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Dietary type (carnivore, herbivore and omnivore) and animal species modulate the nutritional metabolome of terrestrial species.

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ABSTRACT

Ecometabolomics could be implemented as a powerful tool in molecular ecology studies, but it is necessary to know the baseline of certain metabolites and understand how different traits could affect the metabolome of the animals. Therefore, the main objective of this study was to provide values for the nutritional metabolome profile of different diet groups and animal species, as well as to study the differences in the metabolomic profile due to the effect of diet type and species. To achieve this goal, blood samples were taken from healthy animals ($n = 43$) of different species: lion (*Panthera leo)*, jaguar (*Panthera onca)*, chimpanzee (*Pan troglodytes)*, bison (*Bison bison)*, gazelle (*Gazella cuvieri)* and fallow deer (*Dama dama*), and with different types of diet (carnivore, herbivore and omnivore). Each blood sample was analysed to determine nutritional metabolites. The main results this study provides are the nutritional metabolic profile of these animals based on the type of diet and the animal species. A significant effect of the dietary type was found on nutritional metabolite levels, with those metabolites related to protein metabolism (total protein and creatine) being higher in carnivores. There is also an effect of the species on nutritional metabolites, observing a metabolome differentiation between lion and jaguar. In the case of herbivores, bison showed higher levels of uric acid and cholesterol, and lower urea levels than gazelle and fallow deer. More molecular ecology studies are needed to further the knowledge of the metabolism of these animals.

1. Introduction

The possibility of using progressively improved metabolomic techniques in ecophysiological studies has opened a new way to advance knowledge of the structure and function of organisms and ecosystems (Sardans et al., 2011). Ecometabolomics is the methodological approach that uses the metabolomic techniques applied to ecological studies (Fiehn, 2002; Schripsema, 2010). This methodology is in constant development, and although it began with plant studies, it has recently also been implemented in animal studies (Mirnezhad et al., 2010; Marín-García et al., 2022b, 2022c; Marín-García et al., 2023). In this sense, ecometabolomics could be used as a powerful tool in molecular ecology studies. Molecular ecology, understood as the application of molecular genetic methods to address 'ecological' questions (Enkerli and Widmer, 2010), represents a successful example of interdisciplinary science, in which the tools and methods of molecular biology have been merged with concepts of evolution and conservation (Andrew et al., 2013).

Ecological nutrition can use molecular analytical tools to address the management of threatened wildlife (Whilde et al., 2017; Chaousis et al., 2018) and determine the relationship between changes in metabolic phenotype and feed availability, which could help understand how nutrient availability affects populations of different species (Rezzi et al., 2007). In this case, energy and protein metabolites could be key to gaining in-depth biological knowledge (Marín-García et al., 2020, 2022a; Marín-García and Llobat, 2021; Llobat and Marín-García, 2022). Thus, it is especially interesting to have reference values for the animal

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species that have not yet been subjected to metabolomic analyses.

A relationship between metabolomics and phylogeny has been described by Gawenda-Kempczyńska et al. (2022) by finding compounds that may serve as chemotaxonomic markers, indicating that particular biosynthetic pathways have been conserved within a taxon, although this study has been limited to a reduced number of species. In this sense, determining whether the metabolome levels recapitulate the phylogenetic relationship of terrestrial animals will help understand the molecular ecology of these animals and, although there are some studies on the effect of dietary type on the metabolome that quantify the effects of adding a protein of animal or plant origin to carnivorous animals (Roques et al., 2020; Cao et al., 2022), no works have been found that compare the metabolomic profile of carnivorous, omnivorous or herbivorous animals.

Certainly, exploring the biochemical and metabolic dimensions across diverse species promises to deepen our insights into the nuanced ways each species navigates its nutritional landscape. However, the intricacies escalate substantially when venturing into the realm of wild animals (Ryser-Degiorgis, 2013). Accessing samples from these elusive creatures presents a multifaceted challenge, compounded by factors such as habitat remoteness, elusive behavior, and ethical considerations. Despite these hurdles, overcoming them holds the key to unlocking invaluable knowledge about the intricate interplay between wild organisms and their nutritional environments: As understanding adaptations, increasing ecological understanding or with biotechnological applications (Treberg et al., 2020).

In this study, it was hypothesised that metabolome profile is affected by diet type (carnivore, herbivore and omnivore) and animal species; lion (*Panthera leo*), jaguar (*Panthera onca*), chimpanzee (*Pan troglodytes*), bison (*Bison bison*), gazelle (*Gazella cuvieri*) and fallow deer (*Dama dama*) in terrestrial animals. Thus, the main objective of this work was (i) to provide values of the nutritional metabolite profile in these diet type groups and animal species, as well as (ii) to study the differences in the metabolic profile due to these effects. These findings will provide insights into the comparative biochemistry across various species and dietary groups.

