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SHORT COMMUNICATION

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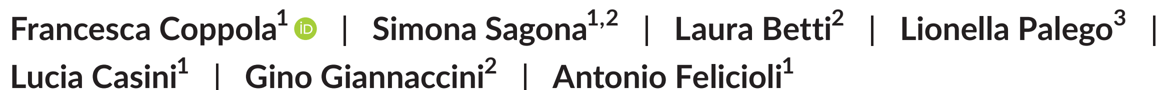
Preliminary investigation on enzymatic activity in saliva of *Hystrix cristata* L., 1758



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Abstract

Mammal's saliva contains a variety of electrolytes and proteins. They carry out an important role in the digestion process, in the antibacterial and antiviral activity, in lubrication and maintenance of oral general health status. It may also contain several enzymes according to dietary habits and general wellness. Sialochemistry is a valid alternative to the haematochemical analysis for the evaluation of animal health and nutritional status. At present, very little knowledge is available on health status and pathology of crested porcupine (*Hystrix cristata*) and no data are yet available on salivary enzymes. Between 2018 and 2020, a preliminary investigation of enzymatic activity on saliva samples was carried out from captured porcupines. In crested porcupine saliva, enzymatic activity of trypsin, chymotrypsin, N-Aminopeptidase, amylase, lignin peroxidise, cellulase and chitinase were recorded. Superoxide dismutase, catalase, glutathione S-transferase and alkaline phosphatase activity was also detected. The superoxide dismutase activity resulted higher (3.13 SD 3.58 U/mg proteins) than those of catalase (130.80 SD 110.65 mU/mg proteins) and glutathione S-transferase (20.21 SD 16.62 mM/mg proteins). Alkaline phosphatase activity resulted lower (5.91 SD 6.12 mU/mg proteins) than acidic phosphatase (19.00 SD 16.16 U/mg proteins) with the highest values of saliva alkaline phosphatases recorded in young individuals. These preliminary data bring new knowledge on crested porcupine saliva enzymes and may provide a useful tool for further investigation on the adaptive response of crested porcupine to different environmental condition and diet. Additional investigation concerning a possible alternative use of saliva enzymes as indicator of health and nutritional status of this rodent are desirable.

KEYWORDS
crested porcupine, digestion, enzymes activity, health, saliva

Francesca Coppola and Simona Sagona contributed equally to the work.

