controlled 24h during the final days before the animals were slaughtered. Studies have shown that over a period of 8 days old traces of microwear are removed and new patterns are recorded on the teeth (Grine, 1986; Teaford and Oyen, 1989). For our trial, the sheep's diet was controlled for at least 10 days prior to death. During this period ewes were

allowed access only to their assigned food-group. Every day, the food for each group was restored. For the barley and dusty alfalfa diets, specific procedures were required. In the barley diet, a ratio of 200 g of grain per kg of straw was supplied; 200 g of sieved clay soil were added per kg of alfalfa to feed the dusty alfalfa diet group.

In coordination with our microwear trial, food and coprolites were collected systematically from each group as a blind test to insure the control of the different diets and as support for the interpretation of the dental microwear. The soil used for the dusty alfalfa diet group was also collected and analysed for the same purpose.

The specimens from each group were slaughtered at the necropsy room of the Faculty of Veterinary by the technical assistant of the Veterinary Faculty of the Autonomous University of Barcelona. The specimens selected for this study were considered cull ewes by the veterinarian researchers (i.e. they were no longer able to be kept by the Farm Service).

2.2. Preparation and casting

All skulls and mandibles were processed – boiled, skinned, eviscerated, and cleaned in peroxidase – before obtaining casts from molars at the Institut Català de Paleoecologia Humana i Evolució Social (IPHES). After this, all molars were cleaned with a dilution of bleach at 3% for 2 h to remove the possible biofilm layer formed on the enamel surface. Later, molars were cleaned again with cotton soaked in 96% ethanol. Once the molars dried properly, high-resolution moulds were made with dental silicone (Heraeus Provil® novo light Light CD and Provil® novo Putty) and positive casts were produced using transparent epoxy resine (C.P. Química CPOX P 1060/A and CPEN 1585/B).

2.3. Microwear analysis

For this study, we focused on the left second molar (m2) following Gordon (1982). In the few cases in which no microwear readings from m2s were possible, we used the third molar (m3). Xafis et al. (2017) has demonstrated that there is no significant difference in terms of microwear between molars.

Casts were observed through a light stereomicroscope (Zeiss Stemi 2000C) at x35 magnification within a standardized squared area of 0.16 mm2 using the refractive properties of the transparent cast to reveal microfeatures on the enamel. We followed the classification of features established by Solounias and Semprebon (2002) and Semprebon et al. (2004) as follows: pits (small, large pits, and gouges), scratches (fine and coarse). For a detailed explanation of each feature, see Solounias and Semprebon (2002).

The number of scratches and pits were recorded for every molar. Qualitative characteristics of the traces (small and large pits, gouges, cross scratches and the scratch width score) were also recorded. In contrast to earlier studies (Rieau, 2014; Solounias and Semprebon, 2002), here

the presence or absence of these qualitative characteristics was not just noted, but also the total amount counted. For instance, if during the analysis of a sample the presence of large pits on the enamel are noticed, the total number was recorded. Using this method, it is possible to analyse in greater detail the variation of conventional qualitative variables between the different diet groups and specimens.

2.4. Analysis of phytoliths, plants, and soil

Samples of fresh coprolites were collected from each of the diet groups in order to obtain the composition and quantification of the phytoliths present. The phytolith extraction procedure was replicated three times to ensure a significant and representative result of each diet group.

The characteristics of the modern coprolite samples made it necessary to introduce a process to eliminate the organic matter prior to analyzing. We followed the dry method, burning three coprolites per sample in a muffle furnace for 3 h at 500 °C. This procedure for the preparation of modern samples does not damage the phytoliths and their multicellular structure (Parr et al., 2001; Jenkins, 2009). After removing the organic matter, coprolite ashes were processed following the rapid phytolith extraction method (Katz et al., 2010). Between 20 and 30 mg of coprolite ashes were processed in a conical centrifuge tube. 50 µl of 6N HCl was added to dissolve the carbonate minerals. When the bubbling had ceased, 450 µl of sodium polytungstate 2.4 g/ml density was added to the solution. After, the sample was sonicated for 10 min and centrifuged for 10 min at 5000 rpm. Then, the supernatant was transferred to a new centrifuge tube and homogenized. An aliquot of 50 µl was placed on a microscope slide. We identified a minimum of 200 individual phytoliths at 400x using a petrographic microscope Olympus BX41 (Albert and Weiner, 2001).

Morphological identification and anatomical and taxonomical adscription of the phytoliths was based on the standard literature (Brown, 1984; Mulholland and Rapp, 1992; Twiss, 1992; Strömberg, 2004; Piperno, 2006) and reference collections (Albert et al., 2000, 2016; Albert and Weiner, 2001; Tsartsidou et al., 2007). Classification of the phytoliths for interpretation was focused on their anatomical ascription, the part of the plant where phytoliths were formed (i.e. inflorescence and leaves in case of grasses), and their taxonomic classification, whenever possible. To quantify the number of phytoliths in a gram of coprolite ash we counted the number of phytoliths present in 20 fields at 200x counted (Katz et al., 2010). The quantitative results were calculated taking into account the loss of organic material in the previous burning process. This extrapolation makes it possible to avoid exaggeration caused by the concentration of phytoliths due to the loss of organic material.

