Effects of a Milk-Based Beverage with Short-chain Fructooligosaccharides on Tolerance and Incidence of Diarrhea in Toddlers

ABSTRACT

Abbott Nutrition conducted a randomized, blinded, controlled study to assess the efficacy of a milk-based beverage supplemented with short-chain fructooligosaccharides (scFOS) on incidence of diarrhea in toddlers. The study was a clinical trial of 283 children ages 10 to 24 months at study entry attending day care in Santiago, Chile. During a 16-week period, the children were assigned to consume a milk-based beverage with approximately 3.4 g scFOS/L (n=139) or the milk-based beverage with no added scFOS (n=144). The beverages were fed *ad libitum* as the child's sole source of milk beverages. Children were encouraged to drink a minimum of 500 mL of the milk beverage each day at day care and at home. Solid foods were permitted. At study entry, children were monitored closely for diarrhea and other significant medical illnesses. Research nurses visited the day care center weekly to ensure study compliance and identify episodes of diarrhea and other illness. At weeks 1, 4, 8, 12, and 16 of the study, the children were evaluated for measures of tolerance, growth, and fecal microbiota. Day care center workers recorded study feeding intake; occurrences of stomach cramps and vomiting associated with feedings; stool frequency, consistency, and characteristics; the amount of gas; and constipation for each child on each day of attendance at the day care center. Parents maintained records of the same tolerance variables, as well as milk intake, during the 3 days prior to each study visit. Weight and length were measured at entry, and at the week 8 and week 16 visits. Fecal samples were collected at baseline, at week 16, and at one additional study visit during the trial for determination of fecal lactobacilli, bifidobacteria, rotavirus, and *Clostridium difficile*. Fecal specimens also were collected from children within two days of the start of an episode of diarrhea and analyzed for fecal pathogens.

A total of 242 children completed the study: 118 in the scFOS group, and 124 in the control group. There were 3 treatment failures in the scFOS group and 6 in the control group. The number of protocol failures in the scFOS and control groups was 18 and 14, respectively. Measures of weight and length were comparable in the two feeding groups throughout the study and normal in both groups. Children in the scFOS group had greater

weight gains than children in the control group, though the gains were not statistically different.

There were no differences between feeding groups in volume or frequency of milk intake. The average intake of scFOS by children in the scFOS-containing milk beverages was approximately 2.5 g at each assessment. There also were no differences between groups in incidence and frequency of stomach cramps and/or vomiting with feedings. Children consuming the scFOS-containing milk had a lower mean rank stool consistency (i.e., softer stools) at week 1 as compared to the control milk group, and a higher percentage of watery stools or stools that were either watery or loose. There were no differences between groups in mean rank stool consistency at baseline or weeks 4, 8, 12, or 16. Children consuming the scFOS-supplemented milk had a greater increase in detectable levels of fecal bifidobacteria as compared to children consuming the control milk, though the beverages had no effects on fecal counts of *Lactobacillus spp*. The incidence of diarrhea did not differ between the scFOS and control groups. Children consuming milk with added scFOS, however, had a shorter mean duration of acute diarrhea episodes (3.91 vs. 4.88 days, p=0.036), lower incidence of otitis media (17 of 131 children vs. 33 of 134 children, p=0.023), and fewer courses of antibiotics to treat otitis media (p=0.039, relative risk of 0.501).

MATERIALS AND METHODS

Study Design

This study was designed to assess the efficacy of addition of the indigestible oligosaccharide scFOS to a beverage for young children on the incidence of diarrhea. A 16-week controlled, randomized clinical trial was conducted at the University of Chile in Santiago, Chile. A total of 283 healthy children were enrolled and fed one of two study beverages: a milk-based control beverage (CON) or an experimental milk-based beverage supplemented with scFOS. All children enrolled in the study attended day care centers (DCC) in the Santiago area.

