

The Difference in Bacteriological Flora Between Cecal Feces and Normal Feces in Guinea Pigs: Animal Experiments

Kobaylarda Sekal Dışkı ile Normal Dışkı Arasındaki Bakteriyolojik Flora Farkı: Hayvan Deneyleri

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ABSTRACT Objective: The purpose of this study was to investigate the differences in bacterial flora between normal feces and caecotrophy in guinea pigs (*Cavia porcellus*). **Material and Methods:** Six adult guinea pigs, housed in pairs, were observed daily from 6.00 pm to 12.00 pm for four weeks. Observations focused on feces and caecotrophy excretion and intake, as well as overall behavior. Fresh samples of feces and caecotrophy were collected from each animal for bacterial analysis, and processed within 12 hours using appropriate agar plates for clinical diagnostics. **Results:** The study found a significant difference in the total bacterial count of *Lactobacillus spp.* between the two types of feces. Both *Bifidobacteria* and *Lactobacillus spp.* were present in both normal feces and caecotrophy. Visual examination of the feces did not reveal any distinctions between the two types of feces for guinea pigs. Behavioral observations showed that active feeding and normal fecal excretion were followed by rest periods during which guinea pigs consumed caecotrophy. Caecotrophy consumption occurred in stages, with animals frequently biting off pieces. When re-offered collected caecotrophy, the guinea pigs consumed it again, while normal feces were rejected. **Conclusion:** These findings confirm the existence of true caecotrophy in guinea pigs, highlighted by significant differences in *Lactobacillus spp.* colony counts and the guinea pigs' selective behavior towards caecotrophy over normal feces. Further research in these areas would contribute to our understanding of the complex interactions between diet, gut microbiota, and host physiology in guinea pigs.

Keywords: Guinea pigs; caecotrophy; bacteriological flora; feces; feeding

ÖZET Amaç: Bu çalışmanın amacı, kobayların (guinea pigs) normal dışkı ile sekotrof dışkı arasındaki bakteriyel floradaki farklılıklarını araştırmaktır. **Gereç ve Yöntemler:** Altı erişkin kobay, çiftler hâlinde barındırıldı ve 4 hafta boyunca her gün saat 18.00-12.00 arasında gözlemlendi. Gözlemler, dışkı ve sekotrof dışkısının atılması ve alınması ile genel davranışlara odaklandı. Her bir hayvandan taze dışkı ve sekotrof örnekleri bakteri analizi için toplandı ve klinik tanı için uygun agar plakaları kullanılarak 12 saat içinde işlendi. **Bulgular:** Çalışmada, iki dışkı türü arasında *Lactobacillus spp.*'nin toplam bakteri sayısında önemli bir fark bulundu. Hem *Bifidobacteria* hem de *Lactobacillus spp.*, normal dışkı ve sekotrof dışkısında mevcuttu. Dışkıların görsel incelemesi, iki tür arasında herhangi bir fark ortaya koymadı. Davranışsal gözlemler, aktif beslenme ve normal dışkı atılımının, kobayların sekotrof tükettiği dinlenme dönemleriyle tarafından takip edildiğini gösterdi. Sekotrof tüketimi aşamalar halinde gerçekleşti ve hayvanlar sık sık parçaları ısırды. Toplanan sekotrof tekrar sunulduğunda, kobaylar bunu tekrar tüketirken, normal dışkı reddedildi. **Sonuç:** Bu bulgular, *Lactobacillus spp.* koloni sayılarındaki önemli farklılıklar ve kobayların normal dışkı yerine sekotrof tercih etme davranışlarıyla vurgulanan, kobaylarda gerçek sekotrof varlığını doğrulamaktadır. Bu alanlarda daha fazla araştırma, kobaylarda diyet, bağırsak mikrobiyotası ve konak fizyolojisi arasındaki karmaşık etkileşimleri anlamamıza katkıda bulunacaktır.