Moreover, representative samples of fresh plants were collected from the pastures used to feed the forage group. Plant community composition and species richness present seasonal variation (Visser et al., 2010). For this reason, plant surveys were carried out at the same moment as herd grazing. In addition, samples of fodder, a mix of monocotyledonous and dicotyledonous plants, used in the barn to complement the grazing were analysed. Taxonomic

identification for all the samples was made using local flora guides (Bolòs et al., 2005), and a binocular microscope when required.

A sample from the sieved soil used to prepare the dusty alfalfa diet was analysed to know the mineral composition of the sample and avoid elements of extreme hardness in the diet. To obtain this information, the granulometry of the soil was analysed (see Table 2 of the supplementary materials for more details about this analysis).

Table 2. Mean of the number of scratches (Mscratches) and pits (Mpits) and their corresponding standard deviation (SD) for the five diet groups. In the last column we show the percentage of specimens in each group that have between 0 and 17 scratches (%0–17). (ALF = alfalfa group; RG = ray-grass group; FOR = forage group; BAR = barley group; DA = dusty alfalfa group).

Group	nº specimens	Mscratches	SD	Mpits	SD	%0-17
ALF	10	11.3	1.97	9.6	5.06	100
RG	10	11.25	1.14	15.05	8.66	100
FOR	10	10.95	3.43	13.2	4.55	100
BAR	10	9.8	3.82	16.8	10.51	90
DA	10	15.65	4.43	12.2	4.12	80

3. Results

3.1. Microwear data

Fig. 1 shows the number of scratches and pits for each individual in each group. There is a general overlap; nevertheless, we can observe differences among the diet types, where every group tends toward a distinct scattering. Specimens from the alfalfa and ray-grass diets tend to have less variation in the number of scratches and a homogeneity in the number of pits, with the exception of one specimen of the ray-grass diet group which clearly have an unusually high amount of pits (detailed information of this specimen (spec. 628) is presented in Table 1 of the supplementary material). Specimens from the forage diet group have fewer pits in comparison to the other groups and a more diverse count of scratches, similar to the two previous groups (but with a slightly higher range). These specimens also plot in the middle of all diet groups, with a standard deviation for both the number of scratches and the number of pits on the average of all the specimens. The barley diet group contains the individuals with the lowest count of scratches, but a high diversity both in the number of scratches and pits among the specimens of this group. The dusty alfalfa diet group has the highest values and variation of number of scratches, but a low count of pits, like the forage diet group. Following Semprebon and Rivals (2007), all groups are considered to be on the browser diet range, because all have more than 72.7% of their specimens with the number of scratches between 0 and 17 (Fig. 1 and Table 2).

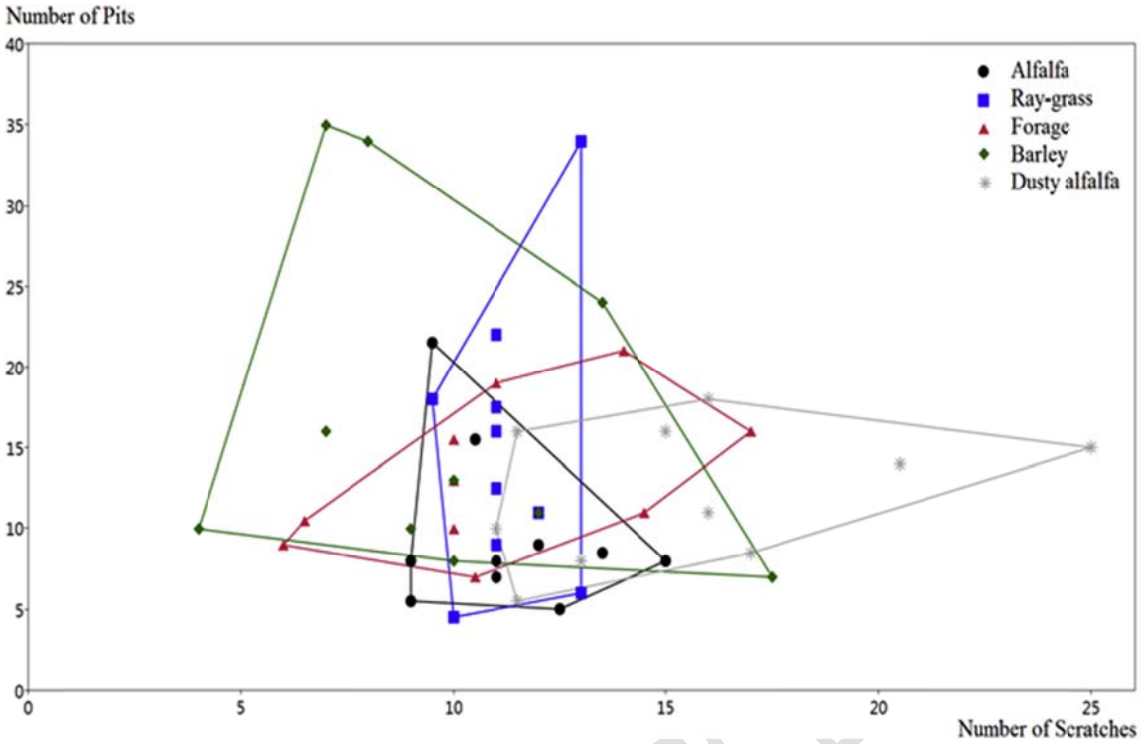

Figure 1. Bivariate plot of the number of scratches and number of pits for the five dietary groups. Convex hulls are drawn around the individuals of each group.

