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The Diversity of Hind-Gut Bacterial Microbiome of House Rats and Cockroaches: An indication of Public Health Risk for Residents of Semiurban and Urban Morogoro, Tanzania



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ABSTRACT

House rats (Rattus rattus) and cockroaches (Periplaneta americana) are important reservoirs of zoonotic bacterial diseases. Understanding these animals' gut bacteria composition is crucial for monitoring and preventing infections. This study aimed to determine the hindgut bacteria composition and diversity of the two hosts sampled from Kilosa and Morogoro districts, in Tanzania. A cross-sectional study design was employed, A total of 114 house rats and 57 cockroaches were caught. The hind guts of trapped host species were dissected and pooled to obtain four pools (two pools represent the hindgut of R. rattus and P. americana from Morogoro municipal and two pools for the hindgut of R. rattus and P. americana from Kilosa district). Genomic DNA was extracted from the pooled samples which was then used in metagenomics sequencing. The observed Shannon and Chao 1 indices indicated higher bacterial species diversity in rats of the Kilosa district and cockroaches of Morogoro municipal. The microbiome diversity in Morogoro municipal was higher for cockroaches than for rats, but it was not statistically significant (p>0.05). In Kilosa district, the microbiome diversity was higher for rats than for cockroaches, but it was not statistically significant (p>0.05) Proteobacteria and Bacteroidetes were the most abundant phyla in the hindgut samples of both animals. However, abundances differed among the host species and areas sampled. Proteobacteria were the most abundant phylum from Rattus rattus from Kilosa district, P. americana and R. rattus from Morogoro municipal, comprising 48%, 41%, and 40%, respectively. Bacteroidetes were plentiful from P. americana from Kilosa district (48%). Bacterial diversity was observed to be higher for R. rattus from Kilosa district and P. americana from Morogoro municipal. Potential pathogenic bacteria were also observed; the highest relative abundance of pathogenic bacteria was observed from P. americana of Kilosa, followed by R. rattus from the Morogoro district. Therefore, this study highlights the composition of hindgut bacteria carried by P. americana and R. rattus, which gives an insight into the different bacteria carried, including the pathogenic ones. This study suggests surveillance of these pests to minimise outbreaks and transmission of zoonotic diseases. Since this study did not focus on factors influencing microbiome composition and diversity, further studies are recommended to be conducted to see the influence of those factors on the gut microbiome.

INTRODUCTION

The house rat (Rattus rattus) and cockroach (Periplaneta americana) are among the world's most prolific and

widespread urban pest species. Both pests inhabit a wide range of habitats including human and animal habitats. The *R. rattus* and *P. americana* gut microbiome comprise

horizontally transmitted and vertically transmitted microbes. Both of their guts harbour a variety of microorganisms, which play an important role in the health and fitness of host animals. The microbes contribute positively to the development and growth of the host by participating in food digestion, host nutrition, protection against pathogens and increasing the immune response (Engel and Moran, 2013a; Huang et al., 2013; Yang et al., 2017). They protect the host against pathogens by inhibiting colonisation and enhancing their immunity (Engel and Moran, 2013a). As reported by (Engel and Moran, 2013b; Brune and Dietrich, 2015; Claus et al., 2016) certain toxins such as pesticides can be metabolised by the gut microbiome. Moreover, recent studies have shown that the microbiome can impact different host behaviours, including frequency of social interactions, mate choice, hyperactivity, anxiety, depression and others (Tinker and Ottesen, 2016).

The assemblage and composition of the bacteria community inhabit the three sections of the alimentary canal, but many studies reported that the hindgut has the highest bacterial density and diversity (Cruden and Markovetz, 1987; Schauer et al., 2012; Bauer et al., 2015; Kakumanu et al., 2018). Like in other animals, the gut microbiome in P. americana and R. rattus is dictated by the interplay of host genetics, early environment, and immediate environment (Tinker and Ottesen, 2016). Typical examples of a host's environment that can cause a major impact on the microbiome include temperature (Sepulveda and Moeller, 2020), diet (Turnbaugh et al., 2009), and/or housing conditions (Ericsson and Franklin, 2015; Caruso et al., 2019). Arguably, the interaction between these and other domestic pests also influences the composition and diversity of the gut microbiome.

Understanding the gut microbial communities is essential as it contributes to understanding their biology and clinical relevance since they are engaged in disseminating pathogens (Kakumanu et al., 2018). Based on the aforementioned, studies have emphasised the urge to acquire an in-depth understanding of host-microbiomeparasites interactions. These interactions are critical for disease transmission. As such, the accrued knowledge will not only establish mechanisms via which gut-microbiome modulates the host's (P. americana and R. rattus) ability to transmit and/or harbour bacterial and possibly other pathogens but also may reveal symbiotic microbes that may be exploited to achieve transmission blocking in disease vectors/reservoirs. Several symbionts have been identified as potential strategies for reducing disease transmission, for example, Wolbachia, Wigglesworthia, Rhodococcus and Serratia (Weiss and Akoy, 2011).

This study aimed to assess the composition of the hindgut bacteria microbiome of cockroaches and house rats since little is documented, especially in Tanzania, about the hindgut bacterial composition of these two pests to ascertain their potential for disease transmission.

MATERIALS AND METHODS

The Ethics Statement

The Ethical approval for the study was given by the ethical committees of Sokoine University of Agriculture, Tanzania, with reference No SUA/AMD/R.1/8/763, approved on 5th January 2022.

Description of the study area

This study was conducted in Morogoro Municipal and Kilosa district (Fig. 1), in the Morogoro region, Tanzania.

Morogoro Municipal covers an area of 260 km². The district has an average minimum and maximum temperature of 16°C and 33°C, respectively. The annual rainfall ranges from 821-1505 mm (Ernest et al., 2017). The main economic activities include subsistence and commercial farming, small-scale enterprises and trade. Kilosa district covers an area of 14,245 km² and experiences rainfall from November to May. The dry season occurs from June to October. The average annual temperature is 24.6°C (Chipwaza et al., 2015). The main economic activities include agriculture and livestock keeping. Both districts experience a high infestation of rodents and cockroaches.

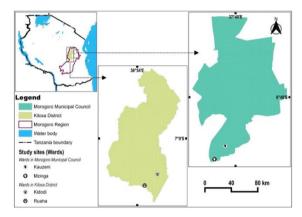


Figure 1. A map showing the selected study sites

Study Design

The study followed a cross-sectional design and was conducted from January 2022 to April 2022. Laboratory analyses for both cockroaches and rodents were conducted mainly in Sokoine University of Agriculture (SUA) laboratories.

Sampling procedures and sample processing

P. americana and R. rattus were collected from four randomly selected wards, two from each district. A total of 57 P. americana and 114 R. rattus were trapped from 62 randomly selected households, 22 A. americana from Morogoro Municipal and 35 from Kilosa District, while 54 R. rattus were from Morogoro Municipal and 60 from Kilosa District. P. americana were manually caught and stored in sterile containers, while R. rattus were trapped in wire cages.

Trapped P. americana and R. rattus were identified based on their morphological features with the guidance of identification keys (Happold et al., 2013; Picker et al., 2004). Both P. americana and R. rattus were sacrificed chloroform. Periplaneta americana individually placed in tubes with 5ml of normal saline (0.9% NaCl) and shacked manually to dislodge bacteria from their body surface; after that soaked in 90% ethanol for 5 minutes and dried to further decontaminate their external body surface. They were then rewashed with sterile normal saline to remove traces of ethanol. Both P. americana and R. rattus were dissected aseptically using scissors to remove the hindgut. The hindgut contents were collected in sterile tubes containing maximum recovery diluent media, stored in a sterile cool box with ice cubes, and transported to Sokoine University of Agriculture for further laboratory analysis.

In aseptic conditions, the hindguts of fifty-seven *P. americana* and 114 *R. rattus* from Morogoro municipal and Kilosa district were dissected and pooled. Four pools were obtained (two representing the hindguts of *P. americana* and *R. rattus* from Morogoro municipal, and two representing the hindgut of *P. americana* and *R. rattus* from Kilosa district). The pools have been abbreviated using the following key: PA1; *P. americana* from Morogoro district, PA2; *P. americana* from Kilosa district, RR1; *R. rattus* from Morogoro district, RR2; *R. rattus* from Kilosa district.

DNA extraction

DNA was extracted from the hindgut content stored in buffered peptone water. Genomic DNA was extracted using the Quick-DNA universal extraction kit (Zymo Research, USA) protocol per the manufacturer's instructions. The quantity and quality of gDNA were then measured using a NanoVue Plus spectrophotometer at a wavelength of $260 \text{nm} (A_{260}/A_{280})$. A ratio between 1.8 and 2.0 indicated a high-quality gDNA.

Library preparation and sequencing

The MinION sequencing techniques of Oxford Nanopore technology were used in this study. The MinION sequencing libraries were generated using the DNA sequencing-barcoding kit (SQK-PCB109-Oxford Nanopore Technologies) following the manufacturer's protocol.

Bioinformatics analysis

Firstly, the raw reads were subjected to quality trimming using Cutadapt V3.4 to remove low-quality reads and trim barcode and adopter sequences. The trimmed reads were then assembled using Megahit V1.2.9 to generate contigs representing the genetic material in the metagenomic samples. These contigs were subsequently binned into individual microbial genomes using Metabat2 V2.15 after aligning the reads to assembled contigs using Bowtie2 V2.5.1; Metabat2 utilises sequence composition and coverage information. The resulting metagenomic bins were annotated using Prokka V1.14.6, which predicted and annotated protein-coding genes within the bins by leveraging the Prodigal gene prediction tool and the NCBI non-redundant database. To determine the taxonomic composition of the metagenomic samples, we performed metagenomic taxonomic classification using Kraken2 V2.1.3 with the RefSeq database.

Statistical analysis

R-studio software (V 4.2.3) was used for statistical analysis; whereby descriptive statistics was used to analyse the relative abundances of bacteria. The Phyloseq package was used for diversity analysis. The Chi-square and t-test were used to compare the abundance and diversity of bacteria genera between the host species and areas sampled. Differences were considered to be significant at the level of p<0.05.

RESULTS

The general characteristics of the sequencing data set are shown in Table 1. The data set consisted of 65 633 and 772 874 sequences for *R. rattus* and *P. americana* samples collected from Morogoro municipal, and 437 465 and 50 859 sequences for *R. rattus* and *P. americana* from Kilosa district. A total of 404 575 818 bases were sequenced from

four pooled samples, most of which were from *R. rattus* from Kilosa (235 119 094) and fewer from *P. americana* from Kilosa (19 708 309).

Table 1. General characteristics of each sequencing data for each host species collected

Sample	Reads	Bases (bp)	Number of bins
RR1	65633	23120071	9
PA1	772874	126628344	19
RR2	437465	235119094	29
PA2	50859	19708309	7

Key; RR1; Rattus rattus from Morogoro municipal, PA1; Periplaneta americana from Morogoro municipal, RR2; Rattus rattus from Kilosa district, PA2; Periplaneta americana from Kilosa district

Bacterial Community

Taxonomic analysis was performed to identify the hindgut bacterial community of the two host species sampled. A total of 27 bacterial phyla were identified Proteobacteria and Bacteroidetes were the most abundant phyla among all host species sampled. Proteobacteria was abundant from R. rattus from Kilosa district (48%) and P. americana (41%) and R. rattus (40%) from Morogoro municipal. Bacteroidetes were also highly abundant from P. americana from Kilosa district (48%) (Fig. 2A & B). To further explore the hind-gut bacterial community of R. rattus and P. americana, the bacterial community at the genus level was analysed and 806 genera were identified. Fifty-one genera with sequence reads above 15 were presented in Table 2. Five bacteria genera shared by pool samples in the two host species (R. rattus and P. americana) were Proteus, Fusobacterium, Escherichia, Citrobacter and Bacteroides.

A chi-square test was performed to determine the significant differences in the number of sequences of bacterial genera that were shared between the two host species (*R. rattus* and *P. americana*). From Kilosa district, the results showed that the number of sequences of *Proteus* (*p*<0.001), *Fusobacterium* (*p*<0.001), *Escherichia* (*p*<0.001), *Citrobacter* (*p*<0.001) and *Bacteroides* (*p*<0.001) were significantly higher in *R. rattus* compared to *P. americana*. For Morogoro municipal, the number of sequences of *Bacteroides* (*p*<0.001), *Citrobacter* (*p*<0.001) and *Proteus* (*p*=0.01) were also significantly higher in *R. rattus* compared to *P. americana*. The number of sequences of *Escherichia* (*p*=0.449) and *Fusobacteria* (*p*=0.087) showed no significant differences between *R. rattus* and *P. americana*.

The significant differences in sequences of shared genera were also determined between the two districts sampled (Kilosa and Morogoro district). The results showed that the number of sequences of *Bacteroides* (p<0.001), *Citrobacter* (p<0.001), *Escherichia* (p<0.001) and *Proteus* (p<0.001) were significantly higher in *R. rattus* from Kilosa than that from Morogoro district but *Fusobacterium* (p=0.414) showed no significant difference. The number of sequences of *Bacteroides* (p<0.001), *Citrobacter* (p<0.001), *Escherichia* (p<0.001), *Fusobacterium* (p<0.001) and *Proteus* (p<0.001) were significantly higher for *P. americana* from Morogoro district than those from Kilosa district.

