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# NICHE PARTITIONING AND COMPETITION BETWEEN DIFFERENT RABBIT BREEDS USING STABLE ISOTOPES

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Abstract: Stable isotope analysis (SIA) is an evolving method for determining diet, understanding food web and resolving biogeochemical issues in the ecosystem. This study aims to trace out ecological niche preferences/partitioning and competition among the lagomorphs, including two different breeds of European rabbit (*Oryctolagus cuniculus*), New Zealand rabbit and American Dutch rabbit, using SIA. Thirty-two samples of tooth enamel were analysed, which were collected from different districts of Punjab, Pakistan, including Okara, Sahiwal and Kasur. Among these samples, 16 belonged to the New Zealand breed (08 male and 08 female rabbits) and 16 to the American Dutch breed (08 male and 08 female rabbits). Significant ($P$<0.001) intergender differences in the isotope content of $\delta^{13}$C in the enamel for New Zealand and American Dutch rabbit were found. The European rabbits showed significant differences for both genders in the stable isotope of oxygen in the enamel ($\delta^{18}$O) values ($P$=0.05). Nitrogen stable isotope results showed no significant intergender differences between American Dutch and New Zealand rabbits ($P$=0.24). The stable isotope results for $\delta^{13}$C, $\delta^{15}$N, and $\delta^{18}$O indicate that the trophic niche partitioning of both breeds overlaps, which can potentially cause competition for resources, whereas the water intake may differ among different genders, which may reflect differential gender-related activities. The archaeological and fossilised data of lagomorphs is present, but there is no significant literature available for living lagomorphs (rabbits). In general, this study provides a basic and first dataset for $\delta^{13}$C, $\delta^{15}$N, and $\delta^{18}$O of living lagomorphs, which can serve as a comparative dataset for future studies.

Key Words: enamel, dietary preference, ecosystem, competition, community ecology, rabbits.

## INTRODUCTION

Stable isotope analysis (SIA) is a rapidly growing method (Walter *et al.*, 2014) for determining diet (Hussey *et al.*, 2012) and environmental implications of animals (Ehleringer and Osmond, 2000), reconstructing individuals' life histories, understanding food web structures (Pecquerie *et al.*, 2010) and resolving biogeochemical issues in ecosystems (Peterson and Fry, 1987). The environmental landscapes of prehistory can be revealed by studying the stable isotopes in the bones and teeth of ancient animals (Koch, 1998; Hedges *et al.*, 2004; Somerville *et al.*, 2020).

Stable isotopes of carbon and nitrogen are widely used in ecological studies, for example on animal migration and the natural food web (Camin *et al.*, 2007; Sandberg *et al.*, 2012). The $\delta^{13}$C values of animal bone tissues correlate with the $\delta^{13}$C values of plants, ingested at the bottom of the food chain (DeNiro and Epstein, 1978; Schoeninger and DeNiro, 1984; Somerville *et al.*, 2018). Carbon from the atmosphere is fixed in plant tissues through the photosynthetic cycle

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(Somerville *et al.*, 2020). The $C_3$ photosynthetic pathway is mostly found in humid shrubs, forbs, trees and leguminous species, which show $\delta^{13}C$ values ranging from –35 to –20‰ (O'Leary, 1988; Kohn, 2010; Somerville *et al.*, 2018). $C_4$ plants are mostly dry seasoned grasses, with $\delta^{13}C$ values ranging from –15 to –7‰ (O'Leary, 1988; Somerville *et al.*, 2018). In turn, CAM (crassulacean acid metabolism) photosynthesis is predominant in xerophytes (such as succulents) and epiphytes (such as orchids and bromeliads), with $\delta^{13}C$ values that are in between $C_4$ and $C_3$ plants (Sternberg *et al.*, 1984; O'Leary, 1988; Somerville *et al.*, 2020).