2. Material and methods

2.1. Animal ethics statement

The authors confirm that the ethical policies of the journal indicated on the author guidelines page have been followed. The samples were taken in Riosafari Elche, Alicante, Spain. The collection of samples was performed by personnel of the zoos. All samples were collected from the serum bank of the institution to health programs, medical checks-ups, preventive programme, surgical interventions or health monitoring in rehabilitation programmes. Under Spanish law, no animal ethics

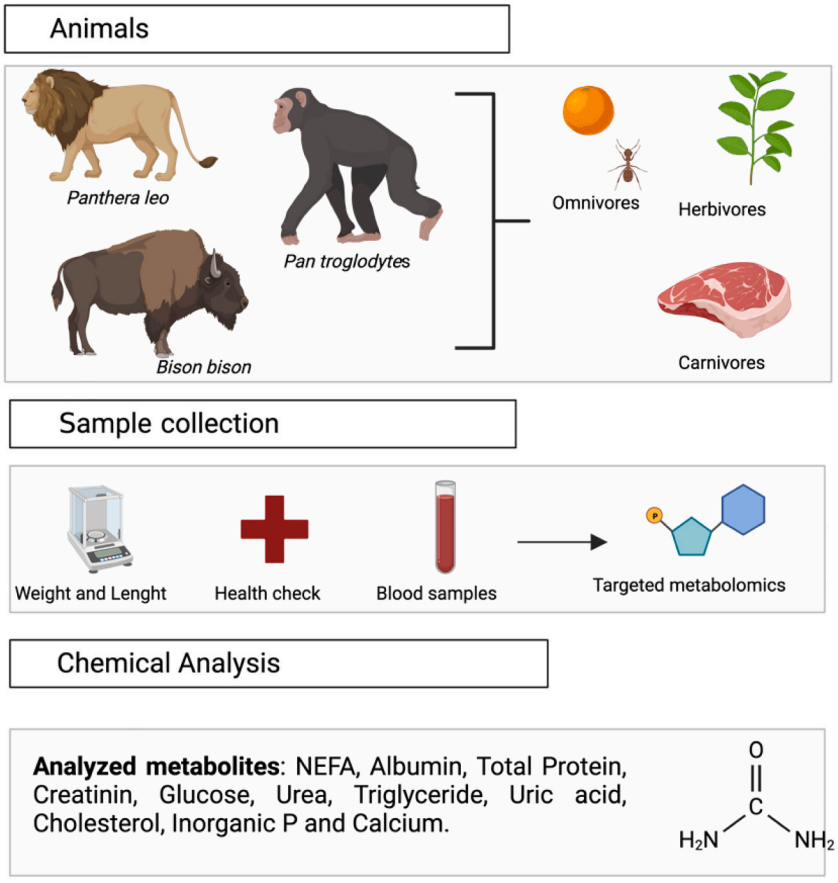

Fig. 1. Experimental design. A total of 43 animals from different species were studied *Classification:* Animals were divided according to their diet: carnivores; herbivores and omnivores. They were also classified according to their species (*Panthera leo*, *Panthera onca*, *Pan troglodytes*, *Bison*, *Gazella cuvier* and *Dama*). Sampling: For each animal that underwent a health check, a biometric measurement and a blood sample was obtained. Blood samples were analysed by targeted metabolomics. NEFA: Non-esterified fatty acid. Only healthy animals were used.

approval was necessary.

2.2. Animals and sampling

A total of 43 terrestrial animals were used in this experiment (Fig. 1). To understand how animal species modulate the nutritional metabolite levels of terrestrial species, the following species were used: lion ($n = 3$), jaguar ($n = 4$), chimpanzee ($n = 5$), bison (n = 5), Cuvier's gazelle ($n = 7$) and fallow deer (n = 5). In addition, to determine the effect of dietary type, samples of African leopard (*Panthera pardus* $n = 1$), tiger (*Panthera tigris*, n = 1), Bornean orangutan (*Pongo pygmaeus*, n = 1), sitatunga (*Tragelaphus spekii*, n = 3), dromedary (*Camus dromedarius*, n = 3), white oryx (*Oryx dammah*, n = 3), Père David's deer (*Elaphurus davidianus*, n = 1) and bull (*Bos taurus,* n = 1) were collected. Due to the small sample size of this second group of animals, the data were only used to characterise the effect of diet on nutritional metabolite levels (and not for analysis of the species effect) and were divided into carnivores ($n = 9$), herbivores ($n = 28$) and omnivores ($n = 6$). Blood samples were extracted by venipuncture in jugular, cephalic or femoral vein depending on the species, with a 5 mL syringe and 21G hypodermic needle (Henry Schein®), and transferred to tubes without anticoagulant (Everest Tecnovet®). Serum samples were obtained after 3000 rpm × 5 min centrifugation and stored at −20 °C until further analysis.

2.3. Chemical analysis

A total of 11 metabolites were selected to study the targeted metabolomic profile of the chosen animals: non-esterified fatty acid (NEFA), albumin, creatinine, total protein, glucose, urea, triglyceride, uric acid, cholesterol, inorganic phosphorous (iP) and calcium. These metabolites were selected because all of them are important in nutrition, have not been previously studied together in these species, and are mainly related to energy and protein metabolism.

NEFA was determined using the Wako, NEFA C ACS-ACOD assay method. The rest of the metabolites were determined by standard procedures (Siemens Diagnostics® Clinical Methods for ADVIA 1800). Analyses were performed using an ADVIA 1800 ®Chemistry System Autoanalyzer (Siemens Medical Solutions, Tarrytown, NY 10591, USA). For non-esterified fatty acid (NEFA), triglyceride and uric acid, intra- and inter CV% variation was respectively below 2.5 and 3.1%. Correspondingly, for albumin, creatinine, total protein, glucose, urea, cholesterol, inorganic phosphorous (iP) and calcium, CV% variation was below 2.2 and 2.8% in all instances.