The Kruskal-Wallis test (Hammer et al., 2001) shows statistical differences in number of scratches between groups ($p = 0.01$). On the contrary, no statistical differences have been documented in relation to pits ($p = 0.17$). We must take into account that each group is comprised of 10 individuals. Therefore, this low statistically significant difference between dietary regimes could mainly be due to the low number of cases per group. At the same time, the variability of each group makes also makes it difficult to find statistical trends between groups.

In all dietary groups the different typologies of pits (small pits, large pits, and gouges) are present but differ in their number and magnitude between the individuals of each group (Table 3 and Fig. 2). Small pits predominate over large pits and gouges in all groups. It must be noted that close to 25% of individuals feeding on barley have a higher number of small pits compared to the rest of the specimens (Fig. 3). In contrast, specimens from the alfalfa diet group exhibit minor variations in the individual number of small pits (Fig. 2).

Table 3. The mean of the number of small pits (SPm), large pits (LPm), gouges (Gm), cross scratches (XSm), fine scratches (FSm) and coarse scratches (CSm) and their corresponding standard deviation (SD) for the five diet groups; ALF (alfalfa), RG (ray-grass), FOR (forage), BAR (barley) and DA (dusty alfalfa).

Group	Nº ind.	SPm	SD	LPm	SD	Gm	SD	XSm	SD	FSm	SD	CSm	SD
ALF	10	7.6	4.84	1.7	2	0.3	0.67	0.55	1.17	10.2	2.41	0.7	1.14
RG	10	13.65	7.93	1	1.22	0.4	0.84	0.2	0.63	9.1	3.14	0.75	1.62
FOR	10	10.4	5.65	1.95	1.59	0.85	1.06	0.2	0.63	9.15	2.47	1.4	1.82
BAR	10	14.2	10.2	1.8	1.81	0.8	1.03	0.2	0.63	9	3.52	0.5	0.97
DA	10	9.55	4.72	1.45	1.72	1.2	1.30	0.45	0.83	12.2	5.01	2.4	2.83

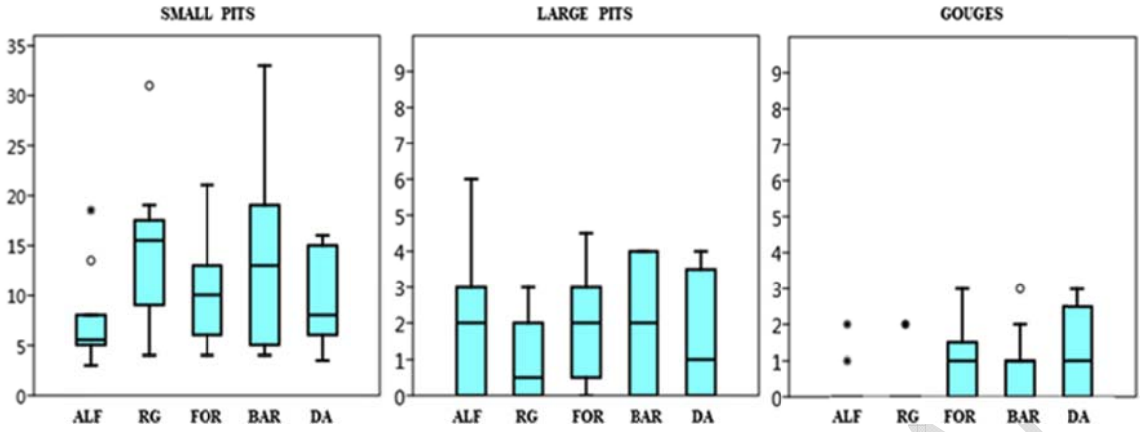

Figure 2. Box plot of individual raw pit data for each diet group with the number of small pits (left), large pits (centre) and gouges (right) for all diet groups; ALF (alfalfa), RG (ray-grass), FOR (forage), BAR (barley) and DA (dusty alfalfa). The centre vertical line marks the median of the sample, the central 50% of the pit's values fall within the range of the box, and the top and bottom bars represent the range of pits values. Values outside the inner fences are shown as circles; values further than 3 times the box height from the box (the "outer fences") are shown as stars.