The variation in $\delta^{15}N$ values within terrestrial environments is more complex due to systematic dynamics (Somerville *et al.*, 2018). Diet can be analysed from a quantitative and qualitative point of view (Marín-García *et al.*, 2023a, b). Dietary nitrogen values affect the mammalian bone collagen $\delta^{15}N$ values (DeNiro and Epstein, 1981; Somerville *et al.*, 2018), over broad altitude, latitude and temporal scales, along with seasonality and small geographic areas. The increase in temperature and aridity enhances the opening of the nitrogen cycle, which relatively increases the enrichment of isotope nitrogen and shows a more positive value (Ambrose, 1991; Gröcke *et al.*, 1997; Amundson *et al.*, 2003; Aranibar *et al.*, 2004; Somerville *et al.*, 2018). Animals in cooler and wet environments show lower $\delta^{15}N$ values than those in hot and dry environments (Heaton *et al.*, 1986; Ambrose, 1991; Gröcke *et al.*, 1997; Amundson *et al.*,2003; Aranibar *et al.*, 2004; Stevens *et al.*, 2006; Somerville *et al.*, 2018). Usually, mean annual temperature is directly proportional to the $\delta^{15}N$ levels of soil and plants and inversely proportional to mean annual precipitation (Martinelli *et al.*, 1999; Amundson *et al.*, 2003; Aranibar *et al.*, 2004; Szpak *et al.*, 2013; Craine *et al.*, 2015; Somerville *et al.*, 2018). This variation is attributed to differences in open and closed environments. An open environment means one in which animals rely on surface water for their body water requirements, whereas a closed environment is one in which animals fulfil most of their water requirements from consumed diets (leafy plants).

Warm and dry open systems are more sensitive to the mineral leaching and denitrification processes of soil nitrogen isotope fractionation, whereas cold and moist closed systems store and regenerate mineral nitrogen more efficiently, resulting in lower $\delta^{15}N$ values (Shearer and Kohl, 1986; Austin and Vitousek, 1998; Handley *et al.*, 1999; Amundson *et al.*, 2003; Somerville *et al.*, 2018).

The stable isotope values reveal bone mineral carbonate and organic bone collagen; however, they also indicate slightly distinct diet features. Animals obtain oxygen through three separate processes: (a) drinking water, (b) water extracted from the diet, (c) inhaling, and water is excreted through sweat, exhalation, and urination (Sponheimer and Lee-Thorp,1999; Koch, 2007; Blumenthal *et al.*, 2017; Waseem *et al.*, 2021a). The temperature, amount of precipitation, latitude and altitude influence the $\delta^{18}O$ values of meteoric water ($\delta^{18}O_{ot}$) and the animals that obtain their water by drinking surface water and from feeding (Dansgaard,1964; Waseem *et al.*, 2021a). The variation of $\delta^{18}O_{enamel}$ values reflects the ecological differences among animals. Lower $\delta^{18}O_{enamel}$ values indicate a preference for closed and humid habitats, such as forests, whereas higher $\delta^{18}O_{enamel}$ values suggest an adaptation to open and arid habitats, such as savannahs or grasslands (Feranec and MacFadden, 2006; Waseem *et al.*, 2021a). Based on physiology and behaviour, animals are categorised into two types: (a) evaporation sensitive (ES) and (b) evaporation insensitive (EI) animals. The ES animals fulfil most of their water requirements from the consumed diet (leafy plants) and mostly show ruminant behaviour ($\delta^{18}O$ values of enamel are interconnected to the evaporation of leaf water), whereas EI animals present non-ruminant behaviour that relies on surface water for their body water requirements ($\delta^{18}O_{enamel}$ is linked to local drinking water sources) (Levin *et al.*, 2006; Waseem *et al.*, 2021a).

