



***Bacillus coagulans* GBI-30, 6086 in Extruded Pet Food Applications**

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Introduction

What are probiotics?

“Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.”

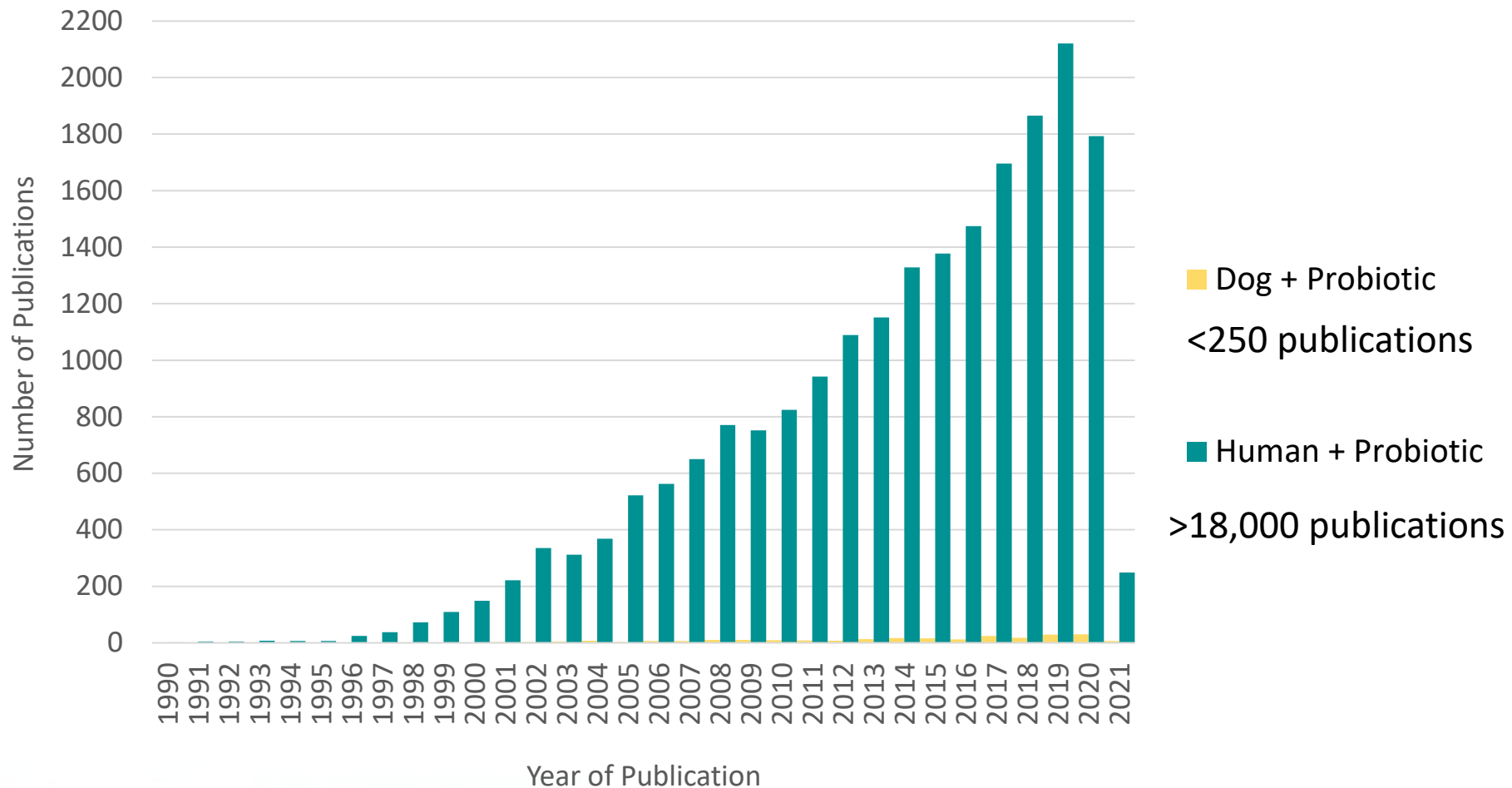
– Hill et al. (2014)

“**Functional**” pet foods, such as those containing probiotics, offer enhanced health benefits beyond supplying essential nutrients when consumed on a regular basis (Di Cerbo, 2017).

- Key growth driver of the \$38.4 billion U.S. market pet foods and treats in the (APPA, 2020).

Introduction

PubMed Open-Access Database Search



Introduction

Reported Health Benefits of Probiotics in Dogs

(Reviews: Vester and Fahey Jr., 2010; Markowiak et al. 2018)



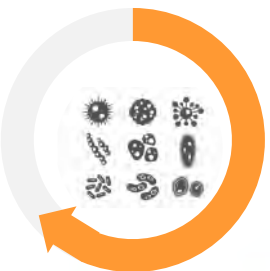
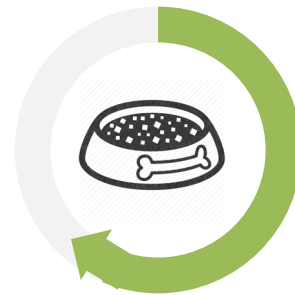
Stool Quality

Reduced incidence of diarrhea, constipation, and improved stool quality



Nutrient Utilization

Improvements to apparent total tract digestibility, weight gain, and epithelium health