2.4. Statistical analysis

Two different statistical analyses were performed to determine the effect of animal species and the effect of dietary type on the nutritional metabolites evaluated. Nutritional metabolites were fitted to a normal distribution and analysed as dependent variables using a GLM model from the Statistical Analysis System (SAS, 2009), including the effect of the animal species (6 levels) or type of diet (3 levels) as a fixed effect. Significance was declared at $p < 0.05$, whereas $p < 0.10$ values were considered as a trend. Least square mean comparisons were performed by *t*-test.

Partial least squares discriminant analysis (PLSR) models were built to determine the metabolites responsible for the differences between experimental groups (6 species or 3 types of diet). PLSR was performed using LatentiX 2.12 (LATENTIX Aps., Gilleleje, Denmark). Model validation was performed using full cross-validation (leave-one-out). Outliers were detected based on the residual variance and the Hotelling's T^2 plot. Models were assessed using the explained variation in Y, plots depicted actual and predicted values, and the proportion of variation explained (R^2). The dendrograms were made with Metaboanalyst using Euclidean distance measure and Ward as a clustering algorithm.

3. Results

The summary of the effect of the diet type on the analysed metabolites is shown in Table 1 and Fig. 2. A clear effect of the diet type is observed, as all the metabolites showed a significant effect ($p < 0.01$; Table 1), except for the albumin. Most metabolites related with protein metabolism such as total protein (+20%), creatinine (+28%), urea (+76%) and calcium (+62%) were higher in carnivores than the average for the other two groups ($p < 0.01$). Herbivores showed higher levels of plasmatic metabolites such as glucose (+39%) and iP (+95%) than omnivores ($p < 0.05$). And omnivores showed higher levels of NEFA, triglycerides, and uric acid than carnivores and herbivores ($p < 0.01$). In addition, cholesterol serum levels were higher in omnivores and carnivores than in herbivores ($p < 0.01$). Fig. 2.a represents the first two principal components of targeted metabolomics by type of diet. The variability associated with these principal components obtained from the metabolic profile (48.6% of the total) can be used to differentiate diet types as there is no overlap between the populations (95% of the probability) of the different types of diet on the nutritional metabolite profiles ($R^2 = 0.897$), causing a clear differentiation between the sample groups, as shown in the dendrogram (Fig. 2.b). It is essential to note that in this instance, we are witnessing an outlier represented by the number 1. This outlier pertains to a female bison whose attributes deviate significantly from the average values of others within its species. Notably, parameters such as NEFA, urea, triglycerides, uric acid and calcium exhibit differences of up to 50% from the average value.

Table 2 and Fig. 3 show the summary of the effect of the species on the analysed metabolites. Although the distribution follows the pattern observed by the type of diet, significant differences were observed in all metabolites studied between the animal species (Table 2). Regarding herbivores, bison showed higher levels of uric acid and cholesterol but lower urea levels than gazelle and fallow deer (+1100% and + 739%; +159% and + 272%; −319% and − 332%, respectively). Within the group of carnivores, NEFA, albumin, creatinine and cholesterol serum levels showed the most relevant differences, where jaguars presented higher levels than lions, which are similar to omnivore levels. In contrast, serum levels of iP were higher in lions than jaguars, which present similar levels to those of omnivores. In addition, serum levels of total protein were higher in jaguars than lions. Fig. 3.a represents the first two principal components of targeted metabolomics by animal species. As shown, the variability associated with these principal components obtained from the nutritional metabolites profile (54,2% of the total) can be used to differentiate species ($R^2 = 0.973$), except for lions and jaguars, where no overlap was observed, whereas there was an overlap on the nutritional metabolite profiles of the herbivores (Fig. 3. b).

4. Discussion

The main aim of this study was to provide values of nutritional metabolites from animals with different diets (carnivores, herbivores and omnivores) and different species (lion, jaguar, chimpanzee, bison, gazelle, and fallow deer), and to determine how these two effects (diet type and species) affect their nutritional metabolite profile.

Our study provides information on serum levels of nutritional metabolites in different wild herbivore, carnivore and omnivore species, with high value from an ecological and nutritional point of view. Next, we proceed to explain the main effects of diet type and species on the nutritional metabolite profile. It is important to recognise that various factors including sex, age, reproductive status, diet, husbandry conditions, time elapsed since feeding or the technique employed for blood sampling, among others, may influence the outcomes achieved (Reilly et al., 2014). Although some of these parameters have been previously studied in the species under investigation, such as lions, (Behera et al., 2013; Larsson et al., 2017), chimpanzees (Reamer et al., 2014), bison (Hawley and Peden, 1982), gazelles (Mohammed et al., 2011) and deer

Table 1
Effect of the diet type on targeted nutritional metabolomic profile (LS means ± Standard Error) of the main analysed metabolites.