The archaeological and fossilised ecological data on lagomorphs is available, but to the best of our knowledge there is no SIA-based ecological literature available for living lagomorphs (rabbits). According to Rizwan *et al.* (2021), there are intergender and intraspecific morphometric/craniometric variations in the two breeds of *Oryctolagus cuniculus*: New Zealand rabbit and American Dutch rabbit. This finding leads to an inquiry regarding their dietary preferences, which may also depend on their differential diet or role in the population. However, there is a dearth of information to testify ecological preferences of living lagomorphs (both interbreed differences and intergender differences) based on SIA. Our study aims to trace out the ecological and dietary preferences of lagomorphs and bridge this gap by analysing the SIA of two different European rabbit breeds (*Oryctolagus cuniculus*), namely New Zealand rabbit and American Dutch rabbit.

## MATERIALS AND METHODS

### *Ethical approval*

Approval for this animal-based study was granted by the Departmental Ethical Committee, Department of Zoology, University of Okara, Pakistan, Letter No. UO/DOZ/2020/195b, Dated: 08-05-2020.

### *Sampling*

Tooth enamel samples of two breeds of (*Oryctolagus cuniculus*), New Zealand rabbit and American Dutch rabbit, were analysed for the estimation of stable isotopes ratio of carbon, oxygen and nitrogen. The animals were captured with animal traps usually used for rodents. Animals were collected from the different areas/locations of the three districts: Okara, Sahiwal and Kasur of Punjab, Pakistan (Figure 1).

### *Data collection*

A collection of 32 European rabbits (*Oryctolagus cuniculus*) having maxillae and mandibles portion in addition to upper and lower incisors were used for SIA. Among this collection of 32 breed samples; 16 belonged to the New Zealand rabbit breed (08 male and 08 female rabbits) and 16 belonged to the American Dutch breed ( 08 male and 08 female rabbits). Google Lens and the ARBA (American Rabbit Breeders Association) website were used to identify the rabbit breeds. Most of the rabbits were collected from different villages, where they were in semi-domesticated conditions and were free to choose their food among the different types of vegetation present in the villages of Punjab, Pakistan.

### *Enamel extraction*

To acquire a drilled sample of tooth enamel, molars were selected. The powdered enamel was extracted by drilling the samples using a M-3 Champion drill machine. In a confined chamber, drilling was done from the lingual side of the tooth from the root to crown direction (bulk samples). A fine-tipped brush was used to collect the powder and transfer it to plastic vials. Fifteen milligrams of drilled enamel powder were processed for further analysis (Waseem *et al.*, 2021b).

### *Pre-treatment and analysis of samples*

Powdered enamel was pre-treated with 10 mL of 2% NaOCl for 1 h to dissolve the organic matter, then decanted and rinsed three times with distilled water. The samples were then treated with 10 mL of 0.1% acetic acid for 1 h to eradicate exogenous carbonates (Waseem *et al.*, 2021b).

The isotopic analysis of $\delta^{13}C_{enamel}$, $\delta^{18}O_{enamel,}$ and $\delta^{15}N_{enamel}$ was subjected to the Isotope Ratio Mass Spectrometer at PINSTECH (Pakistan Institute of Nuclear Science and Technology), Islamabad. The isotope measurements are calibrated based on repeated measurements of NBS-19 and NBS-18, and precision is 0.08‰ for $\delta^{13}C$ (V-PDB), 0.11‰ for $\delta^{18}O$ (V-PDB) (Waseem *et al.*, 2021b) and 0.2‰ for $\delta^{15}N$ ($N_2$). ANOVA (analysis of variance) was applied using SPSS version 16.00 for statistical analysis.

