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Original Article

Effects of yogurt dietary supplementation on the intestinal ecosystem of a population of Emperor tamarins (*Saguinus imperator*)

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Summary. Providing hidden food is a method of nutritional and environmental enrichment for captive animals and yogurt is sometimes used with this purpose for non-human primates. Objective of the present study was to evaluate the effect of feeding fresh yogurt on the intestinal ecosystem of Emperor tamarins (*Saguinus imperator*). A population of nine adult/juvenile emperor tamarins received during the whole trial a diet mainly consisting of different fruits. During the first 30 d, the diet did not contain any yogurt; during the following 28 d, every two days, a total of 300 g of fresh fruit yogurt was provided to the animals. A fresh fecal sample was collected from each animal the day before administration of yogurt started (Day 0) and again after 21 and 28 days for chemical and bacterial determinations. Throughout the study, all tamarins remained in good health and no clinical signs of intestinal discomfort were observed. During yogurt supplementation, fecal pH, moisture and ammonia resulted unchanged respect to the beginning of the study. Similarly, fecal volatile fatty acids were not affected by the yogurt intake. On the contrary, fecal spermine concentration resulted significantly decreased at Day 28 respect to Day 0 (4.4 *vs*. 30.1 nmol/g of feces; $P < 0.05$). Furthermore, the consumption of yogurt resulted in reduced fecal concentrations of coliforms, enterococci and lactobacilli on Rogosa Agar (respectively, -1.9, -1.5 and -2.8 log CFU/g of feces; $P < 0.05$). Results from the present study showed that emperor tamarins can tolerate high amounts of yogurt in their diet without showing any signs of lactose malabsorption (for example, soft feces or diarrhea). On the other hand, yogurt ingestion failed to exert any major influence on the animals' intestinal microbiota.

Key words: Intestinal ecosystem, lactose, *Saguinus imperator*, tamarins, yogurt.

Introduction

During the last decades, yogurt and similar fermented milk products have gained popularity in human nutrition for their beneficial properties and, today, they are considered as functional foods. In fact, yogurt, besides being an excellent source of calcium and vitamins, can exert probiotic effects as it contains live lactic acid bacteria (LAB). Yogurt LAB are in general represented by cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (1) that, if found alive in adequate numbers, might improve consumer's intestinal health acting as probiotic strains (2). Lactose is the disaccharide that is found in milk and can be absorbed only after hydrolysis to glucose and galactose by β-galactosidase (lactase), an enzyme produced in the intestinal mucosa of young mammals. However, lactase production strongly decreases after weaning in mammals and it is believed that only some human populations retain the ability to digest lactose during adulthood (3). Before fermentation, yogurt contains about 6% of lactose which is only partially (20-30%) hydrolyzed by bacteria during the fermentation process (4). As such, yogurt ingestion by individuals lacking lactase might lead to lactose maldigestion and intolerance. At the same time, incomplete or absent digestion of lactose may provide a natural

prebiotic contributing to improved balance of the intestinal microbiota (5).

Environmental enrichment has become a fundamental part of management of captive animals, in order to enhance their physical and psychological well-being. Environmental enrichment has been defined as "an improvement in the biological functioning of captive animals resulting from modifications to their environment" (6) and involves the practice of increasing the physical, social and temporal complexity of captive environments (7). More recently, the Enrichment Working Group of the Behavior and Husbandry Advisory Group, a scientific advisory group of the American Zoo and Aquarium Association, defined enrichment as "a dynamic process in which changes to structures and husbandry practices are made with the goal of increasing behavioral choices available to animals and drawing out their species-appropriate behaviors and abilities, thus enhancing animal welfare" (8). Providing hidden food is one of the methods that can be used for environmental enrichment. Furthermore, a feeding enrichment program might include foods that are not always available and are occasionally added to a stable, nutritionally complete diet. For example, yogurt is sometimes used with this purpose for non-human primates. Nevertheless, at present, little is known about the effect of feeding food containing lactose to adult monkeys and apes and about the ability of these animals to tolerate lactose in their diet.

The objective of the present study was to evaluate the effect of feeding fresh yogurt on the intestinal ecosystem of a population of emperor tamarins (*Saguinus imperator*).

Materials and methods

The Ethical Committee of the University of Bologna reviewed and approved the experimental protocol.

The present study was conducted at Parco Natura Viva, Bussolengo (Italy) and involved a population of eleven emperor tamarins.