Metabolites Analyzed[1]	Carnivores (n = 9)			Hervibores (n = 28)			Omnivores (n = 6)			p_value
NEFA (μ eqv./L)	612^{b}	±	71.9	295^{a}	±	40.8	1029^{c}	±	88.1	<0.001
Albumin (g/L)	35.0	±	1.31	36.1	±	0.74	39.0	±	1.61	0.1645
Total Protein (g/L)	76.49^{c}	±	2.27	61.3^{a}	±	1.29	66.3^{b}	±	2.78	0.0166
Creatinine (μM)	208^{b}	±	27.9	121^{a}	±	15.8	95.3^{a}	±	34.1	<0.001
Glucose (mM)	7.24^{ab}	±	0.96	8.46^{b}	±	0.54	4.93^{a}	±	1.18	0.0295
Urea (mM)	10.1^{b}	±	1.32	8.28^{b}	±	0.75	3.22^{a}	±	1.62	0.0069
Triglyceride (mM)	0.36^{a}	±	0.06	0.20^{a}	±	0.04	1.07^{b}	±	0.08	<0.001
Uric acid (μM)	12.2^{a}	±	8.0	17.7^{a}	±	4.53	90.8^{b}	±	9.80	<0.001
Cholesterol (mM)	3.55^{b}	±	0.43	1.40^{a}	±	0.24	3.19^{b}	±	0.53	<0.001
Inorganic P (mM)	1.64^{b}	±	0.20	1.93^{b}	±	0.11	0.99^{a}	±	0.25	0.0045
Calcium (mM)	2.44^{b}	±	0.19	1.78^{a}	±	0.11	1.24^{a}	±	0.25	0.0013

[1] NEFA: Non-esterified fatty acid. ^{a,b,c} Means within a row with different letter were significantly different at $P < 0.05$.

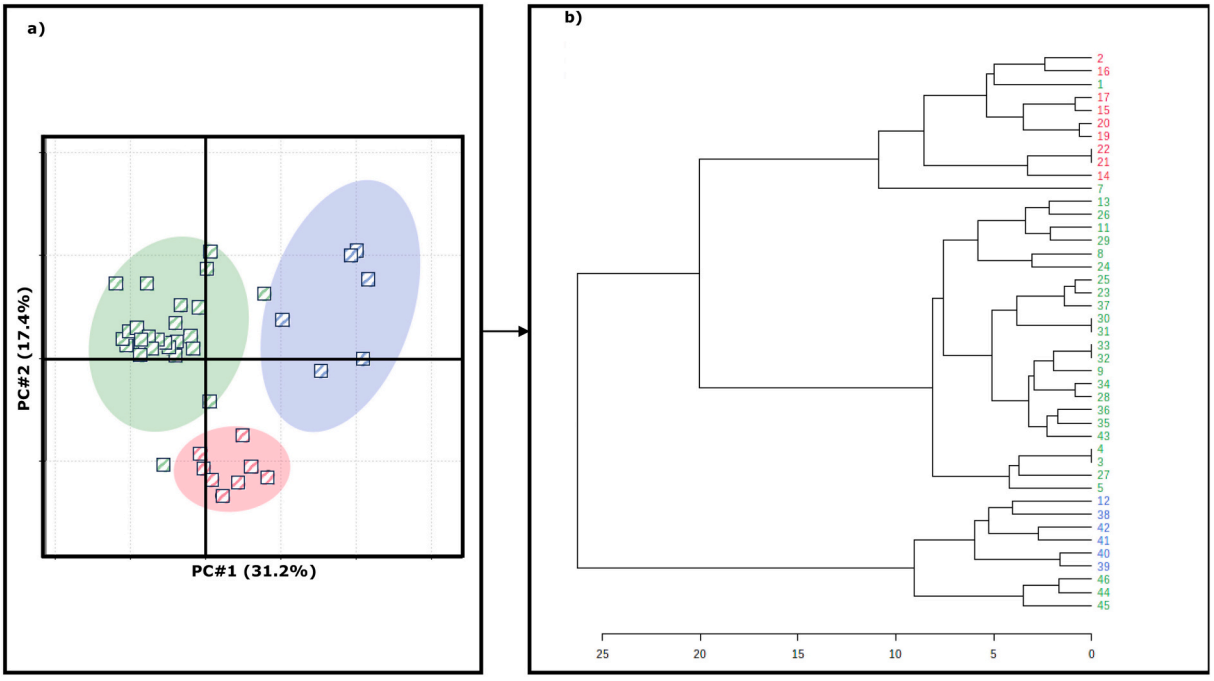

Fig. 2. Effect of diet type (carnivores; herbivores and omnivores) on targeted nutritional metabolomic profile 2.a) PLS mode of plasma, the colours correspond to the 95% probability distribution of diet type. 2.b) Dendrogram of the different samples.

Table 2
Effect of species on targeted nutritional metabolomic profile (LS means ± Standard Error) of the main analysed metabolites.

