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Title: Determining changes in the nutritional condition of red deer in Mediterranean ecosystems: effects of environmental, management and demographic factors

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Abstract: Monitoring changes in the nutritional status of wild populations is crucial to understanding how species respond to natural or human-mediated variations in food availability and quality. It is a fundamental step for decision-making in wildlife management. Many studies on nutritional ecology still tend to rely on the use of single indicators. Nonetheless, nutrition has multiple interdependent facets that should be combined to better understand how organisms interact with their environment through feeding. Here, data from a large-scale cross-sectional survey conducted between 2010 and 2013 were used to examine the influence of intrinsic traits, environmental conditions and management practices on the variation of faecal nitrogen content (FN, index of dietary quality) and kidney fat index (KFI, indicator of nutritional condition) in red deer *Cervus elaphus* in the Mediterranean ecosystems of Iberia. Our results showed that FN levels were statistically and positively correlated with vegetation primary productivity. In contrast, the average altitude of the study areas was negatively associated with FN concentrations. The KFI was related to sex, dietary quality and faecal parasite counts, as well as to changes in vegetation productivity and latitude. The effect of high-quality diets on deer kidney fat deposits was gradually lower as the population density increased. This result was more marked in non-food-supplemented populations. Both FN and KFI were statistically higher during the winter in relation to the autumn. This study shows that FN can be used to monitor and predict changes in the dietary quality, and the KFI in the nutritional status, in red deer in Mediterranean environments, reliably. Since both indicators showed different sensitivity to intrinsic, ecological and management determinants, this research also highlights their value and complementary use when studying populations of large wild herbivores.

Aveiro, May 3rd 2017

Editor-in-Chief
Ecological Indicators

Dear Prof. João Carlos Marques

On behalf of all the authors, I would like to ask you to consider the manuscript entitled “Determining changes in the nutritional condition of red deer in Mediterranean ecosystems: effects of environmental, management and demographic factors” for publication in your journal as an original research paper.

In this study, we used data from a large-scale cross-sectional survey, conducted during three consecutive hunting seasons, in order to assess the influence of environmental conditions, management practices and population densities on the quality of the diet and nutritional condition of wild deer in different populations found across the Mediterranean Iberian Peninsula. Moreover, we examined variations in the nutritional condition in relation to the sex, age, parasite load and dietary attributes of the deer. The amount of nitrogen excreted in faeces (faecal nitrogen, FN) was used as an indicator of the diet quality, in terms of the protein content, while the kidney fat index (KFI) was used as an indicator of the nutritional status of the deer (*i.e.* amount of energy reserves in an animal’s body). We demonstrated that FN and KFI can be used as reliable indicators to monitor changes in the dietary quality and nutritional status, respectively, in red deer in Mediterranean habitats, since they were sensitive to variations in environmental conditions and management measures. Moreover, as both indicators responded differently to the ecological and intrinsic factors tested, this study highlights the value of using them in a complementary way to better understand the nutritional ecology of large wild herbivore populations, in order to adopt more efficient and suitable management practices.

Considering that our paper integrates the monitoring and assessment of ecological indicators with management purposes, we believe that our research fits the main aims and scope of your journal.

This manuscript has not been published and is not under consideration for publication elsewhere. All the authors have read the manuscript, have approved this submission and report no conflicts of interest.

We hope that the editorial board will agree on the interest of this study.

I look forward to hearing from you.

Yours sincerely,

João Santos

Highlights

- The effects of intrinsic and ecological factors on nutritional indicators were assessed
- FN can predict changes in the dietary quality of red deer in Mediterranean habitats
- KFI can be used to monitor deer body condition under different management systems
- Both FN and KFI are valuable indicators for monitoring and managing deer populations

1 **Determining changes in the nutritional condition of red deer in Mediterranean**
2 **ecosystems: effects of environmental, management and demographic factors**

3

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22

23 [†] Our dear friend and colleague Miriam Villamuelas passed away on March 20th 2016

24

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35 **Abstract**

36

37 Monitoring changes in the nutritional status of wild populations is crucial to
38 understanding how species respond to natural or human-mediated variations in food
39 availability and quality. It is a fundamental step for decision-making in wildlife
40 management. Many studies on nutritional ecology still tend to rely on the use of
41 single indicators. Nonetheless, nutrition has multiple interdependent facets that should
42 be combined to better understand how organisms interact with their environment
43 through feeding. Here, data from a large-scale cross-sectional survey conducted
44 between 2010 and 2013 were used to examine the influence of intrinsic traits,
45 environmental conditions and management practices on the variation of faecal
46 nitrogen content (FN, index of dietary quality) and kidney fat index (KFI, indicator of
47 nutritional condition) in red deer *Cervus elaphus* in the Mediterranean ecosystems of
48 Iberia. Our results showed that FN levels were statistically and positively correlated
49 with vegetation primary productivity. In contrast, the average altitude of the study
50 areas was negatively **associated** with FN concentrations. The KFI was **related** to sex,
51 dietary quality and faecal parasite counts, as well as to changes in vegetation
52 productivity and latitude. The effect of high-quality diets on deer kidney fat deposits
53 was gradually lower as the population density increased. This result was more marked
54 in non-food-supplemented populations. Both FN and KFI were statistically higher
55 during the winter in relation to the autumn. This study shows that FN can be used to
56 monitor and predict changes in the dietary quality, and the KFI in the nutritional
57 status, in red deer in Mediterranean environments, reliably. Since both indicators
58 showed different sensitivity to intrinsic, ecological and management determinants,
59 this research also highlights their value and complementary use when studying
60 populations of large wild herbivores.

61

62 **Keywords:** *Cervus elaphus*, faecal nitrogen, Iberian Peninsula, kidney fat index,
63 nutritional ecology, nutritional indicators

64

65 1. Introduction

66

67 Nutritional condition (*sensu* [Harder and Kirkpatrick, 1994](#)) is a major determinant
68 of animals' performance, influencing diverse aspects of their life history such as
69 growth, reproduction and survival ([Barboza et al., 2009](#)) and therefore with
70 consequent implications for population dynamics ([Owen-Smith and Mills, 2006](#)).
71 From a physiological perspective, it can be viewed as an outcome of dynamic
72 processes that involve allocation and mobilisation of somatic reserves, which, in turn,
73 are regulated by a combination of intrinsic traits, environmental factors and ecological
74 interactions ([Barboza et al., 2009](#); [Parker et al., 2009](#)).

75 In sexual dimorphic species, such as most wild ungulates, nutritional or energy
76 requirements may vary according to differences in body size and digestive efficiency
77 ([Barboza and Bowyer, 2000](#)), activity time ([Long et al., 2014](#)), as well as with sex,
78 age, and reproductive status ([Forsyth et al., 2005](#); [Santos et al., 2013](#)). Furthermore,
79 pathogens can negatively affect both nutrient metabolism and absorption ([Coop and](#)
80 [Kyriazakis, 1999](#); [Gálvez-Cerón et al., 2015](#)), therefore increasing the host's
81 nutritional demands and consequently its susceptibility to environmental constraints
82 ([Ezenwa, 2004](#)).

83 Natural populations, inhabiting seasonally changing environments, typically face
84 annual cycles of forage availability and quality, which are strongly influenced by
85 climatic conditions. The way animals cope with seasonal nutritional constraints is
86 context-dependent ([Moen et al., 2006](#); [Parker et al., 2009](#)), thus highlighting the
87 importance of considering environmental conditions on different geographical scales
88 to understand the nutritional ecology of large herbivores ([Martínez-Jauregui et al.,](#)
89 [2009](#)). The influence of environmental variation on both diet and nutritional condition
90 of wild ungulates is broadly documented for populations living in northern latitudes
91 (*e.g.*, [Cook et al., 2013](#); [Pettorelli et al., 2005a](#)). However, fewer efforts have been
92 made to elucidate how seasonal changes affect the feeding ecology and nutritional
93 condition of wild deer in the Mediterranean ecosystems ([Miranda et al., 2012](#);
94 [Rodriguez-Hidalgo et al., 2010](#)), where the summer drought is the most critical factor
95 influencing the availability and nutritive value of forage ([Bugalho and Milne, 2003](#)).

96 Demographic factors have also been shown to be important drivers of nutritional
97 condition among ungulates. Population densities above the ecological carrying
98 capacity (*sensu* [Caughley, 1979](#)) often result in competition for food resources

99 (Stewart et al., 2005), with additional negative impacts on plant communities and the
100 nutritive value of vegetation (Blanchard et al., 2003; Perea et al., 2014). Moreover,
101 the interactive effects between high densities and unfavourable environmental
102 conditions affecting plant productivity may also have profound short- and long-term
103 consequences on the animals' condition and, ultimately, on the dynamics of wild
104 populations (Rodríguez-Hidalgo et al., 2010; Simard et al., 2010). In recent decades,
105 ecological and socio-economic changes have contributed to a significant increase of
106 deer populations in many temperate regions of the Northern Hemisphere (Apollonio
107 et al., 2010), including in the Mediterranean areas of southwestern Europe (Acevedo
108 et al., 2011). Despite this, the impact of population densities on the nutritional status
109 and dietary quality of wild deer remains essentially unknown for this region.

110 Management practices can also affect the nutritional condition of large herbivores
111 in numerous ways, either through interventions in the structure and composition of
112 habitats (Simard et al., 2014; WallisDeVries, 1998), or by directly providing
113 supplemental food, a practice traditionally used in areas managed for hunting (Milner
114 et al., 2014; Putman and Staines, 2004). In the Iberian Peninsula, where big game
115 hunting represents an important economic activity (Martínez-Jauregui et al., 2011),
116 supplementary feeding practices are usually related to the maintenance of artificially
117 high population densities and/or production of high-quality trophies. Although
118 management practices are recognised as important factors influencing the nutritional
119 condition of ungulate species, their effects have rarely been studied in detail in wild
120 populations, especially in Mediterranean environments (but see Rodríguez-Hidalgo et
121 al., 2010; Santos et al., 2013).

122 For wild ungulates, nutrition and condition result from complex interactions with
123 biophysical and social factors (e.g., Parker et al., 2009), which should be considered
124 simultaneously for an overall assessment of their effects. In this study, a large-scale,
125 cross-sectional approach was used in order to assess the influence of environmental
126 factors, population densities and management practices on the dietary quality and
127 nutritional status of red deer *Cervus elaphus* in the Mediterranean habitats of the
128 Iberian Peninsula, during the seasons following the period of greatest food restriction.
129 The red deer is one of the most widespread ungulates in Iberia. Due to its economic
130 and ecological relevance (Martínez-Jauregui et al., 2011; Smit and Putman, 2011),
131 knowledge of the factors that may affect the nutritional condition of this species is
132 therefore fundamental to developing appropriate management practices. Two

133 complementary nutritional indicators were used: faecal nitrogen (FN) as a proxy for
134 dietary quality (Leslie et al., 2008) and kidney fat index (KFI) as an indicator of the
135 amount of energy reserves in an animal's body (Santos et al., 2013). While the FN
136 reflects short-term dietary aspects related to the nutritional value of the plants
137 consumed and the digestibility of materials, the KFI, on the other hand, reflects more
138 long-term dietary factors and cumulative energy balance over time. The following
139 hypotheses were tested: (H1) periods of increased vegetation productivity, which are
140 greatly influenced by accumulated rainfall during the autumn and winter in
141 Mediterranean areas (Rodriguez-Hidalgo et al., 2010), are expected to affect
142 positively both FN and KFI (Blanchard et al., 2003; Hamel et al., 2009; Kucera,
143 1997); (H2) significant effects of habitat composition and supplementary feeding
144 practices are also predictable, with high-quality habitats and increased forage supply
145 having positive effects on deer nutritional condition (Santos et al., 2013); (H3) high
146 population densities are expected to have a negative effect on deer nutritional
147 condition (Blanchard et al., 2003; Stewart et al., 2005). Finally, the effects of sex, age
148 and parasite load on the measured nutritional indicators were also analysed.

149

150 2. Materials and methods

151

152 2.1. Study areas and red deer populations

153

154 The present study was conducted in eight areas which are representative of the
155 Mediterranean environments in the Iberian Peninsula (Fig. 1): *Lombada* National
156 Hunting Area and *Sierra de la Culebra* Regional Hunting Reserve (LSC); *Lousã*
157 Mountain (LOU); *Cubeira* Tourist Hunting Area (CUB); *Negrita Norte* Tourist
158 Hunting Area (NEG); *Doñana* National Park (DN); *Quintos de Mora* (QM); *Montes*
159 *Universales* Hunting Reserve (RCMU); *Caspe-Fraga* Social Hunting Area (CF). The
160 sampling sites are predominantly characterised by a Mediterranean climate, but show
161 different temperature and precipitation regimes throughout the year (Fig. 1). The
162 types of vegetation and land-use patterns found at each location result from the
163 combination of environmental determinants and land management practices carried
164 out by landowners (public or private entities, or both; Table 1). Red deer densities
165 vary among study areas (Table 1) and reflect the type of management applied. In most
166 of the populations studied, management objectives are oriented to deer hunting.

