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Nutritional ecology of European rabbit (*Oryctolagus cuniculus*): Factors affecting chemical composition of gastric content

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Abstract

Nutritional ecology seeks to unravel the extensive web of nutritional links that directs animals in their interactions with their ecological and social environments. European rabbit (*Oryctolagus cuniculus*) populations its endemic locations are declining and it is considered a keystone species of the Mediterranean ecosystem prompteing the interest in its conservation. The main aim of this study was to determine the nutritional composition of the diet of European rabbits through the relative and absolute chemical composition of the gastric content. To address this objective, gastric content was collected from 80 European rabbits in a Mediterranean area for the analysis of its chemical composition. To this end, gastric content was analyzed for dry matter (DM), organic matter (OM), ash, crude protein (CP), highly digestible nonnitrogenous nutrients (HDNN), neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin. The rabbits were divided into two groups: EMPTY and FULL, depending on the level of stomach filling, directly related to food intake. Our results revealed a positive correlation between the rabbits weight with DM in the gastric content, total gastric content with DM in the gastric content, and DM in gastric content with all chemical parameters analysed. The mean relative values obtained were 8.8%, 25.5%, 40.4% and 25.4%, for ash, CP, NDF and HDNN, respectively. Moreover, EMPTY rabbits had both a proportional (+19%, $p = 0.002$ and −40%; $p = 0.004$, on NDF and HDNN, respectively) and absolute (−38%, $p = 0.014$, −52%; $p = 0.012$, −52%; $p = 0.011$ and +83%; $p = 0.008$ for OM, ash, HDNN, and lignin, respectively) different proportion of nutrients in gastric contents than FULL animals. Since there is a connection between this availability and the fitness of this species, understanding the chemical composition of the rabbit's diet can be utilised to delve into its biology. Our study provides information that will help elucidate the factors affecting the chemical composition of the gastric content of European rabbits to assist land use planners and conservationists in identifying sites for conservation in Mediterranean ecosystems.

KEYWORDS

biodiversity, dry matter, energy, fibre, mediterranean ecosystem, organic matter, protein

1 | INTRODUCTION

A key goal of conservation physiology is to understand the physiological responses of organisms to changing environments (Seebacher & Franklin, 2012; Wikelski & Cooke, 2006) and provide a detailed mechanistic understanding of the factors that cause conservation problems. Nutritional ecology aims at unravelling the extensive web of nutritional links that guide animals in their interactions with their ecological and social environments (Parker, 2003; Raubenheimer et al., 2009). Both disciplines focus on understanding animal responses to changing environments' patterns, mechanisms and consequences (Raubenheimer et al., 2012).

The wild European rabbit (*Oryctolagus cuniculus*) embodies the conservation paradox of the 21 century, since it is on the rise in introduced areas but its populations in endemic locations are declining (Lees & Bell, 2008). In the endemic locations, due to irruptions of different diseases and habitat fragmentation (Delibes-Mateos et al., 2008; Moreno & Villafuerte, 1995), rabbits have recently been classed as 'endangered' by the International Union for the Conservation of Nature (IUCN) within their native range (Ferreira & Alves, 2009; Villafuerte et al., 2019). Together with the fact that it is considered a keystone species of the Mediterranean ecosystem (Cortés-Avizanda et al., 2015), there is growing interest in its conservation. Several authors have already highlighted the importance of feed availability (also quantity and quality) for rabbit density (Delibes-Mateos et al., 2008; Marín-García & Llobat, 2021; Moreno & Villafuerte, 1995; Villafuerte et al., 1997), and at this point both conservation physiology and nutritional ecology could play a crucial role in the conservation of this species; as nutrition is the single most important environmental factor influencing reproduction and population growth (Boyd & Bray, 1989).

Emerging evidence shows has shown that the dietary composition of rabbits is variable across seasons (Martins et al., 2002); this study across seasons, suggests a strategy by rabbits that aims to maintain the quality of the diet (Pinheiro et al., 2018). However, there are few specific studies that allow direct quantification of the chemical composition of European rabbit diet. Some studies have analysed the composition of wild rabbit diet through microhistological analysis of faecal pellets but this method presents some limitations, such as those related to the fact that its presence in the faeces is affected by the digestion of these animals. Measuring the relationships between wild herbivorous mammals and their feed availability remains a difficult field of research (Myers & Bults, 1977), as there is a relationship between this availability and the correlation with health, productivity and survival of animals (Klein, 1965).