Anahtar Kelimeler: Kobaylar; sekotrof; bakteriyolojik flora; dışkı; beslenme

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Caecotrophy, the reabsorption of one's own appendix feces, is a vital physiological process that has been clearly proven, especially in rabbits.¹ This behavior involves the consumption of caecotrophs, which are soft, nutrient-rich fecal pellets. In guinea pigs, the practice of caecotrophy is frequently discussed in the literature, with considerable discrepancies regarding its significance and characteristics. While coprophagy, the general consumption of feces, has been unequivocally established in guinea pigs, there remains no conclusive evidence of a significant difference between the two types of feces (caecotrophs and regular feces) in terms of appearance, nutritional composition, and bacterial flora. No studies have specifically examined the bacterial flora differences between these two types of feces in guinea pigs. However, another study was studied the microbial differences between the two types of rabbit feces and found no significant difference in bacterial diversity between them.² Hildebrand et al. investigated the microbial flora in the feces of 60 guinea pigs and compared it to human samples.³ This study found that the intestinal flora of both guinea pigs and humans is predominantly composed of bacteria from the phyla *Bacteroidetes* and *Firmicutes*. An earlier study examined the cecal contents, intestinal contents, and feces of guinea pigs, revealing that the cecal contents were mainly dominated by Gram-positive rod bacteria.⁴ Within the intestinal milieu of guinea pigs, the discerned microbial diversity is principally constituted by phyla encompassing *Bacteroidetes*, *Firmicutes*, *Fibrobacteres*, *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Tenericutes*.^{5,6} Notably, no *Escherichia coli* was detected in the remaining intestinal contents or feces. This absence of *E. coli* was also confirmed who noted a lack of *Clostridium spp.* as well.⁷ Takahashi and Sakaguchi studied the transport of bacteria in the gastrointestinal tract of guinea pigs using flow cytometry and fluorescently labeled viable bacteria.⁸ They demonstrated that some bacteria were retrogradely transported back into the appendix. The microbial composition of the guinea pig's intestinal flora mainly consists of anaerobes and Gram-positive germs.⁷ Further studies also described a

predominantly Gram-positive intestinal flora, primarily composed of sporeless rods such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, along with spore-forming protein decomposers.^{9,11} Huerkamp et al. also noted that juvenile guinea pigs ingest caecotrophs from their mothers to colonize their gastrointestinal tract with *Lactobacillus* and other Gram-positive bacteria, which is crucial for establishing a healthy gut microbiota early in life.¹¹ The hypothesis of the study is that guinea pigs exhibit true caecotrophy, selectively consuming nutrient-rich caecal pellets as part of a digestive strategy to optimize nutrient absorption, rather than indiscriminately engaging in coprophagia. The hypothesis further suggests that if true caecotrophy is present, guinea pigs will consistently select specific types of feces for consumption, while rejecting others. Alternatively, the hypothesis posits that if no such pattern is observed, guinea pigs may engage solely in coprophagia or show no discernible preference in their fecal consumption behavior. The aim of this study is to investigate whether the bacterial composition of feces consumed by guinea pigs differs from that of normal feces.

MATERIAL AND METHODS

Experimental Animals: Six guinea pigs of varying sexes and ages were used. Four animals (2-4 years old) were from the same household, while two animals (1.5 years old) were purchased from a private household. In this study, where W represents female guinea pigs, Mk represents neutered female guinea pigs, and M represents male guinea pigs, The guinea pigs were housed in pairs, forming three groups: Group A: Animal 1 (W) and Animal 2 (Mk), Group B: Animal 3 (W) and Animal 4 (W) and Group C: Animal 5 (M) and Animal 6 (M). Each pair was kept in a cage (100x60x50 cm) with wood chip bedding, shelters, drinkers, and food balls. All animal experiments were conducted following the ethical guidelines and with the approval of the Ethical Committee of the Vienna Veterinary University. This study was conducted in accordance with the principles of the Helsinki Declaration.

Adaptation and Feeding: A 4-week adaptation period began on January 1, 2013. During this time, all animals were fed twice daily with fresh vegetables (cucumber, peppers, lettuce) and commercially available food, along with hay and water provided ad libitum. They received around 30 g commercial feed and 200 g vegetables. The commercial feed was “Vitakraft Vita special”. It was contained 18% crude fiber, 0.4% Phosphorus, 0.7% calcium and ingredients are vegetable by-products, cereals 15%, oil 2.1%, minerals, *Yucca schidigera*-extract, Additives Vit A, D₃, C, 45 mg Se, 0.45 mg J, 60.75 mg Zn, 60.75 Mn, 101.25 Fe, 16.2 mg Cu.

Fecal Collection: Originally, the plan involved using Type IV macrolon cages for fecal collection, but due to high stress levels observed, the experiment continued in the original cages by removing the shelters. Fecal collection started on February 1, 2013, with daily observations from 6 p.m. to midnight. Feces were collected using gloves and placed in 20 mL tubes. Cecal feces were collected by observing the animals’ posture and collecting directly from the anal area.