The family group, comprised of a single breeding adult male and female, along with the independent (seven juvenile subjects) and dependent (two infants) offspring of the breeding pair, was housed in a facility consisting of an indoor area connected (via guillotine doorways) to an outdoor enclosure. The indoor-outdoor enclosure measured more than 60 m^2 (the outdoor being 27 m^2) and was approximately 8 m high. The enclosure allowed and promoted a full range of naturalistic behaviors, social interactions, and locomotion patterns. The primary furnishing for these animals' housing were natural tree branches that were arranged to provide a network of pathways by which the animals can move. Branches are particularly important because tamarins use these as the normal substrate for scent marking. Food and water were made available on a feeding platform and in bowls placed high in the cage in a location that prevented contamination by urine and feces. Prior to the study, adult and juvenile animals received a diet mainly consisting of different fruits (about 250 g of fruit per animal per day, divided in two meals: 150 g in the morning and 100 g in the afternoon). Moreover, animals received each day live larvae of *Tenebrio molitor* or, alternatively, *Zophobas moiro* as a source of dietary protein (about a table spoon of larvae for each animal) and gum arabic (about a table spoon for each animal). Both live larvae (placed in paper cups) and gum arabic (dispensed into holes of a wooden structure in order to simulate the presence of tree exudates) were hidden in order to encourage search for food by the tamarins. Once or twice a week, animals received other foodstuffs including a mineral-vitamin supplement for primates, carbohydrates (noodles, rice or legumes) and protein (eggs, turkey meat, fresh cheese or yogurt) sources and vegetables (carrots, tomatoes, cucumbers and more).

During the whole study, tamarins kept receiving the same diet that has already been described but, during the first 30 d, the diet did not contain any yogurt, fresh cheese or other lactose-containing ingredient. During the following 28 d, every two days, a total of 300 g of fresh fruit yogurt was provided to the animals within 20 paper cups (each paper cup contained approximately 15 g of yogurt). Once the yogurt had been dispensed, paper cups were sealed with paper tape and placed all over the outdoor enclosure. Paper cups were removed after 24 h. Fresh fruit yogurt was a commercial product (containing 21% strawberries and cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*) that was provided by the seller every 10 d (during which it was stored at 0-4°C) in a 5 kg container (in total, 3 containers were used throughout the study). At the opening, a sample of yogurt from each container was collected and stored at 4°C for bacterial (lactobacilli and streptococci) determination.

A fresh fecal sample was collected from each adult and juvenile animal the day before administration of yogurt started (Day 0) and again after 21 and 28 days. Individual fecal samples were collected in sterile containers and frozen at -18°C within 20 min after excretion for chemical (pH, moisture, ammonia, volatile fatty acids, biogenic amines) and bacterial determinations. No fecal samples were collected from infant tamarins.

Chemical and microbiological analysis

Fecal moisture was determined according to AOAC standard method (9). Fecal ammonia was measured using a commercial kit (Urea/BUN – Color, BioSystems S.A., Barcelona, Spain). Volatile fatty acids (VFA) in feces were analyzed by gas chromatography (10). For the determination of biogenic amines, samples were diluted 1:5 with perchloric acid (0.3 M); biogenic amines were later separated by high performance liquid chromatography and quantified through fluorimetry, according to the method proposed by Stefanelli et al. (11).

The day after collection, yogurt samples were serially diluted with Wilkins-Chalgren Anaerobe Broth (WCAB, Oxoid LTD, Basingstoke, Hampshire, UK), added with L-cysteine HCl (0.5 g/L) and plated in triplicate onto selective media: MRS Agar (Oxoid LTD) for lactobacilli and ST Agar (12) for streptococci. Average bacterial counts were 5.72 and 5.46 log CFU/ml of yogurt on MRS Agar and ST Agar, respectively.

Within 10 days from collection, individual fecal samples were homogenized, serially diluted with anaerobe half-strength WCAB and plated in triplicate onto selective media: MacConkey Agar (Merck, Darmstadt, Germany) for coliforms, OPSP Agar (Oxoid LTD) for *C. perfringens*, LAMVAB Agar (13) and Rogosa Agar (Oxoid LTD) for lactobacilli, and Azide Maltose Agar (Biolife, Milano, Italy; added with triphenyl tetrazolium chloride at 10 ml/L) for enterococci. MacConkey Agar and Azide Maltose Agar plates were incubated aerobically at 37°C for 24 and 48 h, respectively; all other media were incubated anaerobically at 37°C for 48 h.

The Fluorescence In Situ Hybridization technique was used to determine counts of bifidobacteria. For this purpose, a ready-to-use commercial kit (BioVisible BV, Groningen, The Netherlands) containing specific FITC-labeled probes for the enumeration of *Bifidobacterium* spp. was used. The slides were evaluated with a Nikon Eclipse E-600 epifluorescence microscope, equipped with an FITC specific filter.

