Standards for Probiotics and Prebiotics

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Abstract: Probiotics and prebiotics in dairy products must be: 1) shown to be safe under their intended conditions of use, 2) shown to provide health benefits at appropriate levels of use, and 3) used at levels that have been demonstrated to have the potential to benefit consumers. Few dairy products use labels which communicate levels of probiotic bacteria to consumers and there are no regulatory standards requiring efficacious formulation levels. This situation leaves consumers in the dark regarding the value of dairy products as delivery vehicles for these functional ingredients. This session will address the science, marketing and regulatory issues of establishing such standards. Efforts in this area by the International Scientific Association for Probiotics and Prebiotics and other groups will be discussed.
Standards

- Are they necessary?
  - Probiotics/prebiotics must be safe
  - They must be shown to provide benefit
  - They must be used in an efficacious manner
  - Current dairy products have unknown or un-communicated efficacy
  - Consumer confidence is at risk

- What should standards entail?
  - Definition of probiotic and prebiotic – what constitutes appropriate use of these terms?
  - Safety
  - Efficacy – substantiation and communication via claims
  - Content (viable cells for probiotics/levels of prebiotics)

- What form should standards take?
  - Government imposed
  - Self-regulated industry
Prebiotic

Nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health.

Gibson and Roberfroid, 1995
Probiotics

Live microorganisms administered in adequate amounts which confer a beneficial health effect on the host

http://www.fao.org/es/ESN/Probio/probio.htm

L. acidophilus NCFM
Photo by Alan Servin

B. longum 536
Photo by Morinaga

At least 9 published definitions – all differ
Safety
Probiotic Microorganisms

Overriding Safety Concern

Potential for direct consumer interaction with live microorganisms
Establishing the Safety of Probiotics

Must consider:

- Characteristics of probiotic organisms
  - Broad range of microbes proposed
  - Narrower range used in foods
- Type of product used to deliver probiotics
- Host factors
Safety Concerns with Probiotics

- **Systemic infections**
  - Pathogenicity; potential for invasion
  - Relatedness to species that produce hemolysins or mammalian toxins
  - Presence of known virulence factors

- **Deleterious metabolic activities**
  - D-lactate acidosis
  - Biogenic amine production
  - Degradation of intestinal mucins
  - Interference with bile acid metabolism

- **Excessive immune stimulation in susceptible individuals**

- **Gene transfer**
  - Sensitivity to therapeutic antibiotics
  - Transferable antibiotic resistance genes
Probiotic Safety Documentation
FAO/WHO Working Group Report

- Identification of the microbe: genotypic and phenotypic techniques
- Determination of antibiotic resistance patterns due to both innate and transferable mechanisms
- Assessment of certain metabolic activities (e.g., D-lactate production, bile salt deconjugation)
- If the strain under evaluation belongs to a species that is a known mammalian toxin producer, it must be tested for toxin production
Probiotic Safety Documentation (cont.)

- If the strain under evaluation belongs to a species with known hemolytic potential, determination of hemolytic activity is required
- Assessment of lack of infectivity by a probiotic strain in immunocompromised animal model
- Adverse incident reporting during human studies
- Post-market surveillance of adverse incidents
Any Ingredient Added to Foods Must Be:

*NOT merely a substance permitted for use as a component of dietary supplements*

Rather...

- A food,
- A prior-sanctioned substance,
- A food additive approved by FDA and authorized in a food additive regulation, or
- A substance that is Generally Recognized as Safe (GRAS)
GRAS

Generally . . .

- among experts qualified by scientific training and experience to evaluate safety
  - consensus--yes  unanimity--no
  - “mere” conflict--OK  “severe” conflict--not OK
GRAS

Recognized . . .

- based on common knowledge
  - general availability of information
  - general acceptance of information
GRAS

As Safe . . .

- reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use
  - Based on the totality of the relevant information
  - NOT “zero risk”
GRAS Requirements

Safety standard is the same as that for food additives, “reasonable certainty of no harm”

Evidence of safety is the same as is required to support approval of a food additive petition
  - breadth and quantity of information
  - quality of information

Information must be publicly available
  - available
  - accepted

May be supported by non-publicly available data
Key Efficacy Studies
What do they tell us about probiotic dose?
Relationship between *Lactobacillus* dose and reduction of diarrhea in children

Fecal LGG concentrations in health human volunteers after daily consumption of LGG in fermented milks
Dose: $2 \times 10^9$ and $2 \times 10^{10}$/d

There was no recovery of LGG in feces at feeding levels of $10^6$ – $10^8$/d. At $10^9$, 2 of 7 volunteers showed LGG in feces. At $10^{10}$ all were colonized. Saxelin et al. 1991. Microbial Ecol Health Dis 4:209-214.