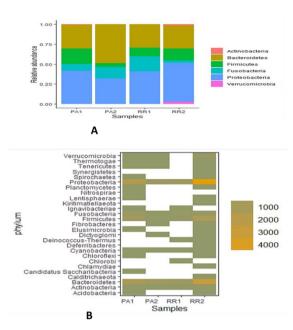


Figure 2. Bacterial composition of different host species (A) and at phylum distribution (B)

Table 2. Taxonomic classification of bacteria at genus level from pools of the two host species sampled

Taxonomy (Phylum; Class; Family; Genus)		Number of sequences			
		Kilosa		Morogoro	
	RR2	PA2	PA1	RR1	
Proteobacteria, Betaproteobacteria, Alcaligenaceae, Achromobacter	15	-	-	-	
Proteobacteria, Gammaproteobacteria, Moraxellaceae, Acinetobacter	203	-	17	-	
Proteobacteria, Gammaproteobacteria, Aeromonadaceae, Aeromonas	16	-	-	-	
Verrucomicrobia, Verrucomicrobiae, Akkermansiaceae, Akkermansia	274	-	-	-	
Proteobacteria, Betaproteobacteria, Alcaligenaceae, Alcaligenes	390	-	60	-	
Bacteroidetes, Bacteroidia, Rikenellaceae, Alistipes	240	-	-	-	
Firmicutes, Clostridia, Lachnospiraceae, Anaerotignum	30	-	-	-	
Firmicutes, Bacilli, Bacillaceae, Bacillus	28	-	26	-	
Bacteroidetes Bacteroidia, Bacteroidaceae, Bacteroides	1838	667	972	456	
Firmicutes, Clostridia, Lachnospiraceae, Blautia	29	-	-	-	
Fusobacteria, Fusobacteriia, Aeromonadaceae, Bordetella	73	-	-	-	
Proteobacteria, Alphaproteobacteria, Caulobacteraceae, Brevundimonas	17	-	-	-	
Proteobacteria, Gammaproteobacteria, Enterobacteriaceae, Citrobacter	537	86	263	42	
Firmicutes, Clostridia Comamonadaceae, Peptostreptococcaceae, Clostridioides	15	-	-	-	
Firmicutes, Clostridia, Clostridiaceae, Clostridium	135	-	126	18	
Proteobacteria, Betaproteobacteria, Comamonas	85	-	-	-	
Proteobacteria, Deltaproteobacteria, Desulfovibrionaceae, Desulfovibrio	70	-	25	-	
Proteobacteria, Gammaproteobacteria, Enterobacteriaceae, Enterobacter	239	-	38	17	
Firmicutes, Bacilli, Enterococcaceae, Enterococcus	23	-	62	-	
Proteobacteria, Gammaproteobacteria, Enterobacteriaceae, Escherichia	302	88	178	164	
Firmicutes, Clostridia, Ruminococcaceae, Faecalibacterium	27	-	-	-	
Bacteroidetes, Flavobacteriia, Flavobacteriaceae, Flavobacterium	20	-	-	-	
Firmicutes, Clostridia, Ruminococcaceae, Flavonifractor	75	-	-	-	
Fusobacteriota, Fusobacteriia, Fusobacteriaceae, Fusobacterium	290	210	354	310	
Firmicutes, Clostridia, Peptostreptococcaceae, Intestinimonas	44	-	-	-	
Proteobacteria, Gammaproteobacteria, Enterobacteriaceae, Klebsiella	265	-	60	18	
Firmicutes, Bacilli, Planococcaceae, Kurthia	23	-	-	-	
Firmicutes, Clostridia, Lachnospiraceae, Lachnoclostridium	327	-	41	-	
Firmicutes, Bacilli, Lactobacillaceae, Lactobacillus	272	-	63	30	

Key; PA1; Periplaneta americana from Morogoro district, PA2; Periplaneta americana from Kilosa district, RR1; Rattus rattus from Morogoro district, RR2; Rattus rattus from Kilosa district. "- "not found.

Table 3. Continued

Taxonomy (Phylum; Class; Family; Genus)		Number of sequences			
		Kilosa		Morogoro	
	RR2	PA2	PA1	RR1	
Firmicutes, Bacilli, Streptococcaceae, Lactococcus	18	-	-	-	
Firmicutes, Bacilli, Bacillaceae, Lysinibacillus	109	-	205	43	
Proteobacteria, Gammaproteobacteria, Morganellaceae, Morganella	203	-	70	-	
Bacteroidetes, Bacteroidia, Muribaculaceae, Muribaculum	30	-	-	-	
Bacteroidetes, Flavobacteriia, Flavobacteriaceae, Myroides	225	-	18	-	
Bacteroidetes, Bacteroidia, Odoribacteraceae, Odoribacter	40	-	-	-	
Firmicutes, Clostridia, Oscillospiraceae, Oscillibacter	24	-	-	-	
Bacteroidetes, Bacteroidia, Tannerellaceae, Parabacteroides	250	-	105	-	
Proteobacteria, Gammaproteobacteria, Morganellaceae, Proteus	396	144	371	306	
Proteobacteria, Gammaproteobacteria, Morganellaceae, Providencia	543	-	139	27	
Proteobacteria, Gammaproteobacteria, Pseudomonadaceae, Pseudomonas	352	-	27	-	
Proteobacteria, Gammaproteobacteria, Moraxellaceae, Psychrobacter	98	-	-	-	
Proteobacteria, Gammaproteobacteria, Enterobacteriaceae, Salmonella	28	-	19	-	
Proteobacteria, Gammaproteobacteria, Yersiniaceae, Serratia	50	-	53	-	
Proteobacteria, Gammaproteobacteria, Shewanellaceae, Shewanella	49	-	98	-	
Bacteroidetes, Sphingobacteriia, Sphingobacteriaceae, Sphingobacterium	60	-	-	-	
Firmicutes, Bacilli, Staphylococcaceae, Staphylococcus	17	-	-	-	
Proteobacteria, Gammaproteobacteria, Xanthomonadaceae, Stenotrophomonas	288	70	48	-	
Firmicutes, Bacilli, Streptococcaceae, Streptococcus	16	-	-	-	
Actinobacteria, Actinobacteria, Streptomycetaceae, Streptomyces	19	-	-	-	
Proteobacteria, Gammaproteobacteria, Vibrionaceae, Vibrio	16	-	-	-	
Firmicutes, Clostridia, Peptostreptococcaceae, Acetoanaerobium	-	-	66	-	

Key; PA1; Periplaneta americana from Morogoro district, PA2; Periplaneta americana from Kilosa district, RR1; Rattus rattus from Morogoro district, RR2; Rattus rattus from Kilosa district. "- "not found."

Potential Pathogenic Bacteria

Potential pathogenic bacteria were identified from the pooled samples of two host species. Only bacteria genera and species with sequence reads above 15 were presented. Twenty-four pathogenic genera were identified with common pathogenic genera, as shown in Table 3. Among pathogenic genera identified, five genera (Proteus, Fusobacterium, Escherichia, Citrobacter Bacteroides) were shared by all host species collected from two areas sampled. Relative abundances of pathogenic bacteria carried by the two host species from each sampled site were observed. The highest relative abundance of pathogenic bacteria was observed from P. americana from Kilosa followed by R. rattus from Morogoro district (Fig 3). Pathogenic bacteria were further

explored at the species level, as shown in Table 4, where *Escherichia coli*, *Citrobacter freundii*, and *Proteus mirabilis* were found in all host species. *Klebsiella pneumoneae* was found in all hosts except in *P. americana* from Kilosa. *Salmonella enterica* was also found only in *P. americana* from Kilosa and *R. rattus* from Morogoro district.

Each bar represents the relative abundance of each bacterial taxa (A). (B). A heatmap representing abundances of bacterial taxon depicted by colour intensity (B). Key; PA1; *P. americana* from Morogoro district, PA2; *P. americana* from Kilosa district, RR1; *R. rattus* from Morogoro district, RR2; *Rattus rattus* from Kilosa district.

Table 4. Common potential pathogenic bacteria genera found in the pooled samples of two host species

RR1	PA1	RR2	PA2
Bacteroides	Bacteroides	Bacteroides	Bacteroides
Citrobacter	Citrobacter	Citrobacter	Citrobacter
Clostridium	Clostridium	Clostridium	Escherichia
Enterobacter	Enterobacter	Enterobacter	Fusobacterium
Escherichia	Enterococcus	Enterococcus	Proteus
Klebsiella	Escherichia	Escherichia	
Fusobacterium	Klebsiella	Klebsiella	
Proteus	Proteus	Proteus	
	Pseudomonas	Pseudomonas	
	Salmonella	Salmonella	
	Fusobacterium	Fusobacterium	
		Staphylococcus	
		Streptococcus	
		Vibrio	

Key; PA1; Periplaneta americana from Morogoro district, PA2; Periplaneta americana from Kilosa district, RR1; Rattus rattus from Morogoro district, RR2; Rattus rattus from Kilosa district

Table 5. Some potential	nathogenic bacte	eria species fo	ound in the pooled	I samples of two hos	species
Table 5. Some bottima	Daulogellie Daeu	ilia succics i	ound in the boolet	i sambics of two nos	i species.

RR1	PA1	RR2	PA2
Bacteroides cellulosilyticus	Citrobacter freundii	Citrobacter freundii	Citrobacter freundii
Citrobacter freundii	Clostridium botulinum	Bacteroides cellulosilyticus	Escherichia coli
Escherichia coli	Escherichia coli	Clostridium botulinum	Fusobacterium ulcerans
Klebsiella pneumoniae Proteus mirabilis	Klebsiella pneumoniae Proteus mirabilis	Enterobacter hormaechei Escherichia coli	Proteus mirabilis
	Salmonella enterica	Klebsiella pneumoniae Proteus mirabilis	
		Pseudomonas aeruginosa	
		Fusobacterium ulcerans Salmonella enterica	

Key; PA1; Periplaneta americana from Morogoro district, PA2; Periplaneta americana from Kilosa district, RR1; Rattus rattus from Morogoro district, RR2; Rattus rattus from Kilosa district.

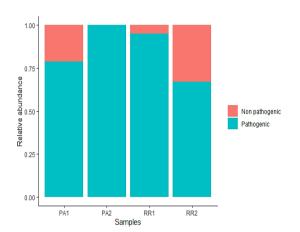


Figure 2. Relative abundances of potential pathogenic bacteria from the pooled samples of two host species. Key; PA1; *Periplaneta americana* from Morogoro district, PA2; *Periplaneta americana* from Kilosa district, RR1; *Rattus rattus* from Morogoro district, RR2; *Rattus rattus* from Kilosa district.

Next, an alpha diversity on all samples was performed. Alpha diversity refers to the diversity of a specific region or ecosystem. The observed Shannon and Chao 1 indices indicated higher species diversity in rats of the Kilosa district and cockroaches of Morogoro municipal (Fig. 4). The microbiome diversity in Morogoro municipal was higher for cockroaches than for rats. However, it was not statistically significant (p>0.05). In Kilosa district, the microbiome diversity was higher for rats than for cockroaches, but it was not statistically significant (p>0.05) (Fig. 5) as well.

In comparing the two districts, the microbiome diversity was high in Kilosa district compared to Morogoro municipal, though the difference was not statistically pronounced (Fig. 6). When beta diversity was assessed. The finding showed that variation of microbial communities between samples existed. However, the composition was similar between cockroaches from Kilosa district and rats from Morogoro municipal (Fig. 7).

The closer the two sample points are, the more similar the bacterial composition of the two samples. Key; PA1; *P. americana* from Morogoro district, PA2; *P. americana* from Kilosa district, RR1; *R. rattus* from Morogoro district, RR2; *R. rattus* from Kilosa district.

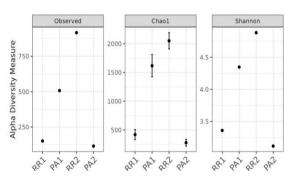


Figure 3: Alpha diversity of collected samples

Key; PA1; Periplaneta americana from Morogoro district, PA2; Periplaneta americana from Kilosa district, RR1; Rattus rattus from Morogoro district, RR2; Rattus rattus from Kilosa district.

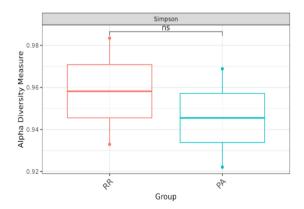


Figure 4: Alpha diversity of collected host species

Key; PA; Periplaneta americana from Morogoro district and Kilosa district, RR; Rattus rattus from Morogoro district and Kilosa district.

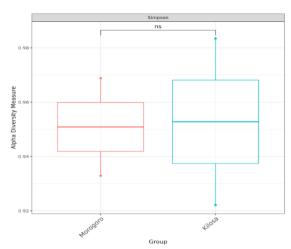


Figure 5: Alpha diversity of collected host species from two sampled areas.