All day care centers participating in the study were licensed to care for infants and young children. An eligible day care center had at least five children participating in the clinical trial and cared for at least ten children. Randomization was stratified by gender and by day care center. Separate randomization lists were prepared for each day care center in order to balance the distribution of the feeding groups.

The study consisted of an entry evaluation period and a 16-week study feeding. At entry, children were placed under active surveillance for diarrhea and other significant medical illnesses. Surveillance included study evaluations at Study Day 7, Day 28, Day 56, Day 84, and Day 112. Research Nurses visited the DCC each week to ensure study compliance and identify episodes of diarrhea and other illnesses.

Subjects

Children enrolled in the study were 10 to 24 months of age at entry and attended licensed day care centers in Santiago five days per week. Parents of children agreed to have their child continue attending the DCC five days a week for the duration of the study.

All eligible subjects were in apparent good health with no clinical evidence of chronic gastroenteritis or diarrhea within seven days prior to enrollment; had no significant chronic or severe renal, liver, or gastrointestinal tract function abnormalities; no history of aspiration pneumonia within three months prior to enrollment; and did not suffer from immune deficiency or receive immunosuppressive therapy. Children participating in another investigational drug study one month prior to enrollment were excluded from entering the study.

The study beverage was fed *ad libitum* as the child's sole source of milk beverages. All subjects were encouraged to consume a minimum average daily intake of 500 mL of the study beverage diet during the 16 week feeding phase. Liquid milk beverages other than the study beverages were restricted. Subjects were allowed to consume solid foods, yogurts, and/or cereals in addition to the assigned study beverage. All children had a

history of ingesting whole cow's milk or cow's milk-based infant formula prior to enrollment and exhibited no symptoms of cow's milk allergy or cow's milk protein intolerance. Parents were required to sign an informed consent form approved by the Ethics Committee of the University of Chile, Santiago.

Dietary Intake and Tolerance

Children were randomized into one of two groups to receive one of two study beverages. The study beverages were powder products with instruction on how to reconstitute with water. The study beverages were a milk-based drink with or without scFOS. The approximate compositions are given in Table 1. The study beverages met the levels of nutrients recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act (1980). Subjects were offered 250 ml of study beverage twice a day while in day care. In addition, subjects were allowed to consume the study beverage *ad libitum* while at home. Intake was recorded by the parents for three days prior to study visits on Day 7, Day 28, Day 56, Day 84 and Day 112 and was recorded by day care center workers for each subject each day the child attended day care. Use of study beverages during diarrhea episodes was continued unless it interfered with the medical management of the illness. The type and duration of rehydration therapy fed (Rehydration salts, Pedialyte, Isomil or other milk products) were recorded by the parents.

To assess study beverage tolerance, records of occurrences of stomach cramps and vomiting associated with feedings; the number, consistency (watery, loose/mushy, soft, formed, or hard), and characteristics of stools; the amount of gas; and constipation were recorded by day care center workers each day the subject attended day care. Records of the same tolerance parameters were kept by parents for three days prior to Study Days 7, Day 28, Day 56, Day 84, and Day 112.

Anthropometric Measurements

Weight and length were measured at entry, and on Study Days 56 and Day 112. Children were weighed unclothed on a calibrated scale; duplicate measurements were made for

each weight. Length was measured once on a recumbent length board for each child at entry, and on Study Day 56 and Day 112.

Routine Stool Specimens

To assess intestinal colonization, stool samples were collected for fecal microbiology (total *Lactobacillus* and total *Bifidobacterium* counts) on all subjects at study entry and at Study Day 112, and at one of the four scheduled study visits during the study feeding period.

Incidence of Illnesses Other Than Diarrhea

Day care center workers and parents of children enrolled in the study were queried at scheduled intervals regarding signs and symptoms of illnesses; i.e., fever, cough, congestion, runny nose, earache or ear discharge, rash, diarrhea, vomiting, and increased irritability. Day care center records and clinic or physician visits were monitored for clinically significant illness: non-specific upper respiratory tract infections, bronchitis, bronchiolitis, bronchopneumonia, otitis media, diarrhea and other significant illnesses. Antibiotic use (duration and reason for use) was also recorded and confirmed by clinic and/or day care center records.