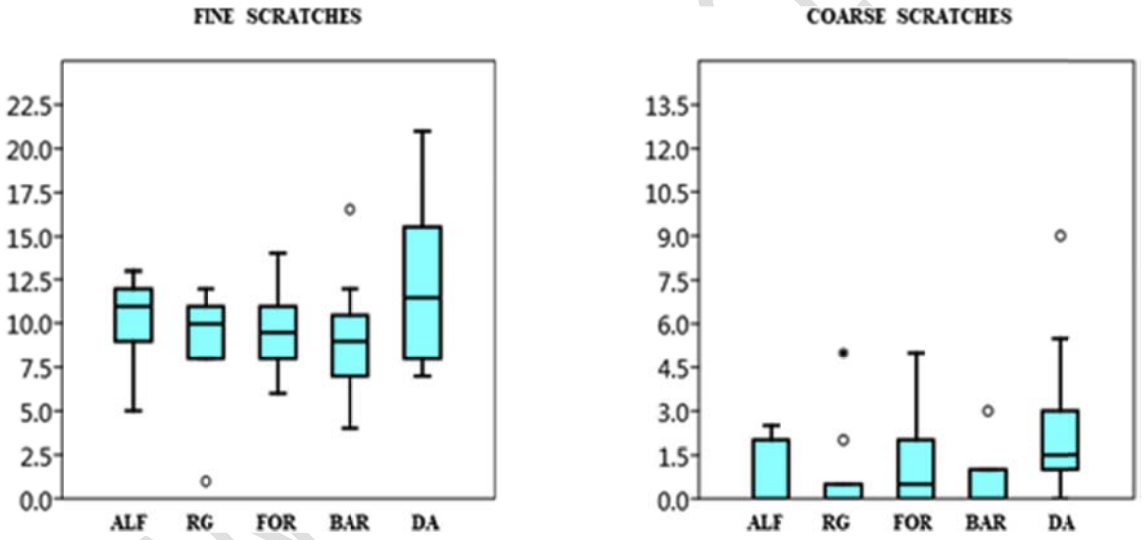

Figure 3. Box plot of individual raw scratch data for each diet group with the number of fine scratches (left) and coarse scratches (right) for all diet groups; (ALF (alfalfa), RG (ray-grass), FOR (forage), BAR (barley) and DA (dusty alfalfa). The centre vertical line marks the median of the sample, the central 50% of the scratch's values fall within the range of the box, and the top and bottom bars represent the range of scratch's values. Values outside the inner fences are shown as circles, values further than 3 times the box height from the box (the "outer fences") are shown as stars.

50% of individuals from all groups have between 0 and 4 large pits, but the mean number from each group is different. 25% of specimens from the alfalfa diet group have a count of 4–6 large pits (Fig. 2).

Gouges are anomalies in both the alfalfa and ray-grass groups, with just 2 and 1 outliers respectively. In the other groups (FOR, BAR and DA), gouges are well documented (75% of the specimens investigated) although never over 3 per ewe.

No significant differences are documented between groups in relation to the presence of the different types of pits.

In regard to the width of the scratches, Fig. 3 and Table 3 demonstrate that fine scratches are predominant over coarse scratches in all groups. On one hand, 50% of the dusty alfalfa diet individuals have more fine scratches; while on the other hand, 25% of the alfalfa and barley groups tend toward the opposite, with specimens that count between 5 and 9 on the alfalfa group, and between 4 and 7 on the barley group (Fig. 3 and Table 3). Only the forage and dusty alfalfa groups have 50% of their specimens exhibiting between 0 and 3 coarse scratches and 25% of them that have up to 5 coarse scratches (Fig. 3 and Table 3).

The Kruskal-Wallis test (Hammer et al., 2001) does not show statistical differences between groups in relation to the presence of the different types of scratches ($p>0.05$).

3.2. Analysis of plants, phytoliths, and soil

The quantitative results of the phytolith analysis show a rich assemblage that varies according to the type of diet (Fig. 4 and Table 2 of the supplementary material). The barley diet has the highest values with an average value of 265 million phytoliths per gram of coprolites. Ray-grass and forage have similar values, around 150 million per gram. The diet groups with the lowest values are those composed by alfalfa (ALF and DA groups), with similar values around 13 million of phytoliths per gram of coprolite.