The aim of this study was to determine the nutritional composition of the diet of European rabbits through the relative and absolute chemical composition of the gastric content. This information will be useful to elucidate the effect of feed intake on the chemical composition of the gastric content.

2 | MATERIAL AND METHODS

2.1 | Animal ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required, as no animals were killed specifically for this study. Samples were collected from wild rabbits legally hunted during the official hunting season in full compliance with the Spanish regulations. No ethical approval by an Institutional Animal Care and Use Committee was deemed necessary.

2.2 | Animals and sampling

A total of 80 wild European rabbits were used in this experiment. All animals were obtained as a product of hunting from different preserves located in the Valencian community during May 2021. The animals were sampled for digestive content (gastric). All samples were obtained during morning hours, at the same time of day (8:00 AM). For each one, sex (male/female), age (young/adult), reproductive stage (Males: nonbreeding and in heat; Females: nonbreeding, pregnant and lactating), state of perirenal thickening, weight, length, the day when the sample was taken and its location were recorded. Then, the digestive content of each individual was extracted and weighed to calculate the gastric content weight (the stomach was weighed in its entirety, i.e., the weight of the stomach and its contents). Gastric contents were stored frozen (−20°C) until further analysis.

2.3 | Chemical analysis

Relative chemical composition of the gastric content was analysed for dry matter (DM), ash, crude protein (CP), neutral detergent fibre (aNDFom), acid detergent fibre (ADFom) and lignin (sa). Samples were analysed according to the methods of AOAC (2000) (AOAC, Arlington, 2000): 934.01 for DM, 942.05 for ash and 976.06 for CP. The aNDFom (assayed with a thermo-stable amylase and expressed exclusive of residual ash), ADFom (expressed exclusive of residual ash) and lignin (determined by solubilisation of cellulose with sulphuric acid, sa) were analysed sequentially (Van Soest et al., 1991). Organic matter (OM) was calculated by subtracting the amount of ash from the DM. Fat, starch and soluble fibre were calculated as the difference by subtracting the amount of ash, CP and NDF from the DM. These chemical components are considered as highly digestible non-nitrogenous nutrients (HDNN). The absolute chemical composition of the gastric contents was calculated from the relative composition and the total DM values observed in the gastric contents.

2.4 | Statistical analysis

Based on the information obtained from the gastric content of the rabbits (gastric content, DM, OM, ash, CP, HDNN, NDF, ADF and lignin), regression equations were fitted, calculating for each of them the equations of the straight line, as well as the Pearson Correlation Coefficient (PCC).

Preliminary statistical analyses were performed to split the population ($n = 80$) into two groups: EMPTY and FULL level of the gastric content, as an indication of previous intake level. To this end, gastric content and ratio between the gastric content and the weight of the animal were analysed.

After assigning the experimental groups, the absolute (calculated as the multiplication between the relative percentages and the gastric content) and relative chemical compositions of the gastric content values were fitted to a normal distribution. Then, an analysis (SAS, 2009) was performed, where gastric content, DM, OM, ash, CP, HDNN, NDF, ADF and Lignin were used as dependent variables, applying a GLM model from the Statistical Analysis System where different groups of previous intake were used as fixed effect.

3 | RESULTS

Table 1 show the chemical composition of the gastric content. Results show a marked differences within the sampled population, including animals with different previous feed intake, different ages, sexes and several others. The coefficient of variation for these traits ranged from 43%–72%.

Correlation between animal's weight and absolute DM of the gastric content was positive (Figure 1a), showing an increase of the DM in the gastric content of animals with higher weight (+4.6 g DM in gastric content per kg of weight; $p < 0.001$). We also found a positive correlation between total gastric content with absolute DM of the gastric content (Figure 1b). In this case, the amount of DM in the gastric content increased proportionally by increasing total weight of the gastric content (+0.14 g DM per g of digestive content). Moreover, all the chemical components increased proportionally with the amount of DM in the gastric content (on av. PCC = 0.783); although in the case of lignin (PCC = 0.410), this correlation was the lowest (Figure 1c–i).

Animals considered with EMPTY gastric content ($n = 12$) had lower gastric content (−42%; $p = 0.052$), and a lower ratio between the gastric content and the weight of the animal (−35%; $p = 0.013$) than those that were considered with a FULL gastric content ($n = 68$). Regarding the relative chemical composition (on DM basis) of the gastric content of the main chemical elements analysed (Table 2), we observed a different relative composition between EMPTY animals (+19%, $p = 0.002$ and −40%; $p = 0.004$, on NDF and HDNN, respectively) compared with FULL animals. Similarly, we observed a different absolute composition of gastric content between EMPTY animals (−38%, $p = 0.014$, −52%; $p = 0.012$, −52%; $p = 0.011$ and +83%; $p = 0.008$ for OM, ash, HDNN, and lignin, respectively) compared with FULL animals (Table 3).