Metabolites Analyzed[1]	*Panthera leo* (n = 3)			*Panthera onca* (n = 4)			*Pan troglodytes* (n = 5)			*Bison bison* (n = 5)			*Gazella cuvieri* (n = 7)			*Dama dama* (n = 5)			p - value
NEFA (μ eqv./L)	434^{a}	±	114	620^{ab}	±	99.0	1001^{b}	±	99.6	242^{a}	±	99.0	333^{a}	±	74.9	208^{a}	±	88.6	<0.001
Albumin (g/L)	30.6^{a}	±	1.87	37.8^{ab}	±	1.61	39.0^{b}	±	1.45	38^{ab}	±	1.61	36.3^{ab}	±	1.22	35.9^{ab}	±	1.44	0.0216
Total Protein (g/L)	75.4^{bc}	±	3.04	78.9^{c}	±	2.63	67.3^{ab}	±	2.35	60.2^{a}	±	2.63	60.9^{a}	±	1.99	60.5^{a}	±	2.35	<0.001
Creatinine (μM)	268^{b}	±	49.4	180^{ab}	±	42.3	86.2^{a}	±	38.3	90.8^{ab}	±	42.8	101^{ab}	±	32.3	99.2^{ab}	±	38.2	0.0318
Glucose (mM)	6.89^{ab}	±	1.33	9.42^{ab}	±	1.15	5.02^{a}	±	1.03	6.36^{a}	±	1.15	7.79^{ab}	±	0.87	10.33^{b}	±	1.03	0.0064
Urea (mM)	11.3^{b}	±	1.86	9.20^{b}	±	1.61	2.44^{a}	±	1.44	2.41^{a}	±	1.61	10.1^{b}	±	1.21	10.43^{b}	±	1.44	0.0002
Triglyceride (mM)	0.42^{a}	±	0.11	0.27^{a}	±	0.09	1.10^{b}	±	0.08	0.36^{a}	±	0.09	0.24^{a}	±	0.06	0.09^{a}	±	0.08	<0.001
Uric acid (μM)	18.7^{a}	±	6.98	10.3^{a}	±	6.04	108^{b}	±	5.40	54.0^{b}	±	6.04	6.43^{a}	±	4.57	4.40^{a}	±	5.40	<0.001
Cholesterol (mM)	2.48^{ab}	±	0.59	4.32^{bc}	±	0.51	3.61^{bc}	±	0.46	3.43^{bc}	±	0.52	0.92^{a}	±	0.39	1.32^{ab}	±	0.46	<0.001
Inorganic P (mM)	1.74^{ab}	±	0.25	1.51^{a}	±	0.22	1.02^{a}	±	0.19	2.17^{d}	±	0.21	1.96^{c}	±	0.16	1.94^{bc}	±	0.19	<0.001
Calcium (mM)	2.27^{b}	±	0.30	2.62^{b}	±	0.26	1.34^{a}	±	0.26	1.02^{a}	±	0.26	2.10^{ab}	±	0.20	2.10^{ab}	±	0.26	0.0025

[1] NEFA: Non-esterified fatty acid.

(Tajchman et al., 2023), in this work the comparisons between the experimental groups are carried out.

In the literature, there is a large number of works in which comparisons of the serum biochemical values conducted in this study were drawn (Umminger, 1975; Kjeld and Ólafsson, 2008; Scanes, 2016). Regarding the type of diet effect, all the carnivores studied (lion and

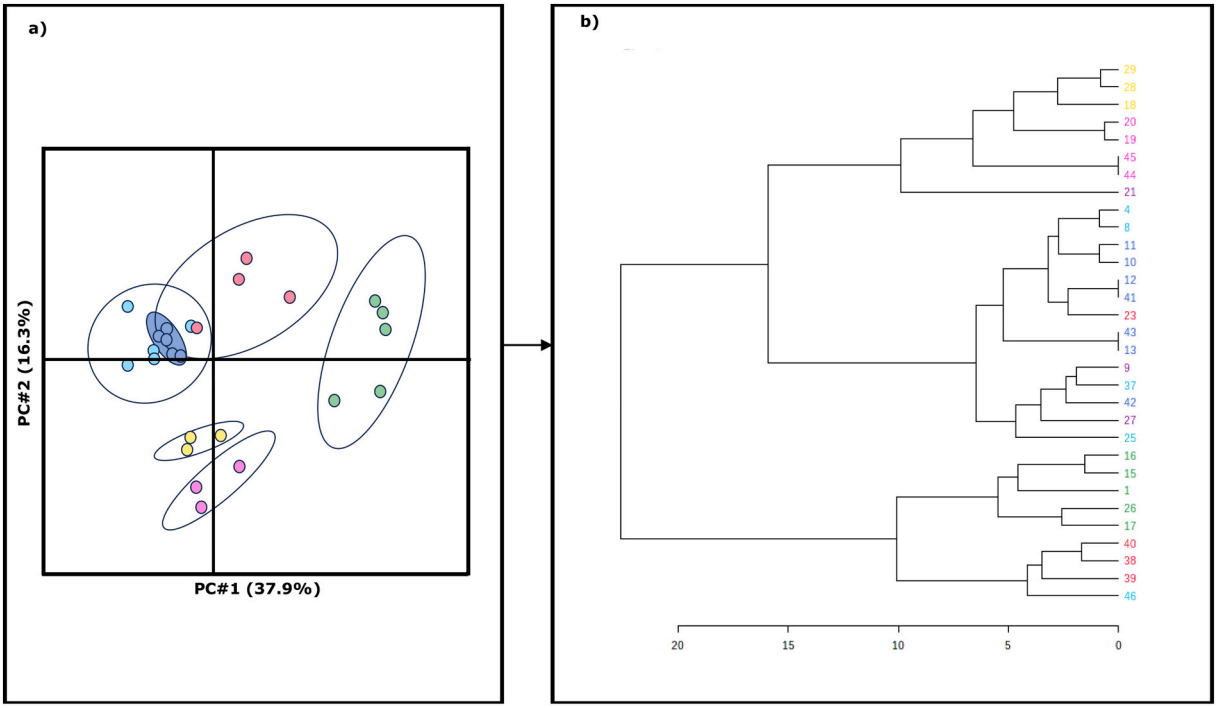

Fig. 3. Effect of species (*Panthera leo*, *Panthera onca*, *Pan troglodytes*, *Bison*, *Gazella cuvier* and *Dama*) on targeted nutritional metabolomic profile 3. a) PLS mode of plasma, the colours correspond to the 95% probability distribution of diet type. 3.b) Dendrogram of the different samples. Missing data are the result of removal outlying samples.

