NUTRIENT COMPOSITION OF PROBIOTICS AND A SURVEY OF THEIR USE IN ZOOLOGICAL INSTITUTIONS

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Introduction

Probiotics are live microbiota, typically lactic acid bacteria and yeast, that when provided in sufficient quantities have a health benefit on the host (World Health Organization). Probiotics are generally regarded as safe and approval by the Food and Drug Administration is not required. Typical characteristics of probiotics include the ability to survive production (*e.g*. heat stable) and the gastrointestinal tract environment, produce inhibitory metabolites, and adhere to the intestinal cell membrane (Smith, 2014). Several studies have identified potential probiotics isolated from animals including dogs, giant panda, and non-human primates (Strompfová *et al*., 2013; Liu *et al*. 2017; Tsuchida *et al.*, 2018); however, little is known about their use in zoological institutions. Probiotics are commonly supplemented for the maintenance of gut health, treatment of chronic or infectious diarrhea, and the prevention of antibiotic-associated diarrhea. Labels of probiotic products consistently include a list of ingredients and the number of colony forming units (CFU) per dose, but the actual nutrient composition is unknown. The main objectives of our study were to 1) identify the nutrient content and quantify the total number of CFU per gram of commercially available probiotic products and 2) use survey results to determine the status of gut health and probiotic use across zoological institutions.

Materials and Methods

Twelve commercially available probiotic products (samples P1-P12) were selected based on peer-reviewed research studies demonstrating efficacy, target species/animal group, and those readily available at the in-house institution. Target groups included 4 human, 2 canine, 1 feline, 1 feline and canine, 1 equine, 1 ruminant, 1 non-human primate, and 1 avian/reptile products. When possible two different lots of the same product were used for analysis. Products were analyzed for nutrient composition by Dairy One Laboratory (Ithaca, NY) and total probiotic counts by Eurofins (New Berlin, WI).

A 10 question survey was generated with SurveyMonkey (surveymonkey.com) and delivered to the Nutrition Advisory Group's listserv to generate responses. The survey included two open-ended statements, 7 multiple choice questions, and 1 fill in the blank statement described below.

Results and Discussion

Total probiotic counts and nutrient composition

Total probiotic counts ranged from $4.0 \times 10^4 - 6.3 \times 10^9$ CFU/g product and the nutrient contents varied by product (Table 1). The greater amounts of crude protein (~50%), iron, zinc, copper, and calcium in products P10 and P11 are likely a result of the added animal digest, ferrous sulfate, zinc proteinate, copper proteinate, and calcium iodate, respectively, in the products. P9 contained the

greatest content of ethanol soluble carbohydrates with sucrose listed as the first ingredient on the label.

Oral gel products had the greatest content of crude fat (Table 1). P1 is a sunflower oil-based oral gel that contains 66% fat, while products P7, P8, and P12 contain soybean oil. The total fatty acid content (dry matter-basis) of P1, P7, P8, and P12 was 61.4%, 59.2%, 61.8%, and 43.0%, respectively. Products containing soybean oil were comprised of similar fatty acid profiles (Figure 1). The content of oleic acid (18:1 *cis*-9) in P1, P7, P8, and P12 was 66.6%, 21.9%, 23.2%, and 22.0%, respectively, while the ratio of n-6 to n-3 fatty acids was 53:1, 8:1, 8:1, and 6:1, respectively. Sunflower oil has previously been shown to have a greater content of oleic acid than soybean oil (Huth *et al*., 2015).

Survey questions and responses

Initially, a total of 11 zoological institutions responded to the survey over a 2-week period. The survey will be disseminated a second time to achieve a greater number of respondents. The first question gave the respondent the option of including the name of their institution, but for privacy purposes this will be excluded from results.

Q2. Please indicate any gut health issues you've observed at your institution.