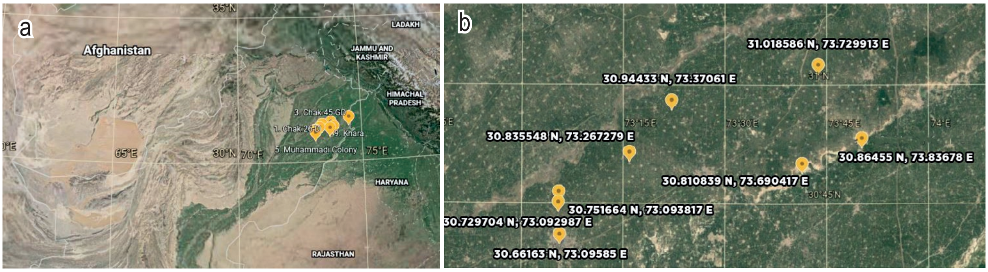


Figure 1: a) Localities of different villages of three districts (Kasur, Okara and Sahiwal) of Punjab Pakistan; b) the coordinates of the localities where the rabbits were collected for current SIA.

## RESULTS

### *Carbon isotopes (δ13C)*

The maximum value for the $\delta^{13}C$ isotope of New Zealand male rabbits was –10.2‰ and the minimum was –10.5‰, with an average value of –10.4‰. In turn, the female population of New Zealand rabbits showed a range from –12.2 to –14.8‰. The average value among the female rabbits was –13.3‰. The mean value and standard deviation of New Zealand male and female rabbits were –10.4±0.1 and –13.3±1.0‰, respectively. The maximum value for American Dutch female rabbits was –13.8‰ and the minimum value was –15.8‰, whereas the mean value was –15.3‰. The mean value and standard deviation of American Dutch male and female rabbits were –13.3±1.2 and –15.3±0.8‰, respectively. The breed-wise comparison for male (New Zealand and American Dutch) and female (New Zealand and American Dutch) rabbits showed mean value and standard deviation of –10.4±0.1 to –13.3±1.2‰ and –13.3±1.0 to –15.3 ±0.8‰, respectively (Table 1). ANOVA showed significant intergender differences in $\delta^{13}C$ values of New Zealand rabbits and American Dutch rabbits ($P<0.001$). The breed-wise comparison also demonstrated significant differences in male rabbits ($P<0.001$), but no significant differences were observed for female New Zealand and American Dutch rabbits ($P=0.06$).

### *Oxygen isotopes (δ18O)*

The $\delta^{18}O_{enamel}$ values of New Zealand male rabbits ranged from +2.9 to +2.5‰ with a mean value of +2.8‰, while the female population of rabbits showed a range from +2.8 to +2.3‰. The mean value among the female rabbits was +2.6‰. The maximum value for $\delta^{18}O_{enamel}$ of American Dutch male rabbits was +3.6‰ and the minimum was +2.3‰ with a mean value of +2.8‰. However, the maximum and minimum value of American Dutch female rabbits was +4.4 and +2.6‰ respectively. The mean value was +3.2‰ between the female rabbits (Table 1). The mean value and standard deviation for both genders of New Zealand and American Dutch rabbits were 2.7±0.2 and 3.2±0.6‰, respectively (Table 1). ANOVA indicated that $\delta^{18}O_{enamel}$ for both genders of New Zealand and American Dutch rabbits were significantly different ($P<0.05$).

### *Nitrogen isotopes (δ15N)*

The maximum value for $\delta^{15}N$ of New Zealand male rabbits was +14.8‰ and the minimum value was +11.4‰, with a mean value of +13.9‰, while the female rabbits showed a range from +14.9 to +12.4‰. The mean value was +13.8‰ between the female rabbits. There was a mean value of +13.5‰ between the maximum value of +15.5‰ and the minimum value of +12.3‰ of American Dutch male rabbits. The maximum value of American Dutch female rabbits was +13.5‰ and the minimum value was +10.5‰. The mean value among the female rabbits

Table 1: Values of $\delta^{13}C_{enamel}$ (‰), $\delta^{18}O_{enamel}$ (‰), and $\delta^{15}N_{enamel}$ (‰) of New Zealand and American Dutch Rabbits, showing the comparative differences in minimum, maximum and mean values for each breed.

