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Nutritional Advantages and the Impact of Bee Pollen in the Feed of Exotic Animals

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1 INTRODUCTION

Bee pollen is the agglutination of flower pollens made by worker honeybees. Humans, at the entrance of hives, can retrieve this substance for consumption. As it is produced without human manipulation, it is considered a wild product.(1) There is a long history of humans using bee pollen. For centuries, medicinal and health promoting properties of it has been recorded, going back thousands of years.(2) An ancient rock painting dating back to 7000 B.C. in Spain shows a man gathering honey.(3) Talmud, the Bible and scrolls from ancient Greece all mention honey and bee pollen as a source of youth and health.(3) Consumption of plant producing seeds (pollen) is mentioned in the Bible, Genesis 1:29 : "and God said, See, I have given you every plant producing seed, on the face of all the earth, and every tree which has fruit producing seed: they will be for your food".(1) There is evidence of the use of pollen by ancient notable Chinese and Egyptian populations.(2) The ancient Egyptians describe pollen as the "life-giving dust". In ancient Greece, Hippocrates, Pliny the Elder, and Pythagoras, whom are considered the "Fathers of western medicine" believed in the healing properties of bee pollen and often prescribed their patients with it. The term "bee bread", used by Aristotle in his Historia animalium to describe bee pollen, was used for many centuries.(1) Sedative properties and beneficial effects on gastric and cardiovascular disorders of it dates back to Middle ages.(2) In folk medicine, the use of it has been noted around the world. The name pollen, which is the Latin word for fine flour or dust, was used for the first time in Historia plantarum in 1686 written by John Ray. The use of bee pollen started on a large scale in humans only after the Second World War, when they found a way to cultivate the pollens by use of pollen traps. (1)

In the last few decades, a substantial amount of research papers have been published on the use and effects of bee pollen among with issues with quality control problems. As people realized the therapeutic effects of bee pollen in scientific basis, apitherapeutics became a frequent topic of interest. One of the most famous therapeutic actions of clinical value is the successful use of pollen based vaccines in clinical trials for desensitization against hay fever.(1) It has been widely used in medicine due to its antimicrobial properties as well as many other benefits upon consumption.

Although there has not been a sufficient amount of research done on the consumption of bee pollen products on animals, apitherapeutics is a growing field, in nutrition of animals as well as of humans. The research articles on the use of bee pollen includes animals that normally consume bee pollen in their natural habitats such as hummingbirds, as well as animals that would not normally consume any bee products in the wild, such as fish. The aim of this thesis article is be to better understand and help the growing field of bee pollen use as an additive in reptiles, specifically tortoises. This thesis project includes article research about bee pollen and its components, the use of bee pollen in different animals and a practical research on the digestibility of bee pollen on tortoises of the Testudo genus of the order Chelonia, which is a reptile that normally does not consume bee pollen in nature. Therefore, the aim of this thesis paper will be to see whether these tortoises are able to digest these particles by checking their fecal samples for any remnants of pollen, as well as a detailed overview of bee pollen as a supplement and the possible uses of it in reptile medicine. To connect these two subjects, as this type of research has never been done before, the thesis will include a comprehensive study of the nutrition and husbandry of tortoises in general, specifically of Testudo species. The study includes nutrition of these animals, and the possible outcome of their nutrition, along with a comprehensive study of bee pollen and its nutritional benefits in other animal species. This research will hopefully open a gate for future studies of this type of additive and its beneficial effects it might have when given in different age groups and for their health status.

1.1 NUTRITION OF THE CHELONIA ORDER

A healthy tortoise should be fed three times a week and hatchlings should be fed daily. Although nutritional requirements of different tortoise species differ slightly, it is possible to estimate their requirements in a general manner. If compared with captive tortoises, wild tortoise diet comprises of forage with a relatively low nutritional value. Giving captive tortoises a diet, which includes 85% vegetables where a majority of them should be dark leafy greens, 10% fruits such as grapes, apples, peaches, melons, and 5% foods with high-protein content such as dry commercial tortoise food, tofu and various cereals, has been successful according to the article “The Turtle care and Husbandry”. Fresh figs can be excellent source of highly

concentrated carbohydrates given before hibernation. As tortoises have a high calcium requirement, captive tortoise diet, at times, should be sprinkled with calcium carbonate. They should also have access to shallow waters for drinking and soaking. Even though there are commercial turtle flakes and plates, these should not be the majority of their diets as they mostly have a low nutritional value and lack dietary fibers, vitamins, minerals and water content. As water content of the commercial diets is minimal, pellets alone may lead to serious renal problems and a risk of dehydration.(4)

1.2 GENUS TESTUDO

Testudo tortoises can be found on every continent except for Australia and Antarctica. Presently, approximately half of the recognized tortoises are located in Africa and the Mediterranean region and six of these are recognized in the genus Testudo. (5)

In the article "Phylogenetic Relationships among the Species of the Genus Testudo (Testudines: Testudinidae) Inferred from Mitochondrial 12S rRNA Gene Sequences", they tested the phylogenetic relationships between the genus Testudo by sequencing a fragment of the mitochondrial 12S rRNA gene of 98 tortoises belonging to Testudo, Indotestudo, and Geochelone. Several methods identify two main clades of Mediterranean tortoises. One clade comprises of species Testudo graeca, Testudo marginata and Testudo kleinmanni and the other comprises of Testudo hermanni, Testudo horsfieldii, and Indotestudo elongata. (5)

Mediterranean tortoises with the scientific name Genus Testudo are members of the order Chelonia, family Testudinidae that comprise of 244 species in 75 genera and 13 families.(6) We call them the Mediterranean tortoise due to their native locations. They are located in the south of Europe and north of Africa, which is in the proximity of the Mediterranean Sea. (7)

This group includes five different tortoises:(7)

- Spur-thighed/Greek tortoise (T. graeca) found in northern Africa and southeast Europe
- Hermann's tortoise (T. hermanni) which is endemic in Italy, France and Spain
- Marginated tortoise (T. marginata) found mainly in Greece

- Egyptian tortoise (T. kleinmanni) found in northern Africa
- Russian/Afghan tortoise (T. horsfieldii) found in southeastern Russia, Iran, Afghanistan and Pakistan.