Fecal *L. reuteri* levels in children after 3 wk feeding

- 14% colonized
- $10^2$/gm feces

- 67% colonized
- $10^4$/gm feces

- 100% colonized
- $10^7$/g feces

Ruiz-Palacios et al. 1996. Abstract
Negative study

- Single center, randomized, placebo-controlled, double blinded, parallel group dietary study
- 64 male and female subjects
- 12 week study
- Test product: Yogurt containing probiotic microbes
  - *L. acidophilus* NCFM
  - *L. rhamnosus* 271
  - *L. paracasei* DN114001
  - *Bifidobacterium* sp. DN BIO
- Dose
  - actual $10^5$-$10^6$/gm yogurt
  - target $10^7$-$10^8$/gm yogurt
Results

- No statistically significant differences in any of the parameters tested
  - Fecal markers: bacteria, SCFA, pH, moisture, genotoxicity of fecal water
  - Blood/serum markers: lipids/lipoproteins, immune markers
<table>
<thead>
<tr>
<th>Health Benefit</th>
<th>Daily Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day care center antibiotic use/infections/dental caries</td>
<td>10⁸</td>
</tr>
<tr>
<td>Antibiotic associated diarrhea</td>
<td>≥2x10⁸</td>
</tr>
<tr>
<td>↑ fecal lactobacilli (LGG)</td>
<td>10⁹</td>
</tr>
<tr>
<td>↑ fecal <em>L. reuteri</em></td>
<td>10⁸-9</td>
</tr>
<tr>
<td>↓ atopic eczema</td>
<td>10¹⁰</td>
</tr>
<tr>
<td>Lactose digestion</td>
<td>10¹⁰</td>
</tr>
<tr>
<td>IBS abdominal pain</td>
<td>2x10¹⁰</td>
</tr>
<tr>
<td>Infant diarrhea</td>
<td>10¹¹</td>
</tr>
<tr>
<td>Remission of pouchitis</td>
<td>10¹²</td>
</tr>
</tbody>
</table>
Milk fermented with yogurt strains and *L. casei* DN-114001; $10^{10}$ CFU per 100 ml serving

88 adults drank Actimel or placebo for 28 days; peripheral blood drawn on days 0, 9, 18, and 28

Blood exposed to 3 microbial antigens:
- Tetanus (bacterial)
- *Candida* (yeast)
- Influenza (viral)

Blood tested for immune response: Proliferation of T-cell and B-cell subsets, Th1 and Th2 cytokines

Significant up-regulation of the proliferation response in the Actimel group, significant only with influenza virus
Dose

- Dose should be determined based on studies which show a physiological benefit
  - Most studies focus on short term clinical benefit
  - What dose is needed for long term, prophylactic effect?
- Dose must be delivered through END of shelf life

How do US dairy products rate?
- No published surveys on probiotic levels in US yogurt
- Products not labeled with levels
Yogurt vs. supplements in the USA 2003 Sales

Yogurt is clearly the key delivery vehicle of probiotics in the USA in terms of \textit{product volume}.

In terms of \textit{total cells delivered}:
- 65% cells - dietary supplements
- 35% - yogurt

As currently formulated, yogurt is lower potency delivery vehicle.

For yogurt or other dairy products to be seriously considered as sources of probiotics, better attention to levels delivered (at end of shelf life), choice of strain and communication to consumer is required.
Why strain is relevant to dose discussion
Same species, different function

Sled dog
- cold-hardy
- good endurance
- tough feet

Guard dog
- protective
- muscular
- strong teeth
- ferocious bark
- courageous

Hunting dog
- soft mouth
- good swimmer

All dogs not ideal for all purposes
Strain effects

- We cannot assume that different strains of even the same species will function the same.
- Levels delivered in a probiotic product should have been shown to improve health.
- These levels may be different for different strains.
- Strains should be documented to be stable throughout shelf life in your product matrix.
Claims – What information on health benefits is allowable?
Nutritional Claims

Food Drug and Cosmetic Act of 1938 (FDCA)

Nutrition Labeling and Education Act of 1990 (NLEA)

Dietary Supplement Health and Education Act of 1994 (DSHEA)

Food and Drug Administration Modernization Act of 1997 (FDAMA)
Statements of Nutritional Support (Structure/Function Claims)

- Benefits related to a classic nutrient deficiency disease;
- Role of a dietary ingredient intended to affect the structure or function of the body;
- Documented mechanism by which a nutrient or dietary ingredient acts to maintain such structure or function; or
- General well-being.
FDA Requirement for a Structure/Function Claim

The manufacturer must have substantiation that the statement is truthful and not misleading.
Establishing Efficacy

Sources of information:

- experience
  - long-standing traditional uses
  - ethnomedical uses
- animal studies
- case reports
- *in vitro* experiments
- clinical trials

Plausible biological mechanism
Health Claim

“All claims made in food labeling that expressly or by implication ... characterizes the relationship of any substance to a disease or health-related condition.”