| | $\delta^{13}C_{enamel}$ (‰) | | | $\delta^{18}O_{enamel}$ (‰) | | | $\delta^{15}N_{enamel}$ (‰) | | |
|---|---|---|---|---|---|---|---|---|---|
| Animal Breed | Min. | Max. | Mean±SD | Min. | Max. | Mean±SD | Min. | Max. | Mean±SD |
| New Zealand male rabbits | –10.5 | –10.2 | –10.4±0.1 | +2.5 | +2.9 | +2.8±0.2 | +11.4 | +14.8 | +13.9±1.1 |
| New Zealand female rabbits | –14.8 | –12.2 | –13.3±1.0 | +2.3 | +2.8 | +2.6±0.2 | +12.4 | +14.9 | +13.4±1.1 |
| American Dutch male rabbits | –14.2 | –11.4 | –13.3±1.2 | +2.3 | +3.6 | +2.8±0.6 | +12.3 | +15.5 | +13.5±1.3 |
| American Dutch female rabbits | –15.8 | –13.8 | –15.3±0.8 | +2.6 | +4.4 | +3.2±0.6 | +10.5 | +13.5 | +11.9±1.3 |

SD: standard deviation.

was +11.9‰ (Table 1). The mean value and standard deviation for both genders of New Zealand and American Dutch rabbits were 13.2±1.1 and 12.7±1.3‰, respectively (Table 1). ANOVA showed that $\delta^{15}N$ for both genders of New Zealand and American Dutch rabbits were not significantly different ($P$= 0.24).

## DISCUSSION

### *Dietary Insights based on SIA*

According to Somerville *et al.* (2020), the three archaeological sites of Pueblo Grande, La Ferreria and La Quemada, respectively, had $\delta^{13}C_{carbonate}$ and $\delta^{13}C_{collagen}$ from *Sylvilagus* that consumed $C_4$ vegetation with little or no CAM or mixed $C_3/C_4$ vegetation (–9.0±1.9 and –16.1±2.9‰, –6.1±2.2 and –12.7±2.3‰ assuming $^{13}C_{enrichment}$=+12.8) (Figure 2). Our findings show that both breeds of *Oryctolagus cuniculus* consumed $C_3$ vegetation, whereas this archaeological $\delta^{13}C_{carbonate}$ *Sylvilagus* consumed $C_4$ vegetation, probably in the form of forestland and woodland, whereas $\delta^{13}C_{collagen}$ of *Sylvilagus* contrasts with our findings due to the dominance of $C_4$ vegetation, with very little CAM or mixed $C_3/C_4$ vegetation. Due to a shortage of food supplies, there is rivalry between New Zealand and American Dutch rabbits. Their foraging habits are another factor, although in this case there is no competition, as there are so many resources available according to Somerville *et al.* (2020).

The present study shows that both breeds of *Oryctolagus cuniculus* consumed $C_3$ vegetation and presented competition for feeding resources. According to Somerville *et al.* (2018), the fossilised cottontail rabbits (–17.9±2.3 and –12.3±2.7‰ assuming $\delta^{13}C_{enrichment}$=+12.8) belonged to the United States and Mexico, which contrasted with our results. The results of Somerville *et al.* (2018) showed that $\delta^{13}C_{collagen}$ of fossilised cottontails rabbits was conquered by CAM flora, whereas $\delta^{13}C_{carbonate}$ of fossilised cottontails rabbits was dominated by $C_4$ vegetation with low moisture, warm temperatures and long periods of sunlight (Ehleringer, 1978; Stowe and Teeri, 1978; Tieszen *et al.*,1979).