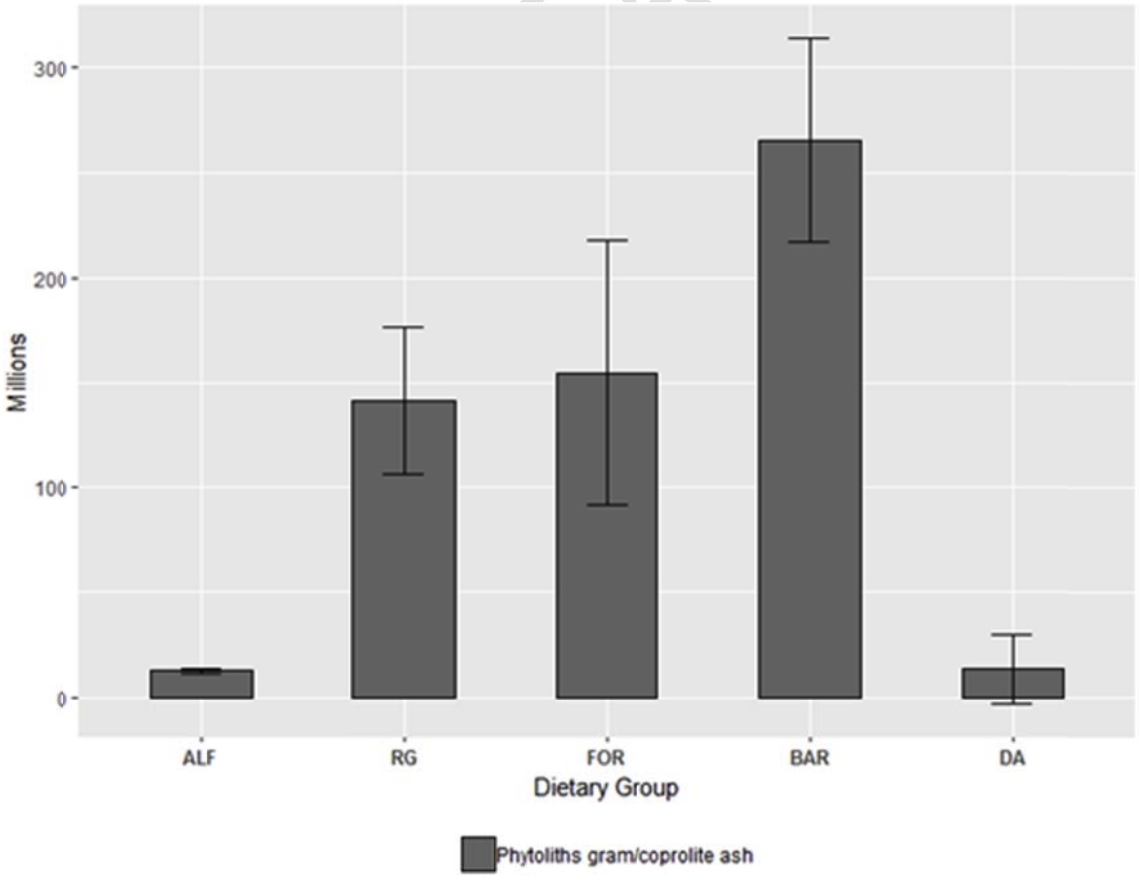

Figure 4. Quantitative results obtained from each of the diet groups. Values expressed in millions of phytoliths per gram of coprolite. Error bars indicate 1σ standard deviation.

The composition of all dietary groups are almost 100% made up of grasses (Table 3, Table 4 of the supplementary material). The anatomical origin of phytoliths, the part of the plant where phytoliths were formed, presents variability between different diet groups. Phytolith assemblages with morphologies without anatomical ascription in plant parts are dominant in the alfalfa and dusty alfalfa groups, with similar percentages of 75% on average (Fig. 5). In the ray-grass diet group, the leaves dominate and in the forage diet group the inflorescence, with average percentages of 63.5 and 60.6, respectively. The group with the most balanced anatomical percentages is the barley diet (38% of grasses without anatomical ascription; 32% of inflorescence; 28% leaves).

Table 4. Identification of plants ate by the forage diet group. Classification of identified taxa between Gramineae and non-Gramineae plants, and marked if were found on the Forage plant and/or Barn plant consumption record.

	Forage	Barn		Forage	Barn
Graminiaea			**non Graminiaea**		
Arundo donax	X	X	*Alyssum simplex*	X	
Avena barbata		X	*Anacyclus clavatus*	X	
Bromus diandrus	X		*Capsella bursa pastoris*	X	
Bromus madritensis		X	*Carduus* sp.	X	
Dactylus glomerata	X		*Cerastium glomeratum*	X	
Festuca sp.	X		*Conyza*	X	
Hordeum murinum	X	X	*Coriaria myrtifolia*	X	
Lolium rigidum		X	*Corylus avellana*	X	
Poa trivialis	X		*Crepis vesicaria*	X	
Sorghum halepense	X		*Cytisus scoparius*	X	
			Ecballium elaterium	X	
			Equisetum telmateia		X
			Erodium cicutarium	X	
			Erodium sp.		X
			Euphorbia serrata	X	
			Foeniculum vulgare		X
			Galium aparine		X
			Pistacea sp.	X	
			Plantago lanceolata	X	
			Plantago media	X	
			Prunus sp.	X	

			Quercus faginea	X	
			Quercus ilex	X	
			Spartium junceum		X
			Silene vulgaris	X	
			Silybum marianum	X	
			Sisymbrium irio	X	
			Tilia cordata		X
			Ulmus minor		X
			Veronica polita	X	
			Vitis vinifera		X
			Vitis sp.	X	