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1 | INTRODUCTION

The crested porcupine (*Hystrix cristata*) is a hystricomorph rodent distributed in North and Central Africa and, among European countries, it is present only in Italy, where it lives as a naturalized species (Vecchio et al., 2018). It is a burrowing and mainly nocturnal mammal that shows occasional diurnal motor activity (Coppola et al., 2019, 2020). It is able to adapt to different habitats (i.e. woods, Mediterranean shrub wood and cultivated open plains) (Santini, 1980). However, deciduous forests (i.e. forests of *Robinia pseudoacacia*, *Quercus cerri*, *Fraxinus ornus* or *Quercus pubescens*) and Mediterranean shrub are the elected habitats for this animal (Orsomando & Pedrotti, 1976). The crested porcupine is a mainly herbivorous rodent, with a marked food preference for roots, bulbs, tubers (also truffles) and rhizomes of many wild and cultivated herbaceous plants (Ori et al., 2018; Pigozzi & Patterson, 1990; Santini, 1980). It also feeds on epigeal parts, leaves, flowers, buds, grass, inflorescences and fallen fruits (Bruno & Riccardi, 1995; Santini, 1980). The crested porcupine eats the root-system and bulbs of plants such as *Arum italicum*, *Tamus communis*, *Iris germanica* and *Cyclamen europaeum*, with no apparent ill effects, although these plants contain various caustic, diuretic, emetic and laxative substances (Santini, 1980). Furthermore, this rodent also feeds on bulbs of Red squill (*Uriginea maritima*), which contains scilliroside, an alkaloid used as a rodenticide (Santini, 1980). In Italy, the crested porcupine adopts a generalist feeding strategy linked to seasonal availability of food (Bruno & Riccardi, 1995; Lovari et al., 2017). The crested porcupine also performs osteophagia and sometimes eats on carrion, thereby occasionally behaving as an omnivorous rodent rather than strictly herbivorous as previously reported (Coppola, Guerrieri, et al., 2020; Santini, 1980). Very little data are available on the health status of this rodent; however, recent studies indicate the crested porcupine as a new potential source in the epidemiology routes of zoonotic diseases as *Giardiasis* and *Leptospirosis* (Cilia et al., 2020; Coppola, Cilia, et al., 2020; Coppola, Maestrini, Berrilli, et al., 2020). Saliva is a watery substance secreted into the mouth produced by exocrine salivary glands and it contains a variety of electrolytes and proteins with an important role in the digestion processes, in the antibacterial and antiviral activities and in lubrication and maintenance of general oral health status (Cappai et al., 2014; Krasteva & Kisselova, 2011; Miletich, 2010). Among salivary chemical components of mammals, several enzymes can be detected, which depend on dietary habits and general wellness (Maciejczyk et al., 2019). The low invasiveness of saliva collection renders sialochemistry a valid alternative to blood sampling for haematochemical analysis to asses health and nutritional status of both humans and animals (Chojnowska et al., 2018; Logan et al., 2020; Zalewska et al., 2014). In crested porcupine, three salivary glands, parotid, mandibular and sublingual glands were described (Obead et al., 2018) but no data is yet available on crested porcupine salivary enzymes. Among rodent species, data on salivary enzymatic pattern are available only for rat, in which a marked activity of acid phosphatase, alkaline phosphatase, pseudo-cholinesterase, β-D-galactosidase, alpha-amylase (Chauncey et al., 1963) and trypsine-like proteases (Ikeno et al., 1986) were detected. Levels of alkaline phosphatases (ALP) in rat saliva may be used as chemical biomarkers for identification of skeletal maturational stages (Pellegrini et al., 2008). In rat, antioxidant system of salivary parotid gland cell (i.e. superoxide dismutase and catalase) was proved to be efficient in the elimination of reactive oxygen species (ROS) during oxidative stress (Zalewska et al., 2014).

In this investigation, the enzymatic activity in saliva of captured crested porcupines was preliminary measured in order to give evidence to the prediction that enzymatic activity in crested porcupine saliva could be similar to other omnivorous rodents. Moreover, results obtained in this investigation could provide new knowledge for further investigation on the adaptive response of this rodent to diet and different environmental condition, as well as for health and nutritional status evaluation.

2 | MATERIALS AND METHODS

2.1 | Samples collection

From February 2018 to June 2020 three porcupines capture campaigns were performed in two experimental site, a hilly area of 2548 ha in Crespina-Lorenzana (86 m a.s.l., 43.57181 Lat.- 10.55348 Long.) and the wildlife hunting reserve Camugliano (24 m a.s.l., 43.60210 Lat - 10.64742 Long.), both located in Pisa Province, Tuscany, Italy. Both experimental sites contain woody cover areas characterized by cops and deciduous forest of *Robinia pseudoacacia*, *Quercus cerris*, *Q. pubescens* and *Q. ilex* with a thick and thriving undergrowth. The undergrowth is composed by a large variety of shrubs and herbaceous plants such as *Sambucus nigra*, *Rubus ulmifolius*, *Laurus nobilis*, *Ruscus aculeatus*, *Juniperus oxycedrus*, *Asparagus acutifolius*, *Cyclamen* sp., *Tamus communis*, *Arum maculatum*, *Rumex* sp. and *Orchis provincialis*. Wheat, sunflower, alfalfa and vegetable crop areas, as well as vineyards and olive groves, surround the woody area. In both study areas, underground storage organs of wild plants (i.e. bulbs, tubers, rhizomes and roots), such as *Cyclamen* sp., *Tamus communis*, *Arum maculatum*, *Rumex* sp., *Asparagus* sp., *Allium* sp. and *Iris* sp. are the main source of food for porcupines and are available throughout the year. Grass inflorescences are an important diet component in spring while, in summer and autumn porcupines feed on fruits and agricultural products. In each study area, six capture traps were placed along pathways mostly used by porcupines. The porcupines trapping and handling was performed according to Coppola et al. (2020a). Captured porcupines were anaesthetized with Zoletil 100® by intramuscular injections in the lumbar region using an air-compressed syringe (Mini-ject 2000, 2 ml), according to the protocol of Coppola et al. (2020b). Porcupine age class was assessed according to the animal body weight: adults (>12 kg),