Bacterial examination: Fecal samples, including both normal and caecal feces, were collected into sterile cups from six guinea pigs the night before and stored in a refrigerator at 5 °C. Each sample weighed approximately 0.3 g. A total of 12 samples were analyzed. For bacterial culture, various agar plates were utilized, including BD™ MacConkey II Agar for *Enterobacteriaceae*, BD™ Columbia Agar with 5% Sheep Blood for a wide range of microorganisms, BD™ Difco Lactobacilli MRS Agar for lactobacilli, and others. Sample preparation involved suspending the fecal samples in peptone water and creating a dilution series from 10⁻¹ to 10⁻⁷ using 0.9% NaCl solution. Each dilution step was carefully performed to ensure accuracy, with subsequent incubation and analysis for bacterial growth and differentiation.

Mold count and other tests: In mold count determination, after incubating the plates under specified conditions, colonies were counted, and their size and morphology were noted, considering factors such as odor, color, surface, and hemolysis. Different

colonies were streaked onto microscope slides using a flamed loop, stained with Gram stain, and assessed using an oil immersion microscope for differentiation between Gram-positive and Gram-negative bacteria. The oxidase test, based on cytochrome C identification, distinguished *Enterobacteriaceae* from other bacteria by inducing a blue-violet color change in oxidase-positive bacteria. For the catalase test, colonies were removed and placed on a slide, then treated with 3% hydrogen peroxide, with bubble formation indicating a positive reaction. The indole test, utilizing Kovacs’ indole reagent, distinguished indole-positive from-negative *Enterobacteriaceae* based on a pink-red color change in the SIM tube. Lastly, the citrate test, using Simmons Citrate Agar, differentiated *Enterobacteriaceae* based on citrate utilization, with a positive reaction resulting in a color change from green to blue due to the pH indicator bromothymol blue.

DATA ANALYSIS

Data were initially examined using descriptive statistic and plots. Normal distribution and homogeneity of the data were evaluated by Kolmogorov-Smirnov tests. To analyze the differences in means, t-tests for dependent samples were used. Normally distributed data are presented as mean±standard deviation (SD), whereas skewed data are presented as median and range. Data were examined using SPSS 20.0 (IBM, 2021, USA) and a value of p<0.05 was considered statistically significant for all analyses.

RESULTS

The observation of the animals during the collection period revealed no noticeable morphological differences between normal feces and cecal feces. Both types exhibited firm consistency, smooth, shiny surfaces, and rope-like structures. Occasional differences were observed, with appendix feces appearing slightly softer and lighter than subsequent normal feces, although this discrepancy was not consistently observed, precluding visual distinction between the two. Recordings were made during resting phases following periods of activity, particularly during feeding. Animals demonstrated

a distinctive “curling” movement to ingest appendix feces, a behavior observed only during periods of rest and well-being. Initially, animals did not exhibit this behavior, but gradually began consuming their appendix feces after acclimating to human presence. Attempts to observe guinea pigs in Type IV macrolon cages proved unsuccessful, as the animals experienced considerable stress from environmental change and separation from their partners, precluding observation of caecotroph intake. Additionally, it was noted that animals consumed appendix feces in multiple stages, often removing partially bitten fecal balls from the anus and continuing consumption upon reoffering.

In the macroscopic and microscopic evaluation, no colony-forming *Enterococci* were observed in either fecal type across all animals, indicating the absence of this genus. Colony morphologies consistent with *Bifidobacterium* were detected in both feces and caecal samples, confirmed by microscopic analysis. *Lactobacillus* colonies were found in feces at a dilution of 10^{-5} and in caecal feces at 10^{-4} , displaying characteristic morphology under microscopy. *Enterobacteriaceae*, specifically *E. coli*, were identified in a caecal feces sample from one animal, supported by colony appearance, microscopic characteristics, and biochemical tests. No typical colonies suggestive of *Clostridium perfringens* or *Campylobacter spp.* were observed in any samples.

As a result, the mean and standard deviation of colony-forming units (CFU) on different culture media are presented in Table 1, with absolute values derived from a sample size of $n=6$. A notable difference between feces and caecal feces was observed on *Lactobacillus* agar, showing significant difference. Conversely, no statistically relevant

TABLE 1: Mean and standard deviation of the colony forming units on the different culture media, absolute values ($n=6$).

	Feces $\bar{X}\pm SD$	Cecal feces $\bar{X}\pm SD$	p-value
Columbia III 5%	$1*10^4\pm 6*10^3$	$5*10^3\pm 5*10^3$	0.03
Lactobacillus	$6*10^4\pm 1*10^6$	$3*10^4\pm 1*10^4$	0.6
Mac Conckey	0 ± 0	$2*10^1\pm 5*10^1$	-

SD: Standard deviation.

TABLE 2: Mean and standard deviation of the colony forming units on the different culture media, log values ($n=6$).