Somerville *et al.* (2017) reported $\delta^{13}C_{collagen}$ results of *Sylvilagus* for different archaeological sites (–19.7 to –16.2‰, –20.1 to –11.2‰, –22.3 to –10.2‰) as well as modern sites (Tlalpan –18.5 to –17‰, Tulancingo –19.9 to –13.9‰) of Teotihuacan consumed $C_4$ vegetation with very little CAM vegetation (Figure 2). The *Sylvilagus* $\delta^{13}C_{collagen}$ ranges for different archaeological sites as well as modern sites contrast with our results. The archaeological sites dominate $C_4$ vegetation with very little CAM vegetation and modern sites dominate CAM/$C_4$ vegetation (grasses).

As reported by Somerville *et al.* (2016), the archaeological results for *Sylvilagus* of Teotihuacan of $\delta^{13}C_{collagen}$ (–13.1 to –4.9‰, –12.9 to 4.9‰, –12.7 to –8.1‰) indicated that *Sylvilagus* fed $C_4$ vegetation with very little CAM, while modern results from different archaeological sites (Tlalpan –13.6 to –11.7‰, Zimapan –11.9 to –10.8‰) showed that *Sylvilagus* dominantly consumed CAM/$C_4$ vegetation (Figure 2). The results of Somerville *et al.* (2016) contrasted with those of our study. This is because our study interpreted that both breeds of *Oryctolagus cuniculus* consumed $C_3$ vegetation and showed competition present between New Zealand and American Dutch rabbits due to a lack of diet resources, and the other possibility is their foraging behaviour.

In our results, a gender-wise comparison of results of New Zealand and American Dutch rabbits indicated that American Dutch rabbits had higher values of oxygen isotope compared to New Zealand rabbits, while breed-wise comparison concluded that both genders of American Dutch rabbits had higher oxygen values compared to those of both genders of New Zealand rabbits (Figure 3). The interpretation of our results is that American Dutch rabbits live in open and arid areas, as they have higher oxygen values. New Zealand rabbits inhabit closed and humid conditions and tend to show lower oxygen values. This suggests that American Dutch

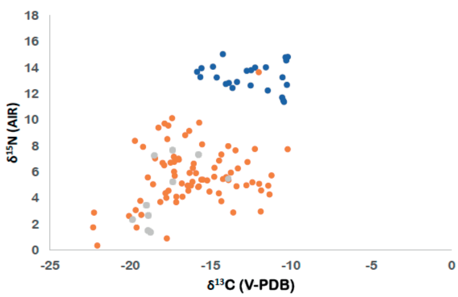


Figure 2: Comparative study between current (*Oryctolagus cuniculus*) data and archaeological and modern *Sylvilagus* data. • Current study; • *Sylvilagus* (Archaelogical data); • *Sylvilagus* (Modern).

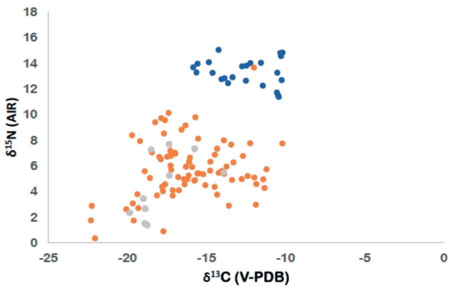


Figure 3: $\delta^{13}C_{enamel}$ versus $\delta^{18}O_{enamel}$ results of two breeds of European rabbits (*Oryctolagus cuniculus*), i.e. New Zealand rabbit and American Dutch rabbit. ● Current study; ● *Sylvilagus* (Archaelogical data); ● *Sylvilagus* (Modern).

rabbits acquire water completely from the vegetation, but New Zealand rabbits utilise freshwater resources (Waseem *et al.*, 2021a). There are different reasons for the higher value of oxygen isotopes which rabbits acquire in water through (i) diet; (ii) stream water sources have high values of oxygen isotopes due to lower evaporation rate and those rabbits which acquired water through stagnant or active water sources have low values of oxygen isotopes due to higher evaporation.