4 | DISCUSSION

The main aim of this work was to provide information on the nutritional composition of the diet of wild rabbits, and its relationship with gastric content. To achieve this goal, it was necessary to use a heterogeneous European rabbit population. This premise was fulfilled by obtaining very high global ranges and variation coefficients of all the studied variables. The high variability may point to the chemical composition's susceptibility to various animal groupings (previous feed intake, sex and age, among others).

Some nutrients and the weight of gastric content have seldom been studied in wild rabbits. Orengo and Gidenne (2007) analysed gastric contents of weaning and growing rabbits and observed that the values for stomach weight in the percentage of live weight (1.4%) was similar to that observed in the present work (1.5%). On the other hand, gastric content weight (36 g) was the same that was observed in the animals at 28 days of age (Orengo & Gidenne, 2007). Nevertheless, compared with the work described in Joseph (1909), the studied wild rabbits in the current work presented lower gastric content (on av. −50%) than domestic animals fed *ad libitum* in farm conditions (data compared with those of animals of the same weight)

TABLE 1 Chemical composition (g) of the gastric content of the experimental population of European rabbit (*Oryctolagus cuniculus*) $n = 78$.

	Range	Values	Number of data	Coefficient of Variation (%)
Fresh matter	2.17–81.71	36.0 ± 2.29	78	56.0
Dry matter	0.45–13.24	5.43 ± 0.34	77	54.5
Organic matter	0.39–12.19	5.29 ± 0.30	70	47.1
Ash	0.04–1.51	0.52 ± 0.04	70	58.3
Crude protein	0.22–4.14	1.43 ± 0.10	72	56.9
Neutral detergent Fibre	0.42–6.57	2.83 ± 0.16	66	47.1
Acid detergent fibre	0.30–2.99	1.61 ± 0.08	66	42.6
Lignin	0.01–1.20	0.40 ± 0.04	66	71.8

Note: (Least square means ± standard errors).