167 However, in some areas, those objectives are related to damage and population
168 control. The provision of supplementary food for red deer is a common management
169 practice only in some study sites, especially in private hunting grounds (Table 1).

170

171 2.2. Sampling and data collection

172

173 The fieldwork was conducted over three consecutive hunting seasons (September-
174 February, from 2010/2011 to 2012/2013). During this period, samples were collected
175 from 272 red deer (147 males and 125 females), which were shot by hunters or game
176 rangers (Table 1). Fresh faecal pellets were taken from the rectal area of each animal
177 sampled, placed in individual plastic zip bags and transported in a cooler box to the
178 laboratory for further analysis. Once in the laboratory, the faecal samples were
179 divided into portions: one portion was frozen at $-20\text{ }^{\circ}\text{C}$ until the determination of the
180 dietary quality, whereas the other portion was maintained in a fridge at $4\text{--}5\text{ }^{\circ}\text{C}$ for a
181 maximum of 48 h before parasitological analysis. The right kidney (along with its
182 perirenal fat) was also collected from each deer and transported under similar
183 conditions for later processing and evaluation of its nutritional condition (see below
184 for detailed laboratory procedures). Whenever the number of animals culled per
185 hunting event was large enough, a subset of individuals of different sex and age was
186 randomly selected for sampling from each population surveyed. Deer age was
187 determined by examining the sequence of eruption and replacement of mandibular
188 teeth in animals up to 2 years old (Sáenz de Buruaga et al., 2001) or by counting
189 annual cementum layers in longitudinal sections of permanent first incisors in
190 individuals older than 2 (Hamlin et al., 2000). Four age classes were subsequently
191 defined according to biological criteria, as in other studies performed previously with
192 red deer (e.g., Santos et al., 2013): calves (< 1 year old, $n = 22$); yearlings (1 year old,
193 $n = 49$); sub-adults (2–3 years old, $n = 38$); adults (≥ 4 years old, $n = 163$).

194

195 2.2.1. Nutritional indicators

196

197 Deer nutritional condition was estimated by calculating the KFI (Riney, 1955). In
198 deer species, the amount of fat surrounding the kidneys is considered a good predictor
199 of the total body fat, i.e., the amount of energy stores in an animal's body (Finger et
200 al., 1981), especially for capital breeders such as the red deer (Serrano et al., 2008).

201 Since KFI values derived from one kidney are equivalent to those obtained by pooling
202 the data from both kidneys (Santos et al., 2013), the KFI was calculated based on the
203 data collected from the right kidney only, as follows: $KFI = (FW / KW) \times 100$, where
204 FW is the weight of the perirenal fat and KW is the weight of the kidney free of fat
205 (Riney, 1955). Both FW and KW were weighed on an electronic precision balance to
206 the nearest 0.1 g.

207 The FN was used as an index of dietary quality among the populations studied
208 (Leslie et al., 2008). In ruminants, the FN depends on the nutritional value in terms of
209 the nitrogen contained in the plants consumed, but can also be greatly affected by
210 other dietary components (*e.g.*, fibres and tannins), which alter the animals' digestive
211 efficiency, thus influencing the amount of FN excreted (Barboza et al., 2009). To take
212 this into account, the insoluble dietary fibre fractions (*i.e.*, structural components of
213 plant cell walls) – neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid
214 detergent lignin (ADL) – were also determined and used as predictors of the nutritive
215 value of the diets and digestibility of the organic matter (Barboza et al., 2009; Gálvez-
216 Cerón et al., 2015). The FN, NDF, ADF, and ADL contents in the faecal samples
217 were estimated by using near infrared reflectance spectroscopy (NIRS) and expressed
218 as a percentage (%) of dry matter (DM) (Dixon and Coates, 2009). A subset of $n = 96$
219 faecal samples (nearly 35% of the total sample size), representative of the same deer
220 populations studied and sampling years, was used to calibrate NIRS instruments and
221 develop equations for estimating the dietary components in the remaining set of
222 samples. The FN content in the samples used for NIRS calibration was initially
223 determined by the Dumas combustion method in a LECO analyser (Villamuelas et al.,
224 2017). The NDF, ADF, and ADL contents were determined sequentially following
225 the detergent method (Van Soest, 1994), using an ANKOM 220 Fibre Analyser, with
226 the application of the filter bag technique. The three main cell wall components
227 (hemicellulose, cellulose, and lignin) were further calculated for all samples from
228 NDF, ADF, and ADL fractions as follows: Hemicellulose (%) = NDF – ADF;
229 Cellulose (%) = ADF – ADL; Lignin (%) = ADL.

230

231 2.2.2. Parasite counts

232

233 The abundance of first-stage larvae (L1) of *Elaphostrongylus cervi* (Nematoda:
234 Protostrongylidae) was quantified in the faeces to account for parasite impacts on

235 animal's condition (Vicente et al., 2007a; 2007b), as well as in the amount of FN
236 excreted (Gálvez-Cerón et al., 2015). This nematode is a highly specific parasite of
237 red deer and the seasonal patterns of L1 excretion have already been described in
238 Mediterranean Iberian Peninsula (Vicente et al., 2005). *E. cervi* L1 were extracted
239 from 8–10 g of faeces using a beaker-modified Baermann technique (Forrester and
240 Lankester, 1997). The larvae were then quantified at the microscope in 1 mL of faecal
241 solution in a Favatti counting chamber and expressed as the number of L1 per gram of
242 faeces (lpg), by multiplying the total number of larvae by the dilution factor of the
243 faecal solution and then dividing by the initial weight of the sample. The
244 identification of *E. cervi* L1 was based on their morphological characteristics and
245 linear dimensions (Vicente and Gortázar, 2001).

246

247 2.2.3. Seasonality, weather, climate and vegetation productivity

248

249 Records of total daily precipitation (mm) and daily temperatures (°C) were
250 obtained from the weather stations closest to each study area for the entire sampling
251 period (years 2010–2013). The Spanish and Portuguese National Meteorology
252 Institutes provided the data. Since the proximate factors that may influence the
253 nutritional condition of red deer were of main interest, variables on a short-time scale
254 were derived from the raw weather databases. Average monthly temperatures and the
255 accumulated rainfall during the last $t - 30$, $t - 60$, and $t - 90$ days in relation to the day
256 on which the animals were culled (t) were calculated. The normalised difference
257 vegetation index (NDVI), which has been found to reflect variation in rainfall
258 occurrence (e.g., Anyamba and Tucker, 2005), was also used in our analyses as an
259 indicator of vegetation productivity (Pettorelli et al., 2005b). The NDVI values for
260 each study area were obtained from satellite images with spatial and temporal
261 resolutions of 250 m and 16 days, respectively, provided by MODIS (Moderate
262 Resolution Imaging Spectroradiometer; <http://modis.gsfc.nasa.gov>) for the nearest
263 periods (approximately 2 weeks) preceding the date on which the deer were shot. In
264 the Iberian Peninsula, there are important regional differences in both plant phenology
265 and productivity, which are associated with geographical and altitudinal gradients
266 (Alcaraz et al., 2006). Hence, the latitude and longitude of geometric centroids were
267 obtained for each study area and used as a measure of the geographic position of the
268 populations studied, as well as a proxy for climatic conditions on a larger spatial

269 scale. Moreover, because interactions between climate and topography may determine
270 asynchronies in plant phenology (Pettorelli et al., 2005a), the average altitude
271 (measured as meters above sea level, m.a.s.l.) of each study site was also determined.
272 Food intake and diet selection may also vary seasonally among sexes in red deer, due
273 to environmental factors and reproductive events (Clutton-Brock et al., 1989; Miranda
274 et al., 2012), and are therefore likely to influence their nutritional condition. For
275 analytical purposes, the sampling months were grouped into two categories according
276 to natural seasonal cycles and the reproductive biology of red deer in Iberia: autumn
277 (September–November) and winter (December–February).

278

279 2.2.4. *Game management practices and habitat composition*

280

281 Information on the management of deer populations at the level of the study site
282 was obtained using standardised questionnaires and/or personal interviews with game
283 managers. Since food supply may affect the nutritional condition of red deer (Santos
284 et al., 2013), our study areas were categorised as having, or not, a supplementary
285 feeding regime (Table 1). In those areas where deer were artificially fed, the food type
286 consisted mainly of grain (especially maize *Zea mays*) and alfalfa *Medicago sativa*.
287 To evaluate the effects of the amount of food supplied on the nutritional status of the
288 deer, two measures were derived from the data provided by managers and from
289 estimates of population size (see below): i) the amount of food supplied per deer per
290 year (feeding/deer/year), and ii) the amount of supplementary food per surface area
291 per year (feeding/Km²/year) (Table 1).

292 Land use and habitat composition may also influence the nutritional condition of
293 deer by offering animals different feeding opportunities and types of diet with
294 different nutritive value (Osborn and Jenks, 1998; Simard et al., 2014). For each study
295 area, the percentage of the main vegetation communities was calculated using a
296 Corine Land Cover map for 2006 (100 m spatial resolution; EEA, 2011). Seven broad
297 categories of vegetation that seemed likely to affect nutritional indicators were
298 defined: coniferous forests, broadleaved forests, mixed forests, scrublands, natural
299 grasslands, agricultural lands, and wetlands (Table 1). Since the creation of food plots
300 for deer was a management option in some areas, an eighth category (defined as ‘food
301 plots for big game’; Table 1) was subsequently derived from the surface area

302 occupied by agricultural land using data provided by the game managers. Food plots
303 were cultivated mainly with leguminous plants and cereals.

304

305 *2.2.5. Deer population densities*

306

307 Vehicle-based spotlight counts combined with distance sampling techniques were
308 conducted annually in each study area in order to estimate red deer population
309 densities (Acevedo et al., 2008). Surveys were carried out following a line transect
310 sampling design (Buckland et al., 2001). Deer counts were done during the species'
311 breeding season (September–October), since this period coincides with the time of the
312 year at which individuals of both sexes are most easily observed. While conducting
313 field surveys, the perpendicular distances of deer 'clusters' (defined as single animals
314 or groups of animals) in relation to the transect lines were measured, and the group
315 sizes recorded as well (Buckland et al., 2001). Red deer density estimates at study site
316 level were obtained by using *Distance 6.0* (Thomas et al., 2010). The most
317 parsimonious models for the detection functions of each study area were selected
318 based on the lowest Akaike's Information Criterion (AIC) value. Detection function
319 model fitting was also evaluated by using chi-squared (χ^2) goodness-of-fit tests and
320 by visually checking the histograms (Buckland et al., 2001; Thomas et al., 2010). Due
321 to the impossibility of conducting deer count surveys in the NEG hunting ground,
322 density estimates were obtained from a neighbouring area with similar characteristics,
323 where a line transect sampling scheme was also applied (Lopes, 2008).

324

325 *2.3. Statistical analyses*

326

327 Two nutritional indicators – faecal nitrogen (FN) and kidney fat index (KFI) –
328 were modelled against a set of intrinsic, environmental, management, and
329 demographic determinants (see Table A.1 for a summary of the explanatory variables
330 used in our analyses; detailed description is given in the previous sections).

331 First, an exploratory analysis was conducted in order to investigate the
332 characteristics of the data (*i.e.*, distribution of the variables, detection of outliers) and
333 the relationship between the variables (Zuur et al., 2010). Multicollinearity between
334 candidate predictors was assessed by means of pairwise correlations and by checking

335 variance inflation factors (VIF). As some of the predictors tested had multiple degrees
336 of freedom (df), adjusted generalised VIF values ($\text{GVIF}_{\text{adj}} = \text{GVIF}^{1/2 * \text{df}}$; [Fox and](#)
337 [Weisberg, 2011](#)) were used preferentially to rule out multicollinearity among
338 explanatory variables. A GVIF_{adj} value of 2.5 was used as a cut-off criterion for
339 identifying the presence of highly correlated predictors. Accordingly, the GVIF
340 values were calculated for the set of variables and the predictor with the largest GVIF
341 was excluded. This process was repeated sequentially until all the predictors reached a
342 GVIF_{adj} smaller than 2.5 ([Zuur et al., 2009; 2010](#)). Parasite abundance data (*E. cervi*
343 L1 counts) were subjected to a natural logarithmic transformation [$\ln(x+1)$] before
344 the application of the statistical models because the distribution was extremely right-
345 skewed ([Zuur et al., 2007](#)).