According to Somerville *et al.* (2020), the mean and standard deviation $\delta^{18}O$ of *Sylvilagus* for three different archaeological sites (Pueblo Grande, La Ferreria and La Quemada) were 29.7±2.9, 25.5±2.3 and 23.9±2.4‰, respectively, which indicated that *Sylvilagus* inhabited arid and open environments and showed a higher rate of evaporation. These *Sylvilagus* results contrast with our results, which have low oxygen values that indicate closed and humid conditions with a lower evaporation rate (Waseem *et al.*, 2021a). In turn, cottontail rabbits in the United States and Mexico with high $\delta^{18}O$ values (26.6±3.1‰) also contradict our results, as they to inhabited open and arid environments with low relative humidity and showed a higher rate of evaporation (Somerville *et al.*, 2018).

Somerville *et al.* (2016), reported the $\delta^{18}O$ ranges 23.7 to 30.2‰, 20.9 to 27.2‰, 21.5 to 28.1‰, 23.7 to 29.6‰ and 21.3 to 30.6‰ for the different archaeological sites Moon Pyramid, Oztoyahualco, Teopancazco, Puerta5 and Cuevas, respectively, for *Sylvilagus* of Teotihuacan. The $\delta^{18}O$ ranges for modern sites Tlalpan 24.6 to 26.7‰, Tulancingo 23.4 to 25.5‰, Zimapan 26.5 to 27.0‰ and San Baltazar Tetela 25.3 to 28.2‰, which contrasts with our results due to the high $\delta^{18}O$ compared to the current study. The archaeological and modern *Sylvilagus* $\delta^{18}O$ inhabited arid and open environments with higher evaporation rates (Waseem *et al.*, 2021a).

For nitrogen stable isotope ($\delta^{15}N$) values, a gender-wise comparison of results for New Zealand and American Dutch rabbits revealed that American Dutch rabbits had higher values of $\delta^{15}N$ compared to New Zealand rabbits, while breed-wise comparison concluded that male American Dutch rabbits had higher $\delta^{15}N$ values compared to the male New Zealand rabbits, and female New Zealand rabbits had higher $\delta^{15}N$ value compared to the female American Dutch rabbits (Figure 4). The interpretation of our results is that male American Dutch rabbits and female New Zealand rabbits both reside in hot and dry environment and come outside for feeding sources from their burrows and showed higher $\delta^{15}N$ values, and male New Zealand rabbits and female American Dutch rabbits both had cold and wet environments with lower $\delta^{15}N$ values.

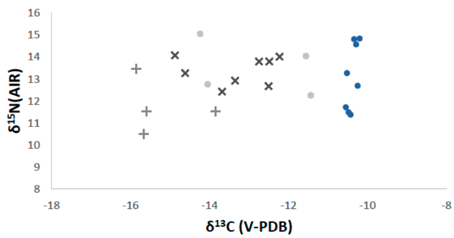


Figure 4: $\delta^{13}C_{enamel}$ versus $\delta^{15}N_{enamel}$ results for two breeds of European rabbits (*Oryctolagus cuniculus*), i.e. New Zealand rabbit and American Dutch rabbit. ● New Zealand rabbit (M); × New Zealand rabbit (F); ● American Dutch rabbit (M); + American Dutch rabbit (F).

According to Somerville *et al.* (2020), the mean and standard deviation $^{15}N_{collagen}$ of *Sylvilagus* for the three different archaeological sites of Pueblo Grande, La Ferreria and La Quemada are 8.0±1.7, 5.8±1.9 and 6.2±1.6‰, respectively, (Figure 2) which contrasts with our results, with lower $^{15}N_{collagen}$ values. The archaeological *Sylvilagus* data show cooler and wetter environments with high mean annual precipitation compared to the New Zealand and American Dutch breeds.