Balance Microflora

After antibiotics, during weaning, or kenneling stress

Immune System

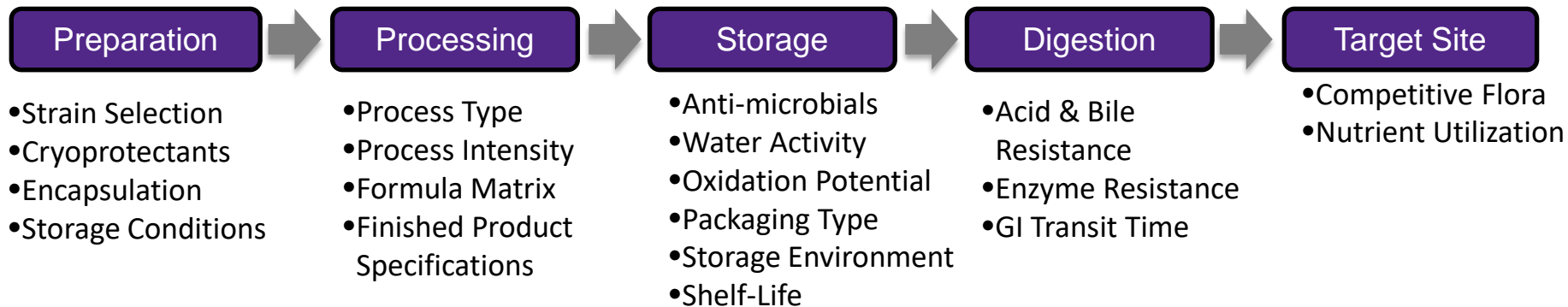
Reduced intestinal inflammation and hypersensitivity to diet



Introduction

Hurdles to Probiotic Viability in Pet Food

(Reviews: Tripathi and Giri, 2014; Terpou et al. 2019)



53%

of sampled commercial pet foods were found to be severely inadequate with respect to strain identity and colony-forming unit guarantees on pet food labels (Weese and Arroyo, 2003).

Introduction

Properties of Vegetative Cells

- Metabolically Active
- Lower thermal tolerance
- Poor survival in processed foods
- Less retention in storage
- Lower Acid/Bile Resistance

Properties of Spores

- Metabolically Dormant
- Higher thermal tolerance
- Higher Acid/Bile Resistance
- Longer retention in storage
- Better survival in processed food?

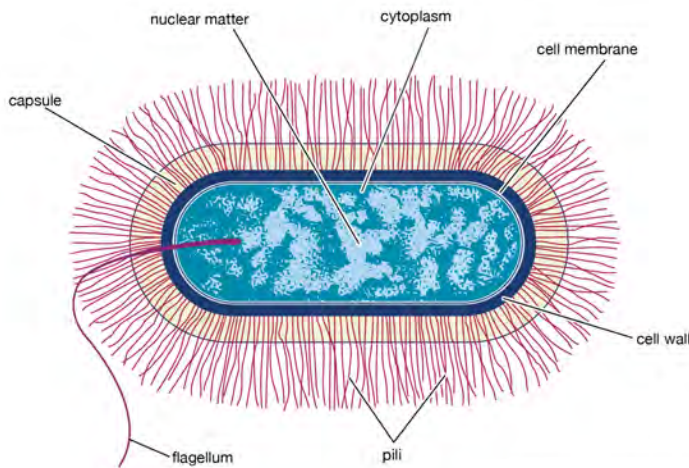
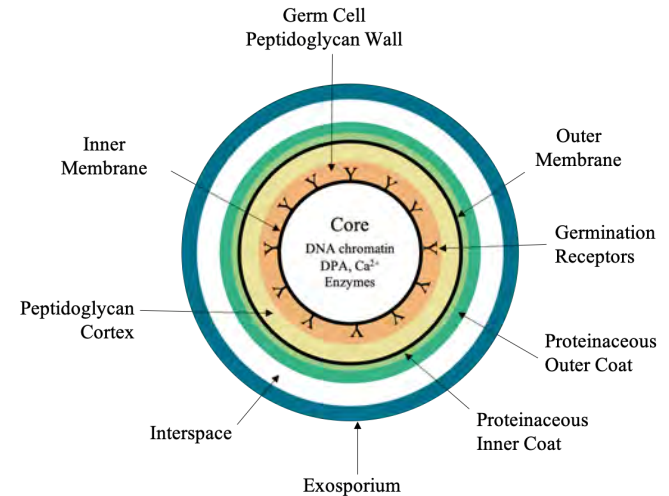


Image source: Encyclopedia Britannica.com



Introduction

***Bacillus coagulans* GBI-30, 6086:**

- GRAS, non-toxigenic at high doses (10^{11} CFU/kg BW in humans)
- Spore-forming
- Lactic acid-producing
- Gram-positive rod
- Transient (non-adhering)
- Microaerophilic
- Optimum growth temperature range is 30 - 50 °C
- Thermotolerant (>90 °C)
- Acid-tolerant