	Feces $\bar{X}\pm SD$	Cecal feces $\bar{X}\pm SD$	p-value*
Columbia III 5%	3.84 ± 0.53	$3.5*10^3\pm 0.54$	0.3
Lactobacillus	5.36 ± 0.63	$4.54*10^4\pm 0.20$	0.02
Mac Conckey	0 ± 0	2.14+	#

*According to the t-test for dependent samples; +Was only detected in one of 6 samples; #no p-value could be calculated for the analysis of McConkey Agar because colony counts were only obtained from a single caecal fecal sample; SD: Standard deviation.

difference was identified on blood agar. Notably, on McConkey Agar, CFUs could only be counted in one caecal fecal sample, precluding determination of a p value (Table 2). These findings indicate potential variations in bacterial growth between fecal types, particularly evident on *Lactobacillus* agar, warranting further investigation into microbial dynamics in guinea pig gastrointestinal flora.

DISCUSSION

The discussion regarding the distinction between two types of feces, particularly the appendix feces and normal feces, as described earlier by Drescher and Hamel, presents an intriguing insight into the digestive behavior of animals, particularly guinea pigs.⁹ While their study partially refuted the distinct characteristics of appendix feces outlined by Drescher and Hamel, it is noteworthy that some observations align with previous findings, regarding the softer consistency and lighter color of appendix feces.^{9,12}

Sakaguchi further adds to this discourse by suggesting that animals primarily engage in caecotrophy during resting phases, consuming softer, water-rich appendix feces, while passing normal feces during active phases.¹² This cyclical pattern of fecal consumption and excretion serves various physiological functions, such as nutrient recycling and maintaining gut health. However, it's important to note the variability in observations across studies. While some instances of lighter color and softer consistency in appendix feces were noted, these characteristics were not consistently observed throughout the collection period. Additionally, the distinction between appendix feces and normal feces was less visually apparent in caecum feces, aligning

with findings by Kamphues et al.¹³ The mechanism of caecal fecal uptake, described by Donnelly and Brown, contrasts with the direct ingestion of appendix feces from the anus, as proposed by Drescher and Hamel.^{9,14} This highlights potential variations in fecal consumption behavior among different species or experimental conditions. The observed rolling movement and repeated biting off of feces align more closely with the process outlined by Drescher and Hamel, suggesting a nuanced approach to fecal consumption in guinea pigs. The observed behavior of guinea pigs making a rolling movement and repeatedly biting off appendix feces directly from the anus offers valuable insight into their feeding behavior. This behavior, as described by Drescher and Hamel, contrasts with previous assumptions were suggested that animals swallow appendix feces whole without chewing.^{9,15}

The differences between findings regarding the timing of cecal feces intake in guinea pigs presents an intriguing aspect of their digestive behavior. Zentek suggests that the maximum intake of cecal feces occurs in the afternoon, while Drescher and Hamel contradict this, referring to caecotrophs as “night feces.”^{9,16} Observations conducted between 6 p.m. and midnight align with the latter assertion, indicating that ingestion of appendix feces predominantly transpires during this nocturnal timeframe. Sakaguchi provides additional context, stating that animals consuming cecal feces do so during resting phases, while normal feces are released primarily during active phases.¹² These findings concur with observed behavioral patterns, revealing a cyclical rhythm characterized by active phases marked by food consumption and subsequent defecation, followed by rest phases during which guinea pigs exhibit behaviors indicative of cecal feces collection. This cyclical pattern repeats multiple times throughout the observation period. Contrary to Sakaguchi’s description, however, the release of normal feces is observed not only during active phases but also during resting phases.¹² This suggests a more nuanced relationship between fecal release and activity level than previously described. Analysis of the test material reveals a significant difference in the content of *Lactobacillus spp.* between feces and

cecal feces. This finding underscores the functional distinction between these two types of feces and highlights the potential role of microbial populations in the digestive processes associated with each.