Chronic diarrhea was the most reported gut health issue across institutions (9 institutions, 81% of total respondents), followed by colic (6 institutions, 54%), constipation and antibiotic-associated diarrhea (4 institutions, 36%). Rumenitis, enteritis, ulcerative colitis, and soft/clumped feces in ungulates were reported by single institutions.

Q3. Which gut health issue is most frequently (i.e., most common) observed at your institution?

Chronic diarrhea was reported as the most common gut health issue observed at 7 institutions (63%), followed by antibiotic-associated diarrhea at 2 institutions (18%). Colic and chronic soft and clumped feces were reported as the most common gut health issue at single institutions.

Q4. Which animal group (s) is/are your biggest concern(s) with regards to the maintenance of normal gut health?

Ruminants were reported to be the most concerning animal group with regards to the maintenance of normal gut health (8 institutions, 73%), followed by primates (5 institutions, 45%), and feline (2 institutions, 18%). Single institutions indicated rabbits, otters, pachyderms, avians, and equids to be of concern.

Q5. What are probiotics used for at your institution (Choose all that apply)?

Antibiotic-associated and chronic diarrhea were the most commonly reported reasons for probiotic use (7 institutions, 64%), followed by ulcerative colitis (3 institutions, 27%). Single institutions reported using probiotics for maintenance of gut health, hand-rearing, before stressful events, and cases of soft/clumped diarrhea.

Q6. Probiotics are ___at improving gut health.

Of the total respondents, 55% stated that probiotics are neither effective nor ineffective at improving gut health, while 45% of respondents found probiotics to be effective.

Q7. Who initiates probiotic supplementation at your institution (Choose all that apply)?
All institutions stated that veterinarians initiate probiotic supplementation, while 6 institutions (54%) stated that both nutritionists and veterinarians are involved in the initiation.

Q8. What type of probiotic is currently used at your institution?
The most common type of probiotic reported to be used is bacteria-based (5 institutions, 45%), while 4 respondents (36%) stated that a combination of yeast and bacteria products are used. Three institutions reported the use of yogurt as a probiotic.

Q9. Please name the probiotic products (brand names are okay) typically used at your institution and which animal species they've been offered to.
A total of 21 different products were listed as typically used products. Eleven institutions used at least 3 different products, while one institution used 2. The most prevalent microbial species was *Lactobacillus acidophilus* in 10 out of 21 total (47%) reported products (Table 2). Seven institutions reported the use of a Probios® (Chr. Hansen, Inc.) product, while 4 institutions reported the use of Purina® Pro Plan® Veterinary Supplements FortiFlora® and PetAg® Bene-Bac products, and 2 reported the use of Culturelle® Kids.

Q10. Which factors contribute to the selection of a probiotic supplement at your institute (Choose all that apply)?
Dose was the most common factor reported by respondents to contribute to the selection of a probiotic supplement (81%), followed by the species of bacteria or yeast (54%), palatability and target species (36%). Peer-discussed efficacy, the use of the website Labdoor.com for label accuracy analysis, cost, presentation of product, and anecdotal efficacy evidence were also factors used by single institutions.

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Next steps
The nutrient profile and total probiotic counts may be considered during the probiotic selection process. The survey will be disseminated a second time to achieve a greater number of respondents. This would enable us to further combine data from a more diverse pool of zoological institutions and have a greater number of respondents. This will better describe current probiotic supplementation practices and provide a better understanding about the gut health status of animals housed under human care.

Literature Cited
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Table 1. Description and nutrient content of select commercially-available probiotics (*n*=12).

