PREBIOTICS HAVE LIMITED EFFECTS ON NUTRIENT DIGESTIBILITY OF A SOYBEAN- MEAL-BASED DIET BY GOLDFISH *Carassius auratus*

A Thesis

by

THIAGO RAGGI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2009

Major Subject: Wildlife and Fisheries Sciences

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Approved by:

Chair of Committee,	Delbert M. Gatlin III
Committee Members,	Christopher A. Bailey
	Luis Orlindo Tedeschi
Head of Department,	Thomas E. Lacher Jr.

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ABSTRACT

Prebiotics Have Limited Effects on Nutrient Digestibility of a Soybean-Meal-Based Diet by Goldfish *Carassius auratus.* (December 2009)

Thiago Raggi, B.S., Universidade Estadual Paulista

Chair of Advisory Committee: Dr. Delbert M. Gatlin, III

Prebiotic compounds comprise a group of dietary supplements defined as non-viable food ingredients that are selectively metabolized to favor beneficial intestinal bacteria. Such bacteria may confer various desirable effects including enhanced disease resistance and nutrient availability to the host.

This study examined the effects of four prebiotics, GroBiotic®-A (a mixture of partially autolyzed brewers yeast, dairy ingredient components and dried fermentation products), mannanoligosaccharide (MOS), galactooligosaccharide (GOS), and the fructooligosaccharide (FOS) inulin on digestibility of soybean-meal-based diets by goldfish. A basal diet was formulated so that 50% of the protein was provided by soybean meal and the other 50% was from menhaden fishmeal. Each prebiotic was supplemented to the basal diet at 1% by weight. A diet containing all of its protein from menhaden fish meal also was prepared as a control diet. Chromic oxide was added to the diets at 1% as an inert marker. Each diet was fed to adult goldfish in duplicate 110-L aquaria for a total of 8 weeks. The dried fecal material from each aquarium was pooled

over time and analyzed for protein, lipid, organic matter and chromium in order to compute coefficients of apparent digestibility. Genomic DNA of gut microbiota also was isolated from the fecal samples of goldfish fed the various diets and subjected to polymerase chain reaction (PCR) using bacteria-specific PCR primers to conserved regions flanking the variable V3 region of 16S rDNA. Then, denaturing gradient gel electrophoresis (DGGE) of the resulting amplicons was conducted as a means of assessing diversity of microbiota in the gastrointestinal (GI) tract.

Results of the present study revealed that none of the prebiotics affected apparent digestibility coefficients of the soybean-meal-based diet compared to the basal diet, although the diet supplemented with MOS consistently yielded the lowest values. In addition, goldfish digested the soybean-meal-based diets as well as the control diet. DGGE analysis revealed no differences in microbiota of goldfish fed the various prebiotics. These results are in contrast to those obtained with carnivorous fish species such as the red drum (*Sciaenops ocellatus*) in which the prebiotics increased digestibility coefficients of soybean-meal-based diets and altered GI tract microbiota.

DEDICATION

To my parents and to tia Edna

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Gatlin, for his attention, guidance and patience. Thank you to my committee members, Dr. Luis Tedeschi and Dr. Bailey, for their support throughout the course of my graduate study.

Thanks also go to my friends and colleagues and the departmental faculty and staff for making my time at Texas A&M University a great experience. Special thanks go to Brian Ray, Dr. Alejandro Buentello, Gary Burr, Annie, Dr. Zhou, Christine Savolainen and Camilo Pohlenz.

Finally, thanks to my family for their encouragement and support during these times.

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INTRODUCTION

Commercial production of baitfish, which primarily occurs in freshwater in the southeastern portion of the U.S., represents an economically valuable component of the U.S. aquaculture industry. The total value of baitfish sold in the U.S. in 2005 was $ 38 million (USDA, 2005). Golden shiners, *Notemigonus crysoleucas*, and goldfish, *Carassius auratus,* are two of three principal species cultured for bait. Many of the concerns of baitfish producers are distinct from those who produce aquatic species for human consumption. In particular, market sizes of baitfishes are relatively small and various sizes are needed for specific purposes, such that repeated grading and handling during production are often required. After harvest, the fish also must withstand the additional demands of distribution, and survive for extended periods before sale. Significant losses typically occur when fish are transported from the production facilities to distribution sites on trucks. A combination of handling stress (harvesting, grading, transporting, etc.) and suboptimal environmental conditions (low dissolved oxygen, sudden changes in water temperature or other impaired water quality conditions, etc.) can result in high mortality when fish are transferred between facilities. In some cases the resulting mortality is due to increased susceptibility to various pathogenic bacteria such as *Aeromonas hydrophila* and *Flavobacterium columnare.*

Therefore, effective management practices that enhance stress and disease resistance as well as prolong survival of baitfishes are critically needed. A number of different dietary

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This thesis follows the style of the journal *Aquaculture*.

supplements may potentially increase baitfish production by increasing immunity and resistance to disease as well as enhancing stress resistance. Among these are the nutrients arginine, vitamin C, vitamin E, and selenium, which have been shown in some cases to confer various benefits to different fish species (Gatlin, 2002). In freshwater fish such as the channel catfish, *Ictalurus punctatus*, megadose levels of vitamin C added to the diet have been shown to improve antibody response, complement activity, and survival following infection with *Edwardsiella tarda* and *E. ictaluri* (Li and Lovell, 1985; Liu et al., 1989). Similar improvements in immune responses of rainbow trout, *Oncorhynchus mykiss*, following dietary supplementation with high levels of vitamin C (Blazer, 1982; Navarre and Halver, 1989; Verhlac et al., 1998) or vitamin E (Blazer and Wolke, 1984a, 1984b; Verhlac et al., 1993) also have been observed. Chen et al. (2003) showed that ascorbic acid prevented deficiency signs and optimized survival of golden shiner in aquaria, while alternative complement activity and resistance to heat stress were increased by elevated levels of dietary vitamin C.