Experiment 1: Processing

Extrusion Processing Parameters

Process Parameter	Extrusion Treatment ¹			SEM ²	P-value ³
	Low SME	Moderate SME	Severe SME		
System Inputs					
Screw Speed (rpm)	401.83 ^c	500.79 ^b	602.17 ^a	0.377	<0.0001
Water Flow (kg/h)	19.58 ^a	11.68 ^b	9.97 ^c	0.096	<0.0001
System Outputs					
In-Barrel Moisture (%)	35.47 ^a	29.13 ^b	27.78 ^c	0.170	<0.0001
Motor Load (%)	41.42 ^c	44.69 ^a	43.38 ^b	0.454	<0.0001
Power (kW)	6.28 ^b	9.43 ^a	9.81 ^a	0.387	<0.0001
Die Exit Temp. (°C)	107.71 ^b	134.34 ^a	138.04 ^a	3.562	<0.0001
Wet Flow Rate (kg/h)	83.18 ^a	73.71 ^b	70.81 ^b	1.390	<0.0001
Barrel Residence Time (s)	91.8	93.3	87.3		
SME (kJ/kg)	122.12 ^b	219.30 ^a	195.12 ^a	8.728	<0.0001

SME = Specific Mechanical Energy

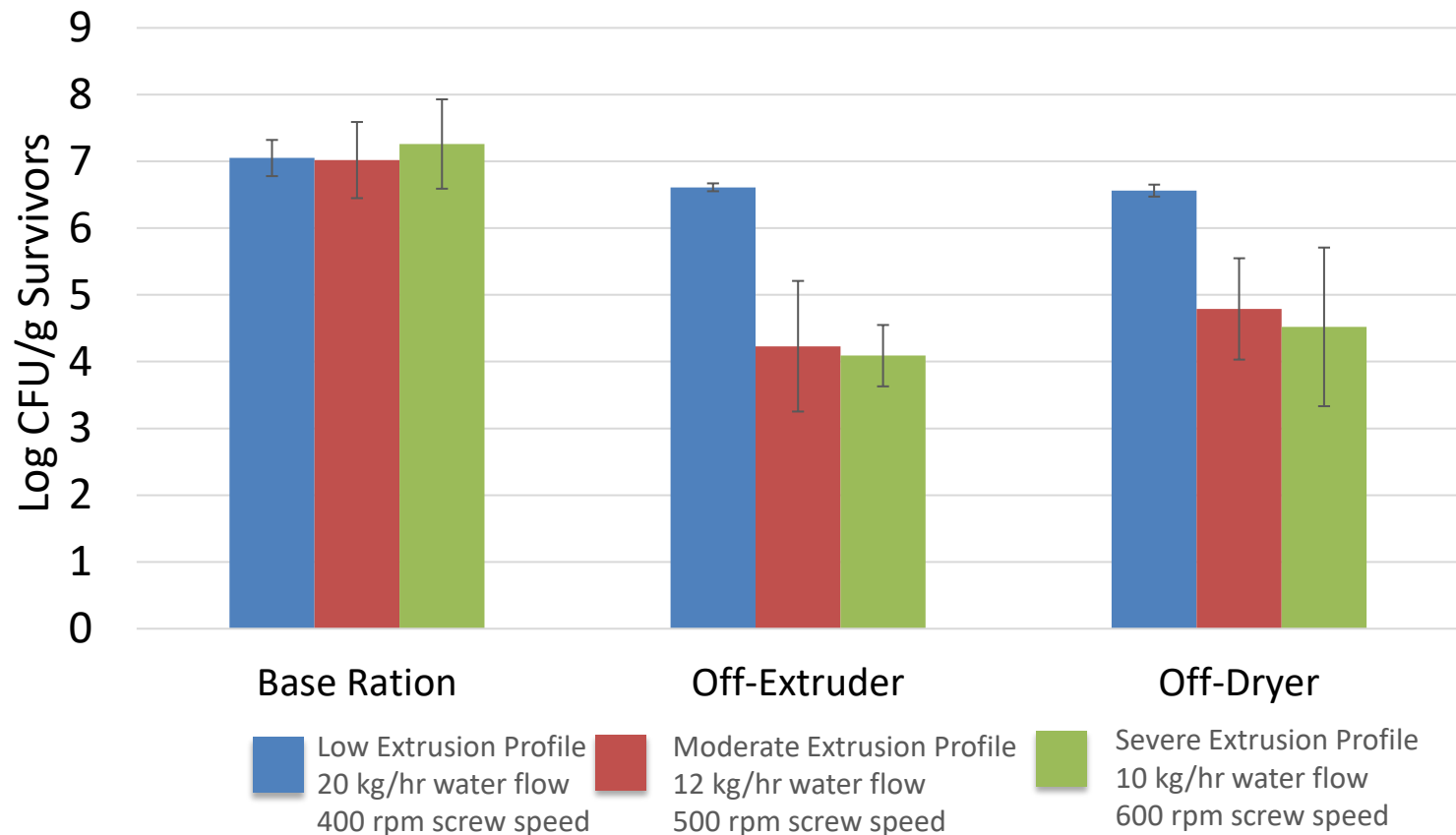
¹ Treatments: Low SME = 20 kg/h extruder water flow with 400 rpm screw speed; Moderate SME = 12 kg/h extruder water flow with 500 rpm screw speed; Severe SME = 10 kg/h extruder water flow with 600 rpm screw speed.

² SEM: standard error of the mean

³ P-values represent Type III fixed effects of extrusion profile.