Diarrheal Episodes

Children were placed on active surveillance for diarrhea on entry into the study and were followed throughout the 16 week feeding period or until they exited from the study. Parents and day care center workers were instructed to notify the Site's research staff when a child developed diarrhea. Diarrhea was defined as three watery or loose stools in a 24 hour period as determined by the parent, guardian, or day care center worker. A Diarrhea Stool Record booklet was completed by the parent for each diarrhea episode. Booklets were used to record the number of stools per day; the consistency (watery, loose/mushy, soft, formed, or hard) of each stool; stool characteristics (presence of blood, mucous, pus, or undigested food); and the amount of gas, if noted (usual amount, less than usual amount, or more than usual amount). Parents also recorded the presence or absence of fever; temperature, if taken; episodes of vomiting; rehydration use;

medications given to the child; and any antibiotics taken by the child during the episode or within 14 days prior to the episode. The type of antibiotic, duration, and reason for use were also recorded. Diarrhea stool specimen collection was done within two days of the onset of diarrhea and analyzed for rotavirus (ELISA), enteric adenovirus, *Clostridium difficile* toxin and Ova and Parasites *(Giardia lamblia* antigen) and for other enteric pathogens *(E. Coil* toxin screen, *Salmonella, Shigella, Vibrio, Yersinia, Aeromonas, and Campylobacter).*

Laboratory Procedures

Stool samples for fecal microbiology were placed in a collection tube containing liquid, modified and pre-reduced Cary-Blair medium. LBS Agar was used for the enumeration of total *Lactobacillus* counts (colony forming units, CFU/g stool). Bifidobacterium iodoacetate medium-25 (BIM-25) was used to culture for total *Bifidobacterium.* Counts for fecal microbiology were reported quantitatively as well as qualitatively, as poor, moderate or heavy counts of CFU/g fecal material.

Study Exit

The Investigators were responsible for assessing the status of each subject on exit from the study. Subjects completing the 112 day study were classified as successful completers. Subjects were classified as treatment failures if they failed to tolerate the assigned study beverage; repeatedly refused to drink the study beverage; or experienced an adverse event related to the study beverage.

Variations from protocol which could classify the subject as a study protocol failure included: failure to provide records of dietary intake, and no information on stooling patterns for any diarrhea episode; failure to comply with scheduled study evaluations and study visits; failure to consume the assigned study beverage as a sole source of liquid cow's milk for the duration of study; failure to consume at least 500 mL of reconstituted study diet for at least 102 days of the 112 day study; failure to provide the Site with the required stool sample(s), as outlined in protocol; or failure to attend the day care center for 70 of the possible 80 days of the study. The onset of any condition (organic, systemic,

etc) other than diarrhea which prevented normal growth and development or necessitated a dietary change during the trial period was also classified as a protocol failure. Parents were allowed to withdraw their child from the study at any time.

Statistical Methods

The sample size was determined assuming a background rate of 48% in diarrhea incidence for the CON group. Therefore a total sample size of 250 subjects (125 per group) will have 80% power to detect an 18% absolute reduction in the incidence of diarrhea in the group fed scFOS. Based on an estimated 20% attrition rate, enrollment of 314 subjects was planned (157 per group).

For continuous outcomes (percent watery stools, percent watery/loose stools, mean rank stool consistency, average number of stools per day, percent feedings, average number of feedings per day, average daily intake) which were measured at several visits, repeated measures analysis of variance was employed. The Mean Rank Stool Consistency (MRSC) was calculated for each infant for each day by assigning a numerical value (rank) to each of the stools consistencies: watery=1, loose/mushy=2, soft=3, formed=4 or hard=5, taking the sum and dividing by the number of stools. The average of the daily mean ranks was computed to find the MRSC for the feeding period; the median is the middle ordered value. For outcomes which were percentages the arcsine of the square root transformation was used prior to analysis.