sub-adults (from 9 to 11 kg), juveniles (from 5 to 8 kg) and porcupettes (<5 kg). The capture activity of porcupines was approved by both the Italian Institute for Environmental Protection and Research (ISPRA) with protocol number 22584 of the 8 May 2017 and protocol number 150071 of the 16 March 2018 and by the Tuscany Region with the Decree n. 14235 of the 3 October 2017 and Decree n. 4842 of the 6 April 2018. Salivary samples were collected by suction method soon after the animal immobilization following a gentile cheek massage to stimulate saliva production. Saliva samples were aspirated directly from the animal oral cavity using a 3 ml plastic Pasteur and kept in 15 ml falcon tubes. Collected samples were stored at −20°C until analysis.

2.2 | Enzymatic assays

All reagents were purchased from Sigma-Aldrich. Spectrophotometric analyses were performed by an EnSpire 2300 Multilabel Reader (PerkinElmer), a Multiskan FC reader (Thermo Scientific) and a Lambda 25 UV/VIS spectrometer (PerkinElmer). Salivary samples were diluted in distilled water before protein quantification and enzymatic analysis.

Salivary samples were diluted 1:40 to measure protein concentration using γ-globulin as standard in accordance with Bradford method (1976). For all analysis, two replicates for each sample were assayed.

Trypsin assay was performed using 20 mM N-α-benzoyldlarginine-p-nitroanilide (BAPNA) as substrate in accordance with Bisswanger (2004). Samples were diluted 1:16. Trypsin (type II from porcine pancreas, EC 3.4.21.4) was used to draw the standard curve. The absorbance was measured at 405 nm. Chymotrypsin assay was performed in accordance with Hummel (1959) and Wirnt (1974) protocols. N-Benzoyl-L-Tyrosine Ethyl Ester (BTEE) 1.18 mM was used as substrate. A solution containing 80 mM Tris/HCl buffer, 100 mM calcium chloride pH 7.8 and sample (1:16) was measured at 256 nm for 10 min. α-chymotrypsin was used to draw the standard curve. Trypsin and Chymotrypsin values were expressed as mU/mg proteins.

Aminopeptidase-N assay was performed using 2.04 mM L-alanine-p-nitroanilide as substrate according to Liu and Wang (2007) and del Valle and Mañanes (2008) and values were expressed as U/mg proteins. SOD activity was determined using Superoxide Dismutase Assay kit (Cayman Chemical Company; No. 706002). SOD values were expressed as U/mg proteins.

Catalase activity was determined using Catalase Assay kit (Cayman Chemical Company; No. 707002). Catalase values were expressed as mU/mg proteins.

Glutathione S-transferase was determined using Glutathione S-transferase Assay kit (Cayman Chemical Company; No. 703302). Glutathione S-transferase values were expressed as mM/mg proteins. Samples for antioxidant enzyme assays were diluted 1:40.

Alkaline and acidic phosphatase activities were determined in accordance with Binder et al. (1987). Alkaline and acidic phosphatases values were expressed as mU/mg proteins and U/mg proteins, respectively. Samples for phosphatase enzymes assays were diluted 1:10.