Statistical analysis

Data were analyzed by repeated measurements ANOVA with time as the main effect; each animal formed an experimental unit. Differences among means of groups were analyzed using the Student-Newman-Keuls test. Differences were considered statistically significant at $P < 0.05$.

Results

Throughout the study, all tamarins remained in good health and no clinical signs of intestinal discomfort were observed (for example, soft feces or diarrhea). Yogurt was always completely consumed by tamarins and no leftovers were found when paper cups were removed from the animals' cage.

Chemical analyses of fecal samples are presented in Table 1 (pH, moisture, ammonia and VFA) and Table 2 (biogenic amines). After 21 and 28 d, fecal pH, moisture and ammonia were not different than before yogurt administration. Similarly, fecal VFA were not influenced by treatment. Among biogenic amines, fecal spermine was significantly lower at 28 d than at trial start (-85%; $P < 0.05$).

Bacterial populations in fecal samples are reported in Figure 1. After 28 d, compared with fecal bacterial populations before yogurt administration started, fecal counts of coliforms, enterococci and lactobacilli on Rogosa Agar were significantly lower (-1.9, -1.5 and -2.8 log CFU/g of feces; $P < 0.05$).

Discussion

For 28 d, each adult/juvenile tamarin received, every two days, about 30-35 g of fresh yogurt. In the present study, lactose content of yogurt was not determined; still, based on literature, lactose concentration in yogurt varies from 25 to 40 g per kg of yogurt (4, 14) which means that, every two days, each monkey presumably ingested an amount of lactose comprised between 0.8 and 1.4 g of lactose. Human beings are considered to be the only

Table 1. pH, moisture and concentration of ammonia and volatile fatty acids (VFA)[1] in feces of emperor tamarins after 0, 21 and 28 d of inclusion of fresh yogurt in their diet.

Item	Day 0	Day 21	Day 28	ANOVA, P value	Pooled SEM
pH	7.42[ab]	7.94[b]	6.78[a]	0.024	0.27
Moisture, g/100 g of feces	81.6[ab]	85.4[b]	79.0[a]	0.041	1.62
Ammonia, μmol/g of feces	119	111	108	0.135	3.81
VFA, μmol/g of feces					
Acetic acid	73.4	66.0	71.7	0.732	6.92
Propionic acid	14.5	10.1	13.7	0.210	1.78
n-Butyric acid	7.41	5.97	6.04	0.720	1.40
iso-Butyric acid	0.83	0.78	0.97	0.888	0.36
Total VFA	95.4	82.6	92.4	0.633	9.57

[1]Values are means of nine animals; [a,b]Within the same row, means without a common letter differ (P < 0.05)

Table 2. Concentration of biogenic amines (nmol/g of feces)[1] in feces of emperor tamarins after 0, 21 and 28 d of inclusion of fresh yogurt in their diet.

Item	Day 0	Day 21	Day 28	ANOVA, P value	Pooled SEM
Putrescine	107	79	132	0.576	34.6
Cadaverine	72.0	19.1	77.6	0.131	21.2
Spermidine	52.8	50.3	42.0	0.400	5.75
Spermine	30.1[a]	16.4[ab]	4.4[b]	0.047	6.60
Total biogenic amines	262	165	256	0.424	56.9

[1]Values are means of nine animals; [a,b]Within the same row, means without a common letter differ (P < 0.05)

mammals that show persistence of lactase in adulthood, whereas in other mammal species lactase activity falls prior to adulthood (15). Nevertheless, to our knowledge, little is known about lactase activity in adult nonhuman primates and, in particular, in tamarins. In human medicine, the lactose load is one method of diagnosis of lactose intolerance and is usually performed in adult patients with amounts of lactose up to 12 g which account for 0.02% of body weight of an adult human being (16). Considering that the average body weight of an adult tamarin is around 300-400 g, in the present study, monkeys were eating a considerable amount of lactose (between 0.3 and 0.5% of their body weight) without showing any clinical signs of malabsorption. In this respect, it has been shown in humans that fermented milk products such as yogurt can improve lactose digestion due to the presence of LAB producing β-galactosidase (17). After 28 d of yogurt supplementation, fecal moisture was significantly lower than at 21 d. Conversely, no difference was observed between fecal moisture detected during the supplementation period and the beginning of the trial. Based on these results, it is not possible to link the variations in fecal moisture content with lactose ingestion.