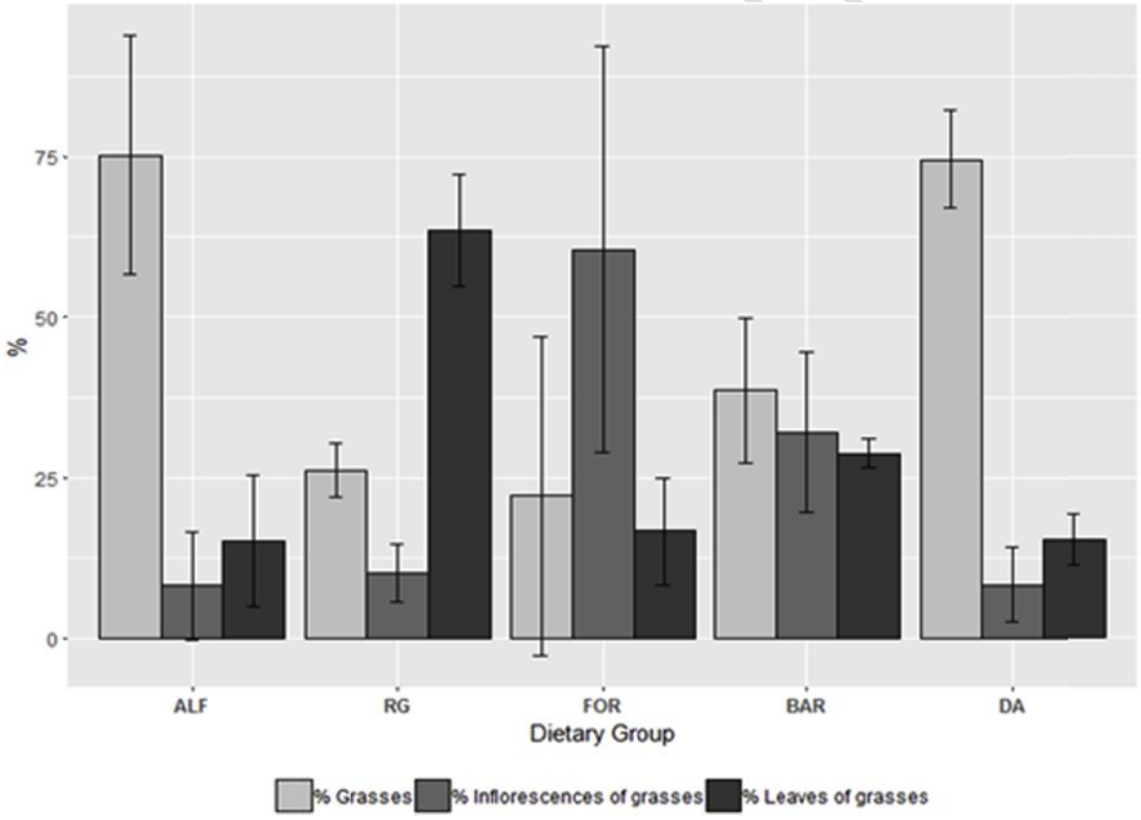

Figure 5. Phytolith morphotype with anatomical origin. Grasses includes all morphologies without anatomical ascription. Error bars indicate 2σ standard deviation.

The plant analysis shows a mixed seasonal composition that the forage diet group was fed in the barn and in the fields and forests that surround the facilities. They ate grasses of the Gramineae family such Bromus diandris (Great brome) or Sorghum halepense (Johnson grass) that grew in the surrounding fields, but the presence of weed and bush species of non Gramineae plants, such as Silybum marianum (Cardus marianus), Anacyclus clavatus

(Camomilla tomentosa) or Vitis sp. (Grapevines) prevail. In the barn, they were fed with a fodder made up of a more balanced mixture of both Gramineae and non-Gramineae grasses. They ate plants such as the grass Lolium rigidum and/or the oat Avena barbata, blended with the common grave vine Vitis vinifera and/or the Fabaceae Spartium junceum (Table 4).

The soil analysis from the sand used to prepare the dusty alfalfa group shows that 87.93% of the sieved dust was composed of fine sand – particles equal or less to 2 mm of diameter – with a small amount of gravel (12.07%) –particles more than 2 mm in diameter mainly composed of silt (31.71%) and clay (25.59%) (Table 2 of the supplementary material).

4. Discussion

In traditional livestock systems, there are several strategies that can be utilized to feed herds. The most economical and least labor-intensive nutrient source is grass, and therefore, the main base of most animal diets. This, however, is not accessible year-round in all locations, which means that it must be complemented with other sources of food. One possibility is the use of forests and less productive lands where the resources that herds would consume are leaves and fine branches (Halstead, 1996). Another resource would be agricultural lands. These areas provide a series of by-products that could be used as livestock resources, such as cereal stubble. The animals can also feed on fodder. It can be produced through agricultural waste, such as the leaves and bark of pruned trees, almonds, olives, vines, walnuts, or fig trees (Forbes, 1998). Damaged food waste, such as cereals or pulses, may also be used. Another type of fodder is hay, which involves the storage and drying of the selected plants to maintain their nutritional value (Lavin et al., 1993).

In this experiment, we attempted to model most of these possible situations. We have simulated free grazing in pastures and forages, a diet mainly based on cereal hay (straw and grains), two diets focused on silage of Legume and a Gramineae) and pasturing on an over-grazed meadow. Each are practices documented during antiquity (Collins et al., 2017).