Amylase assay was performed according to Hassanabatar et al. (2013) with some modifications. Rice starch in 0.1 M Phosphate buffer pH 6.8 was used as substrate. 0.5 ml of substrate and 0.2 ml 1% NaCl were incubated at 37°C for 10 min. Afterwards, 0.3 ml of saliva sample (1:20), was added and incubated at 37°C for 15 min. The reaction was stopped adding 1ml of dinitro salicylic reagent (1% of 3,5 Dinitro salicylic acid in 0.4 M NaOH, 1.06 M $KNaC_4H_4O_6{\cdot}4H_2O$) and left boiling in water for 10 min. The solution was cooled and 10 ml of distilled water was added. The colour developed was measured at 520 nm. Maltose was used for the standard curve. Values were expressed as mg maltose produced/mg proteins.

Lignin peroxidase activity (LiP) was determined in accordance with Gomes et al. (2009) and expressed as mU/mg proteins.

Cellulase activity was determined using carboxymethylcellulose (CMC) as substrate according to Gusakov et al. (2011) and expressed as U/mg proteins.

Chitinase assay was carried out using chitin azure as substrate according to Byrne et al. (2001) with some modifications. Twenty-five μl of sample was incubated at 25°C with 25 μl of chitin azure and 200 μl of 50 mM sodium acetate buffer pH 5.0 for gradually longer periods of time (e.g. 0, 1, 2, 3 and 5 min). The reaction was stopped by adding of 2 M HCl. The solution was cooled, centrifuged and the supernatant was read at 550 nm. Values were expressed as ΔAbs/min/mg proteins.

2.3 | Statistical analysis

Data are presented as the mean, standard deviation and range (min and max values) for each enzyme activity. Statistical analysis for SOD, catalase, glutathione S-transferase, alkaline phosphatase, acidic phosphatase, lignin peroxidase and chitinase activities, grouping data in males vs females and adults vs youngsters (i.e. sub-adults, juveniles and porcupettes) were performed using JMP software (SAS Institute, 2008). Normal distribution of data was assessed by Shapiro–Wilk test. Since alkaline phosphatase, acidic phosphatase, lignin peroxidase and chitinase activities values resulted normally distributed, differences among parameters investigated were assessed by the ANOVA test. While, SOD, catalase, glutathione S-transferase activities resulted non-normally distributed, thereby differences on enzymatic activity, between males vs females and adults vs youngster, were assessed using the non-parametric Kruskal-Wallis *H*-test. Differences were considered significant if associated with a $p < 0.05$.

3 | RESULTS

Overall, 16 crested porcupines, 5 adult females, 5 adult males, 3 sub-adult females and 3 porcupette females were captured. From

TABLE 1 Enzymatic assays on saliva samples of captured crested porcupines grouped based on proteases, antioxidant enzymes, phosphatase enzymes and enzymes which hydrolyse carbohydrates. For each enzyme the mean, SD, minimum (Min), maximum (Max) value and measurement unit are reported

n	Enzymes	Mean (SD)	Min	Max	Units
Proteases					
4	Trypsin	228.35 (59.64)	160.35	332.87	mU/mg proteins
3	Chymotrypsin	389.57 (230.44)	123.02	701.44	mU/mg proteins
3	N-Aminopeptidase	4.01 (2.99)	1.96	7.44	U/mg proteins
Antioxidant enzymes					
10	Superoxide dismutase	3.13 (3.58)	0.39	12.76	U/mg proteins
10	Catalase	130.80 (110.65)	9.87	380.37	mU/mg proteins
10	Glutathione S-Transferase	20.21 (16.62)	4.92	63.27	mM/mg proteins
Phosphatase enzymes					
9	Alkaline phosphatase	5.91 (6.12)	1.06	19.96	mU/mg proteins
8	Acidic phosphatase	19.00 (16.16)	7.11	52.78	U/mg proteins
Carbohydrate-hydrolysing enzymes					
3	Amylase	49.61 (21.09)	31.66	80.81	mg of maltose produced/mg proteins
7	Lignin peroxidase	0.46 (0.28)	0.28	0.61	mU/mg proteins
3	Cellulase	28.78 (17.30)	1.06	45.59	U/mg proteins
7	Chitinase	6.32 (7.48)	0.55	21.62	ΔAbs/min/mg proteins