The number of cases of diarrhea was analyzed by the marginal approach to multivariate survival analysis. In defining an episode of diarrhea, at least seven days must elapse between episodes of diarrhea or it is considered a single two-stage episode. Time to the first episode of diarrhea was analyzed by the Cox Regression with the robust estimator of the variance. A multivariate regression was performed comparing the number of episodes of diarrhea in the feeding groups. This analysis counts all episodes of diarrhea, including repeated episodes. The marginal approach of Lin, Wei and Weisfield was used to analyze the data. A Cox regression analysis was also performed for first episodes, ignoring repeat

episodes. Analysis was also done for all episodes that occurred > 8 days of study feeding that met the protocol definition of diarrhea (at least 3 watery stools in a 24 hour period). Duration of diarrhea was analyzed by the log rank test. Number of stools per episode, number of watery stools per episode and maximum daily stool number were analyzed by the Wilcoxon rank sum tests.

RESULTS

Subjects

Two hundred eighty-three children were enrolled in the study. A total of 242 children completed the study. Of the 139 children enrolled in the scFOS group, 118 completed the study. Three subjects were classified as treatment failures, and 18 were classified as protocol failures. Of the 144 children enrolled in the CON group, 124 completed the study. In the group, six subjects were classified as treatment failures, and 14 were classified as protocol failures.

Day Care Center Attendance

Day care attendance during both phases of the study was lower for both feeding groups than had been anticipated. Children in the scFOS group attended day care an average of 27.36 hrs of an average maximum of 40 hr per week for the 16 week study (8 hr per day for 5 days). Children in the CON group attended day care an average of 26.70 hr of an average maximum of 40 hr per week. No differences were seen between the groups in the average or median number of days, or the average or median number of hours, children attended day care during the 16-week study.

Growth

Weight and length were measured at Entry, at Study Day 56 and at Study Day 112. All children grew normally. No differences were observed between groups in weight or length at entry, Study Day 56, or Study Day 112 (Table 2). The median weight gain between Entry and Study Day 56 was 9.2 g per day for children in the scFOS group and 8.5 g per day for children in the CON group. Between Study Day 56 and 112, the median weight gain was 6.1 grams per day for children in the scFOS group and 5.5 grams per day

for children in the CON group. Although the increased weight gain per day for children consuming the scFOS containing beverage was consistently greater than for children consuming the CON beverage, the greater weight gain was not determined to be statistically significantly different.

Intake and Tolerance

To assess beverage intake and tolerance, daily records from the day care centers, and parent 3-day dairies (booklets) for Entry, Study Day 7, Day 28, Day 56, Day 84, and Day 112 were evaluated. Study groups did not differ in the mean number of feedings per day or the median volume consumed (Table 3). The incidence and frequency of stomach cramps and/or vomiting associated with feedings were also similar between the two groups.

For children consuming the scFOS-containing beverage, the average MRSC at Entry (prior to starting study beverage) was 3.3; at Day 28 was 3.3; Day 56 was 3.3; at Day 84 was 3.4; and at Day 112 the average MRSC was 3.5. For children fed the CON beverage, the average for the MRSC was 3.2 at Entry (prior to starting study beverage); at Day 28 was 3.2; at Day 56 was 3.3; at Day 84 was 3.4; and was 3.4 at Day 112 (Table 3). At Study Day 7, the scFOS group had a significantly lower ($p=0.03$) MRSC compared to the group receiving the CON beverage.

To determine the impact of stool characteristics on the statistically significant difference in MRSC seen between the feeding groups at Study Day 7, the percentage of stools (mean $\pm$ SEM) by characteristic at each study visit was determined. When the percent of stools that were watery or the percent of stools that were watery or loose, at Day 7 was determined, a statistically significant difference was seen between the two feeding groups. Subjects consuming the scFOS beverage had a statistically higher percentage of watery stools (10.6 ± 2.0) compared to the CON group (5.7 ± 1.4, $p=0.05$), and a statistically higher percent stools that were either watery or loose (scFOS, 27.1 ± 2.7; CON, 19.4 ± 2.4; $p=0.04$). Thus, there were more loose or watery stools in the scFOS group at Study Day 7 which is reflected in the significant difference in MRSC.