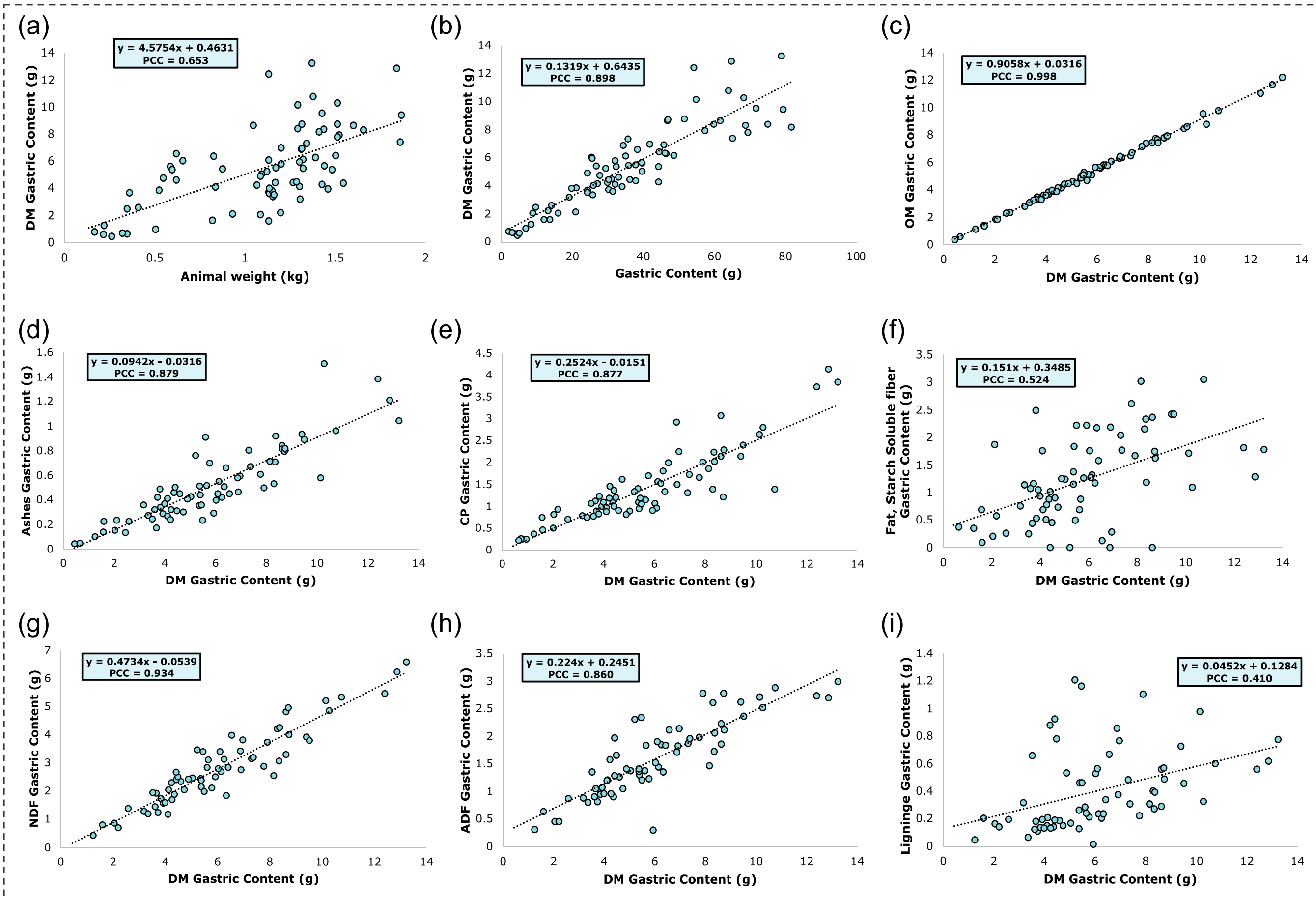

FIGURE 1 Correlationship between the animal's weight and absolute dry matter (DM) of the gastric content (a). Correlationship between total gastric content with absolute DM of the gastric content (b). Correlationship between absolute DM of the gastric content with absolute organic matter (OM), ash, crude protein (CP), fat, starch and soluble fibre, neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin of the gastric content (c–i), respectively. Samples obtained in gastric content of European rabbit $n = 80$ (*Oryctolagus cuniculus*). PCC, pearson correlation coefficient. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Total values, and effect of the level of previous feed intake on relative chemical composition (% on dry matter) of the gastric content of European rabbit $n = 78$ (*Oryctolagus cuniculus*); (Least square means ± standard errors).

	Total	Empty	Normal	*p* Value
Ashes	8.75 ± 0.28	8.86 ± 0.75	8.73 ± 0.31	0.7136
Crude protein	25.52 ± 0.78	28.00 ± 2.20	25.17 ± 0.83	0.1558
Neutral detergent fiber	40.37 ± 0.94	47.13 ± 2.55	39.45 ± 0.93	0.0023
Fat, starch and soluble fiber	25.35 ± 1.83	16.01 ± 3.23	26.65 ± 1.89	0.0036
Total	100	100	100	

Abbreviations: EMPTY, Animals with low levels of stomach filling, considered with low prior feed intake; FULL, Animals with high levels of stomach filling considered with normal prior feed intake.

(Joseph, 1909). This may be due to genetic selection, or the type of food, consisting mainly of plants with a lower density, which would cause lower weights at the same filling level.

Gastric content was collected to measure of previous feed intake, indicating gastric filling and feed availability. The literature shows that the weight of gastric content reflects the diurnal rhythm of intake, conditioned by the nutritional behaviour of this species (Bellier et al., 1995; Prud'Hon et al., 1975). The passage of feed throught the rabbit's stomach is relatively slow and varies between 3–6 h. In our case, gastric content samples were taken at 08:00 h before to the moment of capture, at which time maximum intake was observed (Gidenne et al., 1985; Nutrition of the Rabbit, 2010). In this context, it can be assumed that gastric content is related to previous feed intake.

The relationship between weight and gastric content was also observed by Myers and Bults (1977), but in this case it was related to

TABLE 3 Total values, and effect of the level of previous feed intake on absolute chemical composition (% on dry matter) of the gastric content of European rabbit *n* = 78 (*Oryctolagus cuniculus*); (Least square means ± standard errors).