each captured individual a saliva sample was collected and analysed (Table 1). The mean enzymatic activity of trypsin, chymotrypsin and N-Aminopeptidase resulted 228.35 SD 59.64 mU/mg proteins, 389.57 SD 230.44 mU/mg proteins and 4.01 SD 2.99 U/mg proteins, respectively. The superoxide dismutase resulted higher (3.13 SD 3.58 U/mg proteins) than those of catalase (130.80 SD 110.65 mU/mg proteins) and glutathione S-transferase (20.21 SD 16.62 mM/mg proteins). Among phosphatase enzymes, alkaline phosphatase activity resulted lower (5.91 SD 6.12 mU/mg proteins) than acidic phosphatase (19.00 SD 16.16 U/mg proteins) with highest values of saliva alkaline phosphatases recorded in youngster individuals. Mean values of amylase activity resulted 49.61 SD 21.09 mg of maltose produced/mg proteins, of lignin peroxidise 0.46 SD 0.28 mU/mg proteins, of cellulase 28.28 SD 17.30 U/mg proteins and of chitinase activity 6.32 SD 7.48 ΔAbs/min/mg proteins.

No significant differences were observed for SOD, catalase, glutathione S-transferase, acidic phosphatase, lignin peroxidase and chitinase activities grouping data in males vs females and in adults vs youngsters, $p > 0.05$.

Alkaline phosphatase activity resulted higher in salivary samples collected from youngsters compared to those collected from adults ($p < 0.02$) while no significant difference was observed between males and females ($p > 0.05$).

4 | DISCUSSION

The enzymatic activity in crested porcupine saliva was here investigated for the first time. Results obtained in this investigation show a rich variety of enzymes in crested porcupine saliva as reported in other rodent species such as *Rattus norvegicus*, *Rattus rattus*, *Cavia porcellus*, *Dasyprocta azarae* and *Cricetus auratus* (Junqueira & Moraes, 1965). In crested porcupine saliva, a remarkable activity level of proteases, antioxidant and phosphatase enzymes, as previously reported in rat saliva by Chauncey et al. (1963), Ikeno et al. (1986) and Zalewska et al. (2014) was recorded.

Proteolytic activity in crested porcupine saliva was recorded as in rodents and humans (Ikeno et al., 1986; Pigman & Reid, 1952). In rodents, a remarkably strong proteolytic activity in the saliva, as well as in salivary glands, was recorded (Junqueira & Moraes, 1965). House mouse saliva proteome includes chemical signal transporters, antibacterial and immunity linked proteins, and many other proteins that are involved in general physiology of the oral cavity (Stopka et al., 2016). Nasal and lacrimal proteins, including odorant binding proteins (OBP) were also detected in house mouse saliva (Stopka et al., 2016). Odorant-binding proteins in nasal tissue of crested porcupine were also detected suggesting a discriminating function of these proteins in the process of odour perception and chemical communication (Felicioli et al., 1993). No data are available on crested porcupine saliva proteome, nevertheless, we cannot exclude that as reported in house mouse, also in porcupine, proteins expressed in olfactory tissues may also be transported to the oral cavity. Therefore, a possible hypothesis is that proteolytic activity in crested porcupine saliva could be linked to high variability in porcupine-biased proteins with a potential role in chemical communication. Conversely, in humans, most of the saliva proteins were identified as part of the first line of defence against inflammatory processes (Vitorino et al., 2004) and high proteolytic activities are at the basis of multiple pathological conditions (Sun et al., 2009).