1.2.1 HUSBANDRY OF TESTUDO SPECIES

Like all reptiles, tortoises are ectotherms. Their immune function, digestion and calcium metabolism are maintained by adequate temperatures. Therefore, Mediterranean tortoises are a challenging group of reptiles to keep due to their need for appropriate micro and macroclimates. They are typically most active approximately when the temperatures are between 22–34 °C. At night, temperatures can go down to between 5 and 10 °C. These species require a minimum basking zone temperature of 30 °C. (8) They are not suited to be in enclosures like vivarium or other restricted indoor spaces and these spaces usually fail to offer suitable thermal environments and frequently subject them to extremes of relative humidity. (9) They show complex behavior patterns and use microclimates to regulate their body temperatures. Therefore, providing them with an environment where they can self-regulate their body temperature is essential. (8)

In a survey done in 2018 in the article "Nutrition and Husbandry conditions of Palearctic tortoises in Captivity", they have gathered information on keeping and nutrition of testudo species in captivity. Out of 1075 owners, more than 75% of them said they housed their tortoises in an outdoors enclosure and gave feeds that were 80% grass during the summer vegetation period, which is considered as the ideal feeding method. Overall, they have concluded that unless the tortoises were kept in a terrarium or were fed with a diet with less than 80% grass during summer, they were unlikely to develop pyramidal growth syndrome, which is used as an indicator for the quality of tortoise care. In total, 18.5% of the tortoise owners in the survey said their tortoises had distinct pyramidal growth syndrome. The survey showed that a growing number of people are keeping Testudo tortoises, most of them being T. hermanni, which is a genus that is known to have a long lifespan with a higher occurrence of pyramidal growth syndrome. The article concluded that the results from the survey showed the keepers' knowledge in husbandry and nutrition was progressive and could be considered species appropriate.(10)

1.2.2 NUTRITION OF TESTUDO SPECIES

In the wild, Mediterranean tortoises consume a very large variety of different plants. According to previous research, a population of T. graeca in Donana Park in southern Spain consumed a minimum of 88 different plant species. Another research recorded a population living in an overgrazed area in the Atlas Mountains in Morocco during spring consumed 34 different plant species. (8)

The testudo tortoise diet in their natural environment is extremely high in fiber content of 30 to 49%, rich in calcium, and low in phosphorus and with protein levels at about 17% (dry matter basis) and 4–6% (as-fed basis). (8)

The high fiber requirement of the Mediterranean tortoises acts as a non-nutritive bulking agent and maintains gut motility, which helps intestinal microflora fermentation. This is essential for their vitamin and mineral absorption. Majority of their diet must have vegetables of leafy nature. Some foods, such as cauliflower, celery, iceberg lettuce, kale, and parsnips, must be given in small amounts as these may lead to conditions such as retarded growth and fluid retention within the limbs due to their iodine-binding natures and cause iodine deficiency and goitre if fed in large amounts. Avocado, cabbage, citrus fruits, ivy, onions, spinach, and strawberry are not ideal for feeding tortoises, as they do not follow the 25 per cent protein and 10 per cent fat content that is adequate for herbivorous reptiles. Banana, due to its sticky texture and high sugar content, as it can lead to secondary infections, should be avoided as it can lead to secondary infections. However, in considerably small amounts, we can offer some fruits with a lower sugar content such as apples and pears, as a fiber source. (11)

The article “Variation in Growth and Potentially Associated Health Status in Hermann’s and Spur-Thighed Tortoise (Testudo hermanni and Testudo graeca)” evaluates the growth rates, based on body weight and age, of free-ranging tortoises compared to captive ones. They have collected information from 65 T. hermanni and 21 T. graeca from various owners and included data of 539 tortoises of different species in their research. They tested the age-body length relations of these animals, to evaluate whether a tortoise with a high body length is more likely

to get diet or growth related disorders. The results showed that there is a high degree of phenotypic flexibility in tortoises related to their growth. They suggest that the various tortoise groups of different keeping conditions makes these results evident. The study later stated that they observed growth related disorders, especially pyramidal growth syndrome, more frequently in younger tortoises and this may limit the life expectancy of these tortoises. (12)

1.3 TESTUDO HORSFIELDII

The Russian tortoise, also known as the four-clawed tortoise, steppe tortoise, or the Afghan tortoise, is a small sized tortoise species that is originally located in most of central Asia. They are easily distinguishable from other Testudo species due to their powerful forelegs with only four toes for burrowing, as other Testudo species have five toes. Due to this difference, they were placed in a monotypic genus, Agrionemys, in 1966. (13)

In the article "The Genus Testudo (Testudines: Testudinidae): Phylogenetic Inferences" they re-evaluate Testudo species on the grounds of their classification based on shared traits. The three Agrionemys horsfieldii subspecies undoubtedly deserve a full species status and this is supportable with the grounds of several features including shell features, manus and skull characters. They conclude that, according to the current hypothesis, the genus Testudo is an unnatural taxon comprising of several lineages and Agrionemys is a valid genus for the horsfieldii complex only (14)

In their natural habitat, the Russian tortoises generally live in steppe-like areas in southern central Asia with sparse vegetation. In the area, transitional periods of extremely hot and dry summer is followed by long and cold winters with fast transitions between summer and winter. Therefore, depending on their geographic location, their annual activity period can be very short with a long hibernation period. (13) They come out of hibernation in early spring, start looking to mate, females start laying eggs in May or June, and then their activity slows down around June or July. (7)

In the wild, the Russian tortoise diet is comprised of herbaceous and succulent vegetation. These include grasses (green and dried), twigs, flowers, fruits and the flesh leaves

and stems of native and cultivated plants. Rainstorms obtain their water requirement and during dry season, they rely on their metabolic water. (7)

In captivity, they feed on different types of cultivated vegetables and plants such as leafy greens, lawn grass, corn, and nonpoisonous tree leaves and flowers. A variety in their diet is essential for their health and longevity. They can have fruits only in small amounts due to their high sugar content as this can lead to digestive problems. (7)

1.4 TESTUDO MARGINATA

The marginated tortoise, the largest European land tortoise, are found throughout southern Greece, southwestern Albania and northern Sardinia. According to several articles, the introduction of these tortoises to Sardinia was probably introduced, as they are taxonomically very similar to Greek tortoises. (15)

They are found in densely vegetated habitats and edges of forests, coarse scrubs and hillsides. (8,16)In captivity, they tolerate arid to moderately damp conditions. They also must have a sufficient amount of heat and sun. (16)

We can identify the marginated tortoise by its obvious flaring of the marginal scutes and soft skin on the upper limbs. They are medium sized tortoises, where both genders weigh about 2kg to 3kg. (16)

There is little research done on the marginated tortoise and its habitats, as it is very similar to some other Mediterranean tortoises like T. hermanni and T. ibera. T. hermanni is the most widespread species of tortoise in mainland Greece whereas T. marginata's distribution is limited to southern regions of Greece and some of the islands in the proximity of the mainland. Compared with T. hermanni, T. marginata is better adapted to heavy terrain and dense vegetation. (17)

In the wild, they feed on herbaceous and succulent plants.(16) In captivity, all of the Mediterranean tortoises can all be fed primarily the same type of diet, although some are from tropical forests, some in grasslands, and others are from desert regions.