21 CFR 101.14(a)(1)
Requirements for FDA Approval of an *Unqualified* Health Claim

FDA will approve a health claim “only when it determines, based on the *totality* of *publicly available* scientific evidence ..., that there is *significant scientific agreement* among experts ... that the claim is supported by such evidence.”

21 CFR 101.14(c)
Interim FDA Policy
Regarding Qualified Health Claims

Need to demonstrate, based on a fair review by scientific experts of the totality of information available, that the “weight of the scientific evidence” or “credible evidence” supports the claim.

In assessing whether food labeling is misleading, FDA will use a “reasonable consumer” standard.

December 18, 2002
Interim FDA Procedures Regarding Qualified Health Claims

B “Although there is scientific evidence supporting the claim, the evidence is not conclusive.”

C “Some scientific evidence suggests... however, FDA has determined that this evidence is limited and not conclusive.”

D “Very limited and preliminary scientific research suggests... FDA concludes that there is little scientific evidence supporting this claim.”

July 10, 2003
Interim Evidence-Based Rating System for Scientific Data

1. Define the substance/disease relationship
2. Collect and submit all relevant studies
3. Classify and rate each study as to type*
4. Rate each study for quality
5. Rate the strength of the total body of evidence*
6. Determine and report the “rank” (A - D)

July 10, 2003
Interim Evidence-Based Rating System for Scientific Data

Classify and rate each study as to type:

Type 1: Randomized controlled intervention trial
Type 2: Prospective observational cohort study
Type 3: Nonrandomized intervention trial with concurrent or historical control
Type 4: Cross-sectional study or case series

N.B. Note absence of meta-analyses, animal studies, *in vitro* studies.
Interim Evidence-Based Rating System for Scientific Data

Rate the strength of the total body of evidence:

**Quantity:**
- Number of studies and number of individuals tested, weighted by study type and quality

**Consistency:**
- Similarity of results from high quality studies of design types 1 and 2

**Relevance:**
- Magnitude of effect (observed in high quality studies of design types 1 and 2) is physiologically meaningful and achievable
Companies must provide non-misleading information about efficacy

- Communicate efficacy information that has been documented for the particular product being consumed
- Don’t communicate pie-in-the-sky stories
- Don’t communicate non-science based findings
- Don’t use review articles on probiotics solely to justify your particular product
- Don’t use papers published on other probiotic strains as evidence that your product is efficacious
Standards

Are they necessary?
- To advance this field, yes
- Standards would provide measure of assurance to consumers that:
  - Products are safe
  - Products have been shown to provide benefit as formulated
Standards

What should standards entail?
- Products using terms “probiotics and prebiotics” must be consistent with definitions
- Safety assessment
- Efficacy dossier – support any health benefit statements made
- Verification that products meet label claims
  - Purity
  - Viability to end of shelf life
  - Genus, species, strain - names of bacteria consistent with “List of Bacterial names with Standing in Nomenclature”
  http://www.bacterio.cict.fr/
Standards

- What form should standards take?
  - Self-regulated industry
  - This will perhaps better ensure needed flexibility in standards to reflect technological advances
Actions on Standards

- FAO/WHO guidelines (www.fao.org)
  - Food Agriculture Organization/World Health Organization
  - Guidelines for the Evaluation of Probiotics in Food IDF – International Dairy Federation
  - Methods to determine certain functional and safety properties outlined in the FAO guidelines for the evaluation of probiotics in food.
Actions on Standards

- **ISAPP** ([www.isapp.net](http://www.isapp.net))
  - International Scientific Association for Probiotics and Prebiotics
  - Industry Advisory Committee and BOD will meet at 2004 meeting in August
  - Discuss standards and establishment of laboratory to develop methods

- **EFFCA** ([http://www.effca.com/anglais/pages/id_links.htm](http://www.effca.com/anglais/pages/id_links.htm))
  - European Food & Feed Culture Association
  - Guidelines for use of probiotics in foods
Conclusions

- Industry should consider establishing standards before they are imposed by government

- Yogurt is a natural fit for ‘live beneficial bacteria’
  - Nutrition of milk + additional health benefits

- Currently, yogurts focus little on:
  - Levels of probiotics
  - Communication about probiotics to consumers

- Although dairy is a key delivery agent for probiotics today, it may not be for long
  - The supplement industry is more dynamic today in this field
Why the Live Active Culture Seal Isn’t Enough
Live Active Culture Seal

- National Yogurt Association
- Minimum level of lactic bacteria – $10^7$/g at end of shelf life
- Doesn’t specifically apply to probiotic content
- Starter cultures usually ~$10^{8-9}$/g
- Probiotic bacteria target ~$10^6$/g, and levels at end of shelf life are not known
- Perhaps a 2nd generation LAC seal that refers to probiotics?