N-aminopeptidase activity in crested porcupine saliva resulted higher than those obtained in the small intestinal tissue of *Mongolian unguiculatus*, an omnivorous rodent (Liu & Wang, 2007) and lower than those reported in the intestinal tissue of *Ctenomys talarum*, an herbivorous rodent (del Valle & Mañanes, 2008). *C. talarum* shows a seasonal plasticity of N-aminopeptidase activity in small and large intestines, probably as an adaptive strategy to face seasonal variations of diet composition and environmental factors (e.g. thermoregulation) (del Valle & Mañanes, 2008). Seasonal differences in the food range available to porcupine occur in both study areas of this investigation. This may lead to hypothesise that in crested porcupine, variation in N-aminopeptidase activity in saliva, may be occurring in relation to seasonal food availability as also reported in *C. talarum* by del Valle and Mañanes (2008). Therefore, seasonal investigations on N-aminopeptidase activity variation in crested porcupine saliva are needed. In the omnivorous rodent *Phyllotis darwini*, N-aminopeptidase activity in small intestinal tissue has been demonstrated to be upregulated by dietary proteins while no effect was observed in the herbivorous rodent *Octodon degus* (Sabat et al., 1999). In crested porcupine, despite the saliva and intestinal enzyme activity across species is not comparable, N-aminopeptidase activity pattern resulted similar to those reported for omnivorous rather than for herbivorous rodents. Variation in N-aminopeptidase activity in crested porcupine saliva could also be related to a physiological state since it has shown scavenging behaviour (Coppola, Guerrieri, et al., 2020). Further investigations focussed on assessing physiological variation in N-aminopeptidase activity in crested porcupine saliva are also desirable.

In crested porcupine saliva, activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione S-transferase was also detected. The detection of glutathione S-transferase activity is intriguing since inducers of glutathione S-transferase are abundant in vegetables such as Cruciferae and Liliaceae which are part of porcupines diet (Santini, 1980). Inclusion of Cruciferae and Liliaceae in human diet determines an increase of glutathione S-transferase activity (Sreerama et al., 1995) while no data are available concerning their effects on enzymatic activity in rodent's saliva as well as in other animal species. However, a possible effect of these vegetal species on glutathione S-transferase activity in relation to their seasonal availability and to crested porcupines feeding behaviour, could be hypothesized.

Among antioxidant enzymes investigated in crested porcupines, the superoxide dismutase (SOD) activity resulted higher than those of catalase and glutathione S-transferase. High levels of SOD activity were recorded in crested porcupine whose permanence in the cage traps exceeded 12 h, suggesting that stress induced by the capture and immobilization event can have altered the levels of antioxidant enzyme activity. These results seem to confirm the key antioxidant role of SOD as the first anti-radical scavenging activity as observed in rats and humans (Nagler et al., 2002; Pigman & Reid, 1952; Zalewska et al., 2014).

Antioxidant system of salivary parotid gland cells was proved to be efficient in the elimination of reactive oxygen species (ROS) during oxidative stress both in rats and humans, with decreasing of total antioxidant status during oxidative stress associated to pathological state (Nagler et al., 2002; Zalewska et al., 2014). Therefore, due to the presence of easily measurable indicators of oxidative processes in saliva, this organic fluid was indicated as an alternative biological substrate for early detection and monitoring of many diseases both in rats and humans (Chojnowska et al., 2018; Zalewska et al., 2014).