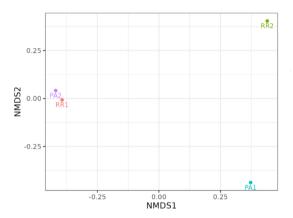


Figure 6: Non metric dimensional scaling (NMDS) using Bray-curtis dissimilarity

DISCUSSION AND CONCLUSION

The animal gut contains many bacterial communities with a complex composition comprising over 500 species. Intestinal microbes are a complex and dynamic ecosystem that coevolves with their host (Han et al., 2020). Previous studies have shown that gut microbiome composition may differ among species based on, diet, environmental factors, genetics and location (Han et al., 2020; Lee et al., 2020). Results showed that the composition of the microbiome did not differ between the hosts collected from the two areas, but their abundances were varied. Proteobacteria and Bacteroidetes were the most abundant phyla compared to other phyla.

Findings from this study regarding the composition of gut microbiome between *P. americana* and *R. rattus* are similar to other previous studies, which showed similar bacteria phyla from *P. americana* and *R. rattus* (Tinker and Ottesen, 2016; Debebe et al., 2017; Han et al., 2020; He et al., 2020). Other previous studies reported on the influence of geographical location on the composition of the gut microbiome (Goertz, Menezes, et al., 2019; Wang, 2022). Findings from this study also revealed that geographical location could influence gut microbiome composition as it was found that Proteobacteria and Bacteroidetes were the most abundant phyla from hosts collected from Kilosa district than that of Morogoro municipal. However, there are other factors reported by

several findings which have more influence on the gut microbiome, including species identity (host genetic), sex, diet, gut pH, age and others (Vicente et al., 2016; Kakumanu et al., 2018; Goertz et al., 2019; Wang, 2022; Tinker and Ottesen, 2016;).

Recently, there have been ongoing studies regarding gut microbiomes since they provide insight into both culturable and non-culturable microbiomes. As reported by several previous studies, symbiont bacteria can be used for pest control, several tactics including manipulation of microbial symbionts have been documented, which results in either minimising pest population or reducing their chance of transmitting pathogens (Shapiro-ilan and Gaugler, 2002; Noman et al., 2019; Pan et al., 2020). For instance, Dillon & Dillon, 2004; Arora and Douglas, 2017 reported that symbiont microorganisms may be genetically modified and in turn, become pathogens and induce effects on the targeted pest. Several bacteria, including Enterobacter species (Arora and Doglas, 2017), Klebsiella species, Proteus species and others (Dillon and Dillon, 2004; Noman et al., 2019) were reported to be involved in the control of various insect pests.

The microbiome diversity observed was higher for species from Kilosa district (the rural area) compared to that of Morogoro municipal (urban area); however, it was not statistically supported. The variation in this diversity is perhaps supported by the fact that in our study area, those hosts collected from rural environments had a environment heterogeneous compared to urban environments. These findings are similar to other previous studies, which also found that hosts from rural areas had higher microbiomes compared to those from urban environments (Aimeric et al., 2018; Gurbanov et al., 2022). This is perhaps due to the fact that in rural areas, there is a diversification of food materials, plus the hygienic status of rural areas is not much improved; thus, hosts are likely exposed to more microbes compared to a host of the urban environment.

Findings from this study also showed the diversity within the district sampled whereby in Morogoro municipal, the microbiome diversity was observed to be higher for *P. americana* than *R. rattus*; this is perhaps because of the fact that *P. americana* of urban areas have a wide range of habitats which is supported by their body size compared to *R. rattus* thus enable them to consume a wide variety of food sources (Kakumanu et al., 2018). On the other hand, rats in Kilosa district had a higher microbiome than cockroaches. This may be supported by the fact that the environmental structure of rural areas favours rats more than cockroaches; thus, rats can easily access a wider variety of food.

This study also presents the pathogenic bacteria carried by the host species sampled. The relative abundance of pathogenic bacteria was high in P. americana of Kilosa, followed by R. rattus of Morogoro district. Some pathogenic bacteria species reported by several previous studies to cause human and animal diseases identified from this study include E. coli, C. freundii and P. mirabilis. Also, other species were S. enterica, K. pneumoneae, and C. botulinum. These findings are similar to previous reports highlighting pathogenic bacteria found in the gut of P. americana and R. rattus (Kakumanu et al., 2018; He et al., 2020; Gurbanov et al., 2022). Several studies in Tanzania have also demonstrated that rats are potential environmental source of zoonotic bacteria and some isolates having multidrug straits (Kimwaga et al. 2023; Ndakidemi et al.,

2023: Mkopi et al., 2024). This is because their biology. behaviours, and habitats contribute to their effectiveness in spreading diseases. These two host species have been numerously reported to be found in indoor environments, and various studies have reported on the potentially carry pathogenic bacteria in their gut and external surfaces. Thus, there is a great chance of these pathogenic bacteria being transmitted to humans and animals through direct contamination and faeces droppings. Controlling their populations is crucial for preventing the spread of infectious diseases. This includes maintaining clean environments, proper waste management, sealing entry points to buildings, and using appropriate pest control methods. Additionally, public awareness and education about the risks associated with these pests are essential for reducing their impact on human health (Kimwaga et al. 2023; Ndakidemi et al., 2023; Mkopi et al., 2024).

Limitation of the Study

The gut microbiome composition observed from this study focused only on the geographical area where the two host species were collected. Other factors, such as species genetics, diet, host parameters, and environmental parameters, which are claimed to impose more influence on the gut microbiome, were not assessed.

This study summarises the composition of the bacteria microbiome between *P. americana* and *R. rattus* collected from two districts (Morogoro municipal, which is an urban area, and Kilosa district, which is a rural area). The bacteria composition was more or less similar to the host species collected from the two areas sampled. Also, the study revealed pathogenic bacteria and symbiont bacteria. This study suggests surveillance of these pests to minimise outbreaks and transmission of zoonotic diseases. Since this study did not focus on factors influencing microbiome composition and diversity, further studies are recommended to be conducted to see the influence of those factors on the gut microbiome.

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Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: BRK., AM., Design: BRC., AM., LM., Data Collection or Processing: BRC., Analysis or Interpretation: BRC., AM., LM., Literature Search: BRC., AM., Writing: BRC., AM., LM.

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REFERENCES

Arora AK, Douglas AE. 2017. Hype or opportunity? Using microbial symbionts in novel strategies for insect pest control. Journal of Insect Physiology, *103*:10-17.

Bauer E, Lampert N, Mikaelyan A, Köhler T, Maekawa K, Brune A. 2015. Physicochemical conditions, metabolites and community structure of the bacterial microbiota in the gut of wood-feeding cockroaches (Blaberidae: Panesthiinae). FEMS Microbiology Ecology, 91(2):1–14

Brune A, Dietrich C. 2015. The Gut Microbiota of Termites: Digesting the Diversity in the Light of Ecology and Evolution. Annual Review of Microbiology, 69(1):145–166.

Caruso R, Ono M, Bunker M. E, Núñez G, Inohara N. 2019. Dynamic and Asymmetric Changes of the Microbial Communities after Cohousing in Laboratory Mice. Cell Reports, 27(11):3401-3412.

Chipwaza B, Mhamphi GG, Ngatunga SD, Selemani M, Amuri M, Mugasa JP, Gwakisa, PS. 2015. Prevalence of bacterial febrile illnesses in children in Kilosa district, Tanzania. PLoS Neglected Tropical Diseases, 9(5): e0003750.

Claus SP, Guillou H, Ellero-Simatos S. 2016. The gut microbiota: A major player in the toxicity of environmental pollutants? Biofilms and Microbiomes, 2(1):1–11.

Cruden DL, Markovetz AJ. 1987. Microbial ecology of the cockroach gut. *Annual* Review of Microbiology, *41*(66): 617–643.

Debebe T, Biagi E, Soverini M, Holtze S, Bernd T, Birkemeyer C, Wyohannis D, Lemma A, Savkovic V, König B, Candela M, Birkenmeier G. 2017. Unraveling the gut microbiome of the long-lived naked mole-rat. Scientific Reports, 7(1): 9590

Dillon RJ, Dillon VM. 2004. The gut bacteria of insects: Nonpathogenic Interactions. Annual Reviews in Entomology 49(1): 71–92.

Engel P, Moran NA. 2013a. Functional and evolutionary insights into the simple yet specific gut microbiota of the honey bee from metagenomic analysis. Gut Microbes, 4(1): 60–65.

Engel P, Moran NA. 2013b. The gut microbiota of insects - diversity in structure and function. FEMS Microbiology Reviews, *37*(5): 699–735.

Ericsson AC, Franklin CL. 2015. Manipulating the gut microbiota: Methods and challenges. ILAR Journal, *56*(2): 205–217.

Ernest S, Nduganda AR, Kashaigili JJ. 2017. Urban Climate Analysis with Remote Sensing and Climate Observations: A Case of Morogoro Municipality in Tanzania. pp. 120–131.

Goertz S, Menezes AB, De Birtles RJ, Id JF, Lowe E, Maccoll ADC, PoulinB, Id SY, Bradley JE, Taylor CH. 2019. Geographical location influences the composition of the gut microbiota in wildhouse mice (Mus musculus domesticus) at a fine spatial scale. Plos one, 14(9):

Gurbanov R, Kabaoğlu U, Yağci T. 2021. Metagenomic profiling of gut microbiota in urban and rural rats: A comparative study. pp. 1-23.

Han X, Shao H, Wang Y, Hu A, Chen R, Chen Q. 2020. Composition of the bacterial community in the gastrointestinal tract of Kunming mice. Electronic Journal of Biotechnology, 43: 16–22.

He W, Xiong Y, Ge J, Chen Y, Chen X, Zhong X, Ou Z, Gao Y, Cheng M, Mo Y, Wen Y, Qiu M, Huo S, Chen

S, Zheng X, He H, Li Y, You F, Zhang M, Chen Q. 2020. Composition of gut and oropharynx bacterial communities in *Rattus norvegicus* and *Suncus murinus* in China. BMC Veterinary Research, *16*(1): 1–11.

Kakumanu Ml, Maritz JM, Carlton JM, Coby S. 2018. Overlapping Community Compositions of Gut and faecal Microbes in lab-reared and Field-collected German Cockroaches. Applied and Environmental Microbiology, 84(17): e01037-18

Kimwaga RB, Mzula A, Mnyone LL. 2023. Antimicrobial Profiles of Pathogenic Enteric Bacteria Isolated from Commensal Rodents and Cockroaches from Morogoro Region in Tanzania: An Environmental Vehicle for Resistance Transmission to Human and Animals. Journal of Health, Medicine and Nursing, 105 (2023): 1-8.

Lee S, Kim JY, Yi M, Lee I., Hye WL, Moon S, Yong D. 2020. Comparative Microbiome Analysis of Three Species of Laboratory-Reared Periplaneta Cockroaches. The Korean Journal of Parasitology, *58*(5): 537–542.

Mkopi JC, Mushi J, Mzula, A. 2024. Occurrence and antimicrobial resistance pattern of Escherichia coli and Salmonella species isolated from domestic and peridomestic rodents. German Journal of Microbiology, 4 (2): 15-28. https://doi.org/10.51585/gjm.2024. 2.0034

Ndakidemi FP, Baravuga ME., Mzula A, Katakweba AS. 2023. Antimicrobial susceptibility patterns and molecular phylogenetics of *Proteus mirabilis* isolated from domestic rats: environmental Tanzania.German Journal of Microbiolology, 3(1):13-23. https://doi.org/10.51585/gjm.2023.1.0022

Noman MS, Liu L, Bai Z, Li Z. 2019. Tephritidae bacterial symbionts: potentials for pest management. Bulletin of Entomological Research, *110*(1): 1-14.

Pan X, Wang X, Zhang F. 2020. New Insights into Cockroach Control: Using Functional Diversity of Blattella germanica Symbionts. Insects, *11*(10): 696.

Schauer C, Thompson CL, Brune A. 2012. The bacterial community in the gut of the cockroach Shelfordella lateralis reflects the close evolutionary relatedness of cockroaches and termites. Applied and Environmental Microbiology, 78(8): 2758–2767.

Sepulveda J, Moeller AH. 2020. The effects of temperature on animal gut microbiomes. Frontiers in Microbiology, *11*: 384.

Shapiro-Ilan DI, Gaugler R. 2002. Production technology for entomopathogenic nematodes and their bacterial symbionts. Journal of Industrial Microbiology and Biotechnology, 28(3): 137-146.

Teyssier A, Rouffaer LO, Hudin NS, Strubbe D, Matthysen E, Lens L, White J. 2018. Inside the guts of the city: urban-induced alterations of the gut microbiota in a wild passerine. Science of the Total Environment, *612*: 1276-1286.

Tinker KA, Ottesen EA. 2016. The core gut microbiome of the American cockroach, Periplaneta americana, is stable and resilient to dietary shifts. Applied and Environmental Microbiology, 82(22): 6603–6610.

Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon, JI. (2009). The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Science Translational Medicine, *I*(6): 6ra14-6ra14.