Results: Extrusion

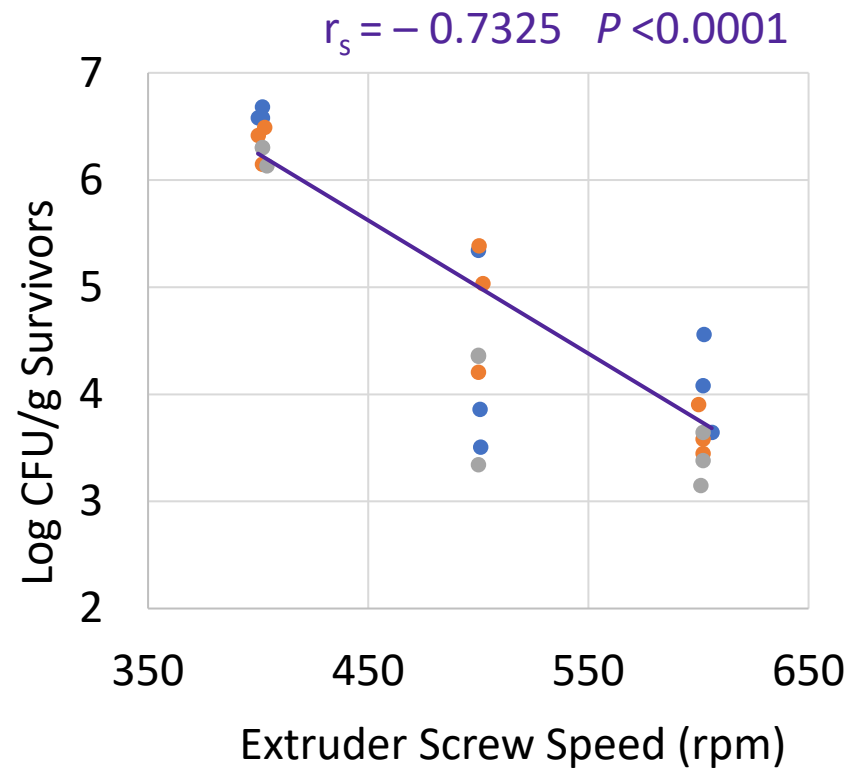
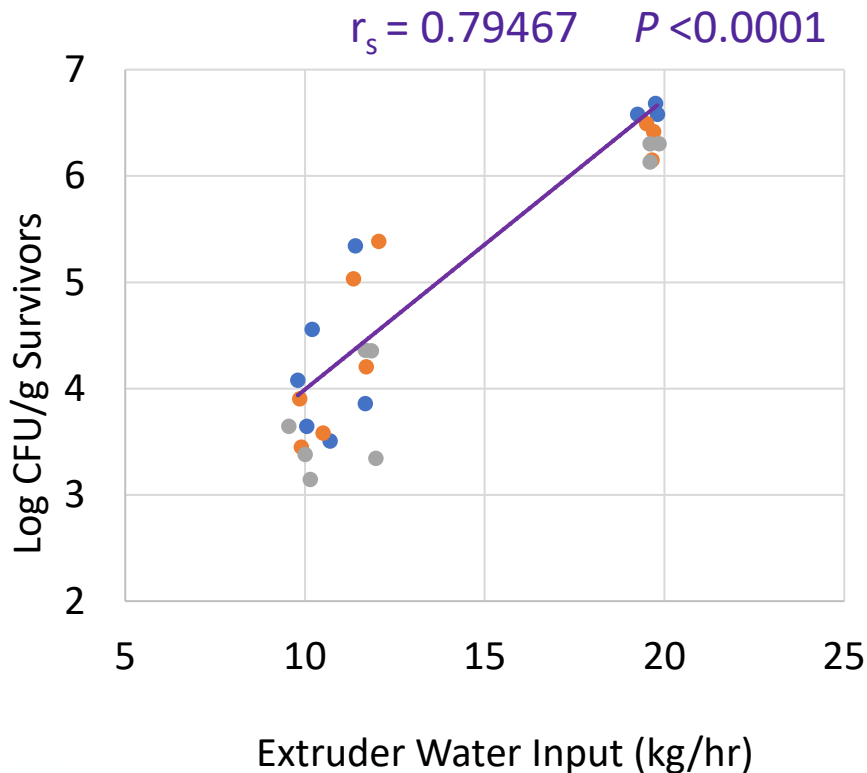
Survival of *Bacillus coagulans* Subjected to 3 Extrusion Profiles