Parameter	**Unit**	**P1**[1]	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**
Target group		avian/ reptile	human	human	human	human	primate	canine	equine	ruminant	canine	feline	canine/ feline
Form		gel	powder	capsule	powder	tablet	tablet	gel	gel	powder	powder	powder	gel
Bacteria		x	x			x	x	x	x	x	x	x	x
Yeast				x	x		x				x	x	
TPC[2]	CFU/g[3]	$4.0x10^7$	$1.8x10^9$	$1.3x10^9$	$6.3x10^9$	$2.4x10^9$	$8.2x10^8$	$8.3x10^5$	$4.0x10^4$	$1.2x10^7$	$2.6x10^8$	$5.2x10^8$	$3.0x10^8$
DM	%	99.9	98.9	98.0	99.0	96.0	95.5	98.6	98.6	99.6	96.6	93.9	99.6
CP	%	0.8	1.6	37.5	13.2	10.6	3.3	1.6	1.1	6.0	50.0	51.6	3.0
NDF	%	24.2	1.2	1.5	0.9	1.6	6.4	21.6	19.3	1.0	1.3	2.0	20.0
ADF	%	9.5	1.0	1.2	0.7	1.2	3.2	7.8	7.2	0.9	1.0	1.6	8.6
Lignin	%	5.0	0.9	0.9	0.6	0.9	1.3	4.8	5.0	0.8	0.8	0.9	6.5
Crude Fat	%	66.0	0.2	0.6	1.0	0.1	1.6	65.7	66.9	0.8	9.9	10.4	45.2
Starch	%	1.9	0.1	0.3	0.1	0.1	0.6	13.7	13.4	0.2	2.2	3.2	0.7
ESC[4]	%	3.3	4.8	12.3	11.7	11.2	7.6	10.1	11.4	24.4	9.7	6.3	n/a
Ash	%	15.10	1.20	9.50	2.30	1.40	0.69	3.10	3.02	8.70	10.50	10.30	39.80
Ca	%	0.01	-	0.04	0.02	0.05	0.11	-	-	0.38	0.34	0.29	0.01
P	%	-	0.10	1.52	0.56	0.35	0.10	-	-	0.45	1.63	1.59	0.03
Mg	%	-	-	0.18	0.05	0.03	0.03	-	-	0.10	0.10	0.10	0.01
K	%	-	0.10	1.67	0.62	0.40	0.12	0.01	0.02	1.19	0.95	0.96	0.10
Na	%	0.01	0.20	0.03	0.01	0.21	0.02	0.02	0.03	1.12	1.70	1.43	0.19
S	%	-	0.01	0.64	0.22	0.10	0.03	-	0.01	0.11	0.80	0.87	0.05
Fe	ppm	-	-	30	5	9	1	3	6	1	1300	847	1590
Zn	ppm	1	2	102	37	7	18	2	3	1	2250	1110	108
Cu	ppm	-	-	43	16	1	1	1	-	-	116	103	5
Mn	ppm	-	78	7	3	159	19	-	-	1	381	252	1.00
Mo	ppm	-	-	0.2	0.1	0.1	-	-	-	0.3	2.3	2.5	-
Se	ppm	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.04	1.74	2.82	0.10
Co	ppm	0.03	0.04	0.76	0.26	0.19	-	-	0.10	0.10	0.46	0.32	2.30

[1]P1, contains *Enterococcus faecium, Lactobacillus acidophilus, L. casei L. plantarum, Bifidobacterium bifidum, Pediococcus acidilactici*; P2, *L. rhamnosus* GG*;* P3 and P4, *Saccharomyces boulardii*; P5, 20 bacterial species, *Lactobacillus* (11 total), *Bifidobacteria* (6), *Leuconostoc* (1), *Streptococcus* (1), *Lactococcus* (1); P6, *L. reuteri, S. boulardii;* P7-9, *E. faecium, L. acidophilus, L. casei, L. plantarum*; P10-11, *E. faecium, S. cerevisiae;* P12, *E. faecium, L. casei, L. acidophilus*

[2]Total Probiotic Count

[3]Colony Forming Units

[4]Ethanol Soluble Carbohydrates

Table 2. Distribution of microbial species across probiotic products used by zoological institutions (*n*=11) surveyed.