1.5 DISEASES OF CHELONIANS

There are 40 living species of tortoises as of now. Most of these animals are experiencing population decline worldwide due to reasons such as pet trade conditions, habitat destruction and use of tortoise as food items by the locals.(18)

Identifying stress and pain can be difficult in chelonians. A clear indication of stress can be withdrawing into their shells and not protruding their heads or legs. Due to their scaly skin, it is also very difficult to determine distress, pain, and emotional response, unlike with birds or mammals. Therefore, it can take long periods for their keepers to notice certain illnesses like rhinitis (runny nose syndrome), abscesses, reproductive diseases, and many others.(19)

Various infectious and non-infectious diseases are susceptible to tortoises that may cause mortality. Studying diseases of free-living tortoises is difficult as by the time they are found, only the hard parts of the animal remains. Therefore, most studies conducted are done on captive tortoise species. Most mortality numbers arise from immature chelonians. In one study, mortality of 51 hatchlings were analyzed and causes of death was determined as sun exposure, cold, decalcification, desiccation, drowning, or other unknown reasons. The adult tortoise diseases and causes of death were compiled as a list of infectious and non-infectious diseases in several articles. A survey done in 1962 at the Zoological Society of London probably has one of the largest examinations done on the disease of tortoises. 144 tortoises of 17 different species were examined postmortem.(18)

1.5.1 INFECTIOUS DISEASES

Like all animals, tortoises are susceptible to numerous virus, bacteria, and parasites. There has been several studies done on infectious diseases on tortoises. Necrotic stomatitis was one of the findings in these studies called “Diseases of tortoises – a review of seventy cases”. Symptoms include anorexia and facial swelling. Samples from some of these tortoises and a bacteriological examination was performed. They found several bacterium such as Aeromonas spp, Pseudomonas spp, Enterobacter sp, E. coli, Klebsiella sp, Corynebacterium,

and several more. Overgrowth of E. coli and Enterococcus organisms causing severe enteritis and peritonitis, and bacterial shell ulceration that was possibly caused by Pseudomonas species, were observed. Most of these animals recovered upon adequate antibiotic treatment. The disease that had infected the largest number of tortoises in this study was gastrointestinal helminthiasis. Although this condition is not a lethal one and mostly they are symptomless, a heavy infestation of ascarids can cause anorexia and vomiting. (20) Other studies also emphasized the common occurrence of Mycoplasma species, which frequently causes upper respiratory tract disease, along with Salmonella species, in which free-living tortoises are known for being important reservoirs. (21)

Fungal infections are also important conditions to mention as various fungal diseases were reported in tortoises in zoological collections worldwide. These types of infections are usually caused by other underlying conditions such as malnutrition, overcrowding and high humidity. Although small, there is a possibility of mortality due to mycotic infections. (18)

Among viruses, one of the important pathogens to mention is herpesvirus infections identified in at least seven genera, including the genus Testudo. Pathological findings include necrotizing lesions in the oral mucosa and tongue, and inclusion bodies in most of the internal organs; mortality rate of this virus is very high. (22)

Tortoise picornavirus with the common name Virus “X” was detected in several tortoise species, including Testudo marginata and Testudo horsfieldii. Studies showed growth retardation, soft carapace and plastron. First symptoms appear at the age of 6-8 weeks after hatching and the majority die 10-14 days later.(23) Other viruses that are worth mentioning are iridovirus, poxvirus and togavirus .(18)

Among with these conditions, there are various others such as mouth rot, shell rot, abscesses, reproductive problems, myiasis, tick infestations, organ failure, and ocular disorders that can be some examples of infectious diseases of tortoises. (24)

1.5.2 NON-INFECTIOUS DISEASES

Various non-infectious diseases are commonly noted in tortoises. Some of these conditions are dehydration and malnutrition, metabolic bone disease, vitamin deficiencies, hypothyroidism, neoplasia, and toxicosis.

There are a number of reasons for disorders involving the shell. The main causes for these diseases are associated with malnutrition, deficiencies, and bad hygienic conditions. An example for these conditions is pyramidal growth syndrome. It is such a common occurrence in captive tortoises that it is considerably rare to find a captive tortoise without any pyramidal shell growth. Possible causes include excess dietary protein, too rapid growth, vitamin or mineral excess or deficiency, metabolic bone disease, inappropriate humidity and species predisposition.(24)

Shell rot which is an infection of the shell, is associated with traumatic injury, unhygienic living conditions, and poor husbandry. It causes erosive lesions involving the upper shell, the lower shell, or both. (24)

Nutritional secondary hyperthyroidism, one of the causes of metabolic bone disease, occurs due to inadequate calcium intake, inappropriate dietary calcium to protein ration, or inadequate sun exposure. (16) It is a common condition that occurs in tortoises kept indoors with inadequate environmental UV light.(11)

Frequent appearance of metabolic bone disease and malnutrition has been recorded in many studies. (17, 25) Metabolic bone disease occurs due to several pathological conditions. These conditions can be prolonged calcium deficiency, phosphorus deficiency, improper ratios of calcium to phosphorus, and vitamin D deficiency. Although there are many studies on the appropriate feeding methods of herbivorous tortoises, little is known about the nutritional requirements of tortoises, and for this reason, most tortoise diets are formulated according to experience, rather than theory or logic. Therefore, the occurrence shell bone thinning, especially in captive tortoises, is very common. Metabolic bone disease symptoms are first seen in the shell, as the skeleton is largely incorporated into the shell. As a result, deficiencies of such sources result in nutritional osteodystrophy and osteopenia. (18)

Among these metabolic conditions, examples for less frequently found non-infectious diseases of tortoises are as follows: (11,18,24)

- Sand impaction mainly observed in newly hatched tortoises;
- Prolapse of the penis, uterus, intestines, or urinary bladder;
- Bladder stones composed of uric acid;
- Iron storage disease which happens from excessive elemental iron accumulation in the Liver; diabetes mellitus which can occur in desert tortoises;
- Hypothyroidism seen in Galapagos tortoises
- Neoplastic diseases
- Post- hibernation anorexia, which is a common condition that happens when they have gone into hibernation with a pre-existing condition such as weight loss, parasite infestation, or inadequate nutrition.
- Gout, an increased build-up of protein in the body, which can be caused by excessive feeding of protein, which leads to high levels of uric acid crystals in the bloodstream that are deposited in the joints and visceral organs such as kidneys.

2 ARTICLE REVIEW

The purpose of this article is to review the nutritional values, benefits and the enzymatic degradation of pollen, specifically in certain reptile species. Due to the scarcity of literature regarding pollen feeding in reptiles, the article will also discuss the nutritional benefits of pollen in different animal classes and species as a general overlook of pollen as a dietary source.

We will be comparing the amount of pollen carried by the pollinator species with the amount of pollen digested by the predators of these species (i.e. reptiles). This comparison will help us determine the value of pollen as a dietary source in the predators. As a result, we will be able to see the difference between pollinator species as a feed source versus giving them bee pollen as a supplement, and therefore establish whether pollen is a valuable feed source in the reptilian diet.

Pollination is the transport of pollen grains, the male gametophyte of flowers, from the anthers of the male seed plants to the female's stigma (26). Flowering plants are able pollinate and fertilize vast areas in many different methods such as wind pollination (anemophily), water, and pollinators such as birds, reptiles, lizards, and insects (26,27).