Routine Fecal Microbiology

Stool specimens were collected on all subjects at entry and Study Day 112 for fecal microbiology. Specimens were also collected on 1/4 of subjects on Study Day 7; and similarly on Study Days 28, 56 and 84. Of the 95 subjects that constituted the evaluable set for analysis, 31 subjects, or 32.6%, had undetectable levels ($<10^4$) of bifidobacteria in their feces on entry into the study [19 subjects (61.3%) in the CON group and 12 (38.7%) in the scFOS group]. The remaining 64 evaluable subjects, or 67.4%, had detectable levels (10^4-10^9) of bifidobacteria in their feces on entry into the study [30 subjects (46.9%) in the CON group and 34 (53.1%) in the scFOS group]. None of the subjects had greater than 10^9 (greater than detectable) Bifidobacteria counts per g feces at study entry.

In the CON group, 13 of 19 subjects (68.4%) with less than detectable levels of bifidobacteria on study entry had detectable levels in their feces by study exit. For the scFOS group, 10 of 12 subjects (83.3%) were found to have changed from undetectable to detectable levels of bifidobacteria in their feces by study exit. Similarly, for the 30 subjects in the CON group with detectable levels of bifidobacteria in their feces at study entry, 10 (33.3%) were found to have greater than detectable counts at study exit. In the scFOS group, 20 of 34 (58.8%) subjects with detectable levels of bifidobacteria at study entry had greater than detectable counts by study exit. These data indicate a statistically significant higher overall rate of improvement in the scFOS group compared to the CON group ($p=0.028$). A higher proportion of subjects in the scFOS group (56.5%) had greater than detectable ($>10^9$) counts of bifidobacteria per g feces than subjects in the CON group (34.7%) at study exit.

Incidence of Diarrhea Episodes

Of the 283 subjects enrolled in the study, 16 exited the study within the first seven days: seven subjects in the scFOS group and nine subjects in the CON group. A total of 267 subjects had diarrhea surveillance beyond seven days on study feeding. Of the 132 children in the scFOS group, 37 subjects had one episode of diarrhea and three subjects had two episodes of diarrhea. Of the 135 children in the CON group, 36 children had one episode of diarrhea; seven children had two episodes of diarrhea; and two children had

three episodes of diarrhea. No diarrhea was reported for 92 children in the scFOS group or 90 children in the CON group.

The duration of diarrhea was determined by the number of days between first and last reported watery or loose stool. Episodes of diarrhea were significantly shorter in subjects consuming the scFOS beverage. The mean duration of diarrhea was 3.91 days for children in the scFOS group and 4.88 days for children in the CON group, p=0.036. Stool specimens were collected from 95 (95.5%) of the 99 episodes of diarrhea. No significant differences were noted between groups in the etiology of diarrhea.

Parents of 18 children reported diarrhea during the first week of study feeding, for 13 of the 113 children in the scFOS group and for 5 of the 108 children in the CON group. No subjects with reported diarrhea during the first week of study feeding exited the study early as treatment failures. Seven of the 13 subjects in the scFOS group and four of the five subjects in the CON group had been given antibiotics within 14 days prior to the onset of diarrhea.

Incidence of Illnesses Other Than Diarrhea

Day care center workers and parents of children enrolled in the study were queried at scheduled intervals regarding signs and symptoms of illness: i.e., fever, cough, congestion, runny nose, earache or ear discharge, rash, diarrhea, vomiting, and increased irritability. No apparent differences were noted between groups in frequency of these signs and symptoms at study evaluations.