The data show a general homogeneity of the microwear pattern observed between groups. This homogeneity was expected because we analysed different diets from one species and not the diet of different species. At the same time, it was also documented in previous studies in which one taxon was to test the differences in tooth microwear with various diets (Mainland, 2003a, 2003b; Merceron et al., 2017). Although there is this general homogeneity, due to the presence of only a single species, we have documented interesting differences between diets that follow below.

The first group that fed on silage was the alfalfa diet group. This hay produced low abrasion and the lowest attrition on the enamel of the specimens documented among the groups of the trial, demonstrating that this wet ensilaged fodder is a soft diet. As a homogeneous fodder, it produced low variation (in quantity of traces and diversity of typology) between the individuals as is reflected in the low number of scratches and low standard deviation.

The dusty alfalfa diet was fed with wet alfalfa combined with soil. Despite been fed with the same equally processed grass, the individuals of this group present a different microwear pattern. Both the mean of number of scratches and pits are lower in the alfalfa diet group compared with the dusty alfalfa diet group. Furthermore, the second diet is characterized by coarser scratches and has the highest number of scratches of all the diet groups. In contrast, these two groups have a similar quantity of phytoliths. We argue that the soil added to the wet ensilaged alfalfa is responsible for the particular scratching of this group, producing more individuals from this diet group that have wide scratches than other groups. A similar trend was observed in previous works (Simpson et al., 1995; Mainland, 1998a, 1998b, 2001; Hoffman et al., 2015) in which more striations in the enamel due to the ingestion of grit were documented. In addition, the microwear differences among the individuals in the dusty alfalfa group could be explained by the differential ingestion of sand mixed with the alfalfa. We propose that this variation in the ingestion is motivated by the selection of the best and most nutritious food morsels –the cleanest parts of the alfalfa– and/or by the different hierarchy of the animals in the order of eating (this behaviour was observed during the feeding time). Sheep that ate more grit had coarser scratches, larger pits, gouges or, in general, more scratching and pitting.

We interpret the results from the barley diet group, which consisted of barley seeds mixed with its dry straw to simulate the administration of dry hay with nutritious seeds of the same plant, to the stalled sheep. As Solounias and Semprebon (2002) have demonstrated, a diet in which seeds are an important part, have an increase in the presence of coarse and mixed scratches, as well as a large quantity of large pits. In our trial, there is great variability in the number of pits and scratches between the individuals of this group. This could be due to the fact that the animals we observed always choose seeds first (more nutritious than straw) during the feeding; only eating the dry straw when there were no seeds available. Similar results were observed in Merceron et al. (2017), in which they linked the ingestion of barley seeds with an increase of the complexity of the tooth microwear. Therefore, the variability documented in this group should be taken as demonstrating the volume of seeds and straw each individual consumed.

As was discussed, the individuals of the forage diet group ate a mixture of Gramineae and non-Gramineae fresh plants. This mixed feed diet has been well represented in our data, with a standard deviation for both the number of scratches and the number of pits falling within the average of all groups. We argue that the small quantity of pits in the forage diet is showing a preferential ingestion of tender parts of the plants instead of stalks, seeds, and fruits. This result fits well with phytolith data, which demonstrates that the flock was eating more inflorescence than stalks or leaves from these plants. Aside from this, the overlap presented by this dietary group in relation to the other groups, fits with the results expounded in Mainland (2003a). In it, the woodland pasture sheep studied on a natural food experiment – fed as our forage diet with a mixture of fresh Graminean and non-Graminean plants – overlapped with the other diets composed by grasslands from the Scottish borders and Greenland (Mainland, 2003a). It also matches with what Winkler et al. (2019) demonstrated in their study. They documented differences in dental wear and phytolith content between fresh and dried plants. This difference is due to the soft properties of fresh plants compared with a dried plant, like our barley diet group. Rieau (2014) also analysed the teeth of sheep who were fed in a semi-

intensive regiment of free forage during the day and hay in the barn during the night. His results, however, cannot be compared with our study case due to the high number of pits and scratches that he recorded (his specimens had a scratches ranging in number from 14 to 27, and pits ranging in number from 36 to 63).

Traditionally, it has been argued that a ray-grass diet group, as a Gramineae plant, should produce more scratches in comparison to non-Gramineae diets (Solounias and Semprebon, 2002). But as we demonstrated above, the ray-grass individuals' trend has a similar quantity of scratches to the alfalfa diet group and has the lowest standard deviation, meaning it is the most homogeneous group in terms of scratches. On the contrary, phytolith results show clear differences between these two groups, with more phytoliths in the ray-grass group. Therefore, we propose that this small quantity of scratches and microwear similarities with the alfalfa diet group must be explained by the technique used to process the fodder for both diets. It probably modified the properties of the plants and their abrasiveness, making them softer but still capable of producing microwear (Daegling et al., 2016).