jaguar, belonging to the Felidae family) are obligate carnivores, so the dietary protein requirements in these animals are higher than those of omnivores and herbivores (Russell et al., 2002), and they do not have the ability to adapt to different levels of protein intake, unlike omnivores and herbivores (Verbrugghe and Bakovic, 2013). For this reason, obligate carnivores present high activity of the amino acid catabolic enzymes in the liver and kidney (Rogers et al., 1977), which could explain their higher levels of total protein and creatinine in serum. Moreover, nitrogen metabolism exhibits variations among animals with diverse dietary patterns (Marín-García et al., 2022a). Serum levels of urea and uric acid are notably elevated in omnivores compared to herbivores and carnivores, with the latter two displaying comparable values. These results agree with the different urea permeability of erythrocytes found in mammals with different diets. Thus, the erythrocytes of herbivores have lower urea permeability than other animals, which is related to different urine osmolarity (Liu et al., 2011). While the erythrocytes of omnivores have an intermediate permeability, the high levels of protein in the diet of carnivores could explain the specific mechanisms that felids possess to metabolise the high levels of nitrogen consumed (Kerr et al., 2011).

In relation to lipid metabolism, omnivores showed higher levels of NEFA and triglycerides than herbivores and carnivores. These results could be explained by the higher efficiency in the use and metabolisation of dietary fats, likely due to a different expression of key genes for lipid metabolism, such as fatty acid degradation (Yizhen et al., 2023). The high levels of protein and fat in omnivore and carnivore diets could explain their higher serum levels of cholesterol. In fact, these different levels of nutrients in the diet are accompanied by metabolic and physiological adaptation, which is also reflected in genomic changes. Thus, herbivores show the absence of triglyceride lipase inhibitor PNLIPRP1, and therefore a consequent improvement in the triglyceride digestion efficiency, whereas carnivores do not present the hormone-receptor pair INSL5-RXFP4, a regulator of glucose homeostasis, related to constant gluconeogenesis (Hecker et al., 2019).

A meta-analysis carried out by Böswald et al. (2008) showed that mechanisms involved in calcium and phosphorous homeostasis are more varied in omnivores, with calcium absorption in the intestine being more efficient. This fact, together with the different intestinal microbiota between the groups studied (omnivores, carnivores and herbivores) and the different levels of mineral intakes in the diet, could explain the results observed in calcium and iP serum levels. Besides, levels of glucose are different between groups, being highest in herbivores, medium in carnivores and lowest in omnivores. This effect could be explained by the higher levels of carbohydrates in herbivores' diets, compared to those of omnivores and carnivores, where the protein and fat are higher.

Regarding the species effect, within the herbivorous animals studied, differences in the serum levels of some metabolites were found. Specifically, bison showed higher levels of cholesterol and uric acid, whereas their levels of urea were lower than those of gazelle and fallow deer. Levels of cholesterol depend on the fat ingested in the diet, and although all the herbivores analysed were ruminants, there are several differences in their diet. Bison are mostly strict grazers (Meagher, 1986), while large cervids such as gazelle and fallow deer include browsing in their natural diet (Gebert and Verheyden-Tixier, 2001). These differences in feeding type may potentially cause changes in rumen characteristics (Clauss et al., 2009) and in the composition of the digestive tract microbiota (Bergmann, 2017), which could explain the different nitrogen metabolism and serum levels of urea and uric acid. Differences attributable to taxonomy have also been determined in other vertebrates, such as glucose metabolism and the disparities observed between camelids and other ruminants (Chandrasena et al., 1979).

Among the carnivorous animals included in this study, information on serum levels of these and other nutritional metabolites is very scarce or non-existent. However, previous studies with some felids reported that, within the same genus, species present differences between the

levels of some metabolites, including those related to lipid and protein metabolism (Crissey et al., 2003). They also observed that different levels of serum metabolites associated with lipid metabolism are not related to a difference in their diets, which provides valuable information about a different metabolism in each of the carnivores belonging to the same genus. As has been observed, there is a distinct biochemical profile when comparing different species and types of diet. These observed differences will allow a deeper understanding of the biological knowledge of these animals and help to determine the complex relationships between metabolism and other biological aspects.

5. Conclusions

The results of this work indicated that there is an effect of the diet type on nutritional metabolome, with the majority of the metabolites related to protein metabolism (total protein and creatine) being higher in carnivores, while omnivores showed the highest levels of NEFA and triglycerides. Furthermore, there is a significant effect of the species on the nutritional metabolome, although this effect was smaller than type of diet effect. More molecular ecology studies are needed to further our knowledge of the effect of environmental factors on the metabolism of these animals and incorporate them into specific programmes for their conservation and biological study.