Vicente CS, Ozawa S, Hasegawa K. 2016. Composition of the cockroach gut microbiome in the presence of parasitic nematodes. Microbes and Environments, *31*(3): 314–320.

Wang Z, Zhang C, Li G, Yi X. 2022. The influence of species identity and geographic locations on the gut microbiota of small rodents. Frontiers in Microbiology, *13*: 983660.

Weiss B, Aksoy S. 2011. Microbiome influences on insect host vector competence. Trends in Parasitology, 27(11): 514–522.



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Otic Microbiome Transplantation in a Dog with Recurrent Otitis Externa: Unraveling Allergic Secrets with Break Through Treatment Methodology



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ABSTRACT

Canine otitis externa (OE) is a common inflammatory disease that is frequently complicated by secondary bacterial and/or yeast infections. The otic microbial population is more complex than appreciated by cytological methods and aerobic culture alone. In the present case report a dog with recuurent OE and newly diagnosed otitis media was enrolled. Due to previous unsuccessful treatment trials with immunosupressive agents and antibacterial choices, with polypharmacy, we decided to perform manupilation of the microbiome in an attempt to investigate otic microecology. For this purpose first author gave his opinion for frontier knowledge and several years of experience for microbiome transplantation. Given literature data regarding ear canal microbiome among dogs with OE, is easily distinguishable to that of healthy ones, we decided to perform otic microbiome transplantation (omT). Microbiome samples were taken prior to and thereafter 10th days of omT, were collected and sent to MiDOG Limited Liability Company (LLC) service for next generation sequencing. Following omT, clinical signs were resolved and regressed, even starting on day 3 with satisfactory results (exudate was disappeared). Microbiome analytes showed Porphyromonas cangingivalis, Peptoclostiridium sp., Pasteurella canis, Malassezia pachydermatis and Aspergillus sp. with increased relative abundances, whereas no more pathogen were evident 10 days after omT. Pathogens were switched and substituted with new microorganisms with potential probiotic facilities. Observed species were increased following omT [prior to omT 47 vs. after omT as 98]. This case report for the first time, showed the efficacy of changing microechology of otic microechology by omT. This treatment modality would be developed by the resarcher group herein dedicated.

INTRODUCTION

Otitis externa (OE) and/or media, are unresolved inflammatory recation for external ear canal and middle ear cavity, respectively, among dogs (Doyle, 2004; de Bellis, 2015; Koch, 2017; Pye, 2018; Bajwa, 2019; Harvey, 2022). Altough veterinary surgeons cope with OE, otitis media keeps its position namely a disease condition that has been underrated in the vast majority veterinary practices (de Bellis, 2015). Given ear canal microbiome among dogs with OE, is easily distinguishable to that of healthy ones (Bradley et al., 2020; Kasai et al., 2020; Tang et al., 2020), we decided to perform otic microbiome transplantation (omT) in a dog with recurrent OE and newly diagnosed otitis media. Following first authors several years experience of both fecal and skin microbiota transplantation, he and his teammates decided to transplate otic microbiome in an unresolved case with

several long duration of antibacterial trials and polypharmacy.

MATERIALS AND METHODS

Case history

A 3 years old dog was referred to several different veterinary private practices and finally the owner was instructed to a veterinary clinic in which an academician was in advisory board with a surgery certificate. However at that clinic 3 different antibacterial choices were prescribed, as was also the case in several clinics. Previously polypharmacy included antibiotics, antihistaminics and immunosupressive drugs. On referral there was bilateral exudate, rare head shaking and decerased hearing. There was no facial nerve paralysis nor Horner syndrome. Signs of OE were evident. Inflammation of the ear canal was strikingly evident. Ear

canal was nearly closed, denoting terminal staging of inflammation. The owner also reported cutaneous adverse food reaction for several months, in which referred veterainary surgeons frequently offered and prescribed diets (none of them were low glycemic index, nor low carbohaydrates ratios).

Ethics statement

Both dogs participated at the present research study were referred to the University of Adnan Menderes, Faculty of Veterinary, Department of Internal Medicine. Both dogs were subjected to physical examination. Matched microbiome samples, 10 days apart prior to and thereafter omT, were collected and used solely for this case report with written owner consent. The present case report, as part of another research/clinical trial was approved by the local ethic committee of Aydın Adnan Menderes University-HADYEK on October 27, 2021 with no: 64583101/2019/022.

Microbiome analytes

A total of 2 seperate cotton swab samples were taken from middle ear, were analyzed. For sample collection, DNA extraction, library preparation, and sequencing brief explanation was follows. Samples were collected with swaps that were prepared and sent previously by MiDOG LLC service (including all neceesary instructions). Following unboxing, aseptical sterile, DNA free swab was handled and subjected gently to the middle ear of recipient. Briefly twisting off and twirling the swab 2-3-times over the ear charge and the swab tip was broken off into previously purchased sterile, sample administration material containing a DNA/RNA preservative, as was previously described (Tang et al., 2020), similarly to preior methodlogy (Ural et al., 2002; Ural et al., 2023) All samples were then shipped for processing to the MiDOG LLC testing center (Irvine, California). Genomic DNA available through ZymoBIOMICSTM-96 DNA kit (Cat. No. D4304, Zymo Research Corp.) (Tang et al., 2020) along with 16S rDNA V1-V3 region was focused against bacterial analysis. Relative abundances regarding fungi were detected suggesting an equivalency of one 16S rDNA copy to one fungal ITS copy. Entire primer sequences were ready on the MiDOG LLC Service facilities. Methodology was previously determined (Tang et al., 2020).

Unenriched otic microbiome transplantation

This untold, entirely new technique was developed by the first author (K.U.) for several stages of scientific organization invoving consideration, planning, preparation and application. For every single moment first author tought like an engineer, who put measuring tape on his shoulder. Donor was a known owner kept dog without stress presentation, as was shown in table 1. Briefly omT was shown in figure 1. With every stages of it. Cotton swap was not subjected to any other condition, directly used fort his purpose. Material was collected from the donor and suddenly transferred to recepient.

Table 1. Stress interpretation, adopted from (Lloyd, 2017) for dogs enrolled herein for omT.

	donor	recipient
Clinical enterance	0	1
Following entrerance to practice	0	0
room at faculty		



Figure 1. Otic microbiome transplantation in a dog with recurrent otitis externa. A) preparation of cotton swap ready for use, b) cotton swap was directed to external ear canal and rotated for entire collection of presumably healthy microbiome, c)material was withdrawn from donor, d) recipient, namely a dog with recurrent otitis externa, was just prior to otic microbiome transplantation, e) cotton swap was directed to recipient's external ear canal and f) rotated for entire contact.

Assessing stress value for both donor and recipient dogs during omT

In an attempt to analyze the stress levels of both donor and recipeint dogs, assessment previously determined (Lloyd, 2017) was used by the reseracher team as follows.

This stress value was based on scoring from 0 [highest friendly attraction, outgoing and mindful/concerned of attention] to 5 [extreme presentation of stress; i., hiding, barking, howling etc].

RESULTS

Photographic evidence of proof was shown below at figure 2 also presenting both the donor and the recipient (before and after omT).



Figure 2. Showing the donor with a healthy ear canal (visible and easily seen/observed with an open tissue appearance), whereas c-d) presented the recipient with recurrent otitis externa that the ear canal is nearly be closed, just prior to terminal stage of allergy. At the bottom photograph recorded at 3rd day of omT. Ear canal was visible and quite opened in contrast to referral day. Inflammation was regressed.

Microbiome analytes

Table 2 and 3 presented all necessary data tabulated by MiDOG LLC service located in USA. Figure 3 depicted results of observed species belonging to microbiota members before and after omT. Following omT Alpha diversity was changed and new species were observed as follows (Table 3)

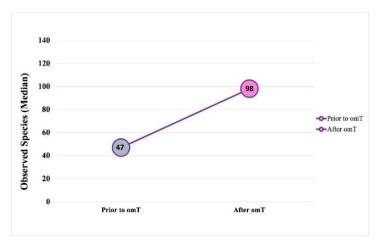


Figure 3. Depicting satisfactory results that was related to omT at ths present case. Observed species belonging to microbiota members were elevated from 47 to 98.

Table 2. *Malassezia pachydermatis*, *Aspergillus sp.*, *Acremonium hyalinulum* relative abundaces were detected as 0.2%, 0.1% and 0.0%, respectively.

	Percei	ntage	Cells per	sample	Normal ranges
Bacteria species detected wit potential clinical relevant disease	h Prior to omT	After omT			
Porphyromonas cangingivalis	25%	2.2%	15.000.000	1.400.000	0-46853
Peptoclostiridium sp.	8.9%	0	5.400.000	0	0-0
Pasteurella canis	1.0%	0%	600.000	0	0-1725
Corynebacterium mastitidis	0.8%	0%	480.000	0	0-770
Truperella bernardiae	0.7%	0%	450.000	0	0-0
Fungi species detected					
Malassezia pachydermatis	0.2%		17		0-22
Aspergillus sp.	0.1%		6		0-30
Acremonium hyalinulum	0.0%		2		0-0

Table 3. New fungal species that were absent prior to omT, which was existed following omT.

New bacterial species (selected ones) observed following omT				
Fusobacterium sp.	19%			
(o) Fusobacteriales sp.	13%			
Bacteroides plebeius	11.6%			
f) Lachnospiraceae sp.	6.9%			
Lachnoclostridium sp.	6.6%			
Megamonas funiformis	5.2%			
Streptococcus equinus-infantarius-luteiensis	4.8%			
New fungal species (selected ones) observed following	omT			
Saccharomyces cerevisiae	21.4%			
Cladosporium sp.	14.3%			
(c)Eurotiomycetes sp.	7.1%			
Lophodermium sp.	7.1%			
Stereum complicatum	7.1%			
Penicillium sp.	7.1%			
Malassezia restricta	4.8%			
Candida sake	4.8%			

DISCUSSION AND CONCLUSION

Clinical findings regarding otitis media among dogs enrolled were frequently accompany OE (shaking of

head shaking, rubbing/scratching the infected ear, appearent exudate within the ear canal) (Doyle, 2004; Harvey, 2022; Koch, 2017, Pye, 2018). In the present

case all aforementioned clinical signs were evident with recurrent presentation, unresponsive to several treatment trials of immunosuppressive and antibacterial choices. Polypharmacy was not accepted by us, and at initial referral with compaints of owner, whom was a teacher, we advised and told her that 'the past is no more concern'. Hence we informed the owner what we tought was 'treat to target' by omT. We, as reseracher group, were luckily in that stress values for both donör and recipient were acceptable, which prompted and motivated us for better application. To our knowledge, prior to transplantation procedure, veterinary surgeons should be aware of stress value for dogs.

In a prior study hypothesized that regarding canine OE; otic microbial echology is more compounded than detected by cytology and aerobic culturing. In that study 30 dogs with OE compared to 10 healthy ears; Staphylococcus spp.; Pseudomonas aeruginosa and Malassezia spp. were isolated with Staphylococcus as most abundant taxa (Bradley et al., 2020). Kasai et al. (2021) investigated alterations within the ear canal microbiota of dogs with OE. In that study ear swab samples were taken from 23 dogs with OE and to those 10 healthy controls. 16S rRNA gene sequenced using Illumina MiSeq and next generation sequencing revealed that otic microbiota in diseased ones exhibited significantly decreased alpha diversity. Elevated relative abundance of the genus Staphylococcus along with other relevant ones suggested that ear canal microbiota of dogs with OE was differing from healthy ones (Kasai et al., 2021). Relatively novel research identified microbiome of healthy/diseased canine ear samples. Out of 589 canine samples, 257 ear swab samples (128 healthy vs. 129 clinically affected) were subjected to next-generation sequencing. According to the results of that research overall loss of microbial diversity and altered relative/absolute abundances of bacteria and fungi were evident in diseased dogs. In comparison to healthy dogs, 78.3% of the diseased ear samples existed microbial overgrowth [i.e. 69.8% bacterial overgrowth, 16.3% fungal overgrowth, and 7.0% both with bacterial/fungal overgrowth]. Within the vast majority taxa enriched in diseased ears were Staphylococcus pseudintermedius, Staphylococcus schleiferi, Malassezia pachydermatis along with Finegoldia magna, Peptostreptococcus canis, and Porphyromonas cangingivalis (Tang et al 2020). In the present article altough only 1 case was involved, same methodlogy was evident by MiDOG LLC testing center California), denoted as next-generation sequencing. In the present case, reported herein Porphyromonas cangingivalis, Peptoclostiridium sp., Pasteurella canis were the most abundant bacterial species with relative abundaces of 25, 8.9 and 1%, respectively. On the other hand mycobiome revealed Malassezia pachydermatis, Aspergillus sp., Acremonium hyalinulum with relative abundaces of 0.2, 0.1 and 0.0%, respectively. Afterwards omT resulted with observed species increased from 47 to 98, as was shown in figure 3. Similar results were deemed available at our case report and research by Tang et al. (2020), in which following omT pathogen bacteria and fungal species were lost, indicating the efficacy of omT.