Note: All treatments were dried at 107 °C for 16 min

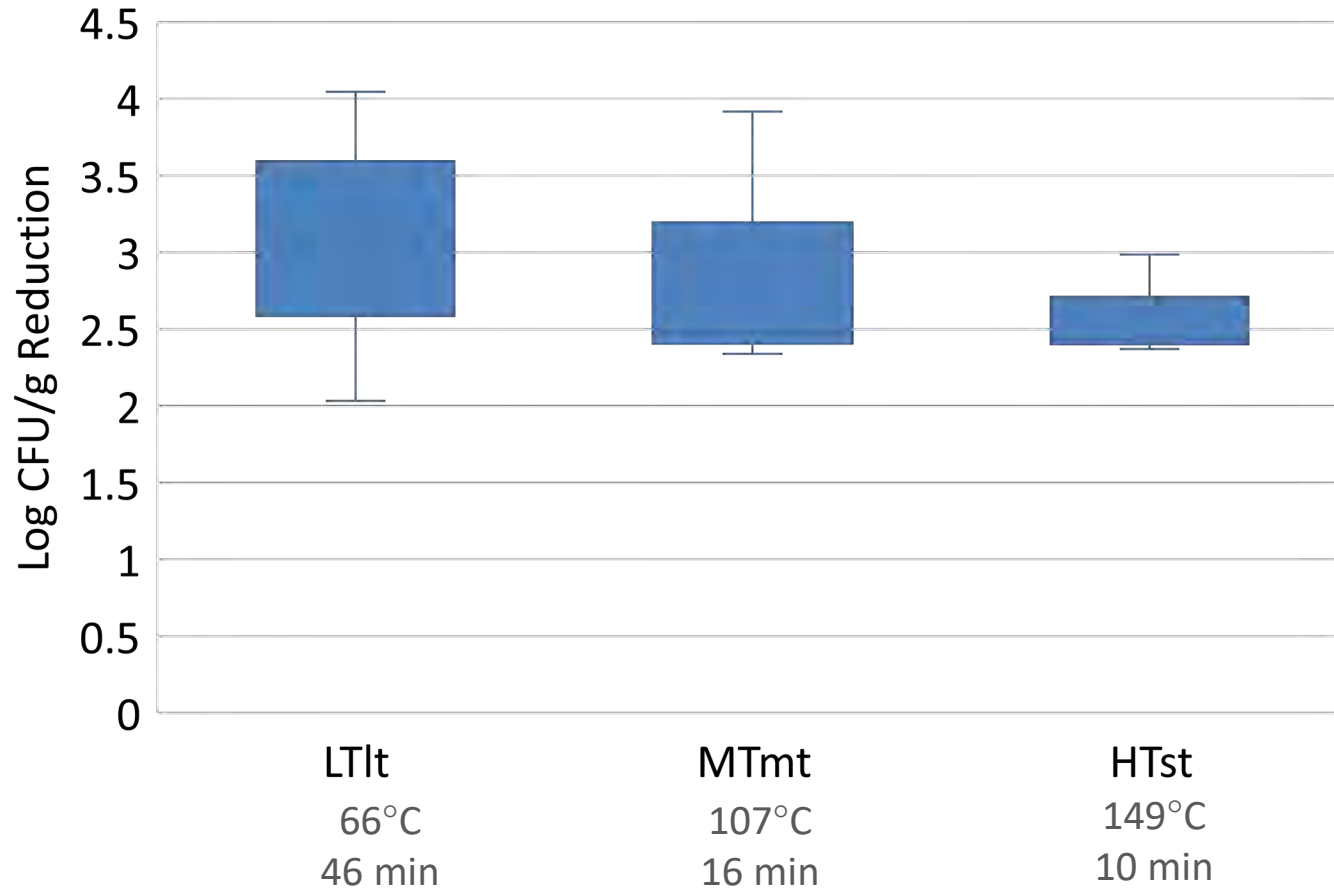
Results: Extrusion

Spearman correlations demonstrating the direction and strength of relationship between extruder water input and extruder screw speed on *Bacillus coagulans* survival.



Results: Dryer

Loss in Viability Under 3 Dryer Conditions



Note: All treatments were produced under moderate extrusion conditions (12kg/hr water flow 500 rpm screw speed)

Experiment 2: Dog Feeding Study

- Institutional Animal Care and Use Committee (IACUC) protocol #4097 and IBC protocol #1187 at Kansas State University
- 10 healthy adult Beagle dogs (3 females/spayed, 7 males/castrated) of similar age (5.75 ± 0.23) and body weight (12.3 ± 1.5 kg) were randomly assigned 5 experimental diets containing graded doses of *Bacillus coagulans*
- Total study duration: 105 days

		Dog ID				
		1	2	3	4	5
Period	1	B	A	E	C	D
	2	D	E	B	A	C
	3	E	C	D	B	A
	4	C	D	A	E	B
	5	A	B	C	D	E

		Dog ID				
		6	7	8	9	10
Period	1	D	E	B	A	C
	2	E	C	D	B	A
	3	B	A	E	C	D
	4	A	B	C	D	E
	5	C	D	A	E	B

ADAPTATION

COLLECTION

Days 1 - 16	17	18	19	20	21
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Experiment 2: Dog Feeding Study

Experimental Diet Ingredient Composition

Ingredients	Amount, %
Chicken Meal	34.635
Peas, Dehydrated	20.000
Sweet Potatoes, Flaked	20.000
Chicken Fat	8.500
Tapioca Flour	5.000
Pea Protein	5.000
Beet Pulp	3.000
Digest Flavoring	1.000
Potassium Chloride	0.500
Salt	0.500
Dicalcium Phosphate	0.500
Titanium Dioxide	0.400
DL-Methionine	0.250
Choline Chloride	0.200
Fish Oil	0.200
Vitamin Premix	0.150
Trace Mineral Premix	0.100
Natural Antioxidant	0.065
<i>B. coagulans</i> (15B CFU/g)	*

Proximate Analysis of Experimental Diet

Nutrient	Analysis, As-Is
Moisture, %	4.92
Crude Protein, %	34.90
Crude Fat, %	15.60
Crude Fiber, %	3.28
Ash, %	9.21
Nitrogen-Free Extract (NFE), %	32.09
Metabolizable Energy, kcal/kg	3,671

¹Formulated to meet the AAFCO Dog Food Nutrient Profiles for adult maintenance (AAFCO, 2020).