Species	Number of products reported	% of total products (n=21)
Bifidobacterium bifidum	5	24%
Bifidobacterium breve	2	9%
Bifidobacterium infantis	2	9%
Enterococcus faecium	9	43%
Lactobacillus acidophilus	10	47%
Lactobacillus casei	6	29%
Lactobacillus plantarum	6	29%
Lactobacillus rhamnosus	3	14%
Pediococcus acidilactici	2	9%
Saccharomyces boulardii[1]	2	9%
Saccharomyces cerevisiae	4	19%
Streptococcus thermophilus	2	9%

[1]The genus *Saccharomyces* is a type of yeast. All other species listed are bacteria.

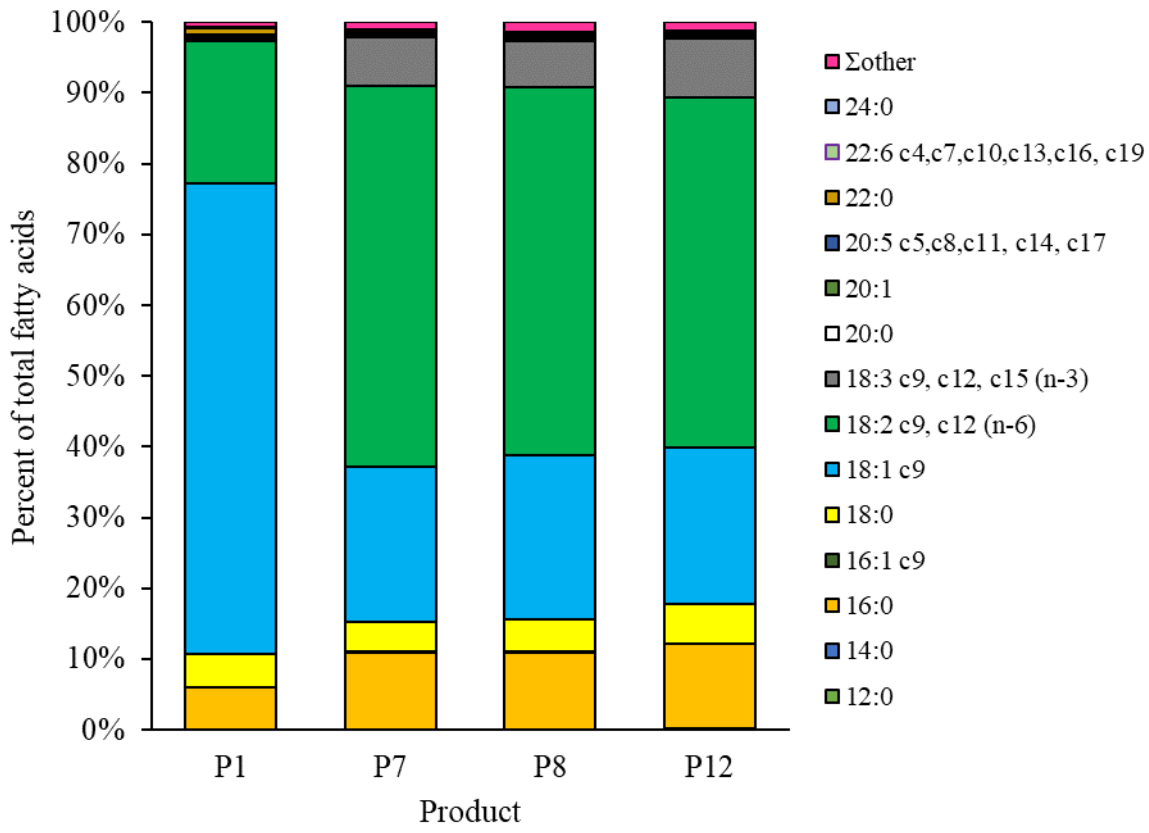

Figure 1. Fatty acid composition of gel-based probiotic products.