Regarding the significance and impact of this case report, for the first time omT was applied at veterinary field, alongwith comperative analyses of the otic microbiota, might provide practical strategy for battling OE and media and provide supportive data for the appropriate treatment of otitis among dogs

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: K.U., Design: K.U., Data Collection or Processing: K.U., H.E., S.E., C.B., Analysis or Interpretation: K.U., H.E., S.E., C.B., Literature Search: K.U., H.E., S.E., C.B., Writing: K.U.

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REFERENCES

Bajwa J. 2019. Canine otitis externa-Treatment and complications. The Canadian Veterinary Journal, 60(1):97.

Bradley CW, Lee FF, Rankin SC, Kalan LR, Horwinski J, Morris DO, Grice EA, Cain CL. 2020. The otic microbiota and mycobiota in a referral population of dogs in eastern USA with otitis externa. Veterinary dermatology, 31(3):225-e49.

De Bellis F. 2015. Management and treatment of otitis externa and media. Veterinary times, January 5, 2015. [The website for the veterinary profession https://www.vettimes.co.uk]

Doyle RS, Skelly C, Bellenger CR. 2004. Surgical management of 43 cases of chronic otitis externa in the dog. Irish veterinary journal, 57(1):1-9.

Harvey R. 2022. A review of recent developments in veterinary otology. Veterinary sciences, 9(4):161.

Kasai T, Fukui Y, Aoki K, Ishii Y, Tateda K. 2021. Changes in the ear canal microbiota of dogs with otitis externa. Journal of applied microbiology, 130(4):1084-1091.

Koch S. 2017. Dermatology details: The challenge of chronic otitis in dogs: From diagnosis to treatment. Today's veterinary practice, 7(3):60-68.

Lloyd JK. 2017. Minimising stress for patients in the veterinary hospital: why it is important and what can be done about it. Veterinary sciences, 4(2):22.

Pye C. 2018. Pseudomonas otitis externa in dogs. The Canadian veterinary journal, 59(11):1231.

Tang S, Prem A, Tjokrosurjo J, Sary M, Van Bel MA, Rodrigues-Hoffmann A, Kavanagah M, Wu G, Van Eden ME, Krumbeck JA. 2020. The canine skin and ear microbiome: A comprehensive survey of pathogens implicated in canine skin and ear infections using a novel next-generation-sequencing-based assay. Veterinary microbiology, 247:108764.

Ural K, Erdogan H, Erdoğan S. 2022. Heterologue skin microbiota transplantation for treatment of sarcoptic manges in two dogs with zoonotic transmission. Bozok veterinary sciences, 3(2):52-56.

Ural K, Erdoğan H, Erdoğan S. 2023. Skin microbiota transplantation by nivea refining clear-up strips could reverse erythema scores in dogs with atopic dermatitis: novel strategy for skin microbiome manuplation: cohort study. Turkiye klinikleri journal of veterinary sciences, 14(1).



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Tracheal Rupture in a Cat

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ABSTRACT

Endotracheal intubation is a common procedure performed during anesthesia in cats. This report describes the diagnosis and treatment of iatrogenic tracheal rupture following a dental scaling of a 4-year-old, female, neutered Scottish Fold cat. Respiratory distress, subcutaneous emphysema and excessive air under the skin in the cervical and head regions were noted on physical and radiographic examination, respectively. The tracheal rupture was successfully repaired using simple interrupted sutures.

INTRODUCTION

Various factors contributing to tracheal ruptures in cats have been discussed in the veterinary literature. Dog bites and motor vehicle accidents are the most common causes of tracheal rupture. Tracheal ruptures associated with intubation in cats have been rarely reported (Fenet et al., 2022). Intubation-related traumatic tracheal injuries can range from minor lacerations to complete ruptures, with the latter causing life-threatening respiratory distress. Partial or small ruptures may present with clinical symptoms such as subcutaneous emphysema and pneumomediastinum, which can be managed with medical treatment (Fenet et al., 2022).

Surgical and medical treatment of tracheal injuries varies depending on the size of the lesion and the severity of clinical symptoms. In the case that the tracheal injury is minor and presents with mild clinical symptoms, conservative medical therapy may be indicated. Conversely, in cases where the rupture is extensive and associated with severe clinical signs, surgical intervention is typically necessitated. The medical treatment regimen includes vigilant monitoring of respiratory rate and effort, enforcement of strict rest within a confined environment, and supplemental oxygen. Additionally, the administration

of sedatives might be considered. Subcutaneous emphysema can be detected via palpation in the cervical region. It is noteworthy that while only mild clinical signs may be evident initially in cases involving tracheal rupture, there exists a potential for acute clinical deterioration within a matter of days (White and Milner, 1995).

MATERIALS AND METHODS

This case report presents a 4-year-old, 3.5 kg neutered female Scottish Fold cat, that was referred to Kırıkkale University Veterinary Faculty Hospital because its breathing worsened after dental scaling procedure under general anesthesia. According to the information we received about the patient's previous situation, clinical examination and hematological parameters were within normal limits before the dental scaling procedure. For dental cleaning procedure, 0.2 mg/kg butorphanol (Butomidor, Richerpharma ag, Austria), 20 µ/kg medetomidine (Domitor, Orion pharma, Finland) and intermittent bolus of propofol (Propofol 2% Fresenius; Fresenius Kabi, Austria) was administered. Atipamezole (Antisedan, Orion pharma, Finland) was administered at the end of the procedure to antagonize medetomidine. It

was extubated when a cough reflex was observed. Subsequent to the procedure, the initial clinical sign observed was swelling of the cat's head and cervical region. The animal was subsequently transferred to our facility upon the onset of open-mouth breathing, an occurrence which persisted despite the provision of supplemental oxygen. In the clinical examination, crepitation was observed on palpation of the head and cervical region, and emphysema was observed in the radiographic image (Figure 1).



Figure 1. L/L radiographic images of subcutaneous emphysema formation in the cervical and cranial regions taken in the preoperative period.

0.2 mg/kg butorphanol (Butomidor, Richerpharma ag, Austria) and propofol (Propofol 2% Fresenius; Fresenius Kabi, Austria) were administered to the patient for the surgical procedure. Lidocaine spray (Xylocaine, AstraZeneca, India) was applied to the larynx for desensitization. Intraoral examination revealed no pathological findings in the larynx region, and the patient was easily intubated and connected to an inhalation device for isofluorane administration (Izofloran, Adeka, Turkey). After the patient's cervical region was shaved, the patient was placed in the dorsal recumbency and a ventral cervical midline incision was performed. After the trachea was exposed, the area was filled with sterile saline to determine the exact location of the tear. The endotracheal tube cuff was deflated, and positive pressure ventilation was administered while monitoring bubble egress. Following aspiration of the administered fluid, the size and location of the lesion were determined (Figure 2).

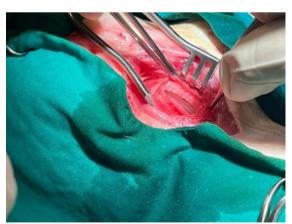


Figure 2. Intraoperative view of tracheal rupture.

The tear was identified dorsally along the trachea, extending longitudinally over an approximate length of 3 cm. As the tear was longitudinal, no tracheal ring resection

was performed; instead, it was repaired using simple interrupted sutures with 4-0 polydiaxanone (PDSII; Ethicon). Subsequently, warm sterile saline was reintroduced into the area, and air bubble formation was monitored to check for leaks. Finally, the surgical site was closed using a standard technique. After the end of surgery subcutaneous air was aspirated by the needle.

RESULTS

The cat's breathing improved after the operation and recovered without any complications. On the 10th postoperative day, radiological examination revealed that most of the subcutaneous emphysema had resolved. The patient was discharged with full recovery (Figure 3a and 3b).

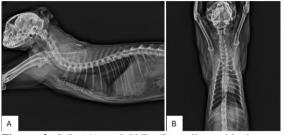


Figure 3. L/L (A) and V/D (B) radiographic images showing resorption of subcutaneous emphysema in the cervical and submandibular region on the postoperative 10th day.

DISCUSSION AND CONCLUSION

The reported rate of tracheal rupture in cats is very low. Repositioning of the tube without cuff deflation or movement of the patient with the cuff inflated are the two most common risk factors of the rupture in cats. Overinflation of the cuff, traumatic intubation with stylet, type of endotracheal tube are the other related causes (Michell et al 2000; Cabell Shannon, 2013). Tracheal rupture is most often a non-letal injury, and medical treatment including strict cage rest and oxygen support is usually successful. Cases that do not respond to conservative treatment should be treated surgically (Bhandal and Kuzma, 2008). In this case, surgical treatment was planned due to onset of clinical symptoms shortly after extubation and worsening respiratory distress despite oxygen therapy.

It is advisable to disconnect the endotracheal tube from the anesthetic circuit when repositioning an animal during a dental procedure to avoid potential tracheal disruption due to rotation of the tube and cuff (Mitchell S. L. et al., 2000). In this case, referring veterinarian reported that the was not connected to the anesthesia device. Possible explanations of the tracheal rupture for this cat can be overinflation of the endotracheal tube cuff or excessive movement of the head during dental cleaning cause the cuff damage the trachea.

Endotracheal intubation in cats requires knowledge and experience. This procedure should only be attempted if jaw tone is lost after induction of anesthesia. If the procedures performed inappropriately it can cause a swallowing reflex and intubation will be more difficult. Cats are exhibit laryngospasm if forced during intubation, making the procedure relatively challenging compared to other pets. Local anesthetic may be used to reduce the incidence of laryngospasm and facilitate endotracheal tube placement. Direct laryngoscopy is useful and allows

observation of movement of the arytenoids so that intubation attempts can be made during inspiration. Confirming tube position via direct laryngoscopy is essential. Failure to adhere to these practices can contribute to tracheal trauma and increase the risk of tracheal rupture (Hofmeister et al., 2007). According to the patient history, lidocaine was not applied to the cat's larvnx before intubation, and coughing occurred upon initial insertion. This may have caused the cuff to move inside due to cough and potentially contributing to rupture risk. Transferring veterinarian mentioned that 3.5 size polyvinyl chloride (PVC) high- volume low-pressure intubation tube was used in this patient. The rigid and inflexible structure of PVC intubation tubes may have facilitated damage. Silicone rubber or armored endotracheal tubes, which tend to be less stiff, may reduce damage occurrence. However, both types of endotracheal tubes are flexible. Endotracheal tubes can be used with a stylet to increase rigidity, but should be used with caution to prevent upper airway damage (Marty-Ane et al., 1995; Mitchell et al., 2000; Hofmeister et al., 2007).

The main clinical signs of tracheal rupture include subcutaneous emphysema, dyspnea, tachypnea, anorexia, cough, lethargy, respiratory stridor, exercise intolerance, cyanosis, vomiting, dehydration, hemoptysis, depression, muffle heart sounds (Berkwitt and Berzon, 1985; Mitchell et al 2000; Bhandal and Kuzma, 2008). The diagnosis of rupture was made based on the worsening of respiration, palpable crepitus in the head and neck region, subcutaneous emphysema observed on radiographs, and the onset of open-mouth breathing after awakening from anesthesia in this case.

Tracheal rupture in cats occurs most frequently in the extrathoracic trachea (Bhandal and Kuzma, 2008), less frequently in the intrathoracic trachea, and rarely in the bronchi (White and Oakley, 2001). Radiographic findings associated with cervical or intrathoracic tracheal rupture include subcutaneous emphysema, pneumomediastinum, pneumoretroperitoneum, hyperinflated lung fields, gasfilled pseudoairway at the rupture site, focal narrowing of the tracheal lumen, and pneumothorax (Bhandal and Kuzma, 2008). When these findings were recorded in intubated cats, immediate radiography is warranted to confirm the diagnosis, and it is concluded that radiography is sufficient for diagnosis. Since the emphysema was recorded in the head and neck regions and no pneumothorax or pneumomediastinum was observed in radiologic examination, it was thought that the tear was in the cervical region.

In conclusion, it was thought that if respiratory deterioration is observed after extubation of the intubated cats, tracheal rupture should be considered, and if clinical symptoms are very severe, surgical treatment should be the first option to repair the tracheal rupture.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: O.M.H., ZP, Design: O.M.H., M.B., Z.P, Data Collection or Processing: O.M.H., Z.P., Analysis or Interpretation: O.M.H., Z.P., Literature Search: O.M.N, Writing: O.M.H., M.B., Z.P

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Ethics statement

This study falls under the category of 'Clinical applications for diagnostic and therapeutic purposes,' as stated in subparagraph (k) of Article 8 of the regulation on the working procedures and principles of animal experimentation ethics committees. Therefore, approval from the Local Ethics Committee for Animal Experiments is not required.

REFERENCES

Berkwitt L and Berzon J L, 1985. Thoracic Trauma. Veterinary Clinics of North America: Small Animal Practice, 15(5): 1031-1039.

Bhandal J and Kuzma A, 2008. Tracheal rupture in a cat: Diagnosis by computed tomography. The Canadian Veterinary Journal, 49(6): 595–597.