Dietary Treatments

Application method and levels of *B. coagulans* in five experimental dietary treatments

<i>B. coagulans</i> Treatment	CON	PEX ¹	PCL ²	PCM ²	PCH ²
Application Method	None	Base Ration	Coating	Coating	Coating
Total CFU/g in Diet	0.00	1.06 x 10 ⁴	5.92 x 10 ⁴	6.86 x 10 ⁵	6.84 x 10 ⁶
Dose (CFU/dog/day) ³	0.00	2.12 x 10 ⁶	1.18 x 10 ⁷	1.37 x 10 ⁸	1.37 x 10 ⁹

¹Diet B contained *B. coagulans* applied in the dry base ration before extrusion and drying.

²Diets C-E were coated simultaneously with *B. coagulans* chicken fat and digest flavoring on the exterior of the kibble after drying.

³Based on an average daily food intake of 200 g/dog/day.

Results: Stool Quality

Food Intake and Stool Quality of Dogs fed diets with differing levels of *Bacillus coagulans*

Parameter	CON	PEX	PCL	PCM	PCH	SEM	P-Value
Food Intake, g/day	189.23	198.82	200.91	197.96	197.40	6.21	0.1364
Fecal Output, g/day	112.60	119.72	126.34	114.25	116.72	5.15	0.1356
Fecal Moisture, %	70.25	69.98	70.30	69.71	70.19	0.45	0.6415
Fecal Dry Matter, %	29.75	30.02	29.70	30.29	29.81	0.45	0.6415
Defecations per Day	2.00	2.12	2.18	2.02	1.98	0.11	0.3041
Fecal Score	3.70	3.71	3.75	3.77	3.68	0.05	0.5508

¹SEM = standard error of the mean

²P-value represents Type 3 Test of Fixed Effects for Diet

Results: Nutrient Digestibility

Apparent total tract digestibility of dogs fed diets with differing levels of *Bacillus coagulans*

Digestibility, %	CON	PEX	PCL	PCM	PCH	SEM	P-Value ¹
Dry Matter	79.04 ^b	79.45 ^b	78.65 ^b	78.75 ^b	81.77 ^a	0.718	0.0034
Organic Matter	83.67 ^b	84.36 ^{ab}	83.51 ^b	84.01 ^{ab}	85.79 ^a	0.553	0.0101
Crude Protein	81.64	81.95	81.70	81.77	83.60	0.547	0.0743
Crude Fat	91.69	90.28	90.96	90.85	91.69	2.097	0.1981
Ash	34.94 ^b	36.23 ^b	33.76 ^b	31.06 ^c	46.31 ^a	2.506	<0.0001
Gross Energy	81.94 ^{ab}	81.66 ^b	82.03 ^{ab}	80.08 ^b	83.96 ^a	0.595	0.0002

^{abc} Means within a row with different superscripts differ (P < 0.05).

¹ Treatments: CON = control; PEX = probiotic applied before extrusion; PCL = probiotic applied as coating at low dose; PCM = probiotic applied as coating at moderate dose; PCH = probiotic applied as coating at high dose.

² P-value represents Type 3 Test of Fixed Effects for Diet

Results: Gut Health Indicators

Fecal fermentation compounds of dogs fed diets with differing levels of *B. coagulans*.

Parameter	CON	PEX	PCL	PCM	PCH	SEM	P-Value
Fecal pH	5.49	5.36	5.44	5.41	5.33	0.06	0.4434
Fecal Ammonia, $\mu\text{mol/g}$ DM feces	99.99	105.49	107.12	104.61	94.30	9.43	0.8414
Total SCFA, ¹ $\mu\text{mol/g}$ DM feces	171.28	183.64	197.20	179.36	192.22	16.68	0.7924
Acetate, %	52.24	54.04	53.10	53.31	53.16	1.22	0.6637
Propionate, %	37.07	36.91	37.75	36.91	37.61	1.37	0.9212
Butyrate, %	10.69	9.05	9.16	9.78	9.23	0.61	0.2327
Total BCFA, ² $\mu\text{mol/g}$ DM feces	11.05	9.02	9.48	12.09	9.61	1.50	0.5781
Isovalerate, %	47.97	52.24	49.53	44.74	46.79	2.08	0.1395
Isobutyrate, %	33.93	32.44	35.67	32.27	36.52	1.59	0.2216
Valerate, %	18.10	15.33	14.81	22.98	16.68	2.47	0.1337

¹Total short-chain fatty acids (acetate + propionate + butyrate); Individual SCFA are expressed as a percent of total SCFA.

²Total branched-chain fatty acids (isobutyrate + isovalerate + valerate); individual BCFA are expressed as a percent of total BCFA.

³P-value represents Type 3 Test of Fixed Effects for Diet

Overall Conclusions

Survival Through Pet Food Extrusion & Drying

- *Bacillus coagulans* GBI-30, 6086 survived extrusion of pet food under three processing profiles, with a 2 – 3 log reduction expected under moderate conditions.
- Viability validation is necessary (process parameters, equipment, formula)
- Dryer conditions did not have a significant effect on retention, however greater variability was observed for the low temperature-long time treatment.