346 Mixed effects linear models ([McCulloch et al., 2008](#)) were used to analyse the
347 relationships between the nutritional indicators of red deer and the explanatory
348 variables selected. Our study sites were assumed to be a random sample of a larger
349 number of wild red deer populations found across the Mediterranean ecosystems of
350 the Iberian Peninsula. Therefore, the ‘study area’ was included as a random factor.
351 Moreover, as our sampling was conducted over three consecutive years, the ‘hunting
352 season’ (as a categorical variable) was also incorporated in the models. However, due
353 to the reduced number of levels available (only 3), it was treated as a fixed effect
354 term, instead of a random effect ([Zuur et al., 2013](#)).

355 All analyses were performed using the R statistical package, version 3.1.2 ([R Core](#)
356 [Team, 2014](#)). The variation in FN content was modelled using linear mixed models
357 (LMM). On the other hand, the KFI was modelled using generalised linear mixed
358 models (GLMM) with a gamma distribution and a log link function ([Zuur et al., 2009;](#)
359 [2013](#)). For each modelling approach and in addition to the main effects of predictor
360 variables, a series of two-way interaction terms that could potentially affect the
361 response variables were incorporated into the **regression** models. Because of the large
362 number of potential explanatory variables, a stepwise variable selection procedure
363 based on AIC was run to help in identifying the most important predictors and find
364 the most parsimonious models. The final models were chosen based on the lowest
365 AIC value ([Burnham and Anderson, 2002](#)). All models (both LMM and GLMM)
366 were run using the ‘lme4’ package, version 1.1-7 ([Bates et al., 2014](#)). Statistical
367 significance was set at $P \leq 0.05$ for all tests. A more complete description of the
368 modelling procedures can be found in [Appendix A](#).

369

370 3. Results

371

372 Throughout the whole study period, the amount of FN varied between 1.51 and
373 3.79% DM (average \pm SE: $2.44 \pm 0.03\%$), while the KFI values ranged from 5.2 to
374 134.7% (average \pm SE: $31.3 \pm 1.4\%$). The two nutritional indices were positively
375 correlated (Pearson's $r = 0.16$, $df = 270$, $P < 0.01$) and their average values varied
376 among the study areas (Fig. 2; Table 1).

377 From the multicollinearity diagnostics, important levels of association were
378 identified among the explanatory variables. Based on VIF analysis, ten predictors
379 were excluded: HConFor; HBIFor; T.mean; Feed.Kg.Km²; LONG; HScrub;
380 SUPPL.01; T.max; Rain.60; Rain.90 (see Table A.1 for variable description).

381 The results of the stepwise model selection procedure and the measures of fit and
382 parsimony of each model are shown in Tables A.2 and A.3.

383

384 3.1. Factors affecting dietary quality of red deer

385

386 The most parsimonious LMM describing the variation in the FN content in red
387 deer included the effects of residual dietary fibre, seasonality, vegetation productivity
388 and topography (Tables 2 and A.2). All faecal fibre components were negatively and
389 significantly associated with the amount of FN excreted (Hemicellulose: $F_{1, 260.511} =$
390 5.124 , $P = 0.024$; Cellulose: $F_{1, 261.993} = 51.307$, $P < 0.0001$; Lignin: $F_{1, 250.818} =$
391 25.783 , $P < 0.0001$). The FN levels were significantly higher in the winter in relation
392 to the autumn ($F_{1, 256.838} = 18.853$, $P < 0.0001$), and a more pronounced negative
393 relationship between FN and the lignin fraction was also observed during the winter
394 period, as revealed by the significant interaction between the season and faecal lignin
395 content ($F_{1, 260.007} = 22.816$, $P < 0.0001$). Increased primary productivity, as measured
396 by the NDVI, was positively correlated with FN ($F_{1, 18.269} = 11.442$, $P = 0.003$), and a
397 negative, marginally significant relationship between FN and the average altitude of
398 the study areas was also found ($F_{1, 3.676} = 7.700$, $P = 0.055$).

399

400 3.2. Factors affecting fat reserves in red deer

401

402 The GLMM with the lowest AIC value included the effects of individual traits,
403 such as sex, dietary attributes and parasite counts, as well as the effects of seasonality,
404 vegetation productivity, latitude, population density and supplementary feeding
405 (Tables 3 and A.3). It was found that females had significantly higher average KFI
406 values than males ($\chi^2(1) = 40.36, P < 0.0001$). Parasite abundance was negatively
407 associated with the KFI, despite the absence of significant effects ($\chi^2(1) = 1.11, P =$
408 0.29). Also at the individual level, the lignin content of the diet had a significant
409 negative relationship with the KFI ($\chi^2(1) = 31.76, P < 0.0001$), while FN associated
410 positively with the amount of fat reserves in red deer ($\chi^2(1) = 6.53, P = 0.01$).
411 Furthermore, the additive effects of FN and deer population density interacted
412 significantly to explain KFI ($\chi^2(1) = 5.61, P = 0.02$), *i.e.*, it was observed that the
413 effect of FN on the KFI was progressively lower with increased deer density (Fig. 3).
414 Fat stores were also positively associated with the amount of supplementary food
415 provided ($\chi^2(1) = 4.15, P = 0.04$), as well as with the NDVI ($\chi^2(1) = 10.45, P =$
416 0.001 ; Fig. 4). In addition, the KFI was significantly affected by the season ($\chi^2(1) =$
417 $7.89, P = 0.005$), with higher average KFI values in the winter, relative to the autumn,
418 and its effect interacted significantly with latitude ($\chi^2(1) = 10.24, P = 0.001$). In this
419 case, the KFI increased slightly with increasing latitude in the autumn, while a
420 stronger inverse relationship was observed in the winter. On the other hand, the
421 interactive effect between the season and the NDVI had no significant influence on
422 KFI ($\chi^2(1) = 2.47, P = 0.12$).

423

424 4. Discussion

425

426 The two nutritional indicators showed different relationships with the predictors
427 tested, especially with those associated with individual characteristics, management
428 and demographic factors, which emphasises the value of their complementary use in
429 studies of nutritional ecology. All faecal fibre components analysed (cell wall
430 polysaccharides and lignin) were negatively related to FN levels, and a negative
431 relationship was also found between the lignin fraction and KFI. In ruminants, forage
432 digestibility is inversely related to dietary fibre content (Van Soest, 1994). Diets
433 containing high fibre content are less digestible because increased levels of these
434 components tend to suppress microbial function in the rumen, thus reducing digestive

435 efficiency. In such situations, animals may consume more food in order to
436 compensate for the decreasing digestibility of their diets. This strategy usually results
437 in more faeces produced and in lower FN concentrations ('dilution effect'; [Barboza et](#)
438 [al., 2009](#)). According to the general principles of forage digestibility and intake, it is
439 also expected that animals relying on diets characterised by high levels of lignin (the
440 most indigestible fraction in plant material), over a relatively large time interval,
441 would have a poorer body condition. Conversely, diets with low fibre content and
442 protein-rich are more digestible, thus allowing animals to extract the maximum
443 nutritional value from the food ingested. High-quality diets are usually associated
444 with increased FN levels and improved body condition. In this study, FN and KFI
445 were associated positively, which was what one would expect considering that the
446 accumulation of fat reserves by animals also depends on the concomitant intake of
447 adequate levels of forage protein ([Barboza et al., 2009](#); [Randall et al., 2002](#)). A
448 similar relationship between FN and KFI has already been described in a population
449 of mule deer *Odocoileus hemionus* ([Kucera, 1997](#)). Furthermore, the concentrations
450 of FN (or faecal crude protein) have been reported to correlate positively with several
451 other measures of condition and performance in populations of large wild herbivores
452 (*e.g.*, [Blanchard et al., 2003](#); [Côté and Festa-Bianchet, 2001](#)).

453 Periods of high-quality forage (protein and energy-rich) coincide with a build up of
454 structural protein and restoration of fat reserves in wild ungulates, thus allowing them
455 to gain body mass ([Parker et al., 2009](#)). Our results indicated that both FN and KFI
456 varied seasonally, showing higher values in the winter in relation to autumn, and were
457 affected positively by the NDVI under Mediterranean conditions. These findings
458 support our first hypothesis, which stated that periods of increased primary
459 productivity would influence positively both nutritional indicators in red deer. In
460 addition, it was predicted that vegetation productivity would be related to
461 accumulated rainfall. For our data, the two variables showed a positive curvilinear
462 relationship. An increasing number of studies have demonstrated the positive effects
463 of time-lagged NDVI or rainfall on the nutritional indicators of wild herbivores
464 inhabiting different environments ([Blanchard et al., 2003](#); [Hamel et al., 2009](#);
465 [Pettorelli et al., 2005a](#)). In the Mediterranean ecosystems of Iberia, the autumn and
466 winter rainfall is key for triggering vegetation regeneration after the summer drought,
467 particularly in promoting the development of the herbaceous layer (mainly dominated
468 by forbs and grasses, with a high protein content and a relatively low degree of

469 lignification), and activating the vegetative growth of shrubs (with a relatively high
470 lignin content and intermediate levels of protein) (Peñuelas et al., 2004;
471 WallisDeVries, 1998). Also in Mediterranean areas dominated by *Quercus*
472 woodlands, wild ungulates usually have access to large amounts of acorns (with a fat-
473 rich content), which may represent a significant proportion of their diets between mid-
474 autumn and early winter (San Miguel et al., 2000). Therefore, after the nutritional
475 constraints of the summer, in which the vegetation available is of low nutritive quality
476 (Bugalho and Milne, 2003), red deer can benefit from the improved feeding
477 conditions along the autumn-winter continuum in order to enhance their body
478 condition. Previous studies conducted during the same seasonal period in south-
479 central Iberia have already demonstrated a progressive increase in the protein content
480 of the diet in red deer (Miranda et al., 2012) and an overall improvement of their
481 nutritional condition (Rodríguez-Hidalgo et al., 2010; Santos et al., 2013). This
482 pattern contrasts with condition cycles documented in ungulate populations living in
483 northern regions of the Holarctic (*e.g.*, Clutton-Brock et al., 1989; Garroway and
484 Broders, 2005), but also with those reported for populations inhabiting high mountain
485 ranges of the Iberian Peninsula (Serrano et al., 2011; Gálvez-Cerón et al., 2013). In
486 these environments, dietary quality and body condition are usually lower during late
487 autumn and winter because of reduced access to high-quality forage and adverse
488 weather conditions.

489 The average altitude of our study areas correlated negatively with the FN levels.
490 Plant phenology patterns can vary substantially with altitude, and even within short
491 spatial distances (Alcaraz et al., 2006; Pettorelli et al., 2005a). At higher altitudes, low
492 temperatures are a limiting factor for vegetation growth during the coldest months of
493 the year. As a consequence, the timing of the peak of vegetation productivity (*i.e.*,
494 when the protein content of plants is higher) occurs later in those areas, typically in
495 the spring months, when temperatures are more favourable (Alcaraz et al., 2006).
496 Likewise, the lower temperatures and reduced photoperiod verified at northern
497 latitudes during the winter, apart from affecting plant growth and availability of food
498 resources for herbivores (WallisDeVries, 1998), also increase the deer's energy
499 demands for body thermoregulation (Randall et al., 2002). Therefore, animals
500 confronted with such conditions are expected to activate catabolic pathways more
501 frequently, in order to maintain their normal metabolic functions, which prevents the
502 accumulation of high amounts of energy reserves. These observations support the

503 pronounced decrease in the reserves of fat in red deer verified during the winter with
504 the increasing latitude of our study region.