Cabell Shannon, 2013. A case of suspected tracheal rupture in 12-year old domestic shorthair cat. CVM Senior Seminars-Seminar SF601.1 2013

Fenet M, Gaillard E, Conchou F, Paredes G P, Jourdan G, Diquelou A and Aumann M, 2022. Cervical tracheal rupture with persistence of a pseudotrachea in a dog. The Canadian Veterinary Journal, 63(8): 830–834.

Hofmeister E H, Trim C M, Kley S and Cornell K, 2007. Traumatic Endotracheal Intubation In The Cat. Veterinary Anaesthesia and Analgesia, 34: 213-216.

Lawrence D T, Lang J, Culvenor J Mischol G, Haynes S and Swinney G, 1999. Intrathoracic tracheal rupture. Journal of Feline Medicine and Surgery, 1(1): 43-51.

Marty-Ane C H, Picard E, Jonquet O and Mary H, 1995. Membranous Tracheal Rupture After Endotracheal Intubation. The Society of Thoracic Surgens, 60: 1367-71.

Mitchell S L, McCarthy R, Rudloff E and Pernell R T, 2000. Tracheal rupture associated with intubation in cats: 20 cases (1996-1998). JAVMA, 216(10): 1592-1595.

Sippel M, Putensen C, Hirner A and Wolff M, 2006. Tracheal Rupture After Endotracheal Intubation: Experience with Management in 13 Cases. Thorac Cardiov Surg. 54: 51-56.

Sparkes A. 2018. Tracheal Disease. In: Norsworthy GD, Restine LM. (Eds.), *The Feline Patient*. 5th ed. Hoboken, New Jersey: John Wiley & Sons; pp. 635–641.

White R N and Milner H R, 1995. Intrathoracic tracheal avulsion in three cars. Journal of Small Animal Practice, 36, 343-347.

White R N and Oakley M R, 2001. Left principal bronchus rupture in a cat. The Journal of Small Animal Practice, 42(10): 495-498.



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Possibility of Using Bee Bread (Perga) as an Alternative Feed Additive in Poultry Nutrition



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ABSTRACT

Livestock industry has an indispensable position in providing a source of protein for humans. It is vital for the future of humanity to produce healthy animal products for the needs of the increasing world population and to offer them for consumption day by day. However, so as to meet the request of animal products rapidly, industrialization has been increased by developing mass production methods, these changes in the sector have worried by some consumers and reservations have even increased with disinformation. Depending on this possible food safety and public health concern, people have become conscious of nutrition with natural products in progress of time and new searches have been embarked. On the other hand, due to the prohibition of the inclusion of antibiotics as feed additives in animal nutrition diets. alternative feed additives to antibiotics have been researched in order to increase performance and eliminate diseases. The fact that the quality and health of products such as meat, milk, eggs obtained from livestock are directly related to nutrition provides a better understanding of the value of feed and feed additives. Indeed, it is seen that the popularity of honey bee product bee bread, which has been known to be used as a curative product since ancient times, has increased in recent years in treatment with bee products called apitherapy. Concordantly, the use of bee bread as an alternative feed additive in animal health and nutrition has become increasingly common in recent years. In this review, it is aimed to give information about the possibility of using bee bread (perga), which has been shown by scientific studies to be natural and rich ingredient, as an alternative feed additive in poultry nutrition.

INTRODUCTION

The use of natural additives in nutrition is gaining importance in animals as well as in human beings due to increase for the demand of chicken meat and eggs from animal foods and the awareness of healthy nutrition in recent years. Although it is known that bee products, which are natural additives, have been being used to improve human, animal and plant health since ancient civilizations, it is seen that their value has increased even more nowadays. The treatment method made with medicinal products such as honey, bee pollen, propolis, bee bread, bee venom, royal jelly and beeswax, which are the products of the honey bee (*Apis Mellifera L.*), is called "Apitherapy" in the medical literature. Apitherapy, which has accerelated since 2014, is a traditional practice that

supports and complements medical treatment in human medicine. These bee products, which are known to have many rich properties such as antioxidant, antibacterial, antiviral, antifungal, anticarcinogenic, antirheumatic, antiallergic, antitumoral. immunomodulator, hepatoprotective, are also very important resources for animal health and nutrition as well as human health. As it is known, with the prohibition of antibiotics use as feed additives in many countries in 2006, research on the using of alternative natural feed additives has become widespread. In this context, various studies are carried out on different application forms, doses and mixtures for the using of bee products in agriculture and food sectors depending on the increase in the reliability of bee products. In studies with bee products, it is seen that it has

positive effects on performance by increasing the appetite, live weight gain and feed efficiency of animals, and also provides great contributions in terms of health. In this review, studies on the use of bee bread (perga) as an alternative feed additive in poultry nutrition, which has a very special place among bee products, but is generally confused with bee pollen and whose value is still not understood enough were evaluated.

Poultry Digestive System and Nutrition

High performing and economical poultry production starts with understanding the nutritional characteristics of the animal, planning a diet suitable for production aspects and characteristics, and having in-depth knowledge of the animal's digestive system and physiology (Kutlu et al., 2005). The digestive system of poultry, which is in the category of granivores (or omnivores) in terms of nutritional characteristics, is quite different from the digestive system of ruminants and more resembles that of reptiles. Poultry animals do not have teeth and their digestive system consists of mouth, crop, stomach, gizzard, small intestine and large intestine (Kutlu, 2015). Even though their digestive systems are shorter than other animals, they have crops and gizzards. It is also striking that there are two large cecum in the digestive system. Poultry with different anatomy and nutrition among other farm animals are animals with higher metabolic rates and energy requirements due to their body temperature of average 41 °C (Çetingül et al., 2018). Poultry animals are more affected by stress factors because of their high metabolic activity (Özdoğan and Sarı, 2001). The fact that the diets of these animals, on which a concentrated feeding program is applied, contain easily digestible, abundant and various nutrients is due to these different characteristics (Çetingül et al., 2018). Digestion and absorption events are the whole of digestive events that are affected by the anatomical structure of the digestive system as well as the structure of basic nutrients such as water, electrolytes, proteins, carbohydrates, fats, minerals and vitamins in the chemistry of the feed taken. On the other hand, benefiting from a feed whose chemical content is known depends on the efficiency of the digestion of the feed, the mechanical and enzymatic breakdown in the digestive system, the rate of intestinal transit and absorption, and the activation of the intestinal microbiome (Kutlu, 2015). Poultry intestines have a complex and energetic structure which mostly made up of bacteria and called the microbiome (Diker and Kaya, 2015). The microbiome provides many affirmative impacts on the host, acts as a protective barrier versus transient bacteria, assists in nourishment breakdown and energy gain from the meal, supplies nutritional intermediates for enterocytes, and plays an effective role in modulating host immunity (Ackerman, 2017). Commensal or pathogenic bacteria in the microbiome are influenced by host elements as well as basal diet and additives (Diker and Kaya, 2015). Thanks to various additives added to the feed, the number of bacteria in the microbiome can be changed in the direction of commensal bacteria, and healthy animals can be raised by increasing the feed efficiency (Şen, 2018).

Feed Additives and Alternative Solutions

Modern poultry farms are jammed and stressful environments at the present time. Even the smallest yield increases in such production areas can bring enormous economic benefits to the herd. For this reason, so as to achieve high productivity and profitability, it is necessary

to provide maximum benefit from the feed as well as to protect animal health. Adding and using feed additives to the diet serves to maximize benfits and improve animal health (Kocabağlı and Alp, 2015). The improvements, expectations and strategies in recent years have created changes in the use of growth promoting substances supplemented to the diet. One of these is the forbidden of using antibiotics for feed additives, and the other is the common using enzymes that increase the nutritional value of feed raw materials and reduce cost of feed (Ravindran, 2012). The determination of the harmful effects of antibiotics and hormones now encourages countries to protect human life and health at the highest level, so directs them to regulatory steps that take into account animal health and welfare, plant health and the environment. Considering that the consumption of organic and natural products for a healthy lifestyle is an unavoidable reality, increasing productivity without having a negative impact on human and animal health will affect both our health and the livestock work economy. In this context, considering the intense global search for alternative additives, it is necessary to explore whether these additives can be used as environmentally friendly additives (Öztürk, 2009). Decreasing supply and ever-increasing raw material prices will make more pressure for getting per unit energy and nutrients from feed ingredients. Accordingly, a series of additives are supposed to play an increasingly important role (Ravindran, 2012).

It is seen in studies that mostly aromatic plants, probiotics, prebiotics, essential oils, organic acids, toxin binders and enzymes are tested as alternative feed additives. Besides it is also known that natural bee products have been used to improve human, animal and plant health since ancient times. Researches conducted in the last few years indicate that bee products whose use in human life (apitherapy) has increased, can be an alternative natural feed additive for emphasized in terms of both animal health and growth factors in the veterinary field (Topal et al., 2015).

Beekeeping and Bee Products

Beekeeping is a profession that produces goods such as honey, propolis, royal jelly, pollen, bee venom and living things such as queen, pack and cluster by using bees, plants and intensive labor for the purpose of health, nutrition, protection and treatment since the birth of human beings (Fıratlı et al., 2000). Beekeeping provides income, employment and healthy nutrition opportunities for rural populations in developing countries. Because of these qualities, beekeeping has a special place in animal husbandry and bee products which are food of animal origin are undoubtedly precious for human's health and diet (Burucu and Gülse Bal, 2017). Türkiye is a country with great potential for beekeeping because of its different natural and climatic conditions, millions of hives, rich lands, and genetic diversity of plant and bee species. Beekeeping which has made rapid progress in Türkiye as in other countries of the world is a rather particular field of activity that ensures the balance of nature and the sustainability and productivity of agriculture. The main factors in bee breeding and production are climatic conditions, geographical structure and honey vegetation, and the presence of 75% of the currently seen honey plant species and varieties in Türkiye is an enormous source of natural wealth (Sıralı, 2010). Bee products like bee pollen, bee bread, honey, propolis, royal jelly, bee venom obtained from beekeeping activities have antimicrobial, antioxidant, anticarcinogen, immune system stimulant etc. qualifications has been used for many years (Premratanachai and Chanchao, 2014). Increasing awareness of natural nutrition in recent years has led to an increase in the attractiveness of the mentioned bee products. Consumers are increasingly showing interest in superfoods and functional foods on account of their great nutritional value as well as their ability to raise and improve human health and well-being. While there is no question that bee pollen and bee bread qualify as superfoods. However, more thorough investigation is still required before bee pollen and bee bread can be used as resources of antimicrobials in clinical exercise and make health assertions (Didaras et al., 2020). There is a requirement to increase the quantity and quality of scientific research supported by the sector for the identification, production, processing and use of bee products in terms of animal health. Using bee products will undoubtedly lead to healthier animal production (Topal et al., 2015).

Chemical Structure and Biological Benefits of Bee Bread

The realm of medicine advocates using as few drugs as possible so that pathogenic microorganisms are less likely to develop resistance. One of the best options in this regard are natural foods that contain life-threatening substances for these microorganisms. One of them is bee bread (perga) whose value is not yet understood enough (Karaman et al., 2016). Bees need food in order to continue their lives, and they meet this directly from pollen and nectar in nature. While bees use nectar to procure their carbohydrate and energy needs, pollen is used to procure their protein, vitamin and lipid needs. Worker bees need a food which has especially characteristics of a winter food source that has a higher nutritive protein and lipid value than pollen. They produce this food namely bee bread by combining pollen they collect from nature with their own secretions and undergoing a series of biochemical changes (Silici, 2015). This bee bread which is the most basic food for the baby bees and the queen bee meets the first nutritional needs of the baby bees for five days after the pupa period, therefore, it is also called "Bee Baby Food" (Karaman et al., 2017). Bee bread is actually a little-known bee product that is often confused with pollen. Bee bread, the main ingredient of which is pollen and known as "Perga" abroad, is a hive product in the form of fresh pollen mixed with nectar and bee secretions, fermented naturally through different chemical processes depending on the effect of certain enzymes, microorganisms, yeasts, humidity and temperature, and stored in honeycomb cells (Karaman et al., 2016; Karaman et al., 2017; Detry et al., 2020; Karlıdağ et al., 2021). Little is also known about how the fermentation process acts on bee pollen ingredients when producing bee bread, and in particular with regard to phenolics, which are the main components of such bee products. There is still necessity for research relating pharmacological ingredients with their mechanisms of action to prove the effectiveness of these nutritionally and medically important bee products (Baky et al., 2023). As far as is reported that due to the presence of lactic acid that provides fermentation, it's acidity rate is higher than pollen and its pH is between 3.8-4.3. Since the pollen walls, which cannot be destroyed by gastrointestinal fluids, are partially destroyed during the fermentation of bee bread, the energy bioavailability and functionality of bee bread is better than bee pollen with rich content