2.1 POLLINATORS

In the Balearic Islands in the Mediterranean, lizards were recorded as true pollinators as they were observed carrying sizable portions of pollen from the shrub Euphorbia dendroides. As it indicates in the article "Pollination of Euphorbia dendroides by lizards and insects:

Spatio-temporal variation in patterns of fower visitation" done by Anna Traveset and Encarna Saez, that this was the first recorded demonstration of a lizard as a pollinator (28).

Although there are varieties of animals that are classified as pollinators, insects are the best-known pollinators (26).

Entomopalynology is the scientific study that deals with the interaction of pollen with insects. Information gathered by entomopalynology can be useful in many different ways. Mainly, researchers use the study of pollen and insects to improve crop management by doing research about the amount and type of pollinators that are needed for the better development of different types of crops. As adult insects feed on different types of flower pollen and nectar, they become contaminated. As a result, we can determine the origins, migration patterns, and feeding routines of these insects (26).

Beetles (Coleoptera), the largest order of insects, are important pollinators in semi-desert regions like South Africa and California. As beetles are large and have large jaws, the flowers they pollinate have evolved to fit their requirements. They tend to be flat shaped and their pollen is easily accessible, and the seeds tend to withstand the force of their jaws (26). These types of flowers are called Cantharophilous flowers such as crab apples (Malus spp.) (26). In other words, probed or chewed flowers by beetles or birds have a more developed system of ovule protection compared to flowers that are sucked by proboscis of wasps, flies, moth and other like

them. Due to these specific requirements and thus the lack of variety of the beetle's target flowers, beetles are often neglected as pollinator (29).

It is important to evaluate the efficiency of a pollinator in many different aspects. When it comes to variation of pollination methods found in plants, the amount of time they spend pollinating in different climatic conditions during the year, flies (Diptera) are one of the most important pollinators. As they are active throughout the year, they are able to pollinate plants that flower in unusual times of the year (26). Although they are very good pollinators when it comes to the time spent pollinating throughout the year, the flowers they pollinate generally don't bloom regularly and are simple with dull color and texture (26).

Lepidoptera, butterflies and moths are other examples of pollinators. Moths are nocturnal and the flowers they pollinate open at night and release a sweet odor during this time. On the other hand, butterflies are diurnal. Their target flowers are open during the day, tend to have very bright colors, and do not have such strong odors. As male moths and butterflies almost only feed on nectars, they involuntarily carry pollen stuck to their proboscis (26).

One of the most important order of insects among all pollinator species are Hymenoptera: bees, wasps and ants. The flowers they target are flat and have open nectars.

Although ants are in this category, they do not pollinate flowers. Their small sizes help them get in and out of the flower easily and get to their target food, sugar. Their size and the fact that they use pollen as a protein source makes them ill equipped for pollen transport (26).

Among all other insect pollinators, bees are the most efficient and effective (26). This is why we mainly focus on bee pollen as a feed source of these animals. Bees, especially social bees, have a very well developed system of pollen transport. Among bee types, social bees are the most active ones. They gather flower pollen and nectar, then connect them into balls with bee saliva substances (30).

Although pollen grains attach to the bees involuntarily most of the time, female bees may collect pollen actively. They have behavioral adaptations that aids them in the transportation of pollen back to their nests (31). A single bee is able of carrying 15,000 pollen grains (26).

In a study done by D. A. Kendall and M. E. Solomon, pollen specimens were collected at different times in 1969 and 1970 to check the quantities of pollen on the bodies of insects visiting apple blossom (32). They insects that had the biggest amount of pollen on them were females of Andrena haemorrhoa and A. coitana and workers of Bombus terrestris+ lucorum, which had over 15 000 grains. Honeybee workers had about 4000 grains. Some wild bee species and the majority syrphid flies had the lowest amount of fruit pollen on them. Various flies and beetles also had very few pollen grains as they are small and have smooth surfaces.

Pollen is an essential product for bees. They use pollen grains as food for their larval stage and use it for their reproduction (31). Pollen supplies them their main source of proteins, amino acids, lipids, starch, sterols, vitamins and minerals (30). Also, it is an important substance that influences their longevity and the production of jelly by young workers to feed other bees. Therefore, pollen is a major factor in their metabolism, immunity, tolerance to pathogens and their resistance to stress.

2.2 BENEFITS OF POLLEN

Therapeutic effects of bee pollen (Apitherapeutics) in different animals and humans has become a widely researched subject (1). As it contains various species of nutritional substances, bee pollen is known to have many pharmacological properties such as antioxidants, antimicrobials, anti-inflammatory, anti-viral effects, hepato-protective (33), anti-carcinogenic effects and can facilitate granulation process of burn wound healing (34,35). The therapeutic effects that the bee pollen is a result of the presence of phenolic acids and flavonoids, which have anti-inflammatory effects. Phytosterols and linoleic acid have anticarcinogenic activity. Polysaccharides stimulate the immune system (36).

In one research article on the antioxidative properties of certain plants named “Antioxidative properties of bee pollen in selected plant species”, they study the bee pollen of 12 plant species and their phenolic constituents (total phenols, phenylpropanoids, flavonols and anthocyanins) and antioxidant ability. They reveal that the antioxidative ability of bee pollen arises for the phenolic compounds it possesses. The results of this research revealed there is a

substantial difference between the phenolic content in pollen of these plant species. In some plant species, a very high antioxidative activity expressed as radical scavenging activity, inhibition of lipid peroxidation and hydroxyl radical scavenging activity were correlated with their high levels of total phenols, phenylpropanoids and flavonols. Hence, they state that phenylpropanoids might be considered as the main phenolic compound responsible for the lipid peroxidation protection in bee pollen tissue.(37)

The article "Antioxidative and Cardioprotective Effects of Schisandra chinensis Bee Pollen Extract on Isoprenaline-Induced Myocardial Infarction in Rats" shows that SCBPE (Schisandra chinensis bee pollen extract) was able to show antioxidative and cardioportective effects on ISO-induced rats with myocardial infarction. The study later stated that the successful outcome of this research could be the basis to use SCBPE as a food to prevent myocardial infarction (7).

There was also research done on the effect of bee pollen on the serum testosterone levels. 120mg/kg of date palm pollen suspension was given for 35 days. The study " Effect of Date Palm Pollen (DPP) on Serum Testosterone Levels in Prepubertal Albino Rats " resulted in an increase of serum testosterone levels with concurrent body weight gain. The study concluded that date palm pollen can be used as a supplementary source in cases where replacement therapy can be influential in adults as well as prepubertal patients (38).

Date Palm Pollen suspension given orally at a dose of 120mg/kg for 35 days to a prepubertal albino rat resulted in an increase serum testosterone levels with concurrent increase in body weight. It is suggested that it may be used as an alternative or adjunctive/supplementary source of increasing the serum testosterone levels in conditions where testosterone replacement therapy is indicated in adults as well as prepubertal subjects (38).