➤ Effects on Gastrointestinal Health Indices in Healthy Dogs:

- Apparent digestibility of DM, OM, Ash, and GE were highest for the 9-log₁₀ dose treatment.
- Crude protein digestibility tended ($P < 0.10$) to increase as probiotic dose increased.
- No significant differences were observed in food intake, stool quality, gastrointestinal health indicators including fecal pH, fecal ammonia, or fecal short chain fatty acids in this study.

Acknowledgements

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Supplementation of Gluten-free Sorghum Flour-based Pet Treat with Soluble Animal Proteins



Presented by:
Krystina Lema Almeida


Advisor:
Dr. Charles Greg Aldrich

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Introduction

- **Pet Treats**
 - Not intended to meet the complete nutritional needs of the animal
 - < 10% daily energy requirement
 - **Global pet treats market projection for 2021 → US\$ 31.37 billion** (Technavio Research, 2017)
 - Hard texture
 - Dental benefits
 - Cereal is the main ingredient
 - Wheat
 - Different shaping technologies
 - Rotary molding




(Davidson, 2018)

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Introduction

- **Sorghum**
 - Globally, the fifth most produced cereal (FAO & ICRISAT, 1996)
 - Environmental and nutritional benefits:
 - Resilient, sustainable, and tolerant crop (Arendt & Zannini, 2013)
 - Rich in dietary fiber, resistant starch, B vitamins, and antioxidant phenolic compounds (Anglani, 1998; Ratnavathi & Komala, 2016; Arendt & Zannini, 2013)
 - Slow starch digestibility (Ratnavathi, 2019)
 - Natural gluten-free cereal (Culliney, 2013)
 - Pet food industry in the U.S. accounts for 2% of sorghum consumption (American Sorghum, 2015)

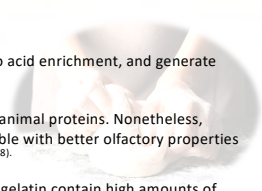



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Introduction

- Proteins
 - Provide dough enhancement, amino acid enrichment, and generate satiety effects (Nogueira & Steel, 2018).
 - Dogs can utilize either vegetable or animal proteins. Nonetheless, animal-proteins can be more palatable with better olfactory properties (Beaver et al., 1992; Brown, 2009; Houpt et al., 1978).
 - Spray-dried plasma, egg whites and gelatin contain high amounts of serum albumin, ovalbumin, and collagen, respectively (Jayathilakan et al., 2012).
 - Water binding ability, gelling strength and emulsifying properties (Pérez-Bosque et al., 2016; Rodríguez et al., 2016).
 - Improve texture and maintain a high degree of cohesion (adhesive) between the ingredients when cooked (Polo et al., 2005).






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Research Questions

- Can we produce a rotary molded sorghum-based (“gluten-free”) treat with the same characteristics as wheat-based product?
- Can the addition of proteins emulate the response of gluten?




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Methods

- Formula (%)

	WWF-GTN	WWS-NC	WWS-SDP	WWS-EP	WWS-GL	WRS-NC	WRS-SDP	WRS-EP	WRS-GL
Whole wheat flour	70.1	-	-	-	-	-	-	-	-
Whole red sorghum flour	-	-	-	-	-	68.6	69.0	65.3	69.8
Whole white sorghum flour	-	68.6	68.9	65.3	69.8	-	-	-	-
Cornmeal	17.5	19.1	12.5	11.8	12.5	19.1	12.5	11.8	12.5
Spray-dried plasma	-	-	6.22	-	-	-	6.23	-	-
Egg protein	-	-	-	11.28	-	-	-	11.28	-
Gelatin	-	-	-	-	5.35	-	-	-	5.35
Water (if added on top of other ingredients)	24.5	41.1	28.9	24.6	31.0	41.1	29.2	27.5	32.8

WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin.
Other ingredients → molasses 5.6%, all-purpose shortening 3.5%, non-fat dry milk 2.2%, salt 0.7%, baking soda 0.4%, sodium bisulfite 0.003%, inactive dry yeast 0.003%.


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Methods

- **Materials**
 - Rotary molded treats (Cookie Cracker Laboratory, AIB International)

WWF-GTN SDP GL EP NC

WWS

WRS

WWF= whole wheat flour, WWS= whole white sorghum, WRS= whole red sorghum, GTN=gluten, NC=no protein, SDP=spray dried plasma, EP=egg protein, GL=gelatin

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Methods

Texture Analysis (three-point bend, TA.XT2 Texture Analyzer) Hardness Fracturability	Dimension Analysis (Digital caliper) Length Width (Body and Tips) Thickness Weight	Animal Evaluation (Li et al., 2017) - IACUC #4277 protocol 12 beagle dogs 4 phases (5 days/each) <ul style="list-style-type: none"> • Acclimation • WWS vs. WWF-GTN • WRS vs. WWF-GTN • WWS-WRS vs. WWF-GTN
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Color Analysis (CR-410 chroma meter) L*, a*, b*
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Results and Discussion

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Functional attributes of carbohydrate hydrocolloids in canned pet food

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2021 March 16

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Introduction to carbohydrate hydrocolloids

- Included in wet pet food formulations to provide structure and filling viscosity.
 - Commonly referred to as “gums” or “gels”
- Can influence nutrient digestibility (Karr-Lilienthal et al., 2002; Zentek et al., 2002).
- Functional effects in wet pet foods are not documented in peer-reviewed literature.
 - Some information related to sausage production, but processing methods are different.