505 Females exhibited better nutritional condition than males, and this difference
506 tended to be more marked at older ages. This fact has been described consistently in
507 Iberian populations of red deer, in particular during the autumn-winter period (Santos
508 et al., 2013; Vicente et al., 2007a; 2007b). Two main factors may explain this result.
509 First, males have larger body sizes and therefore greater energy demands in absolute
510 terms (Parker et al., 2009; Randall et al. 2002). Second, hinds begin to recover their
511 condition earlier in the autumn, during late lactation/early gestation, and when forage
512 conditions become more favourable (Rodriguez-Hidalgo et al., 2010), while stags are
513 investing much of their energy on reproductive activities during the rut. Only after
514 this period (normally in late autumn), do males start to regain condition. Concerning
515 dietary aspects, our results agree, in general, with those of Miranda et al. (2012), who
516 found no differences in dietary components between sexes and age classes from
517 October to March; the only exception was in September, when adult males relied
518 essentially on a poor-quality diet based on senescent grasses and forbs, while hinds
519 and juveniles of both sexes fed preferentially on shrubs, which are more nutritive than
520 the herbaceous layer in late summer and early autumn (Bugalho and Milne, 2003). In
521 our study, these differences may have been masked by the different grouping of
522 sampling months. The results obtained therefore suggest an overall dietary overlap
523 between sexes and age classes in the autumn and winter. The apparent absence of
524 sexual trophic segregation could probably be due to the improved feeding conditions
525 during that period in Mediterranean environments and the concomitant need of both
526 sexes to regain condition (Miranda et al., 2012). According to some hypotheses,
527 dietary sexual segregation in dimorphic cervids is more likely to occur when high-
528 quality food resources are scarce (Barboza and Bowyer, 2000) and, as already
529 mentioned, this period coincides with the summer in our study region (Bugalho et al.,
530 2001; Bugalho and Milne, 2003). Our findings also indicate a change in the diet of red
531 deer between the two seasons analysed. In the winter, faecal fractions revealed higher
532 concentrations of lignin and nitrogen, possibly related to an increased consumption of
533 shrubs (Miranda et al., 2012; San Miguel et al., 2000), which usually become more
534 nutritive with the approach of springtime.

535 Deer in food-supplemented populations had higher levels of energy reserves than
536 those in non-supplemented populations, and their nutritional status increased as a

537 function of the amount of food provided by game managers. These results are
538 consistent with our second hypothesis and are in agreement with previous research
539 conducted under experimental and natural field conditions (*e.g.*, Santos et al., 2013;
540 Vicente et al., 2007a). Intuitively, such situation would be expected, since animals
541 that are less subjected to food restrictions are more likely to maintain a better
542 nutritional condition over time. Similar conclusions were derived from studies in
543 which body mass was used as a measure of condition (Putman and Langbein, 1992;
544 Rodríguez-Hidalgo et al., 2010). On the other hand, no significant effect of
545 supplementary feeding practices on dietary quality was observed in our study. It is
546 suspected that this result arises from the fact that the high-quality forage naturally
547 available in the Mediterranean habitats during our sampling period (autumn-winter),
548 tended to attenuate the differences between food-supplemented and non-
549 supplemented populations. Different results were reported in other studies with wild
550 herbivores that were conducted in different environments (*e.g.*, Osborn and Jenks,
551 1998). These authors reported significant increases in FN concentrations in
552 populations where animals had access to supplementary forage. However, such
553 effects were detected during periods of natural food shortage, which contrasts with
554 our observations.

555 With respect to our last hypothesis, high population densities alone were not
556 statistically associated with decreases in the nutritional status in red deer. However,
557 there was an evident negative density-dependent response of deer body reserves when
558 considering the combined effects of population density and dietary quality, especially
559 in populations without a supplementary feeding regime. The adverse effects of
560 density dependence on an animal's nutritional status are usually exacerbated in
561 natural populations because high-quality forage tends to become scarce faster due to
562 increased intraspecific competition (Blanchard et al., 2003; Stewart et al., 2005),
563 consequently leading to increased nutritional stress (J.P.V. Santos, unpublished data).
564 In contrast, these negative effects are less likely to be observed in populations where
565 supplementary food is provided artificially (Rodríguez-Hidalgo et al., 2010; Vicente
566 et al., 2007a). Nevertheless, despite the apparent effectiveness of such practices in the
567 maintenance or improvement of individuals' condition and overall performance, they
568 are frequently linked to the existence of overabundant populations, which, in turn, can
569 have severe undesirable effects on the ecosystems, apart from the increased risks of

570 disease transmission and several other demographic and physiological effects (*e.g.*,
571 [Gortázar et al., 2006](#); [Milner et al., 2014](#)).

572

573 *4.1. Conclusions and implications for population management*

574

575 This research represents an important contribution for understanding the complex
576 links between nutrition and condition in wild herbivores. The nutritional indicators
577 used in the present study proved to be sensitive to seasonal changes in vegetation
578 productivity, thus demonstrating its potential for evaluating the responses of red deer
579 to changes in the quality of habitats in Mediterranean ecosystems, at least during the
580 autumn and winter. Under our study conditions, FN levels correlated positively with
581 periods of high-quality forage and predicted changes in the body condition of red
582 deer. The KFI of red deer was closely associated with game management practices.
583 Deer in populations with a supplementary feeding regime showed a better nutritional
584 status and were less affected by the effects of density dependence. **Despite this, it is**
585 important to stress that the implementation of such practices has associated risks, with
586 numerous implications at the population and ecosystem levels. Therefore, it is
587 recommended that game managers consider these real problems and weigh the pros
588 and cons of maintaining such management options. Future research should focus on
589 more complex issues, in particular on estimates of the sustainable carrying capacity of
590 habitats according to the specific context. Although hard to achieve, it would provide
591 a firmer basis for management decisions. Certainly, this will require integrative
592 approaches linking data on: the nutritional requirements of species, indicators of
593 physiological change, quantitative measures of resource availability and nutritive
594 value of plants, as well as demographic information.

595

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616

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Table 1. Main characteristics of study areas regarding management of red deer *Cervus elaphus* populations and land cover (% of occupancy of habitat types). Sample size (*n*) and results of descriptive statistics (average \pm standard errors, SE) for the variables related to supplementary feeding practices, population densities, dietary components, nutritional condition, and deer parasite load obtained for each study site are also shown. Statistics refer to data collected during a three-year sampling period (game seasons from 2010/2011 to 2012/2013). Study areas correspond with those in Fig. 1.

	Descriptor	Study areas							
		LSC	LOU	CUB	NEG	DN	QM	RCMU	CF
Game management	Area (ha)	48,740	32,517	1,561	1,722	50,720	6,864	49,778	31,327
	Type of management	Public	Public/Private	Private	Private	Public	Public	Public	Private
	Supplementary feeding	Yes	Yes	Yes	Yes	No	No	No	No
	Feeding (Kg/deer/year \pm SE)	12.3 \pm 0.93	4.6 \pm 0.46	57.3 \pm 6.50	63.0 \pm 4.59	0.0	0.0	0.0	0.0
	Feeding (Kg/Km ² /year \pm SE)	37.6 \pm 3.40	28.8 \pm 2.03	2071.3 \pm 170.83	975.6 \pm 0.00	0.0	0.0	0.0	0.0
	Population density (deer/Km ² \pm SE)	3.1 \pm 0.08	6.3 \pm 0.33	36.5 \pm 2.39	15.7 \pm 1.20*	10.1 \pm 0.85	27.9 \pm 1.85	7.3 \pm 0.24	6.4 \pm 0.89
Land use and habitat types	Coniferous forests (%)	23.0	16.7	0.0	6.3	9.7	35.4	54.1	23.7
	Broadleaved forests (%)	5.6	13.9	71.3	44.5	0.4	26.6	3.1	0.0
	Mixed forests (%)	3.3	19.3	0.0	0.0	1.1	9.3	1.4	0.0
	Scrublands (%)	56.8	39.6	0.0**	0.0**	23.3	13.7	25.3	30.7
	Natural grasslands (%)	1.1	0.0	12.4	0.0	1.5	0.0	4.2	0.0
	Agricultural lands (%)	9.4	9.0	10.4	28.8	4.6	9.2	11.5	45.4
	Food plots for big game (%)	< 1.0	< 1.0	3.5	20.3	0.0	5.8	0.0	0.0
	Wetlands (%)	0.0	0.0	0.0	0.0	39.2	0.0	0.0	0.0
Diet components and deer condition	Sample size (<i>n</i>)	17	38	28	45	18	39	42	45
	KFI (% \pm SE)	35.6 \pm 4.06	26.5 \pm 3.28	30.7 \pm 3.35	54.6 \pm 3.92	17.2 \pm 2.21	23.2 \pm 2.15	34.7 \pm 4.05	20.1 \pm 1.51
	FN (% \pm SE)	2.0 \pm 0.04	2.3 \pm 0.07	2.6 \pm 0.07	2.7 \pm 0.05	2.4 \pm 0.08	2.6 \pm 0.04	2.1 \pm 0.07	2.6 \pm 0.04
	Hemicellulose (% \pm SE)	14.1 \pm 0.67	12.4 \pm 0.30	14.8 \pm 0.29	14.3 \pm 0.26	15.6 \pm 0.72	14.1 \pm 0.24	15.7 \pm 0.27	12.7 \pm 0.19
	Cellulose (% \pm SE)	21.3 \pm 1.33	18.9 \pm 0.60	19.1 \pm 0.85	21.1 \pm 0.64	21.5 \pm 1.08	16.8 \pm 0.57	20.0 \pm 0.43	16.7 \pm 0.36
	Lignin (% \pm SE)	13.1 \pm 1.43	20.9 \pm 1.05	10.4 \pm 0.42	11.3 \pm 0.40	17.0 \pm 1.02	15.9 \pm 0.48	16.0 \pm 0.44	15.5 \pm 0.41
	<i>Elaphostrongylus cervi</i> (lpg \pm SE)	1079.2 \pm 279.77	195.4 \pm 49.43	47.6 \pm 13.12	73.5 \pm 16.07	97.4 \pm 41.45	43.4 \pm 11.17	108.4 \pm 27.03	0.0

* Deer population density for the NEG hunting ground was derived from Lopes (2008).

** Scrublands dominated by *Cistus* spp. occur mainly associated with broadleaved forests of *Quercus* spp.

Table 2. Parameter estimates^(P) for the most parsimonious linear mixed-effect model (LMM) explaining the variation in the faecal nitrogen content (FN, expressed as a % of dry matter and used as an indicator of protein quality of the diet) in red deer *Cervus elaphus*, during three consecutive hunting seasons, in the Mediterranean environments of the Iberian Peninsula. The variable ‘hunting season’ was used in the model as a fixed factor and the variable ‘study area’ was included as a random factor. Significant results are highlighted in bold. Marginal significance is indicated by a superscript ‘#’.

Dependent variable: Faecal nitrogen (FN, % DM)				
Model terms	Estimate	Std. Error	t value	p value
Intercept	3.004e+00	3.490e-01	8.609	<0.0001
Hunting season (2011-2012)	- 7.171e-02	4.140e-02	- 1.732	0.084 [#]
Hunting season (2012-2013)	- 3.901e-02	9.409e-02	- 0.415	0.679
<i>Positive coefficients</i>				
Season (Winter)	5.580e-01	1.285e-01	4.342	<0.0001
NDVI	1.631e+00	4.822e-01	3.383	0.003
<i>Negative coefficients</i>				
Average altitude	- 5.161e-04	1.860e-04	- 2.775	0.055 [#]
Hemicellulose	- 2.265e-02	1.001e-02	- 2.264	0.024
Cellulose	- 3.588e-02	5.009e-03	- 7.163	<0.0001
Lignin	- 6.391e-03	6.470e-03	- 0.988	0.324
Season (Winter) * Lignin	- 3.855e-02	8.071e-03	- 4.777	<0.0001

^(P) Parameter estimates for the levels of fixed factors were computed by considering a reference value of 0 for: level ‘2010-2011’ for hunting season; and level ‘Autumn’ for season.