Somerville *et al.* (2018), said that the $^{15}N_{collagen}$ range (5.6±2.8‰) for cottontail rabbits in the United States and Mexico indicated that they inhabited cooler and wetter environments with lower $^{15}N_{collagen}$ values which contrasted with our results. The cottontail rabbits of the

United States and Mexico have low $^{15}N_{collagen}$ values compared to current data on New Zealand and American Dutch breeds. The New Zealand and American Dutch breeds inhabit a hot and dry environment with higher $^{15}N_{collagen}$ values. Other environmental factors, such as mean annual precipitation, also indirectly influence the $^{15}N_{collagen}$ values. So, cottontail rabbits of the United States and Mexico experience higher mean annual precipitation than stated in the current study data.

Somerville *et al.* (2017), reported the $\delta^{15}N_{collagen}$ ranges from 1.7 to 10.1‰, 0.9 to 9.8‰, 2.9 to 6.9‰, 0.3 to 8.0‰ and 4.4 to 13.7‰ for the different archaeological sites Cuevas, Moon Pyramid, Oztoyahualco, Puerta5 and Teopancazco, respectively, for *Sylvilagus* of Teotihuacan and $\delta^{15}N_{collagen}$ ranges for the modern sites Tlalpan 5.2 to 7.3‰, Tulancingo 2.3 to 5.5‰, Zimapan 7.3 to 7.7‰ and San Baltazar Tetela 1.4 to 3.4‰ for *Sylvilagus* show cooler and wetter environments with high mean annual precipitation due to lower $^{15}N_{collagen}$ values (Figure 2). The reason is that *Sylvilagus* do not come outside for feeding. The *Sylvilagus* $\delta^{15}N_{collagen}$ values for different archaeological sites as well as modern sites contrast with our results. The current data indicate that our New Zealand and American Dutch breeds inhabit a hot and dry environment and come outside their burrows to feed, with higher $\delta^{15}N$ in their body. We compared our study data with previously available archaeological literature, as there is no present study on living lagomorphs.

### *Niche partitioning and competition*

According to our results, gender-wise and breed-wise comparison of results of New Zealand and American Dutch rabbits revealed that male rabbits had higher carbon values compared to female rabbits (Figure 3). New Zealand and American Dutch rabbits are in competition with one another for resources. Lack of food supplies is the cause, while their foraging habits are another potential factor. The findings imply that both breeds ingested $C_3$ plants such as rice, oats, wheat, berseem (*Trifolium alehandrinum*) and alfalfa.

## CONCLUSION

The current results $\delta^{13}C$ conclude that both New Zealand and American Dutch breeds of European rabbit consumed $C_3$ vegetation. The New Zealand breed of *Oryctolagus cuniculus* had higher $\delta^{13}C$ values compared to American Dutch rabbits. This indicates that New Zealand rabbits have an open vegetation dietary niche, whereas the American Dutch breed has shown a semiclosed dietary niche. The oxygen isotope values reveal that American Dutch rabbits fulfil their water requirements completely from the vegetation, whereas New Zealand rabbits tend towards water resources for water acquisition. Nitrogen stable isotope results showed no significant differences between the values for American Dutch and New Zealand rabbits. This indicates that both breeds inhabit a similar environment, consume food grown on similar soils, and feed at the same trophic level. The archaeological and fossilised data on lagomorphs is present, but there is no literature available for living lagomorphs (rabbit). This is the initial study on the dietary preference of living *Oryctolagus cuniculus* breeds (New Zealand and American Dutch rabbits) on the basis of SIA. These are two common breeds in Pakistan, although according to the ARBA there are 48 breeds worldwide. We can achieve a complete picture of dietary preference if we use this technique on all *Oryctolagus cuniculus* breeds, and can compare the dietary patterns of our two breeds with those of other *Oryctolagus cuniculus* breeds using SIA.

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Authors contribution: Shouket U.: data curation, formal analysis and writing–original draft. Ahmad R.M.: conceptualization and supervision. Waseem M.T.: formal analysis and writing–original draft. Khan A.M.: validation and writing–review & editing. Zubaid S.: investigation and methodology.

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