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Hypothesis

- Different combinations of carbohydrate hydrocolloids will thicken batters, decrease heat penetration, and alter color and texture of wet pet food.

Objective

- Determine the effects of carbohydrate hydrocolloids on batter development, heat penetration, color, and texture of wet pet food.

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Experimental treatments

Ingredient ¹ , %	D ²	DG ²	KCG ²	LBG ²	XGG ²
Dextrose	1.00	0.50	-	-	-
Guar gum	-	0.50	0.50	0.50	0.50
Kappa carrageenan	-	-	0.50	-	-
Locust bean gum	-	-	-	0.50	-
Xanthan gum	-	-	-	-	0.50

¹Other ingredients: 56.00% chicken, 38.35% water, 3.00% brewer's rice, 0.50% potassium chloride, 0.50% spray dried egg white, 0.50% sunflower oil, 0.10% vitamin premix, and 0.05% trace mineral premix

²D = 1% dextrose; DG = 0.5% dextrose and 0.5% guar gum; KCG = 0.5% guar gum and 0.5% kappa carrageenan; LBG = 0.5% guar gum and 0.5% locust bean gum; XGG = 0.5% guar gum and 0.5% xanthan gum

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Treatment production

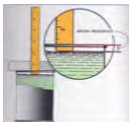
1. Chicken and water were heated to 40 °C.
2. Brewer's rice, spray dried egg white, sunflower oil, potassium chloride, and premixes were added to batter.
3. Batter was heated to 60 °C.
4. Dextrose and/or carbohydrate hydrocolloids were added and mixed for 5 minutes.
5. Cans were filled with 405 grams of batter and lids were seamed.
6. Cans were held in water baths until all treatments were ready for retort processing.



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Analysis during production

- Batter consistency (viscosity), pH, and water activity (a_w)
- Can fill weight
- Gross headspace
- Internal "cold spot" temperature



Source: "Canned Foods", 2015



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F₀ and Cook Value calculations

$$Value = \sum_{i=1}^t (t_i - t_{i-1}) * 10^{\frac{T_i - T_R}{z}}$$

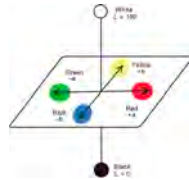
	F ₀	Cook value
Item of concern	<i>Clostridium botulinum</i>	Thiamine
T _R , °C	121.11	100
z, °C	10	33

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CIELAB color space values

- Konica Minolta colorimeter
- L* = lightness/darkness
- a* = red/green
- b* = yellow/blue



Source: degruyter.com

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Statistical analysis

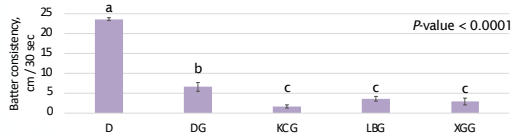
- Randomized complete block design (RCBD)
 - Fixed effect: treatment
 - Random effect: production day
- One-way analysis of variance
- Means separation using Fisher's LSD
- P-values less than 0.05 were considered significant



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Batter consistency and other characteristics



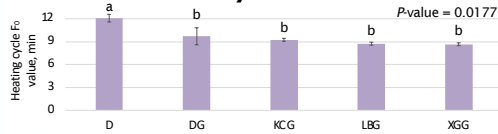
^{abc} Means that do not share a common superscript within the same chart are different ($P < 0.05$).
¹ D = 1% dextrose; DG = 0.5% guar gum and 0.5% dextrose; KCG = 0.5% guar gum and 0.5% kappa carrageenan; LBG = 0.5% guar gum and 0.5% locust bean gum; XGG = 0.5% guar gum and 0.5% xanthan gum.
 Error bars represent the standard error.

- pH and a_w were not different ($P > 0.05$) and averaged 5.94 and 0.990, respectively.



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Heat penetration during heating cycle



^{ab} Means that do not share a common superscript within the same chart are different ($P < 0.05$).
¹ D = 1% dextrose; DG = 0.5% guar gum and 0.5% dextrose; KCG = 0.5% guar gum and 0.5% kappa carrageenan; LBG = 0.5% guar gum and 0.5% locust bean gum; XGG = 0.5% guar gum and 0.5% xanthan gum.
 Error bars represent the standard error.

- Same relationship with heating cycle cook value.
 - D = 137.01 min; average of all others = 117.24 min

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Changes in color

	D	DG	KCG	LBG	XGG	SEM	P-value
L*	53.61 ^c	56.88 ^b	57.59 ^{ab}	59.09 ^a	58.65 ^{ab}	1.044	0.0023
a*	8.18 ^a	8.56 ^a	4.03 ^b	4.68 ^b	4.51 ^b	1.180	0.0108
b*	21.40 ^a	22.69 ^a	14.64 ^b	15.93 ^b	15.59 ^b	1.511	< 0.0001

^{abc} Means that do not share a superscript are different ($P < 0.05$).



From left to right: D, DG, KCG, LBG, and XGG


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Conclusions

- Carbohydrate hydrocolloids thicken batters, slow heat penetration, and provide structure in finished products.
- Dextrose affects product color by darkening and increasing red and yellow hues.


Future work

- Texture analysis
- Expressible moisture
- Evaluation of novel gums, gels, and functional ingredients in wet pet foods



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