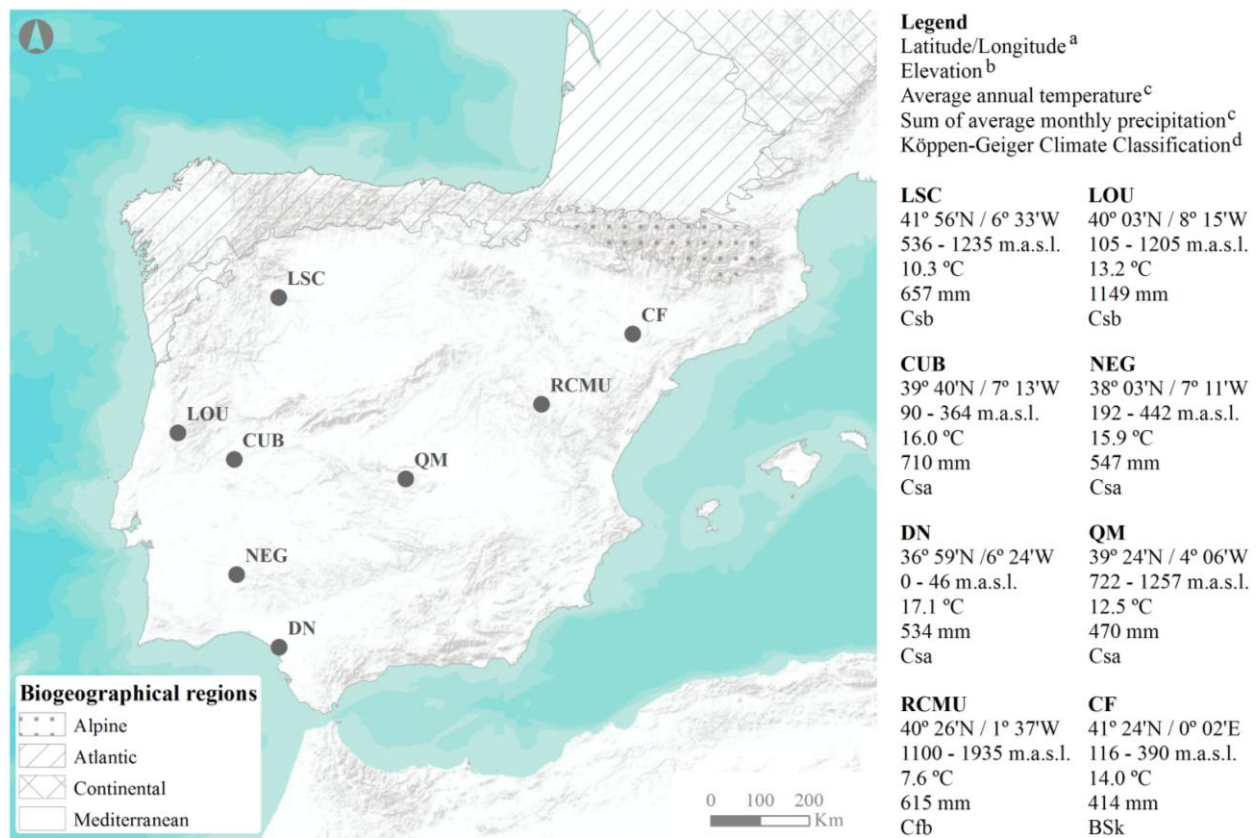
Table 3. Parameter estimates^(Ψ) for the most parsimonious generalised linear mixed model (GLMM) explaining the variation in the kidney fat index (KFI, expressed as % and used as an indicator of the nutritional condition) in red deer *Cervus elaphus*, during three consecutive hunting seasons, in different Mediterranean environments of the Iberian Peninsula. The variable ‘hunting season’ was used in the model as a fixed factor and the variable ‘study area’ was included as a random factor. Significant results are highlighted in bold. Marginal significance is indicated by a superscript ‘#’.

Dependent variable: Kidney fat index (KFI, %)

Model terms	Estimate	Std. Error	t value	p value
Intercept	- 1.517	1.582	- 0.959	0.338
Hunting season (2011-2012)	- 0.114	0.067	- 1.711	0.087 [#]
Hunting season (2012-2013)	- 0.017	0.113	- 0.153	0.878
<i>Positive coefficients</i>				
Sex (Females)	0.395	0.060	6.615	<0.0001
Season (Winter)	9.185	2.957	3.106	0.002
NDVI	0.452	0.456	0.992	0.321
Latitude	0.116	0.037	3.102	0.002
Food supply, Kg/deer/year	0.005	0.003	2.164	0.030
Deer density	0.029	0.021	1.400	0.161
Faecal nitrogen	0.373	0.140	2.665	0.008
Season (Winter) * NDVI	1.372	0.823	1.666	0.096 [#]
<i>Negative coefficients</i>				
Lignin	- 0.053	0.009	- 5.931	<0.0001
Log _e (E.cervi.lpg + 1)	- 0.019	0.018	- 1.059	0.290
Deer density * Faecal nitrogen	- 0.020	0.008	- 2.436	0.015
Season (Winter) * Latitude	- 0.248	0.068	- 3.661	0.0003

^(Ψ) Parameter estimates for the levels of fixed factors were computed by considering a reference value of 0 for: level ‘2010-2011’ for hunting season; level ‘Males’ for sex; and level ‘Autumn’ for season.

Fig. 1. Location of the study areas in the Iberian Peninsula, within the Mediterranean biogeographic region (EEA, 2011). Specific climatic and topographical features of each sampling site are also shown (right panel).



^a Centroid geographic coordinates (degrees, minutes).

^b Elevation ranges (expressed as meters above sea level, m.a.s.l.) were derived either from the 25x25 m resolution Digital Elevation Model (DEM) produced by the Spanish Geographical National Institute (CNIG) or from the 1:25,000 topographic maps (series M888) from the Portuguese Army Geographical Institute (IGeoE).

^c Local temperature (°C) and precipitation (mm) regimes were obtained from WorldClim (v.1.4, release 3; Hijmans et al., 2005).

^d Köppen-Geiger climate classification (Kottek et al., 2006).

Fig. 2. (a) Average values (\pm SE) of the kidney fat index (KFI, %; in blue) and faecal nitrogen (FN, % of dry matter; in green) of red deer *Cervus elaphus* in eight study areas located in the Mediterranean Iberian Peninsula during three consecutive hunting seasons (from September to February, 2010/2011 – 2012/2013). Deer populations are ordered by latitude, from south to north. Light and dark blue colours in the KFI bars differentiate non-supplemented from food-supplemented populations, respectively. (b) Linear relationship between FN and KFI for the entire set of red deer populations studied. Solid black line: fitted regression line ($y = 8.55x + 10.36$). Dashed black line: estimated confidence intervals for the mean.

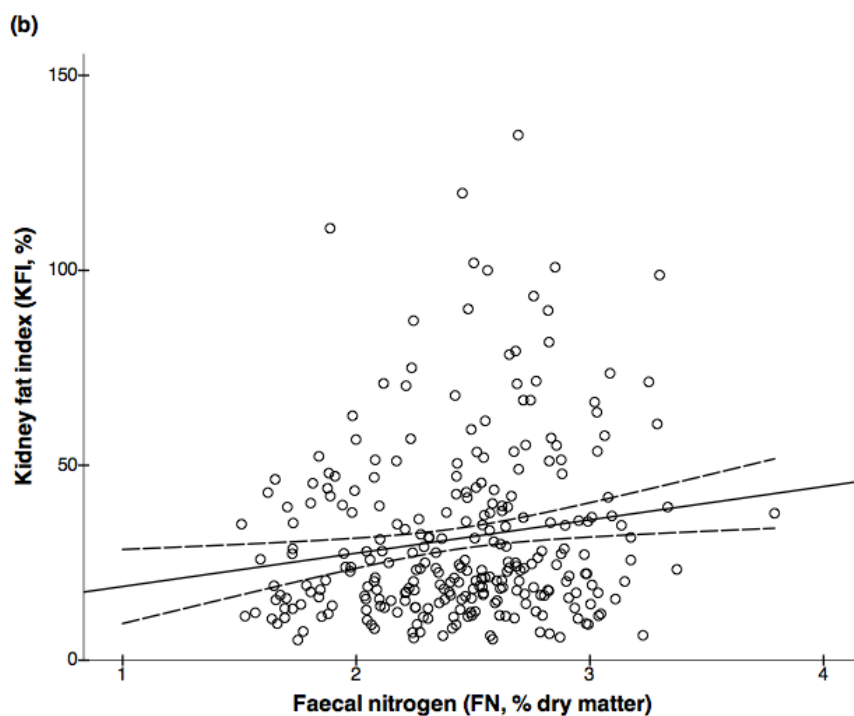
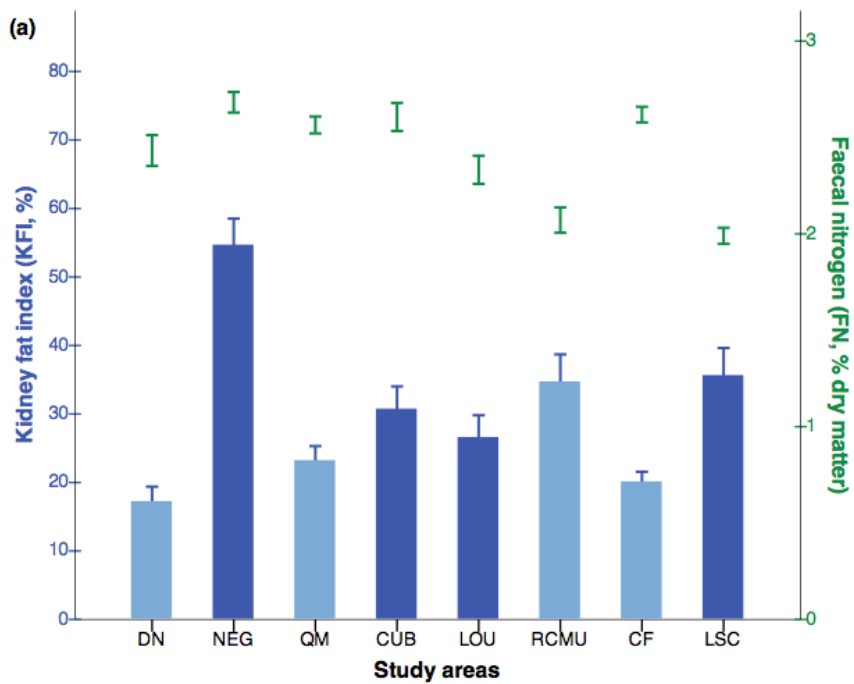


Fig. 3. Conditional plot showing the relationship between faecal nitrogen (expressed as % of dry matter, DM) and kidney fat index (KFI, %) for different red deer *Cervus elaphus* density ranges (divided into low, medium, and high, and expressed as deer per Km²), and different supplementary feeding regimes (this is represented in terms of the presence or absence of supplementary feeding practices for clarity).

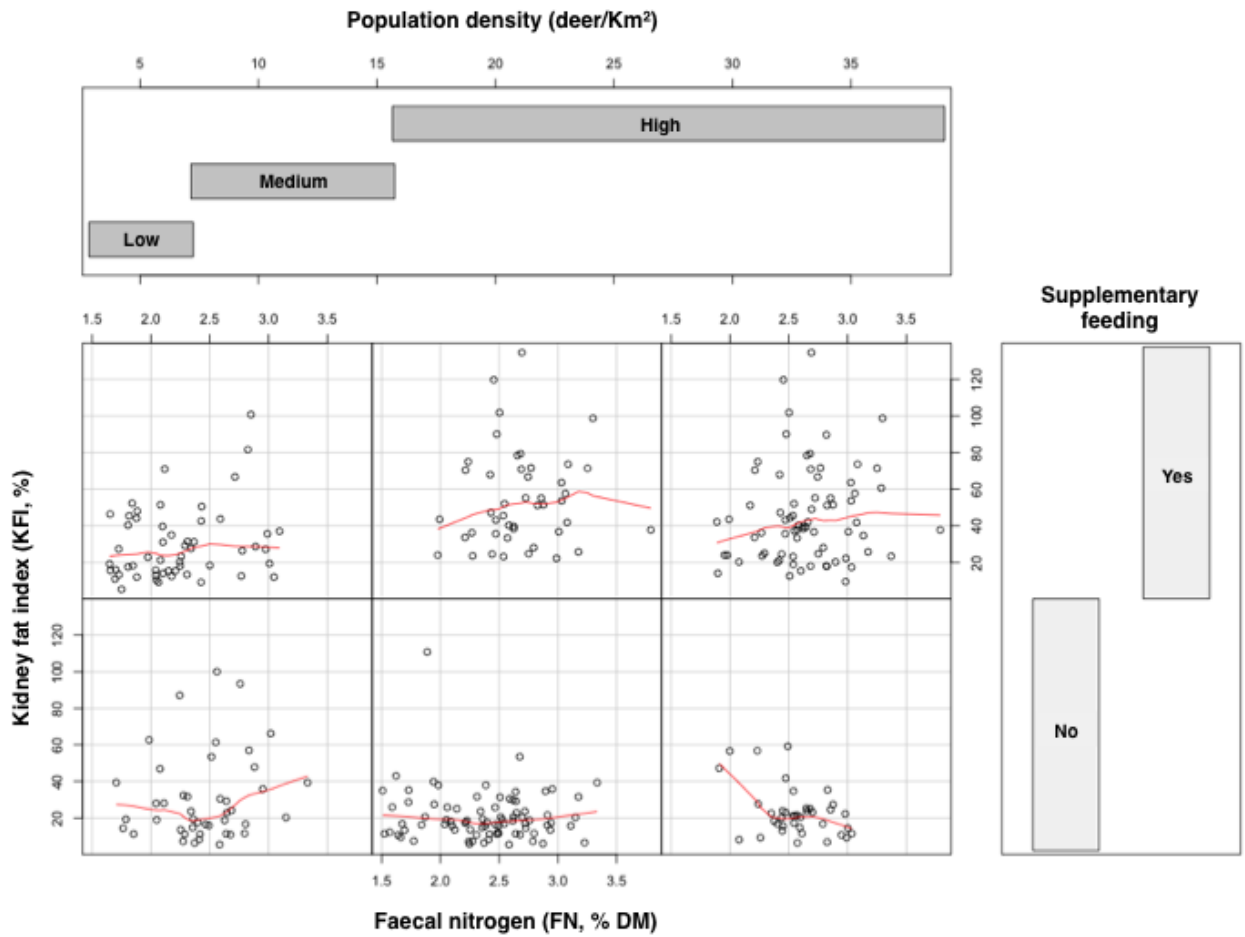


Fig. 4. Relationship between the normalised difference vegetation index (NDVI) and kidney fat index (KFI, %) in red deer *Cervus elaphus*.