	Total	Empy	Full	*p* Value
Dry matter	5.43 ± 0.34	3.80 ± 0.92	5.68 ± 0.36	0.0616
Organic matter	5.30 ± 0.30	3.48 ± 0.78	5.60 ± 0.31	0.0137
Ash	0.516 ± 0.036	0.326 ± 0.09	0.547 ± 0.037	0.0303
Crude protein	1.43 ± 0.10	1.06 ± 0.27	1.48 ± 0.10	0.1502
Fat, starch and soluble fibre	1.56 ± 0.11	0.79 ± 0.31	1.66 ± 0.11	0.0116
Neutral detergent fibre	2.47 ± 0.13	2.04 ± 0.40	2.52 ± 0.15	0.2596
Acid detergent fibre	1.50 ± 0.08	1.38 ± 0.36	1.51 ± 0.67	0.5879
Lignin	0.33 ± 0.03	0.55 ± 0.09	0.30 ± 0.03	0.0077

Abbreviations: EMPTY, Animals with low levels of stomach filling, considered with low prior feed intake; FULL, Animals with high levels of stomach filling considered with normal prior feed intake.

age (Myers & Bults, 1977). Cooke (2014) estimated 81.4 g of DM intake per kilogramme of metabolic weight (Cooke, 2014), Rommers et al., (2002) demonstrated that heavy does consume more feed per day (+45 g/day, P0.001) than small does (Rommers et al., 2002), and even growing rabbits have shown a correlation between weight and gastric content. This is because gastric content can be a measure of previous feed intake (Marín-García et al., 2020a). On the other hand, there is a positive correlation between the gastric content, DM and the rest of the chemical components, although it is not proportional in all the variables studies. This is the case of HDNN, which showed lower correlation values (PCC = 0.524). These lower correlation values could be explained by a possible control of rabbit caecal fermentation and rate of passage according to the type of dietary starch (Gidenne & Perez, 1993), as the animals were collected from different locations. The lowest correlation was observed in the case of lignin. These different levels in gastric content could be explained by the fact that an increase in lignin content in diets for growing rabbits is associated with a faster digestive transit (Caîsin et al., 2020; Gidenne et al., 1994; Gidenne et al., 2001) and a lower relative weight of caecal digesta (García et al., 2002; Nicodemus et al., 1999).

We also observed low relative DM values of the gastric content (15.62 ± 0.47). This DM percentage in gastric content was lower (19.1%) than that observed in domestic animals (Orengo & Gidenne, 2007). This may be due to the variability of forage plants consumed by these animals (Llobat & Marín-García, 2022) with relative DM levels much lower (e.g., 38.5%, 45.5%, 45.5%, 24.4%, 27.8%, 32.7% and 25.9% of DM for *Agrostis capillatis*, *Dactiys glomerata*, *Festuca arundinaceae*, *Lotus corniculatus*, *Rumex acetosa*, *Sanguisorba minor*, *and Trifolium patense*, respectively (Reiné et al., 2020)) than the average levels of the diets used for this species (for example, 90.3%, 90.7% and 92.1% (Marín-García, Ródenas et al., 2020), Marín-García, López-Luján et al., 2020). This causes a proportional correction in all chemical components, and should be considered as a possible limiting factor in the nutrition of herbivores.

Regarding the different chemical components, CP levels (i.e., 25.52%) were similar to those found elsewhere (e.g., 20% [Myers & Bults, 1977]). It seems that chemical composition of the gastric content could determine the quality of the food consumed by rabbits, and it can be predicted from the quality of the recently ingested food in the stomach (in accordance with this work, the total CP intake by these animals was around 35 g of CP/DM), as there is a significant correlation between CP in the food and in the stomach ($p < 0.001$) (Myers & Bults, 1977). Although studies that contrast these values in gastric content are neglected, Gidenne & Ruckebusch, 1989 analysed some chemical components in the ileum, hard faeces and soft faeces (Gidenne et al., 1989). Data on NDF, CP and OM was similar to that obtained in the ileum (45.5%), intermediate to that obtained in hard faeces (15.5%) and soft faeces (31.7%) and slightly higher than the mean of all measures (on av. 86%), respectively (Gidenne et al., 1989).

Overall, we found low values of relative DM in the gastric content, as very low absolute DM values of the gastric content (5.43 ± 0.34) were observed. These values were also especially low for all the chemical components analysed, showing a distinct total composition of gastric content of EMPTY animals (−38%, $p = 0.0137$, −40%; $p = 0.0303$, −52%; $p = 0.0116$ and +83% for OM, ash, HDNN and lignin, respectively) in comparison to FULL animals. These differences could be explained as factors mentioned above, since there is a relationship between the global composition and the relative values.