As a dietary supplement, pollen is associated with the regulation and enhancement ability of protein metabolism at the molecular level. Therefore, it is used in cases of recovery from malnutrition, especially in older patients. It is able compensate for the high requirements of essential amino acids, essential fatty acids, and micronutrients. Using protein-rich bee pollen has been shown to be particularly beneficial for patients after surgeries, and old, long-term patients where malnutrition can result in mortality. Dietary supplementation of bee pollen is

also favorable in patients with high physical or mental work, as it has been shown to strengthen muscle function and increase body length (2).

Another article that studied the effects of bee pollen on animals is the “Effects of supplementary bee pollen and its polysaccharides on nutrient digestibility and serum biochemical parameters in Holstein calves”. The study was conducted on 25 female calves that were 14 to 70 days old and were supplemented with dietary bee pollen or its polysaccharides and how these affect their growth performance, serum biochemical parameters, and apparent nutrient digestibility. After 56 days of offering them five different diets with a milk replacer with different levels of bee pollen contents which ranged from no pollen to 50g/day bee pollen, they were able to observe the differences each calve had. The study showed that the calves fed with bee pollen showed a much greater bodyweight gain, a higher dry matter intake, and they had significant increase in dry matter digestibility. The results concluded that dietary supplementation of 25g/day or 5g/day bee pollen improved the nutrient digestibility greatly. In another study mentioned by this article stated that this supplement improved egg productivity, feed utilization efficiency, immunity, and resistance to disease in chicken. In addition, in chicken, they were able to reduce the use of other dietary supplements such as lysine, vitamins, enzyme preparations and coloring agents by using bee pollen supplementation. Similar results were also studied in pigs, where they observed increased growth rates by giving bee pollen as a supplement. (39)

In the study “Effect of bee pollen on the development of digestive gland of broilers”, 144 broilers that were one day old were divided into two groups, one of them were not given any bee pollen and the others were given 0.5% bee pollen for 6 weeks. After slaughtering and dissecting randomly selected chickens each week, they observed significant changes. The main differences they observed was that the chicken that was given bee pollen had a much clearer structures of hepatocytes and hepatic sinusoids, the length of small intestine was significantly longer and the Kupffer cell volume and gland alveolus of pancreas were bigger. Hence, the results showed bee pollen supplements can promote the development of liver, pancreas and small intestine in broiler chicken. (40)

2.2.1 APITHERAPY IN HUMAN MEDICINE

Apitherapy is the science of maintaining health with the use of honeybee products. These are honey, bee pollen, propolis, royal jelly, and bee venom.(3) Each of these products have been widely studied and are being used in different fields of medicine.

Bee venom therapy (BVT) is a centuries old treatment that is used for rheumatoid arthritis. Bee venom includes more than 50 identifiable components, most of which have anti-inflammatory and antimicrobial effects and act as antioxidants. By using either a live honeybee or a transdermal injection, it was used to treat multiple sclerosis for decades, as there is documentation of bee venom treatment with beneficial aspects to the patients. (3)

Propolis, a beehive component, contains more than 180 components including waxes, essential oils, vitamins and enyzmes. It has been used in various periodonotal diseases, otitis externa and otitis media, psoriasis among with other autoimmune diseases. Also, it has antibacterial, antiparasitic, and anticancer properties. (3)

Honey is the most known product of honeybees. The honey produced in the patient's living area can be used in allergy treatment. It is also used for induction of cough, wound healing and gastrointestinal diseases. (3)

Historically, queens such as Cleopatra used royal jelly for its beautifying effects, and is still used in cosmetics today for its rich bioactive compounds.(41) It has various health benefits such as antioxidant, anti-inflammatory, neurotrophic, hypotensive, antidiabetic, antilipidemic, antirheumatic, anticarcinogenic, anti-fatigue, and antimicrobial activities.(41) For this reason, it is used for many conditions like diabetes, hypertension, hyperlipidemia, cancer, skin diseases, and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.(41) Royal jelly's antibacterial and antifungal agents and has shown great results in wound healing and tissue repair.(3) It also has lowering capability of lipid levels in both animals and humans.(3) Although in small amounts, royalisin, jelleines, and aspimin are in royal jelly, with Major Royal Jelly proteins (MRJP) which constitutes more than 80% of royal jelly protein content.(41) These substances show strong antimicrobial and bactericidal activities against some of the most drug-resistant bacterial strains such as methicillin-resistant

Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, vancomycin-resistant Enterococci, as well as extended-spectrum-lactamase-producing Proteus mirabilis and Escherichia coli.(41)

Bee pollen, a nutritious and high quality food, which contains all the nutritional requirements of humans. It can be used as an anti-depressant, it can also increase endurance in sports, and improve brain, heart, liver and prostate functions. It is used in autoimmune disease patients such as multiple sclerosis, thyroiditis, lupus, and celiac disease. Also, there is number of studies of bee pollen for benign prostatic hypertrophy, memory loss, and cancer. (3)

In the article, "Results of Treatment with Pollen Extract in Chronic Prostitis and Prostatodynia", they conduct a prospective study with 90 patients that have chronic prostatitis syndrome. Patients that were divided to a group that has complicating factors and another with no complications were given Cernilton N which is a pollen extract. The group without complications showed a 78% improvement after 6 months of treatment and the group with complications showed little to no response to the treatment. This study showed that the pollen extract Cernilton N has a great therapeutic significance in the treatment of these two conditions. (42)

Further medical research on the effects of bee pollen as a complementary treatment for different diseases can be seen in the article "Prospective observational study to evaluate the efficacy and safety of the pollen extract Serelys) in the management of women with menopausal symptoms". As the hormones used for menopausal patients have many contraindications, there's a high demand on herbal treatments for it. Upon giving Serelys which contains pollen extract, there was a significant decrease of various menopausal symptoms such as reduced hot flashes, sleep disturbance, depression, vaginal dryness and muscle and joint pain, 12 weeks after starting the treatment and had very low side effects.(43)

2.3 NUTRITIONAL ANALYSIS

Bee pollen has approximately 250 substances in its composition. These include amino acids, lipids (triglycerides, phospholipids), vitamins, macronutrients, micronutrients, and flavonoids (34). Many scientific articles review the nutrients and the chemical composition of pollen grains. As it is very difficult to obtain easily by hand, it is difficult to prepare a chemical analysis of pollen. Most summaries are based on a few taxa which doesn't properly measure an average of the nutrient contents. The chemical composition can vary between different bee pollen as the contents of it changes according to the bee type and the plant types of the environment they are in (44).

One study done by Jean-Noël Tasei and Pierrick Aupinel, "Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers", shows how different botanical origins change the parameters of the bee pollen. They tested 15 single pollens and pollen mixes and checked how that effected the larvae produced by bumblebee workers. They chose the pollens that constituted 32% of the mix by volume as the main genera. The result was that the highest nitrogen content of % dry weight was the pure pollens: Papaver being 3.98% and Castanea being 3.25%. The lowest nitrogen content was in pastes of Cistus being 2.31% and Helianthus being 2.3% (45).