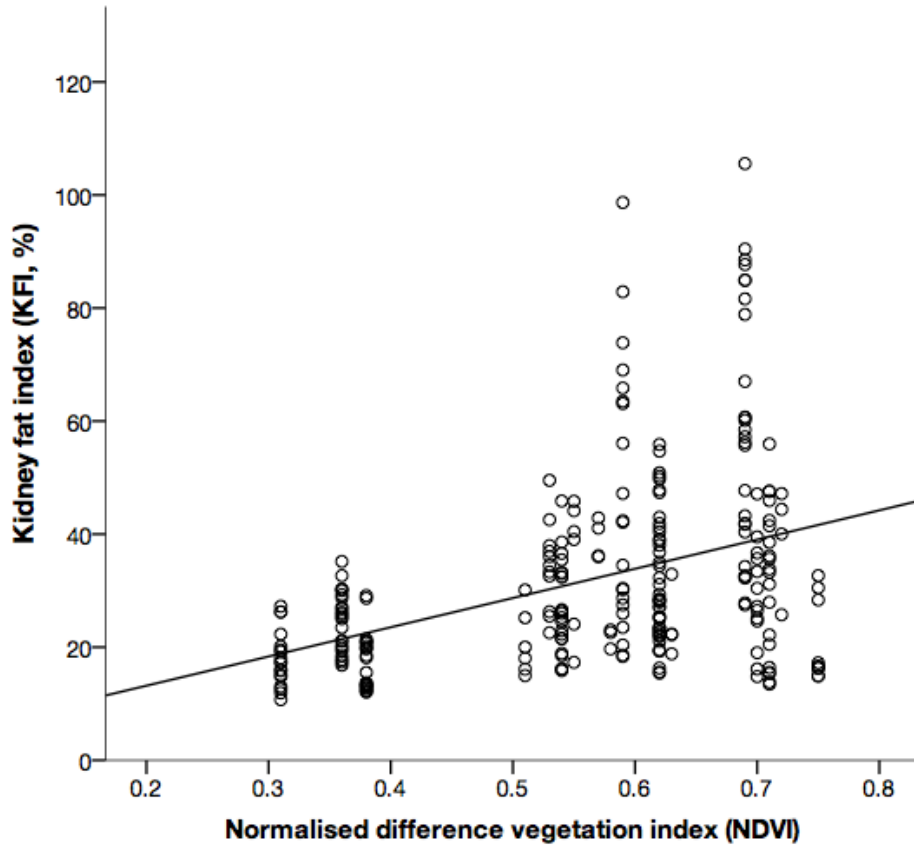
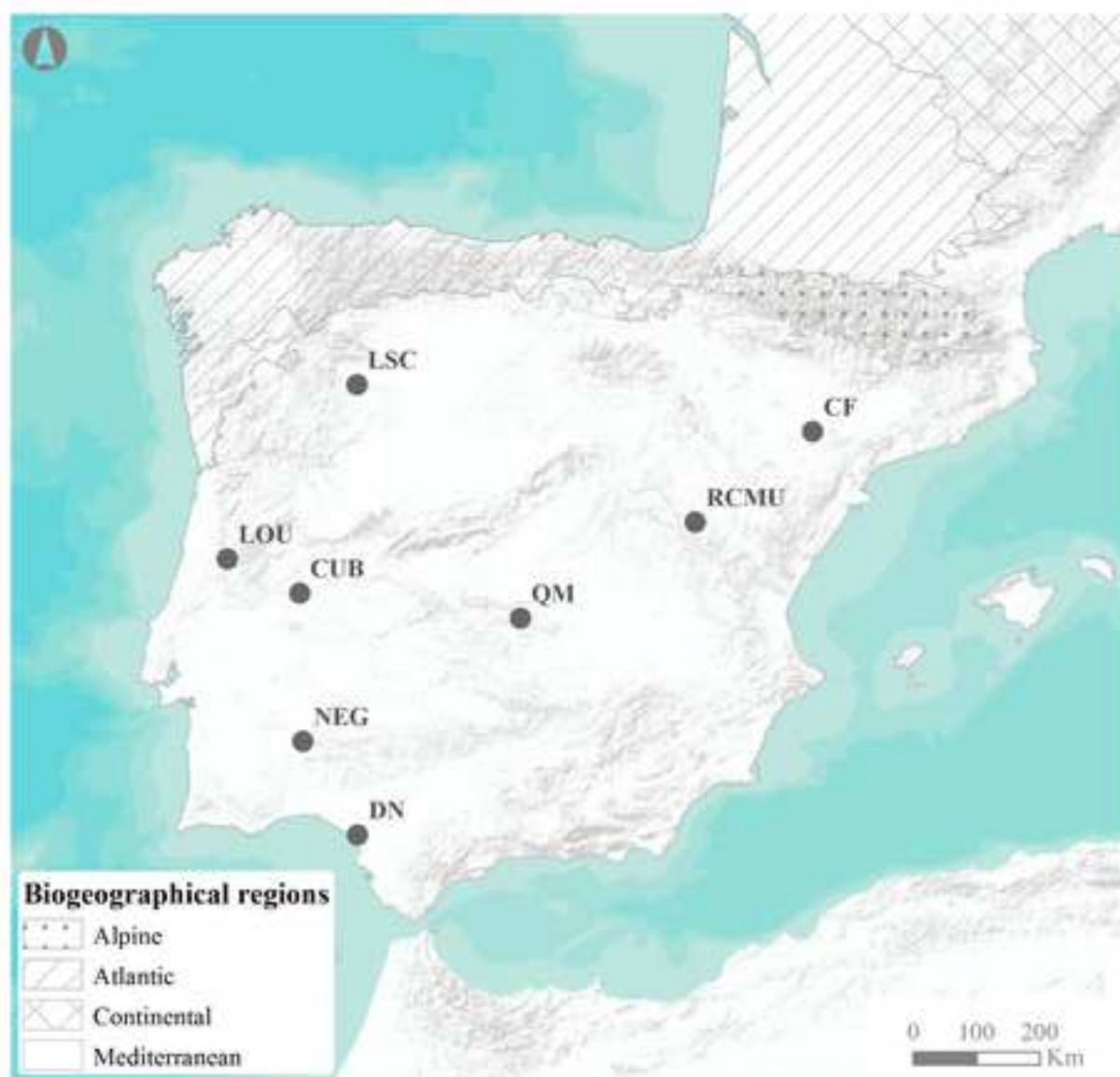


Figure 1
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Legend

Latitude/Longitude^a

Elevation^b

Average annual temperature^c

Sum of average monthly precipitation^c

Köppen-Geiger Climate Classification^d

LSC

41° 56'N / 6° 33'W

536 - 1235 m.a.s.l.

10.3 °C

657 mm

Csb

LOU

40° 03'N / 8° 15'W

105 - 1205 m.a.s.l.

13.2 °C

1149 mm

Csb

CUB

39° 40'N / 7° 13'W

90 - 364 m.a.s.l.

16.0 °C

710 mm

Csa

NEG

38° 03'N / 7° 11'W

192 - 442 m.a.s.l.

15.9 °C

547 mm

Csa

DN

36° 59'N / 6° 24'W

0 - 46 m.a.s.l.

17.1 °C

534 mm

Csa

QM

39° 24'N / 4° 06'W

722 - 1257 m.a.s.l.

12.5 °C

470 mm

Csa

RCMU

40° 26'N / 1° 37'W

1100 - 1935 m.a.s.l.

7.6 °C

615 mm

Cfb

CF

41° 24'N / 0° 02'E

116 - 390 m.a.s.l.

14.0 °C

414 mm

BSk

Biogeographical regions

Alpine

Atlantic

Continental

Mediterranean

0 100 200
 Km

Figure 2a
[Click here to download high resolution image](#)