(Campos et al., 1997; Fatrcová-Šramková et al., 2010). Lactobacillus and Bifidobacterium bacteria which are the most important representatives of lactic acid bacteria widely used as probiotics in human and animal health were isolated from digestive system of honey bees, bee bread and pollen. Lactobacillus was the dominant bacteria of 90.9% of all bacteria in honey, 74.6% in pollen, 83.9% in bee bread and 93.3% in royal jelly (Asama et al., 2015). Bee bread stored in honeycomb cells is fixed to the hive with honey and beeswax (Barene et al., 2014; Zuluaga et al., 2015). The moisture content of bee bread may decrease to 13-14% depending on post-harvest drying (Mutsaers et al., 2005). Bee bread, which is a product resulting from the lactic acid fermentation of pollen collected by bees from different plant sources, ordinarily contains carbohydrates, proteins, amino acids, fatty acids, organic acids, enzymes, vitamins, phenolic compounds, minerals, phytochemicals, carotenoids and volatiles (Baky et al., 2023; Şimşek et al., 2023). The chemical composition of bee bread may vary based on botanical, geographical and seasonal origin. The chemical and fatty acid composition of five citrus bee bread samples obtained from various geographical regions in Türkiye were examined, and the ash content was found between 1.86-2.4%, moisture 11.0-16.4%, protein 18.6-21.6% and fat 7.0-13.4%. Palmitic, arachidic, oleic, stearic, erucic, linoleic, and eicosinoic acids were the most common acids in all samples, and a total of 37 fatty acids (FA) were identified. The ratios of unsaturated and saturated fatty acids varied between 1.28 and 2.23. This result showed that citrus bee bread is a good source of unsaturated fatty acids (Kaplan et al., 2019). Čeksterytė et al. (2008) determined 24 fatty acids in bee bread collected in spring and summer, subsequently reported that the most common unsaturated fatty acids were arachidonic and oleic acids. Although polyunsaturated fatty acids (PUFA) such as Alpha-linolenic (ALA), Eicosapentaenoic (EPA) and Docosahexaenoic (DHA) are essential in the human diet, the human body cannot synthesize these acids in the digestive system (Maclean et al., 2004). Studies on the beneficial effects of unsaturated fatty acids in humans have been reported that ethyl esters of DHA and EPA reduce the level of triglycerides in serum (Von Schacky and Harris, 2007). It has been stated that DHA and EPA which reduce triglyceride and cholesterol in the blood, have antiinflammatory, cardioprotective, antiarrhythmic and antithrombotic effects due to these abilities (Artemis and Simopoulos, 2004). As it can be seen from the existing literature studies, bee pollen and bee bread are excellent sources of polyunsaturated fatty acids (PUFA) which are very valuable for human dietary and cannot be produced by the body. Bee bread which has a different chemical composition compared to plant pollen (Silici, 2015) contains simple sugars, amino acids, a wide variety of vitamins and minerals, enzymes and plant hormones in its basic structure apart from fatty acids. About 20-22% protein, 1.6% fat, 24-35% carbohydrate, 35% sugar, 2.43% mineral, 1.6% lipid and 3.5% lactic acid are all present in bee bread (Bildir et al., 2022). In addition to essential amino acids that the human body cannot biosynthesize, there are proteins, vitamins such as C, B, B2, E, H, P, nicotinic acid, folic acid, pantothenic acid, pigments, enzymes such as sucrose, amylase, phosphatase, flavonoids, carotenoids and hormones. These complexes are extremely effective and beneficial for the body's immune system (Haydak and Vivino, 1950; Isidorov et al., 2009). Bee bread proteins containing less protein than bee pollen are easier to digest. Bee bread contains six times more lactic acid than bee pollen. This feature ensures its own structure preserved and not yeast growth exposed as in pollen. Moreover, bee bread which has better taste qualities compared to bee pollen is easily absorbed through the body (Mutsaers et al., 2005). Bee bread's biological active compounds content such as isorhamnetin, apigenin, kaempferol, two forms of quercetin, p-coumaric were carried out by means of high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) analysis methods (Baltrušaitytė et al., 2007b; Isidorov et al., 2009). In addition to previously referred compounds, Isidorov et al. (2009) identified little amount of chrysin and naringenin, ferulic and caffeic acids. Similarly, Markiewicz-Żukowska et al. (2013) identified small amounts of phenol compounds such as kaempferol and apigenin, Tavdidishvili et al. (2014) identified quercetin, naringin and rutin as 20% of the total flavonoids in Georgian bee bread, Suleiman et al. (2021) identified totally nine phenolic compounds including phenolic acids such as trans-ferulic acid, gallic acid, caffeic acid, trans 3-hydroxycinnamic acid, caffeic acid, 2hydroxycinnamic acid, and including flavonoids such as mangiferin, quercetin, apigenin, kaempferol via HPLC analysis method. On the other hand, Hryniewicka et al. (2016) determined lipophilic antioxidants such as coenzyme Q10 and α-tocopherol which play essential regulatory and metabolic functions in each cell of living organisms. It has been showed that bee pollen and bee bread of diverse botanic origins have different properties and their antioxidant limit are more linked with certain flavone and phenolic profiles rather than amount of entire flavones (Almaraz-Abarca et al., 2004). Therefore, flavonoids can severely represent as biochemical identifiers of the spesific plant origins (Čeksterytė et al., 2016). There have been various publications and reviews addressing methodological studies and extraction techniques on the chemical structure of beekeeping products such as honey, bee pollen and propolis in recent times (Gómez-Caravaca et al., 2006; Sulaiman et al., 2011; Da Silva Frozza et al., 2013; Markiewicz-Żukowska et al. 2012; Mohdaly et al., 2015). However, the chemical composition and antioxidant profile of bee bread samples have still not been sufficiently determined and examined homogeneously. Nevertheless, studies have clearly shown so far that bee bread is characterized by different structure, richer chemical composition, higher nutritional value and better digestibility than pollen (Anđelković et al., 2012; Habryka et al., 2016). Although research on bee bread has been quite limited so far (Didaras et al., 2020), there has been an increasing attention in the antimicrobial properties of bee pollen and bee bread in recent years since antimicrobial resistance emerging by pathogens. Bee pollen or bee bread have been shown to own hepatoprotective, immunomodulatory, anti-radiation, adaptogenic (Bogdanov, 2015), antibacterial (Baltrušaitytė et al., 2007a; Abouda et al., 2011), antioxidant (Nagai et al., 2004; Zuluaga et al., 2015; Tomás et al., 2017), antitumoral (Markiewicz-Żukowska et al., 2013; Sobral et al., 2017), anti-inflammatory, antidiyabetic, antiobestic, anticancer (Aylanc et al., 2021) characteristics in in vitro and in vivo studies. Bee bread can be used more in the future not only in medicine and pharmacology, but also in the food industry and nutrition. Further studies are required to confirm its chemical composition and biological beneficts, and in vivo tests to evaluate the bioactive components and digestibility properties stabily (Markiewicz-Żukowska et al., 2013;

Ivanišová et al., 2015). In summary, with developing analysis techniques, the value of every bee product, notably bee bread, will increase day by day as it is better researched and discovered (Arigül et al., 2023). Researches on bee products show so far that they can be used not only for humans but also for producing natural animal foods and treating animals. The possibility of using bee products in poultry has shed light on many studies and created opportunities for new ones (Akın and Çelen, 2019).

Use of Bee Bread in Poultry Nutrition

In a study conducted by Awad et al. (2013), the supplementation of bee bread at levels of 0.5, 1.0, 1.5 g/kg to the diets of experimental groups consisting of local Sinai hens during laying period (24-47 weeks), it was reported that bee bread can be used in the diet up to 1.5 g/kg as a growth supporter and natural antioxidant in order to improve egg performance, egg quality, hatchability, nutrient digestibility without negative effects on the economic efficiency and viability. Arafa et al. (2016), similar to previous study on laying hens, supplemented different proportions of bee bread, bee pollen and their mixtures as antioxidants to the diet of laying period (27-44 weeks). According to results of the study, it was recorded that 0.2 % bee bread, 0.15 % bee bread, 0.1 % bee pollen, 0.1 % bee bread ratios can be used to improve production performance, nutritional egg quality and welfare in Hyline hens. Fahim (2018) also examined semen quality and fertilizing ability of Sinai cocks with the supplementation as natural feed additive bee bread to the diet. During the experiment (24-40 weeks of age), it was supplemented 0.5, 1.00, 1.50 g/kg to the diet of Sinai cocks and reported that bee bread supplementation up to 1.5 g/kg to cock diet had positive effects on sperm quality, reproductive performance and hatchability of Sinai cocks.

Pavelková et al. (2020) observed that the use of 2, 4, 6 mg/kg bee bread powder to the diets of Japanese quails during 56-day feeding period had no positive impact on the meat performance. They hence suggested that extra alternative solutions to their application dose should be tested for poultry species including quails. In another study conducted on quails, Hanusová et al. (2021) stated that the addition of bee bread of 2, 4, 6 g/kg into the diets of Japanese quails during laying period (15-27 weeks) positively affected the laying indicators concerning egg quality. However, it was pointed out that the weight of seperately egg albumen and yolk as well as the total egg was meaningfully affected by the age of the quail.

CONCLUSION

Türkiye, which is already in good condition in poultry production, is even rich in bee breeding and production of bee products. Despite this situation, let alone the use of other bee products other than honey in apitherapy studies, even their production has unfortunately not reached the desired level. Nowadays, the importance of healthy and natural nutrition is increasing day by day. More original research is needed on the production and consumption of residue-free bee products and their use in medicine, veterinary and animal husbandry in Türkiye. Thus, it will be possible to benefit more from natural bee products that are affordable, healthy and have no adverse effects (Topal et al., 2015). In conclusion, Although it is seen that there are positive effects of bee bread in the literature reviews on the effect on poultry animals regarding both its numerous chemical components and its high bioactive usefulness, the available information is limited. It is thought that there are not enough studies on poultry nutrition based in bee bread, therefore, new scientific studies supporting previous similiar studies should be conducted in a row and the use of bee bread as an alternative natural feed additive should be clarified for poultry. It had better not forget that the bee bread added to the poultry diet is pure, natural and fresh, as well as the amount, form and duration of use are of great importance in obtaining the highest benefit.

Conflict of Interest

The authors declare that they have no competing interests.

Authorship contributions

Concept: F.G., M.A.K., Design: F.G., M.A.K., Data Collection or Processing: F.G., M.A.K., Analysis or Interpretation: F.G., M.A.K., Literature Search: F.G., M.A.K., Writing: F.G., M.A.K.

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REFERENCES

Abouda Z, Zerdani I, Kalalou I, Faid M, Ahami MT. 2011. The antibacterial activity of Moroccan bee bread and bee-pollen (fresh and dried) against pathogenic bacteria. Research Journal of Microbiology, 6(4): 376-384.

Ackerman N. 2017. The canine microbiome. Veterinary Nursing Journal, 8(1): 12-16.

Akın Y, Çelen MF. 2019. Arı ürünlerinin kanatlı hayvan yetiştiriciliğinde kullanım olanakları. The 2nd International Science and Academic Congress, Konya, Türkiye, 19-20 April, pp. 515-526.

Almaraz-Abarca N, Campos MDG, Antonio Ávila-Reyes J, Naranjo-Jiménez N, Herrera-Corral J, González-Valdez LS. 2004. Variability of antioxidant activity among honeybee-collected pollen of different botanical origin. Interciencia, 29(10): 574-578.

Anđelković B, Jevtić G, Mladenović M, Marković J, Petrović M, Nedić N. 2012. Quality of pollen and honey bee bread collected in spring. Journal of Hygienic Engineering and Design, 1; 275-277.

Arafa SA, Omara II, Beshara MM, Abdel-Azyme MG. 2016. Effect of adding bee bread and bee pollen as antioxidants on productive performance and functional properties of Hy-Line hens strain. Egyptian Poultry Science Journal, 36(4): 905-929.

Arıgül AM, Zorba M, Kayaboynu Ü. 2023. Bal arısı ve bal arısı ürünleri. Sinop Üniversitesi Fen Bilimleri Dergisi, 6(2): 202-223.

Artemis P, Simopoulos MD. 2004. Omega-6/Omega-3 essential fatty acid ratio and chronic diseases. Food Reviews International, 20(1): 77-90.

Asama T, Arima TH., Gomi T, Keishi T, Tani H, Kimura Y, Tatefuji T, Hashimoto K. 2015. Lactobacillus kunkeei YB38 from honeybee products enhances IgA production in healthy adults. Journal of Applied Microbiology, 119(3): 818-826.

Awad AL, Beshara MM, Ibrahim AF, Fahim HN. 2013. Effect of using bee bread as natural supplement on productive and physiological performance of local Sinai hens. Egyptian Poultry Science Journal, 33(IV): 889-913.

Aylanc V, Falcão SI, Ertosun S, Vilas-Boas M. 2021. From the hive to the table: Nutrition value, digestibility and bioavailability of the dietary phytochemicals present in the bee pollen and bee bread. Trends in Food Science and Technology, 109: 464-481.

Baky MH, Abouelela MB, Wang K, Farag MA. 2023. Bee pollen and bread as a super-food: A comparative review of their metabolome composition and quality assessment in the context of best recovery conditions. Molecules, 28(2): 715.