CRediT authorship contribution statement

Lola Llobat: Writing – original draft, Conceptualization. **Pilar Soriano:** Writing – review & editing, Data curation. **Francesco Bordignon:** Conceptualization. **Trinidad de Evan:** Conceptualization. **Torben Larsen:** Conceptualization. **Pablo Jesús Marín-García:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

Authors declare no conflicto of Interest.

Data availability

Data will be made available on request.

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References

Andrew, R.L., Bernatchez, L., Bonin, A., Buerkle, C.A., Carstens, B.C., Emerson, B.C., Garant, D., Giraud, T., Kane, N.C., Rogers, S.M., Slate, J., Smith, H., Sork, V.L., Stone, G.N., Vines, T.H., Waits, L., Widmer, A., Rieseberg, L.H., 2013. A road map for molecular ecology. Mol. Ecol. 22, 2605–2626.

Behera, P., Dash, D., Senapati, M., Bisoi, Parija, 2013. Effect of sex and season on serum biochemical constituents and enzymes of *Panthera leo* of Nandankanan Zoological Park. IOSR J. Agri. Vet. Sci. 4, 68–72.

Bergmann, G.T., 2017. Microbial community composition along the digestive tract in forage- and grain-fed bison. BMC Vet. Res. 13, 253.

Cao, Y., Gao, Q., Li, X., Zhou, Y., Dong, S., Wang, Y., Dai, Z., 2022. Integrated analysis of metabolomics and transcriptomics for assessing effects of fish meal and fish oil replacement on the metabolism of rainbow trout (*Oncorhynchus mykiss*). Front. Mar. Sci. 9, 843637.

Chandrasena, L.G., Emmanuel, B., Gilanpour, H., 1979. A comparative study of glucose metabolism between the camel (*Camelus dromedarius*) and the sheep (*Ovis aries*). Comp. Biochem. Physiol. A Physiol. 62, 837–840.

Chaousis, S., Leusch, F.D.L., Van De Merwe, J.P., 2018. Charting a path towards non-destructive biomarkers in threatened wildlife: A systematic quantitative literature review. Environ. Pollut. 234, 59–70.

Clauss, M., Fritz, J., Bayer, D., Nygren, K., Hammer, S., Hatt, J.-M., Südekum, K.-H., Hummel, J., 2009. Physical characteristics of rumen contents in four large ruminants of different feeding type, the addax (*Addax nasomaculatus*), bison (*Bison bison*), red deer (*Cervus elaphus*) and moose (*Alces alces*). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 152, 398–406.

Crissey, S.D., Ange, K.D., Jacobsen, K.L., Slifka, K.A., Bowen, P.E., Stacewicz-Sapuntzakis, M., Langman, C.B., Sadler, W., Kahn, S., Ward, A., 2003. Serum concentrations of lipids, vitamin d metabolites, retinol, retinyl esters, tocopherols and selected carotenoids in twelve captive wild felid species at four zoos. J. Nutr. 133, 160–166.

Enkerli, J., Widmer, F., 2010. Molecular ecology of fungal entomopathogens: molecular genetic tools and their applications in population and fate studies. BioControl 55, 17–37.

Fiehn, O., 2002. No title found. Plant Mol. Biol. 48, 155–171.

Gebert, C., Verheyden-Tixier, H., 2001. Variations of diet composition of Red Deer (*Cervus elaphus* L.) in Europe. Mammal Rev. 31, 189–201.

Hawley, A.W.L., Peden, D.G., 1982. Effects of ration, season and animal handling on composition of bison and cattle blood. J. Wildl. Dis. 18, 321–338.

Hecker, N., Sharma, V., Hiller, M., 2019. Convergent gene losses illuminate metabolic and physiological changes in herbivores and carnivores. Proc. Natl. Acad. Sci. USA 116, 3036–3041.

Kerr, K.R., Beloshapka, A.N., Morris, C.L., Swanson, K.S., 2011. Nitrogen metabolism of four raw meat diets in domestic cats. Br. J. Nutr. 106 (Suppl. 1), S174–S177.

Kjeld, M., Ólafsson, Ö., 2008. Allometry (scaling) of blood components in mammals: connection with economy of energy? Can. J. Zool. 86, 890–899.

Larsson, M.H.M.A., Flores, A.S., Fedullo, J.D.L., Teixeira, R.H.F., Mirandola, R.M.S., Ito, F.H., Pessoa, R.B., Itikawa, P.H., 2017. Biochemical parameters of wild felids (*Panthera leo* and *Panthera tigris altaica*) kept in captivity. SCA 38, 791.

Liu, L., Lei, T., Bankir, L., Zhao, D., Gai, X., Zhao, X., Yang, B., 2011. Erythrocyte permeability to urea and water: comparative study in rodents, ruminants, carnivores, humans, and birds. J. Comp. Physiol. B. 181, 65–72.

Llobat, L., Marín-García, P.J., 2022. Application of protein nutrition in natural ecosystem management for European rabbit (*Oryctolagus cuniculus*) conservation. Biodivers. Conserv. 31, 1435–1444.

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 11, 2453.

Marín-García, P.J., López-Luján, M.D.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 Sci. 28, 63.