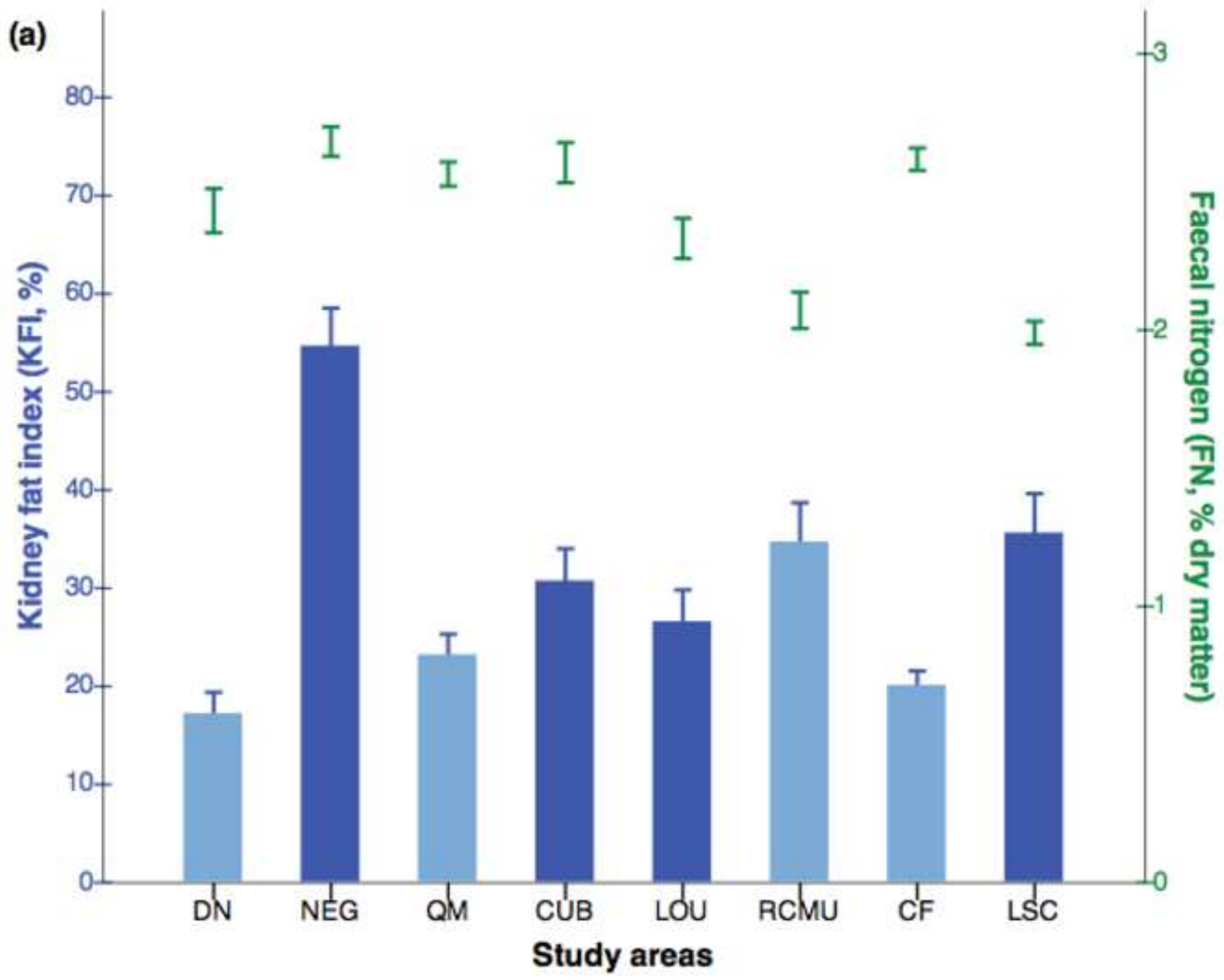


Figure 2b
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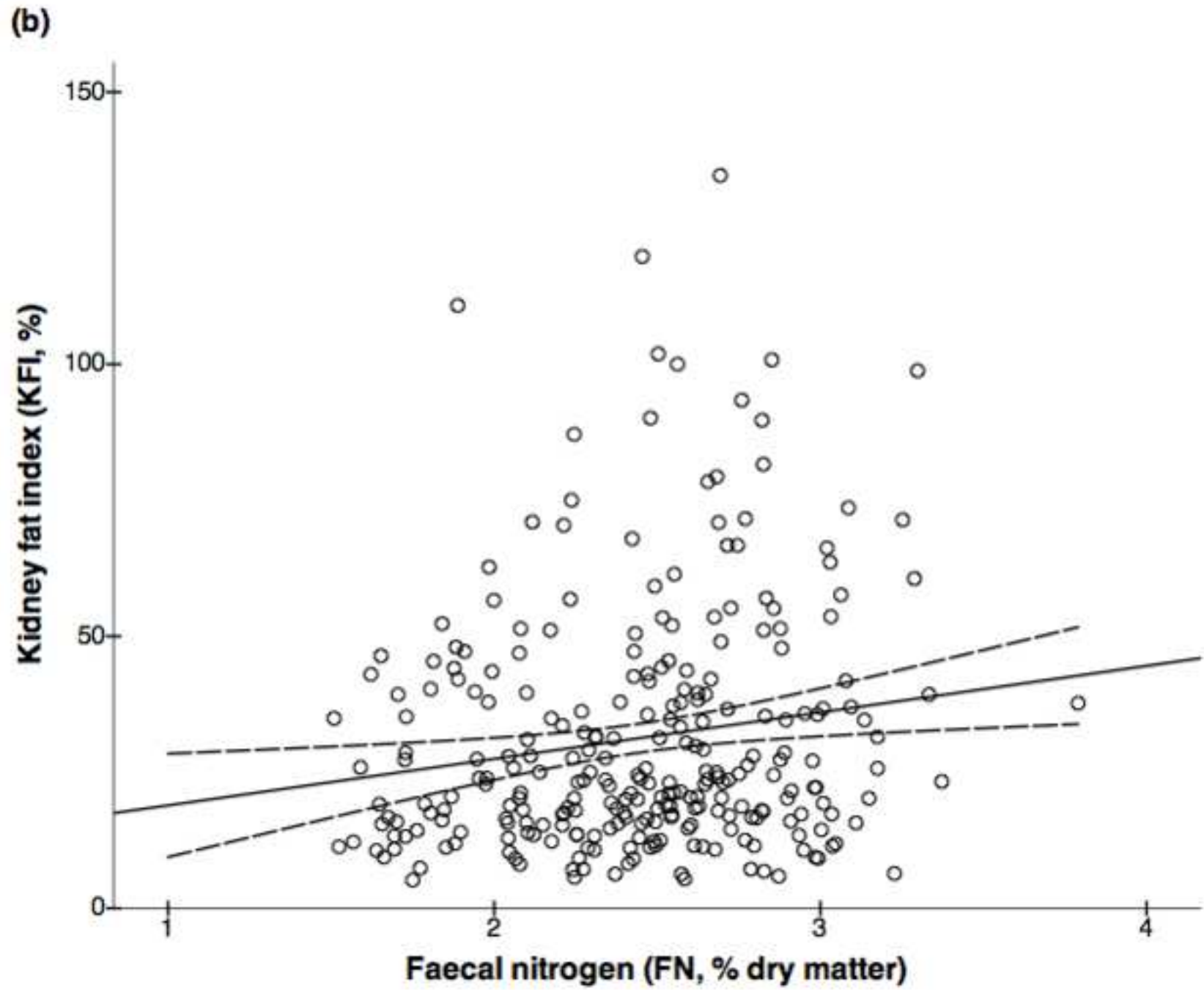


Figure 3
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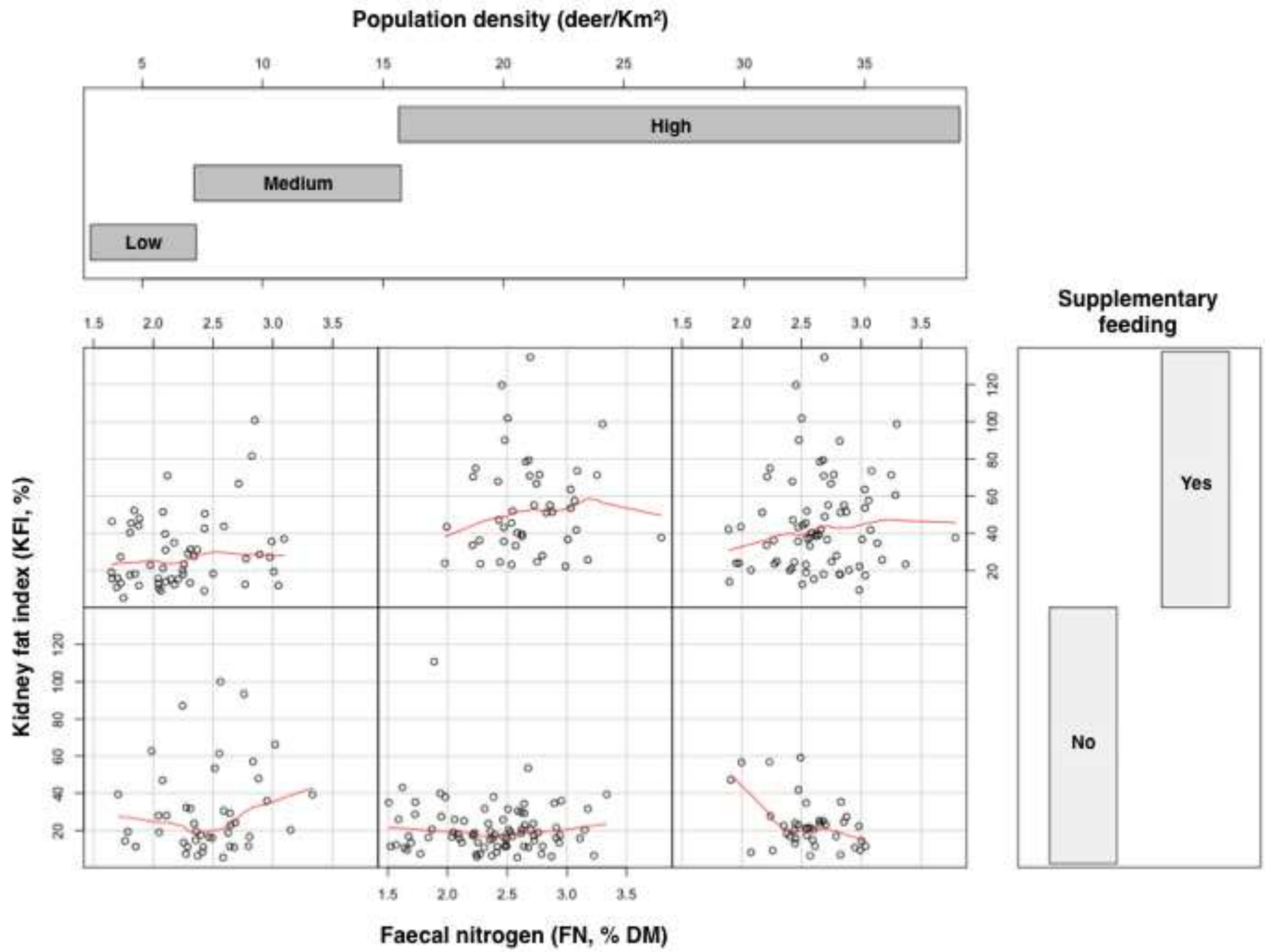
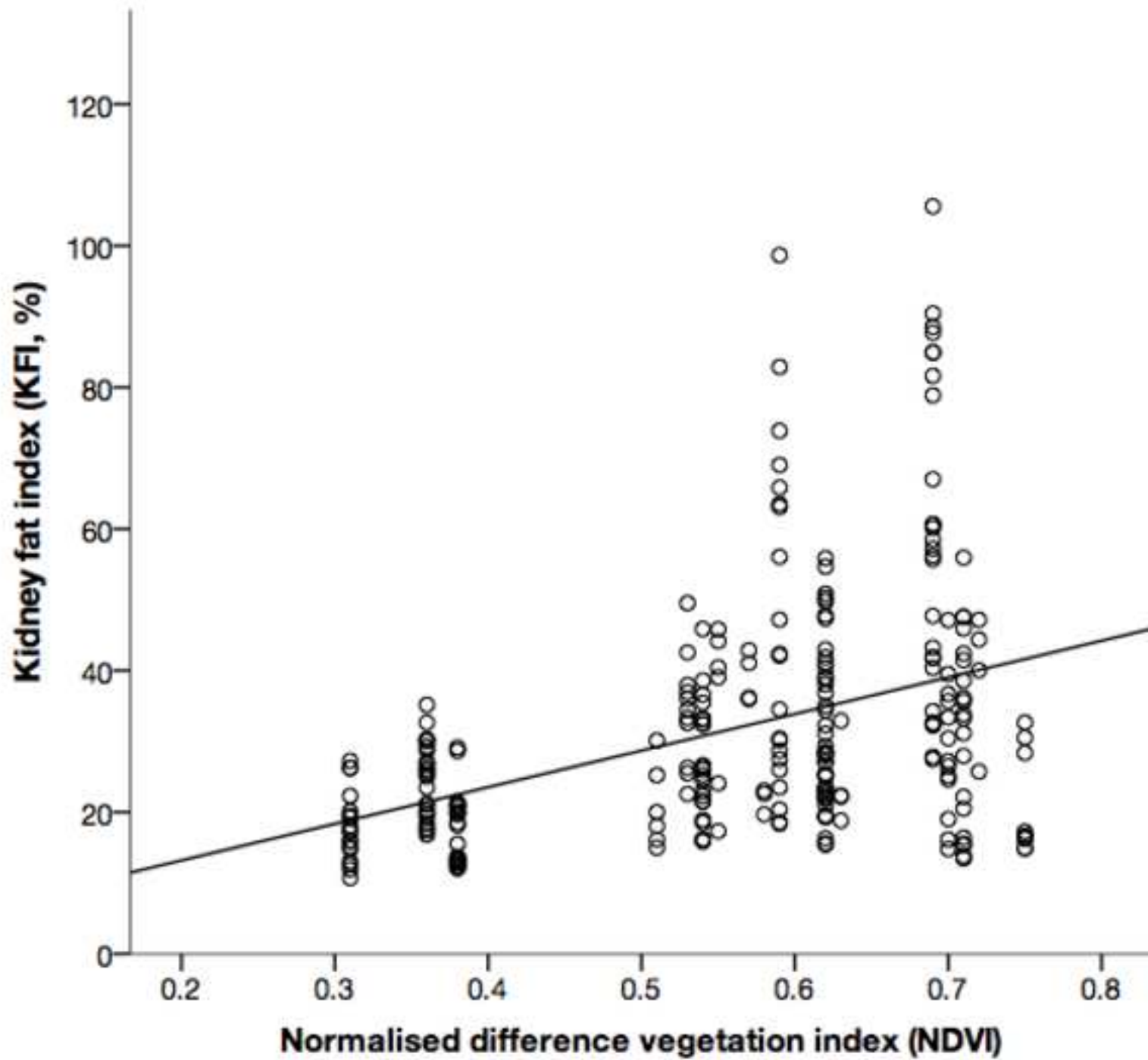


Figure 4
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Appendix A

Variables, statistical procedures and models

Table A.1. Summary of the variables used to model the dietary quality (measured as the amount of nitrogen excreted in faeces, FN) and nutritional condition (measured using the kidney fat index, KFI) of wild red deer *Cervus elaphus* in the Mediterranean ecosystems of the Iberian Peninsula.