The different grasses used to carry out this experiment were processed with distinct techniques before being supplied to the animals. The alfalfa and the ray-grass were ensilaged, wrapping them in plastic bags to create a wet fermentation of the plants. The opposite was done for the barley, which was dried and stored before finally being served to the animals as a dried hay. The pastures were, of course, fresh. We consider the similarity between the results of the ray-grass and the alfalfa diets can be explained by the preparation process of these fodders. Therefore, the plants that were wet ensilaged, left fewer pits and scratches on the enamel of the individuals than the dry and fresh plants, independent of the plant (Gramineae or non-Gramineae).

Once discussed the data, we have summarized the microwear variables scored in each group and the type of feeding management system that can be gleaned from them in Table 5, in order to characterize each dietary group, and therefore, use the information to interpret archaeological data.

Table 5. Summary of microwear feature patterns for the five diets where the variations between diets are described.

Feeding strategies	**Hay**		**Free alimentation**	**Dry fodder with seeds**	**Over-grazed pastures**
Diets	**Alfalfa**	**Ray-Grass**	**Forage**	**Barley**	**Dusty Alfalfa**
Average Scratch Count	Moderate	Moderate	Variable	Variable	High
Average Pit Count	Moderate	Moderate	Low	Variable	Low
Small Pits	Low	Moderate	Moderate	Variable	Moderate

Large Pits	Low	Low	Low	Low	Low
Gouges	Nearly absent	Nearly absent	Low	Low	Low
Fine Scratches	Moderate	Moderate	Moderate	Moderate	High
Coarse Scratches	Low	Low	Low	Low	Variable
Cross Scratches	Nearly absent	Nearly absent	Nearly absent	Nearly absent	Nearly absent

This table shows that the two wet ensilaged diets are defined by a moderate count of scratches and pits, where most scratches are fine and pits are small, with a low computation of large pits and a near absence of gouges and coarse scratches. The wet ensilaged process done on the vegetation homogenized the abrasive properties of the plants, even when they were from different species groups such as Gramineae (ray-grass) and Fabaceae (alfalfa) used to make these two fodders.

The forage diet group is characterized by a varied count of number of scratches and low number of pits. There is a small quantity of large pits, gouges and coarse scratches, while cross scratches are nearly absent (Table 5). These results are due to the heterogeneity of the fresh vegetables eaten by the animals. They preferentially choose the inflorescence of each vegetable, which in turn implies a lower sediment intake because the sheep did not feed close to the ground.

The barley diet group has a varied number of scratches and pits, and a differing number of small pits. The other variables show low numbers of features, except a moderate number of fine scratches and almost no cross scratches (Table 5). All of this is due to the presence of seeds mixed with the dry stalk, where the ingestion of these seeds produces more small pits and fine scratches.

The dusty alfalfa diet group is characterized by a high number of scratches and low number of pits. There is a moderate number of small pits and a low quantity of large pits and gouges, while the scratches are mostly fine scratches with a differing amount of coarse scratches; cross scratches are nearly absent (Table 5). The presence of a large amount of soil during feed intake produced this high abrasion characterized by the presence of wider scratches.

Therefore, from this information we argue that a diet based on wet ensiled fodder is characterized by a moderate number of pits and scratches. Free grazing in fresh pastures and forages produces a variable count of scratches and a low quantification of pits. On the contrary, a diet mainly consisting of dry hay with seeds, would produce a larger number of scratches and pits. Finally, a food-system based on over-grazed meadows is characterized by a high number of scratches and a low number of pits.

5. Conclusions

This paper presents the first dental microwear reference collection, created from a controlled-food trial in which the diet was designed prior the experiment. The results can be applied to the study of ancient feeding management systems of ovine herds. The results obtained through this study, simulating different dietary ranges of a single domestic species, show that different diets of sheep produce different microwear patterns. Therefore, we have demonstrated that controlled-food experimental studies are necessary to better interpret archaeological data when dealing with domestic species.

This new system we employed to record and quantify the microwear features has allowed us to characterize, in terms of scratches and pits, a diet based on wet ensilaged fodder, a free grazing mainly in fresh pastures and forests, a diet mainly focused on cereal products (dry hay with seeds), and a feeding-system based on over-grazed meadows. With this dietary characterization, we present a useful framework to properly interpret the archaeological record.

The data presented here have also shown that there are other factors that may influence or obscure the formation of the dental microwear pattern, rather than solely the variables investigated previously in studies focused on wild species. We demonstrate that the technique used to produce the fodder supplied to the herds (dry hay or wet ensilaged) must be taken into account, as it can affect the microwear pattern. Other aspects must also be taken in consideration, such as the internal hierarchy among the animals of the same flock, the environment in which the domestic animals eat (differences in and out of the barn in relation to humidity and dust concentrations), or the parts of the plant provided to the animals or eaten directly by them (seeds, leaves, stems, stalks).

Further research is needed. As a first consideration, more individuals from each group are required to properly contrast the data presented here. Secondly, a deeper integration with phytolith and plant data would be suitable for a better understanding of the microwear patterns. Finally, the investigation of other dietary ranges, such as processing the same fodder with different techniques, or administrating different parts of the same plant to different specimens to compare the results, would be useful to increase the hypothetical dietary ranges used in the past.

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Appendix A. Supplementary data

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