The ingestion of yogurt, a foodstuff containing a combination of viable LAB and lactose (a potential synbiotic, according to the definition given by Schrezenmeir and De Vrese (18)), was expected to reduce bacterial proteolysis, thus reducing fecal ammonia concentrations and pH. The latter effect was expected also as the consequence of lactose fermentation by LAB with production of lactic acid and VFA (19). However, in the present study, ingestion of yogurt did not influence fecal pH and ammonia concentrations. In fact, after 28 d of yogurt administration, fecal pH, moisture and ammonia were not different from values obtained at the end of the adaptation phase (Day 0), during which animals had received for 30 d a diet not containing yogurt or any other source of lactose.

In the present study, fecal concentrations of VFA were not influenced by yogurt ingestion. Among VFA, acetic acid accounted for more than 75% of total VFA and, throughout the study, the acetic to propionic acids ratio was comprised between 5:1 and 6:1. This high ra-

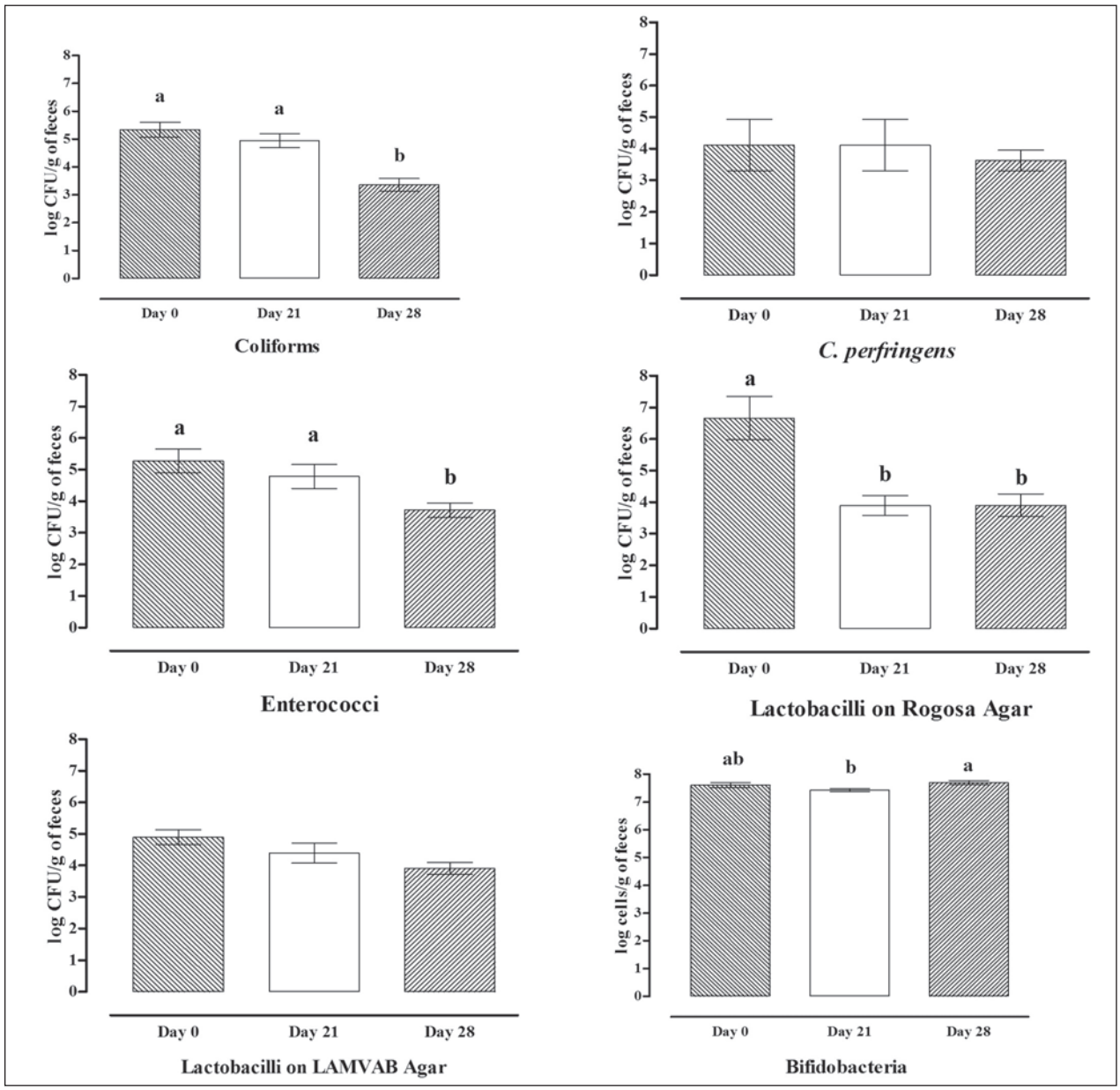

Figure 1. Bacterial populations in feces from emperor tamarins after 0, 21 and 28 d of inclusion of fresh yogurt in their diet.
[a,b]Within the same graph, columns without a common letter differ (P < 0.05).