The ingestion of caecotrophs, particularly by juvenile guinea pigs, serves a crucial role in colonizing their gastrointestinal tract with beneficial bacteria, as noted by Huerkamp et al.¹¹ These caecotrophs, obtained from the mother, facilitate the establishment of *Lactobacillus* and other Gram-positive bacteria in the juvenile guinea pigs’ digestive system. However, in the study at hand, fecal collection was limited to adult animals, indicating that *Lactobacillus* is indeed a component of the bacterial flora present in both feces and cecal feces of adult guinea pigs. Drescher and Hammel assert that the predominant Gram-positive flora in guinea pigs includes *B. bifidum* and *Lactobacillus acidophilus*.⁹ While this study confirms the presence of *Lactobacillus*, the identification of *Bifidobacterium* was not explicitly mentioned. Nevertheless, the presence of *Lactobacillus* corroborates the findings of Drescher and Hammel.⁹ Even attempts to introduce *Lactobacillus* through diet failed due to the hostile acidic environment of the rabbit’s stomach, which effectively eliminates introduced bacteria. This discrepancy highlights species-specific differences in intestinal flora and their interactions with dietary components. Harkness and Wagner note the near absence of *E. coli* and *Clostridium spp.* in the intestinal flora of guinea pigs.⁷ Drescher and Hammel further support this observation, attributing the low pH value (6.0 to 6.8) in the guinea pig’s colon to the inhibition of colonization by Gram-negative coliform bacteria.⁹ Consequently, these bacteria are not considered part of the guinea pig’s physiological flora.

The application of probiotics has yielded substantial enhancements in the production performance of various animals, marked by elevated growth rates, improved feed conversion efficiency.¹⁰ McLean and Boquest conducted an examination of the intestinal contents, cecal contents, and feces of guinea pigs to assess the presence of *E. coli*.⁴ Their findings supported the notion that guinea pigs lack *E. coli* in their physiological bacterial intestinal,

appendix, and fecal flora. However, contrasting results were also reported and suggested that *E. coli* and *Clostridium* are present at low levels in the intestinal flora of guinea pig.¹¹

In the present study, attempts were made to corroborate these findings. However, isolation of *Clostridium spp.* from the blood agar was unsuccessful, and *E. coli* was detected only in the cecal feces of one animal on MacConkey Agar. Subsequent analyses of the cecal feces from the same animal revealed no presence of *E. coli*. This suggests that the initial detection of *E. coli* could have been attributed to contamination of the feces or feed, highlighting the importance of rigorous control measures in microbiological studies. However, *E. coli* ingested through contaminated feed is excreted in the feces, which aligns with the absence of *E. coli* in the fecal flora of guinea pigs observed in a previous study.⁹ Additionally, previous studies were asserted that the intestinal flora of guinea pigs is primarily composed of anaerobes and Gram-positive bacteria.^{5,7,9} This aligns with the findings of the current study, where colonies were predominantly counted on anaerobically incubated *Lactobacillus* agar, blood agar, and bifido agar plates, confirming the prevalence of anaerobes in the intestinal flora of guinea pigs. Furthermore, the absence of *Clostridium spp.* in the randomly selected colonies from blood agar, despite its use for *Clostridium* detection, consistently yielded Gram-positive rods in the Gram stain. This discrepancy suggests potential limitations in the detection methods used or supports the assertion that *Clostridium* is not a significant component of the intestinal flora in guinea pigs.

In summary, the significant difference in the total colony number of *Lactobacillus* supports the assumption of caecotrophy in guinea pigs. However, discrepancies in the presence of *E. coli* and *Clostridium* warrant further investigation to elucidate the composition and dynamics of the intestinal flora in guinea pigs.

CONCLUSION

This study aimed to assess whether the bacterial composition of feces consumed by guinea pigs differs

from that of normal feces. Our investigation was prompted by the need to clarify whether guinea pigs exhibit true caecotrophy or solely engage in coprophagia, or if there is no discernible pattern regarding their fecal consumption. Our findings reveal a clear presence of coprophagia in guinea pigs, consistent with previous observations. However, our analysis did not uncover any significant discrepancy in bacterial composition between recovered feces and normal feces. Thus, it can be concluded that guinea pigs do not demonstrate a preference for consuming feces based on bacterial flora, supporting the notion that they do not differentiate between normal feces and caecal feces. Further investigations are warranted to explore additional factors that may influence fecal consumption behavior in guinea pigs. In conclusion, this study contributes to our understanding of guinea pig fecal characteristics, highlighting nuances in the visual, microbial, and behavioral aspects of caecotrophy. Further research may elucidate the factors contributing to the variability observed and refine our understanding of guinea pig gastrointestinal dynamics.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Gülşah Kaya Karasu; **Design:** Gülşah Kaya Karasu; **Control/Supervision:** Gülşah Kaya Karasu; **Data Collection and/or Processing:** Gülşah Kaya Karasu; **Analysis and/or Interpretation:** Gülşah Kaya Karasu; **Literature Review:** Gülşah Kaya Karasu; **Writing the Article:** Gülşah Kaya Karasu; **Critical Review:** Gülşah Kaya Karasu; **References and Fundings:** Gülşah Kaya Karasu; **Materials:** Sandra Schmidt.

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