This work delves into the approach consisting of chemically analysing the stomach contents to gain information on the food ingested, providing information not previously given on the chemical components of the digestive content and their relationships (Marín-García et al., 2023). Nevertheless, more studies are needed to elucidate the relationships between this amount and the correlation with the conservation physiology, health and survival of this species. More work is needed to move conservation physiology and nutritional ecology forward, establishing methodologies that allow quantification of the intake of each nutrient. Although this work provides information on the chemical composition of the rabbit diet, it is interesting to increase the analytics and try to find other important interactions with nutrition.

5 | CONCLUSIONS

This work provides both relative and absolute chemical composition values of the gastric contents of European wild rabbits. From these data, the following conclusions can be drawn: (i) The mean relative values obtained were 8.51%, 24.81%, 44.38% and 22.29%, for ash, CP, NDF and HDNN. (ii) There is a positive correlation between the animal's weight and DM in the gastric content, a positive correlation between total gastric content with DM in the gastric content, and a positive correlation between DM content in gastric content with all the chemical parameters analysed. (iii) EMPTY animals presented both a proportional (+19%, $p = 0.0023$ and −40%; $p = 0.0036$, on NDF and, HDNN, respectively) and absolute (−38%, $p = 0.0137$, −52%; $p = 0.0116$, −52%; $p = 0.0114$ and +83%; $p = 0.0077$ for OM, ash, HDNN, and lignin, respectively) different proportion of nutrients in gastric contents than FULL animals. As a key lesson learnt, chemical composition of the gastric content could be used in the nutritional ecology of European rabbits. There exists an important relationship between the parameters analysed (weight, length and different chemical components) that indicate an important interaction between nutrition and ecology of this species.

AUTHOR CONTRIBUTIONS

Pablo Jesús Marín-García, Carlos Rouco, Lola Llobat, María Cambra-López-López, Enrique Blas and Pascual A. Juan José designed the study. Pablo Jesús Marin García, Carlos Rouco, Juan Antonio Aguayo-Adán collected rabbit samples. Pablo Jesús Marín-García analysed rabbit samples. Pablo Jesús Marín-García completed statistical analysis and wrote a first draft. Pablo Jesús Marín-García led the writing of the final version of the manuscript with inputs from the rest of the authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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REFERENCES

AOAC. (2000). *Official methods of analysis of the association of official analytical chemists. 18th, Arlington, USA*.

Bellier, R., Gidenne, T., Vernay, M., & Colin, M. (1995). In vivo study of circadian variations of the cecal fermentation pattern in postweaned and adult rabbits. *Journal of Animal Science*, *73*, 128. https://doi.org/10.2527/1995.731128x

Boyd, I. L. & Bray, C. J. (1989). Nutritional ecology of the wild rabbit—an input to the timing of reproduction. *Proceedings of the Nutrition Society*, *48*, 81–91. https://doi.org/10.1079/PNS19890012

Caîsin, L., Martínez-Paredes, E., Ródenas, L., Moya, V. J., Pascual, J. J., Cervera, C., Blas, E., & Pascual, M. (2020). Effect of increasing lignin in isoenergetic diets at two soluble fibre levels on digestion, performance and carcass quality of growing rabbits. *Animal Feed Science and Technology*, *262*, 114396. https://doi.org/10.1016/j.anifeedsci.2020.114396

Cooke, B. D. (2014). Daily Food Intake of Free-Ranging Wild Rabbits in Semiarid South Australia. *Wildlife Research*, *41*, 141. https://doi.org/10.1071/WR14003

Cortés-Avizanda, A., Colomer, M. À., Margalida, A., Ceballos, O., & Donázar, J. A. (2015). Modeling the consequences of the demise and potential recovery of a keystone-species: Wild rabbits and avian scavengers in mediterranean landscapes. *Scientific Reports*, *5*, 17033. https://doi.org/10.1038/srep17033

de Blas, C. & Wiseman, J. (Eds.). Nutrition of the Rabbit. (2010). (2nd ed.). CABI.