tio may be explained by the fact that tamarins consumed a diet containing high amounts of fruits. In fact, bacterial fermentation of soluble fiber (such as pectins) that is found in fruits leads in general to the production of acetic acid, as already observed in humans (20), rats (21) and dogs (22). On the contrary, fermentation of lactose by LAB usually results in increased concentrations of lactic acid (23) which is later converted to propionic acid by lactate utilizers (24).

Consumption of yogurt for 28 d resulted in reduced fecal spermine, but did not influence concentrations of other biogenic amines. Since biogenic amines are produced during protein fermentation, reduction of spermine may be interpreted as a sign of reduced intestinal bacterial proteolysis.

In general, findings from the present study regarding the effect of yogurt supplementation on fecal bacterial metabolites seem to highlight that the presence in

the tamarins' diet of large amounts of soluble highly fermentable fiber from fruits may overwhelm the influence of yogurt on the animals' intestinal ecosystem. Moreover, it is known that concentrations of bacterial metabolites that are absorbed by the intestinal mucosa decrease while digesta move along the intestine. As such, feces might not reflect the changes in the concentration of ammonia, VFA and biogenic amines that yogurt might have induced in the hindgut (25).

In other animal species, the consumption of yogurt or similar fermented dairy products resulted in changes of metabolic activities of the intestinal microbiota, as reported by some authors. In a study by Djouzi et al. (26), human flora-associated rats fed for six weeks a diet containing 30% of fermented milk (in presence of a strain of *L. casei* and yogurt starters) showed a significant increase of fecal acetate, propionate and butyrate. In another study (27), the consumption of 125 g/d of fresh yogurt for one month by healthy infants, did not affect fecal pH, water content, and concentrations of VFA but resulted in decreased concentrations of branched-chain fatty acids, the latter being considered a marker of proteolytic fermentation.

There is enough evidence that the utilization of probiotic bacteria and prebiotic substances represents an effective strategy to modulate the gastrointestinal ecosystem of humans (28, 29) and mono-gastric animals (30), increasing the abundance of beneficial bacteria and reducing the presence of undesired microbes. In the present study, after 28 d of yogurt supplementation, compared with counts at trial start, fecal coliforms were significantly lower whereas *C. perfringens* counts were not affected. Coliform bacteria, including *Escherichia coli*, are microorganisms that are commonly found in the intestine of humans and animals where, usually, they are not harmful; however, coliforms are undesired microbes as they include pathogens. In a study with human volunteers conducted by Chen et al. (31), the ingestion of yogurt increased the counts of anaerobic bacteria, suppressed aerobic bacteria and significantly elevated the bifidus to coliform ratio. Furthermore, the elevated bifidus to coliform ratio gradually diminished after yogurt consumption was discontinued. Beneficial effects deriving from yogurt consumption on fecal microflora composition were reported by other authors, who observed increased concentrations of LAB (32), enterococci and lactobacilli (27) and bifidobacteria (33, 34). In the present study, compared with values at trial start, fecal counts of enterococci and lactobacilli grown on Rogosa agar showed a significant reduction at 28 d. This result is surprising considering the fact that fresh yogurt used in the present study was a source of viable cells of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Furthermore, after 28 d of yogurt administration, fecal counts of bifidobacteria were slightly higher than at 21 d but unchanged respect to the beginning of the trial. Nowadays, modern molecular identification methods have been developed to achieve a better understanding of the composition of intestinal microbiota of animals; however, Azide Maltose Agar (also known as KF Streptococcal Agar) is still used for the selective isolation and enumeration of enterococci and fecal streptococci (35), whereas Rogosa (1) and LAMVAB (36) agar are still proposed for the enumeration of lactobacilli.

Conclusions

Results from the present study showed that adult and juvenile emperor tamarins can tolerate very high amounts of yogurt in their diet without showing any signs of lactose malabsorption. However, ingestion of yogurt, despite resulting in decreased fecal concentrations of spermine and coliform bacteria, failed to exert any major influence on the animals' intestinal microbiota.

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