Baltrušaitytė V, Venskutonis PR, Čeksterytė V. 2007a. Antibacterial activity of honey and beebread of different origin against S. aureus and S. epidermidis. Food Technology and Biotechnology, 45(2): 201-208.

Baltrušaitytė V, Venskutonis PR, Čeksterytė V. 2007b. Radical scavenging activity of different floral origin honey and beebread phenolic extracts. Food Chemistry, 101(2): 502-14.

Barene I, Daberte I, Siksna S. 2014. Investigation of bee bread and development of its dosage forms. Medicinos Teorija Ir Praktika, 21(1): 16-22.

Bıldır B, Demirkan Z, Kaya B. 2022. Biosynthesis, characterization and determination of sun protection factor (spf) of iron nanoparticles with bee bread. Türk Doğa ve Fen Dergisi, 11(3): 110-117.

Bogdanov S. 2015. Pollen: production, nutrition and health: A review. Bee Product Science, 2: 1-30.

Burucu V, Gülse Bal HS. 2017. Türkiye'de arıcılığın mevcut durumu ve bal üretim öngörüsü. Tarım Ekonomisi Araştırmaları Dergisi, 3(1): 28-37.

Campos MG, Cunha A, Markham KR. 1997. Beepollen: Composition, properties, and applications, in: Mizrahi A, Lensky Y. (Eds), Bee Products. 1st ed. Springer, New York, pp. 93-100.

Čeksterytė V, Navakauskienė R, Treigytė G, Jansen E, Kurtinaitienė B, Dabkevičienė G, Balžekas J. 2016. Fatty acid profiles of monofloral clover beebread and pollen and proteomics of red clover (Trifolium pratense) pollen. Bioscience, Biotechnology, and Biochemistry, 80(11): 2100-2108.

Čeksterytė V, Račys J, Kaškonienė V, Venskutonis PR. 2008. Fatty acid composition in beebread. Biologija, 54(4): 253-257.

Çetingül İS, Gültepe EE, Uyarlar C, Iqbal A, Bayram İ. 2018. Afetzede Kanatlı Hayvanların Beslenmesi. International Animal Rescue Conference, Afyonkarahisar, Türkiye, 7 July, pp. 80-86.

Da Silva Frozza CO, Celi Garcia CS, Gambato G, Oliveira de Souza MD, Salvador M, Moura S, Padilha FF, Seixas FK, Collares T, Borsuk S, Dellagostin OA, Henriques JAP, Roesch-Ely M. 2013. Chemical characterization, antioxidant and cytotoxic activities of Brazilian red propolis. Food and Chemical Toxicology, 52: 137-142.

Detry R, Simon-Delso N, Bruneau E, Daniel HM. 2020. Specialisation of yeast genera in different phases of bee bread maturation. Microorganisms, 8(11): 1789.

Didaras NA, Karatasou K, Dimitriou TG, Amoutzias GD, Mossialos D. 2020. Antimicrobial activity of beecollected pollen and beebread: State of the art and future perspectives. Antibiotics, 9(11): 811.

Diker KS, Kaya IB. 2015. Kanathlarda Mikrobiyom ve Hastalık İlişkisi. The 3rd International Poultry Meat Congress, Antalya, Türkiye, 22-26 April, pp. 404-406.

Fahim HN. 2018. Semen quality and fertilizing ability of Sinai cocks fed bee bread as a natural supplement to the diet. Journal of Animal and Poultry Production, 9(2): 77-83.

Fatrcová-Šramková K, Nôžková J, Ostrovský R. 2010. Nutričné vlastnosti včelieho peľu (Nutritional properties of bee pollen). Potravinarstvo, 4(special issue): 24-32.

Fıratlı Ç, Genç F, Karacaoğlu M, Gencer HV. 2000. Türkiye Arıcılığının Karşılaştırmalı Analizi, Sorunlar-Öneriler. Türkiye Ziraat Mühendisliği V. Teknik Kongresi, Ankara, Türkiye, 17-21 Ocak, pp. 811-826.

Gómez-Caravaca AM, Gómez-Romero M, Arráez-Román D, Segura-Carretero A, Fernández-Gutiérrez A. 2006. Advances in the analysis of phenolic compounds in products derived from bees. Journal of Pharmaceutical and Biomedical Analysis, 41(4): 1220-1234.

Habryka C, Kruczek M, Drygaś B. 2016. Bee products used in apitherapy. World Scientific News, (48): 254-258.

Hanusová E, Oravcová M, Hrnčár C, Hanus A, Kalafová A, Capcarová M. 2021. The effect of bee bread on the egg quality of Japanese quails during laying period. Journal of Microbiology, Biotechnology and Food Sciences, 11(3): e4092.

Haydak MH, Vivino AE. 1950. The changes in thiamine, riboflavin, niacin and pantothenic acid content in the food of female honeybees during growth with a note on the vitamin K activity of royal jelly and bee bread. Annals of the Entomological Society of America, 43: 361-367.

Hryniewicka M, Karpinska A, Kijewska M, Turkowicz MJ, Karpinska J. 2016. LC/MS/MS analysis of α-tocopherol and coenzyme Q10 content in lyophilized royal jelly, beebread and drone homogenate. Journal of Mass Spectrometry, 51(11): 1023-1029.

Isidorov VA, Isidorova AG, Sczczepaniak L, Czyżewska U. 2009. Gas chromatographic-mass spectrometric investigation of the chemical composition of beebread. Food Chemistry, 115(3): 1056-1063.

Ivanišová E, Kačániová M, Frančáková H, Petrová J, Hutková J, Brovarskyi V, Velychko S, Adamchuk L, Schubertová Z, Musilová J. 2015. Bee bread-Perspective source of bioactive compounds for future. Potravinarstvo, 9(1): 592-598.

Kaplan M, Karaoğlu O, Silici S. 2019. An evaluation on bee bread: Chemical and palynological analysis. Mellifera, 19(1): 21-29.

Karaman MR, Artık N, Küçükersan K, Halıcı Z, Çelik M. 2017. Sağlıklı beslenme ve apiterapi için değerli bir arı ürünü: Perga (bee bread). Gıda 2000 Gıda Teknolojisi ve Tarım Dergisi, 180: 1-8.

Karaman MR, Artık N, Küçükersan MK. 2016. Perga (Bee Bread) Composition and Health Benefit. The 2nd International Turkic World Conference on Chemical Sciences and Technologies, Skopje, Macedonia, 26-30 October.

Karlıdağ S, Keskin M, Keskin Ş, Özkök A, Karabulut E, Akyol A, Yılmaz I. 2021. Doğal ve fermente polenin biyokimyasal karakterizasyonu. İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksekokulu Dergisi, 9(3): 1094-1104.

Kocabağlı N, Alp M. 2015. Kanatlı beslemede kullanılan yem katkı maddeleri. Turkiye Klinikleri Animal Nutrition and Nutritional Diseases-Special Topics, 1(2): 17-24.

Kutlu HR, Görgülü M, Çelik LB. 2005. Çukurova Üniversitesi Ziraat Fakültesi Zootekni Bölümü Yemler ve Hayvan Besleme Anabilim Dalı. Genel hayvan besleme ders notu. https://www.ruminantbesleme.com/wp-

content/uploads/2018/09/GENEL-HAYVAN-BESLEME.pdf. Erişim Tarihi: 01.06.2023.

Kutlu HR. 2015. Çukurova Üniversitesi Ziraat Fakültesi Zootekni Bölümü Yemler ve Hayvan Besleme Anabilim Dalı. Kanatlı hayvan besleme ders notu. https://doczz.biz.tr/doc/4250/kanatl% C4% B1-hayvan-besleme---cu-department-of-animal-science-.... Erişim Tarihi: 01.06.2023.

MacLean CH, Mojica WA, Morton SC, Pencharz J, Hasenfeld Garland R, Tu W, Newberry SJ, Jungvig LK, Grossman J, Khanna P, Rhodes S, Shekelle P. 2004. Effects of omega-3 fatty acids on lipids and glycemic control in type II diabetes and the metabolic syndrome and on inflammatory bowel disease, rheumatoid arthritis, renal disease, systemic lupus erythematosus, and osteoporosis. Evidence Report/Technology Assessment (Summary), (89): 1-4.

Markiewicz-Żukowska R, Car H, Naliwajko SK, Sawicka D, Szynaka B, Chyczewski L, Isidorov V, Borawska MH. 2012. Ethanolic extract of propolis, chrysin, CAPE inhibit human astroglia cells. Advances in Medical Sciences, 57(2): 208-216.

Markiewicz-Żukowska R, Naliwajko SK, Bartosiuk E, Moskwa J, Isidorov V, Soroczyńska J, Borawska MH. 2013. Chemical composition and antioxidant activity of beebread, and its influence on the Glioblastoma cell line (U87MG). Journal of Apicultural Science, 57(2): 147-257.

Mohdaly AAA, Mahmoud AA, Roby MHH, Smetanska I, Ramadan MF. 2015. Phenolic extract from propolis and bee pollen: Composition, antioxidant and antibacterial activities. Journal of Food Biochemistry, 39(5): 538-547.

Mutsaers M. Blitterswijk HV, Leven LV, Kerkvliet JD, Waerdt JV. 2005. Bee Products: Properties, Processing and Marketing, first ed. Agrodok 42, Wageningen.

Nagai T, Nagashima T, Myoda T, Inoue R. 2004. Preparation and functional properties of extracts from bee bread. Food/Nahrung, 48(3): 226-229.

Özdoğan M, Sarı M. 2001. Kanatlı rasyonlarına yağ katkısı. Hayvansal Üretim, 42(1): 28-34.

Öztürk E. 2009. Kanatlı Hayvan Beslemede Alternatif Yem Katkı Maddeleri ile Yapılan Çalışmalar. V. Ulusal Hayvan Besleme Kongresi, Çorlu, Türkiye, 30 Eylül-3 Ekim, pp. 397-402.

Pavelková A, Haščík P, Capcarová M, Kalafová A, Hanusová E, Tkáčová J, Bobko M, Čuboň J, Čech M, Kačániová M. 2020. Meat performance of Japanese quails after the application of bee bread powder. Potravinarstvo Slovak Journal of Food Sciences, 14: 735-743.

Premratanachai P, Chanchao C. 2014. Review of the anticancer activities of bee products. Asian Pacific Journal of Tropical Biomedicine, 4(5): 337-344.

Ravindran V. 2012. Advances and future directions in poultry nutrition: An overview. Korean Journal of Poultry Science, 39(1): 53-62.

Sıralı R. 2010. Arıcılığın Türkiye için önemi. Arıcılık Araştırma Dergisi, 2(4): 3-5.

Silici S. 2015. Arı poleni ve arı ekmeği. Uludağ Arıcılık Dergisi, 14(2): 99-105.

Sobral F, Calhelha RC, Barros L, Dueñas M, Tomás A, Santos-Buelga C, Vilas-Boas M, Ferreira ICFR. 2017. Flavonoid composition and antitumor activity of bee bread collected in Northeast Portugal. Molecules, 22(2): 248.

Sulaiman GM, Al Sammarrae KW, Ad'hiah AH, Zucchetti M, Frapolli R, Bello E, Erba E, D'Incalci M, Bagnati R. 2011. Chemical characterization of Iraqi propolis samples and assessing their antioxidant

potentials. Food and Chemical Toxicology, 49(9): 2415-2421.

Suleiman JB, Mohamed M, Abu Bakar AB, Nna VU, Zakaria Z, Othman ZA, Aroyehun AB. 2021. Chemical profile, antioxidant properties and antimicrobial activities of Malaysian Heterotrigona itama bee bread. Molecules, 26(16): 4943.

Şen G. 2018. Broyler rasyonlarında üzüm posası ile inülin kullanımının performans, karkas randımanı, barsak viskozitezi, bağışıklık ve antioksidan durum üzerine etkileri. Doktora tezi, Kırıkkale Üniversitesi, Sağlık Bilimleri Enstitüsü, Kırıkkale.

Şimşek F, Çetin B, Mutlu C. 2023. Arı ekmeğinin bazı fiziksel ve kimyasal özellikleri ve sağlık üzerine etkileri. Gıda, 48(4): 807-818.

Tavdidishvili D, Khutsidze T, Pkhakadze M, Vanidze M, Kalandia A. 2014. Flavonoids in Georgian beebread and bee pollen. Journal of Chemistry and Chemical Engineering, 8(7): 676-681.

Tomás A, Falcão SI, Russo-Almeida P, Vilas-Boas M. 2017. Potentialities of beebread as a food supplement and source of nutraceuticals: Botanical origin, nutritional composition and antioxidant activity. Journal of Apicultural Research, 56(3): 219-230.

Topal E, Yücel B, Köseoglu M. 2015. Arı ürünlerinin hayvancılık sektöründe kullanımı. Hayvansal Üretim, 56(2): 48-53.

Von Schacky C, Harris WS. 2007. Cardiovascular benefits of omega-3 fatty acids. Cardiovascular Research, 73(2): 310-315.

Zuluaga CM, Serrato JC, Quicazan MC. 2015. Chemical, nutritional and bioactive characterization of Colombian bee-bread. Chemical Engineering Transactions, 43: 175-180.