Delibes-Mateos, M., Ferreras, P., & Villafuerte, R. (2008). Rabbit populations and game management: The situation after 15 years of rabbit haemorrhagic disease in Central-Southern Spain. *Biodiversity and Conservation*, *17*, 559–574. https://doi.org/10.1007/s10531-007-9272-5

Ferreira, C. & Alves, P. C. (2009). Influence of habitat management on the abundance and diet of wild rabbit (Oryctolagus Cuniculus Algirus) populations in mediterranean ecosystems. *European Journal of Wildlife Research*, *55*, 487–496. https://doi.org/10.1007/s10344-009-0257-4

García, J., Gidenne, T., Luisa, F.-e-C., & de Blas, C. (2002). Identification of the main factors that influence caecal fermentation traits in growing rabbits. *Animal Research*, *51*, 165–173. https://doi.org/10.1051/animres:2002011

Gidenne, T., Arveux, P., & Madec, O. (2001). The effect of the quality of dietary lignocellulose on digestion, zootechnical performance and health of the growing rabbit. *Animal Science*, *73*, 97–104. https://doi.org/10.1017/S1357729800058094

Gidenne, T., Perez, J., Lapanouse, A., & Ségura, M. (1994). Apports de lignines et alimentation du lapin en croissance. I. Conséquences sur la digestion et le transit. *Annales De Zootechnie*, *43*, 313–322.

Gidenne, T. & Perez, J. M. (1993). Effect of dietary starch origin on digestion in the rabbit. 2. Starch hydrolysis in the small intestine, cell wall degradation and rate of passage measurements. *Animal Feed Science and Technology*, *42*, 249–257. https://doi.org/10.1016/0377-8401(93)90102-P

Gidenne, T., Poncet, C., & Lapanouse, A. (1985). Digestion, Chez Le Lapin En Croissance, d'une Ration à Taux Élevé de constituants Pariétaux: Étude méthodologique pour le calcul de digestibilité apparente, par segment digestif. *Annales De Zootechnie*, *34*, 429–446. https://doi.org/10.1051/animres:19850404

Gidenne, T., Ruckebusch, Y., Lapanouse, A., & Segura, M. (1989). Flow and passage rate studies at the ileal level in the rabbit. *Reproduction Nutrition Développement*, *29*, 403–412. https://doi.org/10.1051/rnd:19890402

Joseph, D. R. (1909). The relation of the weight of the contents of stomach and cecum to the body-weight in rabbits. *Journal of Experimental Medicine*, *11*, 36–40. https://doi.org/10.1084/jem.11.1.36

Klein, D. R. (1965). Ecology of deer range in Alaska. *Ecological Monographs*, *35*, 259–284. https://doi.org/10.2307/1942139

Lees, A. C. & Bell, D. J. (2008). A conservation paradox for the 21st century: The European Wild Rabbit *Oryctolagus Cuniculus*, an invasive alien and an endangered native species. *Mammal Review*, *38*, 304–320. https://doi.org/10.1111/j.1365-2907.2008.00116.x

Llobat, L. & Marín-García, P. J. (2022). Application of protein nutrition in natural ecosystem management for european rabbit (*Oryctolagus Cuniculus*) conservation. *Biodiversity and Conservation*, *31*, 1435–1444. https://doi.org/10.1007/s10531-022-02426-5

Marín-García, P. J. & Llobat, L. (2021). What are the keys to the adaptive success of european wild rabbit (*Oryctolagus Cuniculus*) in the Iberian Peninsula? *Animals: An Open Access Journal from MDPI*, *11*, 2453. https://doi.org/10.3390/ani11082453

Marín-García, P. J., Llobat, L., Aguayo-Adán, J. A., Larsen, T., Cambra-López, M., Blas, E., Pascual, J. J., & Rouco, C. (2023). The nutritional strategy of European rabbits is affected by age and sex: Females eat more and have better nutrient optimisation. *Journal of Animal Physiology and Animal Nutrition*, 1–8. https://doi.org/10.1111/jpn.13826

Marín-García, P. J., López-Luján, M., del, C., Ródenas, L., Martínez-Paredes, E. M., Blas, E., & Pascual, J. J. (2020). Plasma urea nitrogen as an indicator of amino acid imbalance in rabbit diets. *World Rabbit Science*, *28*, 63. https://doi.org/10.4995/wrs.2020.12781

Marín-García, P. J., López-Luján, M. C., Ródenas, L., Martínez-Paredes, E., Cambra-López, M., Blas, E., & Pascual, J. J. (2021). Do growing rabbits with a high growth rate require diets with high levels of essential amino acids? A choice-feeding trial. *Animals: An Open Access Journal from MDPI*, *11*, 824. https://doi.org/10.3390/ani11030824

Marín-García, P. J., Ródenas, L., Martínez-Paredes, E., Cambra-López, M., Blas, E., & Pascual, J. J. (2020). A moderate protein diet does not cover the requirements of growing rabbits with high growth rate. *Animal Feed Science and Technology*, *264*, 114495. https://doi.org/10.1016/j.anifeedsci.2020.114495