A total of 72 episodes of non-specific upper respiratory illness were diagnosed: 40 in the scFOS group and 32 in the CON group. Subjects in the scFOS group had a total of 134 episodes of bronchitis, while subjects in the CON group had 151 episodes diagnosed. Antibiotics were given to 97 of the 131 subjects with medication records in the scFOS group and to 111 of the 134 subjects in the CON group. Subjects in the scFOS group received a total of 241 courses of antibiotics while subjects in the CON group received a total of 306 courses of antibiotics. No apparent differences were noted between groups

for illnesses or antibiotic use other than otitis media. Children consuming scFOS had a reduced incidence of otitis media (17 of 131 children vs 33 of 134 children, p=0.023) and fewer courses of antibiotics to treat otitis media (p=0.039, relative risk of 0.501)

DISCUSSION

In the present study, a feeding effect was seen for the scFOS-containing beverage on the level of bifidobacteria in fecal samples. On entry into the study, approximately one-third of children did not have detectable levels of bifidobacteria in their stools. After 16-weeks of study feeding, approximately two-thirds of children in the control group were found to have detectable levels of bifidobacteria in the feces, while greater than 80% of the children consuming the scFOS beverage had bifidobacteria present at detectable levels. After stratifying for baseline levels of bifidobacteria, the data indicate a statistically significantly higher overall improvement in subjects in the scFOS group. No differences were seen for effect of feeding on *Lactobacillus* counts.

The average daily intake of the scFOS beverage during the 16-week feeding period was 740 mL. Thus, children were consuming, on average, 2.5 g scFOS per day, or 0.22g of the indigestible oligosaccharide per kg body weight per day. Consumption of a nutritional beverage containing scFOS by young children as part of their daily diet resulted in comparable growth to those consuming the beverage without scFOS. For the diarrheal episodes that did occur, the episodes were statistically shorter in duration in children consuming the beverage with scFOS. For other medically important illnesses, incidence of otitis media were significantly lower in children consuming the scFOS beverage as part of their daily diet, and subjects randomized to the scFOS group had statistically fewer courses of antibiotics prescribed for otitis media compared to children receiving the control formula.

TABLE 1. Composition of study beverage with or without scFOS[1]

	Control Beverage	scFOS Beverage
Protein, g	15.8	15.7
% Kcal from protein	9.2	9.0
Fat, g	35.8	37.3
Carbohydrate, g	74.7	74.7
Vitamin A, IU	3266	3397
Vitamin E, IU	13	14
Thiamin, mcg	163	167
Vitamin B6, mcg	546	513
Vitamin C, mg	133	134
Calcium, mg	967	972
Phosphorus, mg	642	638
Magnesium, mg	81.8	81.4
Iron, mg	14.9	14.7
Sodium, mg	211	212
Potassium, mg	1053	1100
Chloride, mg	571	572
Energy (Kcal)	685	698
Beta-Carotene, mcg	444	446
FOS, g	0	3.4

[1]Units per liter

TABLE 2. Disposition and growth of infants enrolled in 16-week study of milk-based beverage with or without scFOS

	Feeding Group	
	Control Beverage	scFOS Beverage
Enrolled [completed]	144 [124]	139 [118]
Treatment failures	6	3
Protocol failures	14	18
	Mean ± SEM	
Weight at: (g)		
Baseline	10,914 ± 120	10,936 ± 134
Midpoint	11,428 ± 127	11,437 ± 136
Study end	11,756 ± 131	11,786 ± 151
Weight gain at: (g/day)		
Midpoint	7.8 ± 0.6	9.6 ± 0.6
Study end	5.7 ± 0.6	6.4 ± 0.6
Length at: (cm)		
Baseline	79.6 ± 0.4	79.9 ± 0.4
Midpoint	81.6 ± 0.4	81.3 ± 0.4
Study end	83.1 ± 0.4	83.0 ± 0.4

TABLE 3. Feeding, tolerance and stool characteristics of children consuming a milk-based beverage with or without scFOS[1]