Variables/Codes	Type of variable	Variable description and units
Individual traits		
Sex	Categorical	2 levels: 1 = Males; 2 = Females
Age.class	Categorical	4 levels: 1 = Calves; 2 = Yearlings; 3 = Sub-adults; 4 = Adults
Parasite load		
E.cervi.lpg ⁽¹⁾	Continuous	Abundance of excreted first-stage larvae (L1) of <i>Elaphostrongylus cervi</i> (L1 per gram of faeces, lpg)
Dietary fibre		
Hemicell ⁽²⁾	Continuous	Hemicellulose content (% of dry matter, DM)
Cell ⁽²⁾	Continuous	Cellulose content (% , DM)
Lign ⁽²⁾	Continuous	Lignin content (% , DM)
Seasonality		
SEASON	Categorical	2 levels: Autumn = (Sep., Oct., Nov.); Winter = (Dec., Jan., Feb.)
Weather and climate		
Rain.30 ⁽³⁾	Continuous	Accumulated rainfall (mm) in the last 30 days
Rain.60 ⁽³⁾	Continuous	Accumulated rainfall (mm) in the last 60 days
Rain.90 ⁽³⁾	Continuous	Accumulated rainfall (mm) in the last 90 days
T.min ⁽³⁾	Continuous	Average monthly minimum temperature (°C)
T.mean ⁽³⁾	Continuous	Average monthly mean temperature (°C)
T.max ⁽³⁾	Continuous	Average monthly maximum temperature (°C)
Vegetation productivity		
NDVI ⁽⁴⁾	Continuous	Normalized Difference Vegetation Index
Geographic		
LAT	Continuous	Latitude (decimal degrees)
LONG	Continuous	Longitude (decimal degrees)
Topographic		
ALT.m	Continuous	Average altitude (meters above sea level, m.a.s.l.)
Habitat composition		
HConFor ⁽⁵⁾	Continuous	Coniferous forests (%)
HBIFor ⁽⁵⁾	Continuous	Broadleaved forests (%)
HMxFor ⁽⁵⁾	Continuous	Mixed forests (%)
HScrub ⁽⁵⁾	Continuous	Scrublands (%)
HNatGrass ⁽⁵⁾	Continuous	Natural grasslands (%)
HAgr ⁽⁵⁾	Continuous	Agricultural lands (%)
HWet ⁽⁵⁾	Continuous	Wetlands (%)
HPlotGame ⁽⁶⁾	Continuous	Food plots for big game (%)
Game management		
SUPPL.01	Categorical	Supplementary feeding, 2 levels: 0 = absence; 1 = presence
Feed.Kg.Deer	Continuous	Amount of food supplied per deer per year (Kg/deer/year)
Feed.Kg.Km2	Continuous	Amount of food supplied per surface area per year (Kg/Km ² /year)
Demographic		
D.deer	Continuous	Population density (red deer/Km ²)

⁽¹⁾ Log transformed: $\ln(x + 1)$.

⁽²⁾ Fractions derived from neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). See section 2.2.1 on Materials and methods for additional information.

⁽³⁾ Data provided by the Spanish and Portuguese National Meteorology Institutes for the weather stations closest to each study area. Accumulated rainfall variables were calculated in relation to the date on which the deer were culled.

⁽⁴⁾ NDVI was calculated from satellite images from MODIS (Moderate Resolution Imaging Spectroradiometer), available at: <http://modis.gsfc.nasa.gov> (see section 2.2.3 on Materials and methods for details).

⁽⁵⁾ Information derived from Corine Land Cover maps for 2006 (EEA, 2011).

⁽⁶⁾ Values were calculated from the percentage of agricultural land in each study area, based on the information provided by game managers.

Detailed information on modelling procedures and packages used in R

Mixed effects linear models (McCulloch et al., 2008) were used to analyse the relationships between the nutritional indicators – FN and KFI – of red deer and the explanatory variables selected (see Table A.1 for a summary). This analytical approach was chosen on the basis of the nature of the data and type of inferences under consideration. Our study sites were assumed to be a random sample of a larger number of wild red deer populations found across the Mediterranean ecosystems of the Iberian Peninsula. Therefore, the ‘study area’ was included as a random factor in all the models developed. Moreover, as our sampling was conducted over three consecutive years, the ‘hunting season’ (as a categorical variable) was also incorporated in the models. However, due to the reduced number of levels available (only 3), it was treated as a fixed effect term, instead of a random effect (Zuur et al., 2013).

All analyses were performed using the R statistical package, version 3.1.2 (R Core Team, 2014). The variation in FN content (normally distributed continuous variable) was modelled using linear mixed models (LMM). On the other hand, the KFI (continuous response variable containing only positive values and with a distribution moderately skewed to the right) was modelled using generalised linear mixed models (GLMM) with a gamma distribution and a log link function (Zuur et al., 2009; 2013). For each modelling approach and in addition to the main effects of predictor variables, a series of two-way interaction terms that could potentially affect the response variables were incorporated into the regression models for testing. These included all possible interactions with biological and/or ecological significance based on our predictions. Because of the large number of potential explanatory variables, a stepwise variable selection procedure based on AIC was run to help in identifying the most important predictors and find the most parsimonious models, *i.e.*, those models having the most favourable trade-off between the number of parameters and model fit (Burnham and Anderson, 2002). All models (both LMM and GLMM) were run using the ‘lme4’ package, version 1.1-7 (Bates et al., 2014). The ‘lmer’ function with restricted maximum likelihood (REML) was used to fit LMM, while the ‘glmer’ function, with the maximum likelihood (ML), was applied in the case of GLMM. For each model fitted, residual plots were checked to ensure that model assumptions were not violated (Zuur et al., 2010). Parameter estimates \pm standard errors (SE) and *t*-tests for the significance of each parameter coefficient, when all other predictors are present in the model, are only given for the LMM and GLMM with the lowest AIC (see Tables 2 and 3 in Results). Significance tests for fixed effects were also

performed to evaluate the importance of predictors in the final models. The ‘anova’ function in the ‘lmerTest’ package, version 2.0-20 (Kuznetsova et al., 2014), was used to calculate approximate denominator degrees of freedom by Satterthwaite's method, as well as F -statistics along with P -values for all LMM fitted (see section 3.1 in Results). In relation to GLMM, likelihood ratio tests (LRT) were used to derive P -values and assess the significance of fixed effects (see section 3.2 in Results); here, the ‘mixed’ function included in the ‘afex’ package, version 0.13-145 (Singmann et al., 2015), was used. Statistical significance was set at $P \leq 0.05$ for all tests.

The results of stepwise model selection and measures of fit and parsimony for each model tested are shown in Tables A.2 and A.3 (see below).

Table A.2. List of the most parsimonious linear mixed models (LMM) obtained to describe the variation in faecal nitrogen content (FN, % of dry matter) in red deer *Cervus elaphus* in the Mediterranean environments of the Iberian Peninsula. A stepwise procedure based on Akaike’s Information Criteria (AIC) was run to help in selecting the most important predictors and find the most parsimonious model. Models are ordered according to AIC. Model parsimony increases with decreasing AIC. All LMM were fit using restricted maximum log-likelihood (REML) estimation: $REML = \text{Log}(\mathcal{L}) * (-2)$, where $\text{Log}(\mathcal{L})$ is the log-likelihood, K is the number of parameters, and ΔAIC is the difference in the AIC value of a given model in relation to the best model (on the top). The ‘study area’ was included in all models as a random factor and is indicated as [RF]. Variable abbreviations are shown in [Table A.1](#).

Models: Faecal Nitrogen, FN [Linear Mixed Models]	Interaction terms	Log (\mathcal{L})	REML	K	AIC	ΔAIC
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Season*Lign + [RF]	1	– 80.0	160.0	12	184.0	0.0
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Season*Lign + Season*NDVI + [RF]	2	– 79.5	159.0	13	185.0	1.0
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Rain.30 + Season*Lign + [RF]	1	– 80.5	161.0	13	187.0	3.0
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + LAT + Season*Lign + [RF]	1	– 81.6	163.1	13	189.1	5.1
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Season*Lign + ALT.m*NDVI + [RF]	2	– 81.6	163.1	13	189.1	5.1
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Sex + Season*Lign + [RF]	1	– 82.4	164.8	13	190.8	6.8
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + D.deer + Season*Lign + [RF]	1	– 83.6	167.2	13	193.2	9.2
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Rain.30 + Season*Lign + NDVI*Rain.30 + [RF]	2	– 82.6	165.3	14	193.3	9.3

Continuation of Table A.2 (previous page)	Interaction terms	Log (\mathcal{L})	REML	K	AIC	ΔAIC
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + LAT + Season*Lign + Season*LAT + [RF]	2	- 82.8	165.6	14	193.6	9.6
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Feed.Kg.Deer + Season*Lign + [RF]	1	- 84.6	169.3	13	195.3	11.3
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + [RF]	0	- 86.8	173.6	11	195.6	11.6
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Sex + Season*Lign + Season*Sex + [RF]	2	- 83.9	167.8	14	195.8	11.8
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + log _e (E.cervi.lpg) + Season*Lign + [RF]	1	- 85.5	171.0	13	197.0	13.0
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Age.class + Season*Lign + [RF]	1	- 84.6	169.2	15	199.2	15.2
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Season*Lign + Season*ALT.m + [RF]	2	- 86.9	173.8	13	199.8	15.8
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Sex + D.deer + Season*Lign + Sex*D.deer + [RF]	2	- 89.9	179.8	15	209.8	25.8
FN = Hunt_season + Season + Hemicell + Cell + Lign + ALT.m + NDVI + Sex + Age.class + Season*Lign + Sex*Age.class + [RF]	2	- 90.2	180.4	19	218.4	34.4

Table A.3. List of the most parsimonious generalised linear mixed models (GLMM) obtained to describe the variation in kidney fat index (KFI, %) in red deer *Cervus elaphus* in the Mediterranean environments of the Iberian Peninsula. A stepwise procedure based on Akaike's Information Criteria (AIC) was run to help in selecting the most important predictors and find the most parsimonious model. Models are ordered according to AIC. Model parsimony increases with decreasing AIC. All GLMM were fit using maximum log-likelihood (ML) estimation: $\text{Log}(\mathcal{L}) = \log\text{-likelihood}$; K = number of parameters; ΔAIC = difference in the AIC value of a given model in relation to the best model (on the top). The 'study area' was included in all models as a random factor and is indicated as [RF]. Variable abbreviations are shown in [Table A.1](#).

Models: KFI [Generalised Linear Mixed Models: gamma distribution + log link function]	Interaction terms	Log (\mathcal{L})	K	AIC	ΔAIC
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + $\log_e(\text{E.cervi.lpg})$ + FN + LAT + Season*NDVI + Season*LAT + D.deer*FN + [RF]	3	-1009.2	17	2052.4	0.0
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + $\log_e(\text{E.cervi.lpg})$ + FN + LAT + Season*LAT + D.deer*FN + D.deer*Feed.Kg.Deer + [RF]	3	-1009.3	17	2052.6	0.2
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + $\log_e(\text{E.cervi.lpg})$ + FN + LAT + Season*LAT + D.deer*FN + [RF]	2	-1010.5	16	2052.9	0.5
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + $\log_e(\text{E.cervi.lpg})$ + FN + LAT + Season*LAT + D.deer*FN + Feed.Kg.Deer* $\log_e(\text{E.cervi.lpg})$ + [RF]	3	-1009.9	17	2053.8	1.4
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + $\log_e(\text{E.cervi.lpg})$ + FN + LAT + Season*NDVI + Season*LAT + D.deer*FN + Sex*Season + [RF]	4	-1009.2	18	2054.4	2.0
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + $\log_e(\text{E.cervi.lpg})$ + FN + LAT + Season*LAT + D.deer*FN + Sex*Season + [RF]	3	-1010.5	17	2054.9	2.5
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + $\log_e(\text{E.cervi.lpg})$ + FN + LAT + Age.class + Season*NDVI + Season*LAT + D.deer*FN + [RF]	3	-1008.9	20	2057.8	5.4

Continuation of Table A.3 (previous page)	Interaction terms	Log (\mathcal{L})	K	AIC	ΔAIC
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + \log_e (E.cervi.lpg) + FN + LAT + Season*NDVI + D.deer*FN + [RF]	2	- 1014.4	16	2060.7	8.3
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + \log_e (E.cervi.lpg) + FN + LAT + Age.class + Season*NDVI + Season*LAT + D.deer*FN + Sex*Age.class + [RF]	4	- 1008.0	23	2061.9	9.5
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + \log_e (E.cervi.lpg) + [RF]	0	- 1019.6	12	2063.1	10.7
KFI = Hunt_season + Sex + Season + NDVI + Lign + D.deer + \log_e (E.cervi.lpg) + [RF]	0	- 1020.6	11	2063.2	10.8
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + \log_e (E.cervi.lpg) + FN + [RF]	0	- 1019.3	13	2064.6	12.2
KFI = Hunt_season + Sex + Season + NDVI + Feed.Kg.Deer + Lign + D.deer + \log_e (E.cervi.lpg) + FN + LAT + [RF]	0	- 1019.3	14	2066.5	14.1

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