The oxidative enzymes also have an important role in thermoregulation. Increased baseline activity of antioxidant enzymes seems to be an evolutionary adaptation of animals to oxidative stress (Blagojevic et al., 2011; Xu et al., 2019). Haim et al. (1990) observed that cape porcupine shows good thermoregulation ability up to 30°C with an increase in overall thermal conductance and respiratory rate. Therefore, despite no data are available for thermoregulation abilities in crested porcupine, it could be hypothesized that oxidative enzymes have a physiological role in radical free metabolism in order to prevent oxidative stress. Since reduction of body temperature is a possible effect of administration of Zoletil®100 during animal immobilization (Fahlman et al., 2006) it could be hypothesized that anaesthesia could affect the levels of antioxidant enzymes activity recorded in crested porcupine. Therefore, investigation of redox enzymes in crested porcupine saliva could provide a valuable monitoring tool for health and disease assessment as well as an indicator of environmental adaptation. As observed in rat saliva by Chauncy et al. (1963), alkaline and acid phosphatases activities were also detected in crested porcupine. Phosphatases in crested porcupine saliva are interesting because their possible effect on teeth decalcification processes may lead to osteophagia practice (Pigman & Reid, 1952; Van Jaarsveld, 1983). In crested porcupine, the average alkaline phosphatase activity resulted lower than those of acid phosphatase as previously observed in human's saliva (Chauncey et al., 1954). Furthermore, alkaline phosphatases in crested porcupine resulted significantly higher in youngsters than adults. Levels of alkaline phosphatases (ALP) in saliva may be used as chemical biomarkers for identification of skeletal maturational stages in rats and humans (Pellegrini et al., 2008; Tarvade et al., 2015), as well as for pubertal growth phase in humans (Irham & Bahirrah, 2018). Therefore, also in crested porcupine, levels of ALP in saliva may be a useful indicator to evaluate systemic bone turnover status or pubertal growth phase identification.

As previously reported in other rodent species such as *R. norvegicus*, *R. rattus*, *C. porcellus*, *D. azarae* and *C. auratus* (Chauncy et al., 1963; Junqueira & Moraes, 1965) and human saliva (Pigman & Reid, 1952) amylase activity was also detected in crested porcupine saliva. In the study areas the porcupine diet is mainly composed by bulbs, tubers and rhizomes of many wild and cultivated plants such as *Cyclamen* sp., mainly available in autumn; *A. maculatum* and *Rumex* sp., available throughout the year; *Asparagus* sp. and *Allium* sp., available in spring and *Solanum tuberosum*, available in summer. Since underground storage organs of all these plants are rich in starch, the detection of amylase activity in porcupine

saliva is not surprising. Results obtained in this investigation showed that also lignin peroxidase, cellulose and chitinase activity were present in crested porcupine saliva. In crested porcupine two strains of obligatory anaerobic cellulolytic bacteria designated as PS7 and PS8, able to degrade cellulose, were isolated from hindgut fluid (Odenyo et al., 1999). Riccardi and Bruno (1996) observed that captive crested porcupines consume 575 g of barks/day. Debarking of saplings by porcupine, in particular of *Fraxinus ornus*, at the base of the trunk mainly occurs in the winter months (Santini, 1980). Therefore, the lignin peroxidase and cellulases enzymatic activity recorded could be related to porcupine feeding habits.

No data are available on chitinase activity in other rodent species, however, the presence of this enzymatic activity in crested porcupine could be a defence mechanism against invasion by chitin-containing pathogens as in humans as suggested by Van Steijn et al. (1999).

In conclusion, the results obtained in this investigation support the prediction that the enzymatic activity in crested porcupine saliva is similar to those of other omnivorous rodent and give a biochemical support to the ethological evidences previously reported by Coppola, Guerrieri, et al. (2020). Moreover, this investigation provides new knowledge on crested porcupine saliva enzymes that may be a useful tool for further investigation on the adaptive response of crested porcupine in relation to different environmental condition and diet and for the evaluation of health and nutritional status of this rodent.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceived the study: AF, FC, SS; Designed the experiment: AF, FC, SS; Performed the field activities and sampling: AF, FC, LC; Performed the laboratory work: SS, LB, PL, GG; Analysed and interpreted data: AF, FC, SS, LB, PL, LC, GG; Wrote the original draft of the manuscript: AF, FC, SS; Reviewed and edited the final version of the manuscript: AF, FC, SS, LB, PL, LC, GG; Supervision: AF.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The capture-marking activity of resident porcupines was approved by the Italian Institute for Environmental Protection and Research (ISPRA) with protocol number 22584 of the 8 May 2017 and protocol number 150071 of the 16 March 2018 and by Tuscany Region with the Decree n. 14235 of the 3 October 2017 and Decree n. 4842 of the 6 April 2018.

DATA AVAILABILITY STATEMENT

All data are available from the corresponding author.

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