Marín-García, P.J., Llobat, L., López-Lujan, M.C., Cambra-López, M., Blas, E., Pascual, J. J., 2022a. Urea nitrogen metabolite can contribute to implementing the ideal protein concept in Monogastric animals. Animals 12, 2344.

Marín-García, P.J., Llobat, L., Rouco, C., Aguayo-Adán, J.A., Larsen, T., Cambra-López, M., Blas, E., Pascual Amorós, J.J., 2022b. Unravelling the role of metabolites for detecting physiological state of wild animals: European Rabbit's (*Oryctolagus cuniculus*) case. Animals 12, 3225.

Marín-García, P.J., Llobat, L., Rouco, C., Aguayo-Adán, J.A., Larsen, T., Cambra-López, M., Blas, E., Pascual, J.J., 2022c. Nutritional metabolites as biomarkers of previous feed intake in European rabbit (*Oryctolagus cuniculus*): applications on conservation. Animals 12, 2608.

Marín-García, P., 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. Anim. Physiol. Nutr. 107, 1294–1301.

Meagher, M., 1986. Bison bison. Mamm. Species 1–8.

Mirnezhad, M., Romero-González, R.R., Leiss, K.A., Choi, Y.H., Verpoorte, R., Klinkhamer, P.G.L., 2010. Metabolomic analysis of host plant resistance to thrips in wild and cultivated tomatoes. Phytochem. Anal. 21, 110–117.

Mohammed, O.B., Omer, S.A., Macasero, W.V., Kock, R.A., 2011. Serum biochemistry reference range values for Arabian mountain gazelle (*Gazella gazella*) and Arabian sand gazelle (*Gazella subgutturosa marica*) at king Khalid wildlife research Centre, Saudi Arabia. Comp. Clin. Pathol. 20, 187–191.

Reamer, L.A., Haller, R.L., Thiele, E.J., Freeman, H.D., Lambeth, S.P., Schapiro, S.J., 2014. Factors affecting initial training success of blood glucose testing in captive chimpanzees (*pan troglodytes*): chimpanzee diabetic treatment training. Zoo Biol. 33, 212–220.

Reilly, S., Seddighi, M.R., Steeil, J.C., Sura, P., Whittemore, J.C., Gompf, R.E., Elliott, S. B., Ramsay, E.C., 2014. Selected clinical, biochemical, and electrolyte alterations in anesthetized captive tigers (*Panthera tigris*) and lions (*Panthera leo*). J. Zoo Wildl. Med. 45, 328–334.

Rezzi, S., Ramadan, Z., Fay, L.B., Kochhar, S., 2007. Nutritional Metabonomics: applications and perspectives. J. Proteome Res. 6, 513–525.

Rogers, Q.R., Morris, J.G., Freedland, R.A., 1977. Lack of hepatic enzymatic adaptation to low and high levels of dietary protein in the adult cat. Enzyme 22, 348–356.

Roques, S., Deborde, C., Richard, N., Skiba-Cassy, S., Moing, A., Fauconneau, B., 2020. Metabolomics and fish nutrition: a review in the context of sustainable feed development. Rev. Aquac. 12, 261–282.

Russell, K., Murgatroyd, P.R., Batt, R.M., 2002. Net protein oxidation is adapted to dietary protein intake in domestic cats (*Felis silvestris* catus). J. Nutr. 132, 456–460.

Ryser-Degiorgis, M.-P., 2013. Wildlife health investigations: needs, challenges and recommendations. BMC Vet. Res. 9, 223.
Sardans, J., Peñuelas, J., Rivas-Ubach, A., 2011. Ecological metabolomics: overview of current developments and future challenges. Chemoecology 21, 191–225.
SAS, 2009. SAS/STAT ® 9.2 User's Guide Version 9. SAS Institute Inc, Cary, NC, USA.
Scanes, C.G., 2016. A re-evaluation of Allometric relationships for circulating concentrations of glucose in mammals. FNS 07, 240–251.
Schripsema, J., 2010. Application of NMR in plant metabolomics: techniques, problems and prospects. Phytochem. Anal. 21, 14–21.
Tajchman, K., Kowalik, S., Janiszewski, P., KLa, Ż.S.-B., 2023. Basic haematological and biochemical parameters of farmed red deer and fallow deer blood. Med. Weter. 79, 6775–2023.
Treberg, J.R., Martyniuk, C.J., Moyes, C.D., 2020. Getting the most out of reductionist approaches in comparative biochemistry and physiology. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 250, 110483.
Umminger, B.L., 1975. Body size and whole blood sugar concentrations in mammals. Comp. Biochem. Physiol. A Physiol. 52, 455–458.
Verbrugghe, A., Bakovic, M., 2013. Peculiarities of one-carbon metabolism in the strict carnivorous cat and the role in feline hepatic lipidosis. Nutrients 5, 2811–2835.
Whilde, J., Martindale, M.Q., Duffy, D.J., 2017. Precision wildlife medicine: applications of the human-centred precision medicine revolution to species conservation. Glob. Chang. Biol. 23, 1792–1805.
Yizhen, Z., Chen, L., Jie, X., Shen, F., Zhang, L., Hou, Y., Li, L., Yan, G., Zhang, X., Yang, Z., 2023. Comparative study of the digestion and metabolism related genes' expression changes during the postnatal food change in different dietary mammals. Front. Genet. 14, 1198977.