Protein content of pollen differs greatly depending on the botanical origin. Free aminoacids comprises 1/10 of it. Seventeen different aminoacids can be found in the bee pollen (46). On average, pollen contains 22.7% protein (34). Among this percentage are 10.4% essential amino acids, which are crucial as they cannot be synthesized by the organism. These are methionine, lysine, threonine, histidine, leucine, isoleucine, valine, phenylalanine, and tryptophan (34). Tryptophan and phenylalanine are known to be very important substances in the reduction of depression and anxiety (46).

30.8% of pollen on average is digestible carbohydrates. Among these, 25.7% is reducing sugars such as fructose and glucose (34). Carbohydrates are one of the main components of bee pollen. The type of carbohydrates they have is mainly polysaccharides such as starch and cell wall material. The different ratios of sugar changes between different plant species but overall fructose, glucose and sucrose are about 90% of all low molecular sugars (46).

Fat composition of bee pollen differ greatly due to different botanical origins. Lipid content is approximately 5.1% (34). These are mainly polar and neutral fats such as mono-, di-, and triglycerides, as well as small amounts of fatty acids, sterines, and hydrocarbons. (46) Results from "Pollen composition and standardisation of analytical methods" done by gas chromatography analysis show that lipids manly consist of linoleic acid, palmitic acid, and oleic acid. Unsaturated fatty acids are on average 70% of the total (46). The most important lipid content are essential fatty acids (EFAs). 0.4% of these are acids such as linoleic acid, γ-linoleic acid and archaic acid. 1.5% has phospholipids. Phenolic compounds are on average 1.6%. These include leukotrienes, catechins, phenolic acids, and flavonoids. Phenolic acids, which are 0.2%, mainly have chlorogenic acid. A major part of bee pollen has triterpene bonds, the most common ones being are oleanolic acids, 3-ursolic acid, and betulin alcohol (34).

Vitamins is one of the significant sources in bee pollen. Like many other parameters, vitamins and minerals differ depending on the pollen type (46). Fat-soluble vitamins constitute 0.1% of the total amount. These include provitamin A, vitamin E, and vitamin D. Water soluble vitamins are 0.7% and these include pantothenic, nicotinic and folic, biotin, rutin, and inositol (34).

Macronutrients and micronutrients together make up about 1.6%. Determination of average number of content is mainly done by atom absorption carried out on pollen ash (46). Macronutrients include calcium, phosphorus, magnesium, sodium, and potassium. Micronutrients, 0.02%, include iron, copper, zinc, manganese, silicon, and selenium (34). The main mineral potassium is about 60% of total mineral content, magnesium is about 20%, and sodium and calcium are about 10% (46).

Water content pollen is determined after drying to a constant weight in a cabinet dryer or by infrared balance (46). In some countries, they have established minimal requirement for dried pollen. For example, in Brazil, it is maximum 4g/100g, in Poland and Switzerland it is maximum 6g/100g, and in Bulgaria it is maximum 10g/100g (44).

It is possible to see the dried bee pollen composition compiled by "Pollen composition and standardisation of analytical methods" below in table 1 (46).

Main Components	Content (g/100g dry weight) Min – Max
Protein	10-40
Lipids	1-13
Total Carbohydrates	13-55
Dietary fiber, pectin	0.3-20
Ash	2-6
Undetermined	2-5

Vitamins	mg/kg
β- Carotene	10-200
Thiamin	6-13
Riboflavin	6-20
Niacin	40-110
Pantothenic acid	5-20
Pyridoxine	2-7
Ascorbic acid	70-560
Biotin	0.5-0.7
Folic acid	3-10
Tocopherol	40-320

Minerals, trace elements	mg/kg
Potassium	4000-20000
Magnesium	200-3000
Calcium	200-3000
Phosphorus	800-6000
Iron	11-170
Zink	30-250
Copper	2-16
Manganese	20-110

Component	Content
Water content - not more than	6-8 g/100g
Total ash content - not more than	6 g/100g
Total protein content - not less than	15 g/100g
Total sugar content - not less than	40 g/100g
Fat - not less than	1.5 g/100g

Table 1. Dried Pollen composition (46)

2.4 ENZYMATIC DEGRADATION

Durability of pollen grains give paleoecologists a significant tool to study the deposits of dead plant material and sediment deposits even after millions of years. This allows them to study ancient floras and paleoclimates. The sturdy structure of pollen causes challenges in the digestion of pollen grains (44).

Pollenkitt is the outermost layer of the pollen grain. This semi-solid coat is comprised mainly of neutral lipid hydrocarbons, terpenoids, and carotenoid pigments. Exine, the structure inside the pollenkitt, is the intricately ridged matrix of the carbohydrate sporopollenin. These structures of exine are the contents that makes the pollen grains resistant to decay and digestion although the germination pores, perforated pores or slits, lead to the inner layer, intine. Intine is mainly composed of cellulose and pectin, which again makes pollen resistant to decay and digestion. It forms the final barrier to the nutrient rich cytoplasm. Hence, the animals that consume pollen must externally probe the pollenkitt and must penetrate or dismantle two resistant pollen wall layers to access the nutrient rich cytoplasm (44,47).

Six basic methods are introduced for animals to extract pollen contents in the article. These are cracking open the pollen wall mechanically, piercing the pollen wall with sharp mouthparts, dissolving the pollen with enzymes, inducing germination or pseudo-germination, bursting open the pollen wall through osmotic shock, and penetrating the pollen wall with digestive enzymes. The article explains that the first three methods are rare and the last three are difficult to distinguish (44).

Three species are known to pierce pollen grains and extract their contents without ingesting the grains: beetles, thrips, and some species of ceratopogonid flies. Studies show that pollen-feeding beetles may crack pollen grains in their digestive tract with the aid of lignified trichomes. Instead of cracking it open with their mouthparts, they ingest both the pollen and the lignified trichomes from the inflorescence of the peach palm. Although trichomes have no nutritional value, they apparently act as gastroliths that crush the grains in the beetle’s digestive tract (44).

Digestive efficiency of bee pollen is calculated as percent of empty pollen grains in the feces of different animals. In birds, efficiency of digestion ranged between zero for some birds such as hummingbirds and the honeyeater, to 80-100% for some bat species, Darwin's finch, and the mouse. Honeybees were able to extract 50-98% of ingested pollen, depending on pollen type and age of the pollen (44).

Overall, investigation of digestion of bee pollen has mainly been done in bees and other arthropods, as well as some bats, birds, and marsupials. The western pygmy possum and the honey possum have been shown to digest up to 100% of pollen, the Queensland blossom bat and the yellow bellied glider digest about 50%, and among birds, purple-crowned lorikeets, New Holland honeyeaters, zebra finches, and budgerigars can digest less than 50% of the pollen grains they eat (48).