	Week 1		Week 4		Week 8		Week 12		Week 16	
Parameter	Control	scFOS	Control	scFOS	Control	scFOS	Control	scFOS	Control	scFOS
Feeding volume, mL/d	736 ± 17	710 ± 17	713 ± 17	757 ± 18	770 ± 17	730 ± 19	745 ± 17	745 ± 19	750 ± 18	766 ± 18
Feeding frequency, No./d	3.5 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.5 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.6 ± 0.1	3.5 ±0.1	3.6 ± 0.1
Tolerance - % feedings with										
Stomach cramps	12.4 ± 2.2	13.4 ± 2.4	10.3 ± 2.1	10.5 ± 2.0	9.1 ± 2.1	6.9 ±1.8	4.5 ± 1.4	4.1 ± 1.3	0.9 ± 0.7	5.4 ± 4.0
Vomit	0.9 ± 0.4	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.5 ± 0.2	0.2 ± 0.1	1.0 ± 0.4	0.2 ± 0.2	0.2 ± 0.2
Stomach cramps & vomit	13.3 ± 2.2	13.7 ± 2.3	10.5 ± 2.1	10.8 ± 2.0	9.3 ±2.1	7.3 ±1.8	4.6 ±1.4	5.0 ±1.4	1.1 ± 1.0	5.6 ± 4.0
Stool characteristics										
Number of stools/d, median	2.0	2.2	2.0	2.0	2.0	1.7	2.0	2.0	2.0	1.7
Number of stools/d	2.0 ± 0.1	2.2 ± 0.1	1.9± 0.1	2.0 ± 0.1	2.2 ± 0.1	1.9 ± 0.1	2.1 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.7 ± 0.1
Consistency										
Mean	3.2 ± 0.1[a]	3.0 ± 0.1[b]	3.2 ± 0.1	3.3 ± 0.1	3.3 ± 0.1	3.3 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.5 ± 0.1
Median	3.2	3.0	3.1	3.2	3.3	3.3	3.4	3.3	3.5	3.6
Consistency, %										
Watery	5.7 ± 1.4[a]	10.6 ± 2.0[b]	5.1 ± 1.3	4.3 ± 1.0	1.9 ± 0.8	4.4 ± 1.2	2.7 ± 0.9	2.2 ± 0.8	2.2 ± 0.8	1.3 ± 0.5
Loose/Mushy	13.6 ± 2.0	16.5 ± 2.0	13.9 ± 2.0	13.2 ± 2.1	16.2 ± 2.2	10.8 ± 1.9	10.2 ± 1.7	11.4 ± 1.9	14.2 ± 2.4	8.7 ± 1.6
Soft	43.8 ± 3.1	40.7 ± 3.0	42.9 ± 3.3	39.3 ± 3.2	37.5 ± 3.0	37.6 ± 3.3	39.5 ± 3.1	35.6 ± 3.2	33.3 ± 3.0	35.8 ± 3.1
Formed	33.0 ± 3.1	27.6 ± 1.0	37.2 ± 3.5	39.0 ± 2.9	39.4 ± 3.2	41.8 ± 3.3	41.8 ± 3.4	44.7 ± 3.2	43.0 ± 1.9	46.2 ± 3.4
Hard	4.0 ± 1.3	3.7 ± 1.0	2.9 ± 0.8	4.0 ± 1.2	4.9 ± 1.3	5.5 ±1.4	5.6 ± 1.3	5.6 ± 1.5	7.0 ± 1.9	6.7 ± 1.6
Watery or loose/mushy	19.4 ± 2.4[a]	27.1 ± 2.7[b]	19.0 ± 2.4	17.4 ± 2.4	18.2 ± 2.4	15.2 ± 2.3	12.9 ± 2.0	13.6 ± 2.1	14.2 ± 2.4	10.0 ± 1.7

[1] Values with different superscripts are significantly different at the $p \leq 0.05$. Unless otherwise stated, values in the table are mean ± SEM.