Martins, H., Milne, J. A., & Rego, F. (2002). Seasonal and spatial variation in the diet of the wild rabbit (*Oryctolagus Cuniculus* L.) in Portugal. *Journal of Zoology*, *258*, 395–404. https://doi.org/10.1017/S0952836902001541

Moreno, S. & Villafuerte, R. (1995). Traditional management of scrubland for the conservation of rabbits oryctolagus cuniculus and their predators in Doñana National Park, Spain. *Biological Conservation*, *73*, 81–85. https://doi.org/10.1016/0006-3207(95)90069-1

Myers, K. & Bults, H. G. (1977). Observations on changes in the quality of food eaten by the wild rabbit. *Austral Ecology*, *2*, 215–229. https://doi.org/10.1111/j.1442-9993.1977.tb01139.x

Nicodemus, N., Carabaño, R., García, J., Méndez, J., & de Blas, C. (1999). performance response of lactating and growing rabbits to dietary lignin content. *Animal Feed Science and Technology*, *80*, 43–54. https://doi.org/10.1016/S0377-8401(99)00042-5

Orengo, J. & Gidenne, T. (2007). Feeding behaviour and caecotrophy in the young rabbit before weaning: An approach by analysing the digestive contents. *Applied Animal Behaviour Science*, *102*, 106–118. https://doi.org/10.1016/j.applanim.2006.03.010

Parker, K. L. (2003). Advances in the nutritional ecology of cervids at different scales. *Écoscience*, *10*, 395–411. https://doi.org/10.1080/11956860.2003.11682788

Pinheiro, V., Outor-Monteiro, D., Mourão, J. L., Cone, J. W., & Lourenço, A. L. (2018). Effects of animal type (Wild vs. Domestic) and diet alfalfa level on intake and digestibility of european adult rabbits (*Oryctolagus Cuniculus*). *Journal of Animal Physiology and Animal Nutrition*, *102*, e460–e467. https://doi.org/10.1111/jpn.12774

Prud'hon, M., Chérubin, M., Goussopoulos, J., & Carles, Y. (1975). Évolution, au cours de la croissance, des caractéristiques de la consommation d'aliments solide et liquide du lapin domestique nourri ad libitum. *Annales De Zootechnie*, *24*, 289–298. https://doi.org/10.1051/animres:19750212

Raubenheimer, D., Simpson, S. J., & Mayntz, D. (2009). Nutrition, ecology and nutritional ecology: toward an integrated framework. *Functional Ecology*, *23*, 4–16. https://doi.org/10.1111/j.1365-2435.2009.01522.x

Raubenheimer, D., Simpson, S. J., & Tait, A. H. (2012). Match and mismatch: Conservation physiology, nutritional ecology and the timescales of biological adaptation. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *367*, 1628–1646. https://doi.org/10.1098/rstb.2012.0007

Reiné, R., Ascaso, J., & Barrantes, O. (2020). Nutritional quality of plant species in pyrenean hay meadows of high diversity. *Agronomy*, *10*, 883. https://doi.org/10.3390/agronomy10060883

Rommers, J. M., Meijerhof, R., Noordhuizen, J. P. T. M., & Kemp, B. (2002). Relationships between body weight at first mating and subsequent body development, feed intake, and reproductive performance of rabbit does. *Journal of Animal Science*, *80*, 2036. https://doi.org/10.2527/2002.8082036x

SAS Institute. (2009). SAS/STAT9.2 user's guide. Sas Institute Inc.

Seebacher, F. & Franklin, C. E. (2012). Determining environmental causes of biological effects: The need for a mechanistic physiological dimension in conservation biology. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, *367*, 1607–1614. https://doi.org/10.1098/rstb.2012.0036

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Villafuerte, R., & Delibes-Mateos, M. (2019). Oryctolagus cuniculus. The IUCN Red List of Threatened Species. https://doi.org/10.2305/IUCN.UK.2019-3.RLTS.T41291A170619657.en

Villafuerte, R., Lazo, A., & Moreno, S. (1997). Influence of food abundance and quality on rabbit fluctuations: Conservation and management implications in Doñana National Park (SW Spain). *Revue d'Écologie (La Terre et La Vie)*, *52*, 345–356.

Wikelski, M. & Cooke, S. J. (2006). Conservation physiology. *Trends in Ecology & Evolution*, *21*, 38–46. https://doi.org/10.1016/j.tree.2005.10.018

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