In the article "Pollen digestibility by hummingbirds and psittacines", it is shown that the adult Anna's hummingbirds were only able to digest 6.9% of the Eucalyptus pollen grains, and Costa's Hummingbirds could not digest any of it. In addition, Moluccan Lorikeets as nestling and as adults, and nestling Cockatiels were given Prunus pollen, which showed less than 15% digestion for all the birds. In all the trials with Prunus pollen, percentage of empty grains before and after feeding were virtually the same, but the percentage of partially full pollen grains were much higher in the fecal samples than in the diet. An average of 64% of Prunus pollen grains were found partially full in the feces compared to the average 9% that was found when the same birds were fed with Eucalyptus pollen (47).

The importance of pollen grain consumption in nectar-eating geckos is seen when we look at their behavior. Although their consumption of pollen is not a topic that is found to be important, at least four species of geckos in the Seychelle Islands have been observed eating pollen from several species of palm and defending their pollen sources from other animals (48).

In the article "Pollen digestion in Galapagos lava lizards", they study the digestion of pollen grains by Galapagos Lava lizards. After taking twenty seven faecal samples from different lava lizards, the results showed that the exine of Opuntia and Tribulus flower pollens were penetrated during passage through the gut of the lizard. Feeding Opuntia pollen showed, on average, 92.4% of the grains were empty in the faeces of the lizard, whereas, for the Tribulus

flower pollen, it was 89.9% empty (48). Also, lava lizards can be considered pollinators. In the study, in three years, nine lava lizards were observed transporting pollen in seven single-island endemics (49)

3 MATERIALS AND METHODS

Bee pollen supplied by the apiary of the University of Veterinary Medicine in Budapest was used to conduct this research. Firstly, all of the tortoises were fed with grass dusted with bee pollen but as the grains did not adhere to the surface sufficiently, we have decided to feed them with a few slices of different summer fruits with bee pollen in them.

Samples for the research were taken from one male and 10 female tortoises, in total 11 different tortoises. Five of these animals were Testudo marginata and six of them were Testudo horsfieldii. All of these animals were imported from Uzbekistan a year ago to the Veterinary University of Budapest.

The male Russian tortoise (Testudo horsfieldii) was medium aged and his Total Carapace Length (TCL) was 12cm and according to the exporters, was 10 years of age. The four female Russian tortoises were believed to be 60 or more years of age and their Total Carapace Length (TLC) ranged between 17 and 20cm.

We have also collected samples from five Testudo marginata species. Three of the marginated tortoises were female and two of them were male. Their total carapace length ranged between 16 to 18 cm and they were all 6 years old.

As the tortoises ranged between sub-adult, medium-aged to very old; this gives us a better understanding of bee pollen digestibility in different age groups of these Testudo species.

In order to check the digestion and the time of digestion of the pollen grains, four fecal samples were taken every 12 hours. In other words, we have taken samples 12, 24, 36 and 48 hours after feeding them with bee pollen to see if there was any trace of pollen grains in any of these time intervals, in the samples we have taken. Samples from the top and the bottom of the

fecal flotation test were used, and then they were stained using the Diff Quick method. Later, the slides were examined under a microscope to see whether there was any trace of undigested pollen grains or, if any of these particles were visible upon inspection.

4 RESULTS

Upon dusting bee pollen powder on fruits for the consumption of 11 tortoises, each tortoise digested bee pollen successfully. Of the 11 tortoises and the 4 fecal samples taken from each tortoise, we were unable to detect any pollen particles in any of the fecal samples even though the samples were taken from both the top and the bottom of the fecal flotation test. These results most probably show they were able to digest most of the bee pollen grains successfully, as the indicator of digestibility of the grain pollen is possible by examining the ratio between empty and full grains. (48) Additionally, the consistency of digestibility of the pollen grains between different age groups and sexes also show there is no difference, in terms of digestibility, between these groups. The healthy tortoises that were in the experiment did not have any behavioral alterations upon putting bee pollen in their diet. They also did not show any change in their eating habits and had the same appetite before and after this experiment.

5 DISCUSSION

The results above may indicate that the digestive system of both tortoise species were able to penetrate the hard exine of the pollen grains. In order to suggest that they were able to absorb all the nutrients in the grain, we would have to do a detailed analysis of the tortoise faeces as it is also possible that some or all of the nutrients could have been excreted. As the tortoises were not given feeds containing bee pollen for a substantial amount of time, it is not possible to suggest that these pollen grains had any long term effect on the health of these tortoises. However, we can now consider the possible side effects or benefits of adding pollen grains to the tortoise diet. Some parts of plants, like pollen, have greater nutrient stores than

others. Most plant-eating reptiles have limited masticatory muscles and thus prefer feeds that are softer and easier to digest. (48)

In the article “Pollen Digestion in Galapagos Lava Lizards”, they observe that most lizards can obtain sufficient amounts of carbohydrates and fats from the young leaves, nectars and fruits. Thus, they suggest that pollen as a nitrogen source in lizards can be given when other protein sources, such as arthropods, are scarce. This approach can also be taken in tortoise diets as pollens can be considered not only a nitrogen source, but also vitamins, amino acids, proteins, and lipids. (48)

There are several published research articles on the digestibility of bee pollen in different animal species, as it is one of the most useful therapeutic products favored by natural medicine scientists. Use of bee pollen, thus, has been studied in a range of animals including food-producing animals such as poultry, fish, and cattle due to its variety of therapeutic features. (39,50)

One such article, “ Honey Bee Pollen in Meagre (Argyrosomus regius) Juvenile Diets: Effects on Growth, Diet Digestibility, Intestinal Traits, and Biochemical Markers Related to Health and Stress”, evaluates the digestibility and possible use of honey bee pollen in reared fish, specifically meagre, in an attempt to improve the welfare and sustainability of animal husbandry. The results showed the addition of honeybee pollen to meagre resulted in the reduction of growth performances and diet digestibility, histological alterations in the intestinal tract, and high levels of biomolecular stress markers. They suggest that this could be due to the inability of monogastric animals to digest the honey bee pollen, as they have complex structures. Their interpretation for such a result was the similarity of pollen walls with indigestible fibrous substances such as suberin and lignin. They assumed that the bee pollen had a pro-inflammatory action on the intestinal mucosa of fish with carnivorous feeding habits like meagre. This study, according to its authors, confirmed the necessity of testing the positive actions and exclude any negative impacts these natural nutraceutical additives might have on the animal’s health. (51)

The article mentioned above proves the importance of detailed and long term analysis of the effects of any additional feed given to tortoises. As bee pollen is not a natural source of

nutrition for any members of the Chelonians, it would be unethical to confirm the use of bee pollen without thorough research.

In the article " Pollen digestibility by Hummingbirds and Psittacines", they mention the difference of pollen digestibility of adults and chicks. Adult lorikeets and Cockatiels were mainly unaffected by passage through the digestive tracts, however, their nestlings were able to digest the pollen particles to a better degree. Nestling lorikeets digested 24% of the Eucalyptus pollen while the adults only digested 7% of it, and nestling Cockatiels digested 38% while the adults digested 17%. (47) Although the results that were obtained in our research did not show any difference between adults and sub-adult tortoises, further research may be required to better observe the differences that may arise in particular age groups of tortoises, especially tortoise hatchlings.

As this is a very newly researched subject and there is no other research article about the consumption of bee pollen by tortoises found on any of the search engines, there are many possibilities these results can take us. We know from various sources that Mediterranean tortoises primarily feed on leafy goods. During summer, it is much easier to find fresh and nutritious greens than in winter. Therefore, it can be much harder to find adequate supplies of leafy greens during wintertime. Feeding them during winter can therefore be much more difficult and they might need further supplements. Consequently, we must know what they require in their diet. While selecting plants for tortoises, we must consider that they need certain amounts of different substances in their diet. Examples for these are Calcium and Phosphorus, Vitamin D, Vitamin A, Thiocyanates, and protein. These requirements form a foundation for choosing specific plants for the consumption of captive tortoises. (52)

Most of these requirements of captive tortoises need for a health nutrition can be found in bee pollen. Therefore, supplementing them with bee pollen in small amounts during winter could possibly have beneficial effects on their health.

Post-hibernation anorexia occurs when the tortoise goes into hibernation with a pre-existing condition like weight loss, parasite infestation, or inadequate nutrition.(11) Upon further research and eliminating the possibility of non-nutritional causes of this condition, we

can consider supplying bee pollen to their diet prior to hibernation, to reassure any possibility of inadequate nutritional causes of this possibly lethal disease.

The diet of tortoises used in captivity include commercial pellets of different animals. An example for this is used at the Wildlife Survival Center, where they give commercial antelope diet, which has 18% protein level among with mixed greens, carrots and apples. Many of these animals develop pyramidal scutes. Although they do not cause severe malformations that is dangerous for the animal's health, it could mechanically interfere with successful copulation if the degree of pyramiding is very severe. Although it is a possibility that the high protein levels that are given to them can be the cause of pyramiding, there may be other factors that might contribute to it such as individual genetic differences, deficiencies or excess of vitamins, minerals and ultraviolet light exposure. (52)

In an experiment done, named "Influence of environmental humidity and dietary protein on pyramidal growth of carapaces in African spurred tortoises (Geochelone sulcata), fifty different hatchling African spurred tortoises were studied in a 5 month period to study the dietary protein level and environmental humidity on the degree of pyramiding. They were given such a diet that would prevent deficiencies and pathogenesis as these are considered possible causes of pyramidal growth syndrome. The study suspected that the dry environmental conditions and comparative high growth rates induced by nutritionally intense diet caused pyramidal growth on these tortoises. However, he results indicated that the level of dietary protein is probably not the main cause of pyramidal growth syndrome, although most of the previous studies suggest otherwise. The conclusion in the article suggested, rather than the fast growth being the reason of pyramiding, environmental humidity played the main role for the condition Their results showed there is no connection between dietary protein level and the degree of pyramiding. They suggest that in order to minimize pyramiding, areas with a humidity of nearly 100% for hiding should be provided at all times. Keeping the level of dietary protein in tortoise diets low can be useful for other protein related diseases such as gout but it is not related to pyramidal growth syndrome. (53)

Even though the above mentioned article suggests that protein supplies do not have any correlation with the degree of pyramidal growth syndrome, this article is one out of many others

that suggests otherwise. Furthermore, as this research was done on one species of tortoise, it is possible that the results will be different in others, for example, Testudo species. However, these results must be kept in mind when considering changing the diet of tortoises for the treatment and prevention of pyramidal growth syndrome.

By doing further research on the addition of bee pollen, the type of diets mentioned above can be replaced by a more appropriate diet with better nutritional values. Pollen nutritional values can vary depending on which plants have been used to form these bee pollens. By studying different types of bee pollen, it is possible to find an appropriate balance of what the tortoises might need during winter or if they need further supplementation other than green goods.

Apart from pyramidal growth syndrome, another important and commonly seen condition is softening of the shell caused by the metabolic bone disease, which is a result of deficiencies of calcium, phosphorus, and Vitamin D.(18) This disease is strongly associated with improper feeding of captive tortoises. When we compare natural forages with commercially available fruits and vegetables, there is an impressive difference in their protein, mineral and energy contents. (54) Considering these finding, upon thorough research, bee pollen supplementation could be a solution to this captive tortoise condition. As we know, most of the vitamins and minerals that cause shell deformities are found in bee pollen. Therefore, it is possible for us to assume that there might be beneficial effects of bee pollen extract to help treat and prevent metabolic bone disease, and other diseases that are associated with inadequate and inappropriate digestion of the tortoises.

6 ABSTRACT

Therapeutic effects of bee pollen (Apitherapeutics) in different animals and humans has become a widely researched subject due to its high concentration of reducing sugars, essential aminoacids, fatty acids, and minerals like Zn, Cu, Fe and high K/Na ratio among with high amounts of several vitamins. (1) The purpose of this thesis article is to study the possibility of using this substance on certain reptiles, specifically tortoises of the Testudo genus. However, there has been little research done on bee pollen digestion and its effects on reptiles. Due to the scarcity of literature regarding pollen feeding in reptiles, the article also discusses the nutritional benefits of pollen in different animal classes and species as a general overlook of pollen as a dietary source among with literature review on bee pollen's benefits, nutritional analysis and enzymatic degradation.

This research includes the digestibility of bee pollen of two different tortoise species to evaluate whether we can consider giving them bee pollen as an additive. Because of the scarcity of research done on the digestibility of bee pollen in such animals, it is hard to determine the favorable or disadvantageous effects of using such additives.

Our research revealed that we could now consider doing further studies as the samples taken from 11 different tortoises of two different species showed no signs of bee pollen particles in their feces. These results most likely suggest that supplying certain tortoises with bee pollen is possible. However, this topic must be studied further to see if there is any negative effects bee pollen might have, as there have been studies on different animals, such as the meagre, that showed supplying them with bee pollen mostly had disadvantageous effects.

E. L. MacQuiddy could find an explanation for such results in the article "The appearance of Pollen in the Stool". They attempted to detect ragweed pollen in stools of people residing in Nebraska, by determining the amount of pollen necessary and giving fifty milligrams of defatted pollen mixed with noon meal mixed with charcoal so the portion of the stool containing the pollen could easily be detected. They could not find any pollen particles upon inspection. However, after giving 75 milligrams of pollen by applying the same method, they were able to detect the pollen granules successfully. They have also dyed the pollens that were

given to these patients with carmine to analyze if any digestion occurred in the intestinal tract. Their results showed that there was little to no trace of the stain. This suggests that some digestion had occurred while pollen was passing through the intestinal tract.(55)

Although we have not done an experiment similar to the article mentioned above, it gives a better understanding for the results gathered. Therefore, it is possible to assume that there is a chance of digestion of bee pollen in the intestinal tract of the tortoises we have used.

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