Other non-nutrient compounds such as nucleotides, chitin, and beta-glucans from yeast and fungi as well as prebiotics, also have been shown to potentially benefit some fish species (Li and Gatlin, 2006). Of particular interest are prebiotics, which are classified as non-digestible food ingredients that beneficially affect the host by stimulating growth and/or activity of a limited number of bacteria in the intestine (Manning and Gibson, 2004). Examples of prebiotics include mannanoligosaccharides (MOS) (White et al., 2002), lactose (Szilagyi, 2002), trans-galactooligosaccharide (TOS) (Ito et al. 1993), as well as oligofructose and inulin (Teitelbaum and Walker, 2002). For a dietary substrate to be classified as a prebiotic, at least three criteria are required: the

substrate must not be hydrolyzed or absorbed in the stomach or small intestine, it must be selective for beneficial commensal bacteria such as Bifidobacteria, and fermentation of the substrate should induce beneficial luminal/systemic effects within the host (Manning and Gibson, 2004).

Reports from various studies have revealed that prebiotics such as FOS and TOS can modify the gastrointestinal (GI) tract microbial community to exert various effects such as enhance nonspecific immune responses (Bailey et al., 1991), increase fermentation products (Smiricky-Tjardes et al., 2003), as well as improve mineral uptake (Bongers and van den Heuvel, 2003) and livestock performance indices such as protein efficiency ratio and feed conversion ratio (Flickinger et al., 2003; Kirkpinar et al., 2004). Prebiotics such as oligofructose have been reported to increase bioavailability of glucose and trace elements in the diet of various animals (Breves et al., 2001; Bongers and van den Heuvel, 2003). Because prebiotics are completely natural, relatively inexpensive, and can be administered for extended periods of time to exert their beneficial effects, they are considered by some to be ideal feed additives. However, research on prebiotics is still in its infancy with fishes, compared to the progress that has been made in development of prebiotics for poultry (Patterson and Burkholder, 2003) and other production animals (Petkevicius et al., 1997; Breves et al., 2001). For example, the common prebiotic FOS was reported to lessen *Salmonella typhimurium* in the GI tract of chickens when included at 0.75% of diet (Bailey et al., 1991). A diet supplemented with FOS also has been reported to lessen infestation of intestinal worms in the GI tract of pigs (Petkevicius et al., 1997).

In recent years there has been a growing body of literature in which the effects of various prebiotics have been reported for several different fish species. Some of first studies were reported by Li and Gatlin (2004, 2005) who found that hybrid striped bass, *Morone saxatilis* x *M. chrysops* fed a diet containing GroBiotic®-A at 2% of diet had enhanced growth performance, higher feed efficiency and significantly lower mortality when challenged with the bacterial pathogens *Streptococcus iniae* and *Mycobacterium marinum*. GroBiotic®-A is a mixture of partially autolyzed brewers yeast, dairy ingredient components and dried fermentation products. Sink et al. (2007) more recently reported beneficial effects of GroBiotic®-A in golden shiners fed a high-fat diet followed by exposure to *Flavobacterium columnare*. Shiners fed a diet supplemented with the prebiotic at 2% by weight had significantly lower mortality compared to fish fed the other diets. In another experiment (Sink and Lochmann, 2008), golden shiners were fed diets with or without supplemental GroBiotic®-A at 2% for 10 weeks and then subjected to handling stress or directly exposed to *Flavobacterium columnare*. Handling stress prior to disease exposure significantly increased mortality of fish, but those fed the diet supplemented with GroBiotic®-A had reduced mortality compared to fish fed the basal diet.

A study with turbot larvae, *Psetta maxima*, showed that dietary supplementation of 2% inulin significantly changed GI microbiota by increasing *Bacillus* species to 14% and decreasing *Vibrio* species (Mahious et al., 2006). Recent in vitro studies (Burr et al., 2008a) confirmed a shift in the intestinal microbial population of red drum, *Sciaenops ocellatus,* in the presence of GroBiotic®-A. The predominant microbial species found in the intestinal inoculum of red drum was of the genus *Lactococcus* based on denaturing

gradient gel electrophoresis (DGGE) and DNA sequencing.

Burr et al. (2008b) also recently demonstrated that nutrient and energy digestibility of soybean-meal-based diets by red drum was enhanced with supplementation of several different prebiotics. A fishmeal-based control diet containing 40% crude protein exclusively from menhaden fishmeal had the highest nutrient and energy digestibility coefficients. However, the experimental diet with approximately half its protein from soybean meal and half from menhaden fishmeal when supplemented with GroBiotic®-A at 1% of diet had similar nutrient digestibility coefficients as the control diet. Also, the GroBiotic®-A- supplemented diet showed the most significant increase in protein and organic matter digestibility compared to the basal diet, although the other prebiotics also tended to increase protein and organic matter digestibility as well. Further studies are needed to determine the mechanisms that altered the protein, lipid, organic matter and lipid digestibility of the prebiotic-supplemented diets in this fish species. In addition, the effective application of prebiotics to aquatic organism will require their microbial community to be better characterized and understood.

Objectives

Based on the preceding information, the objective of this study was to determine if prebiotics commonly used for terrestrial animals and one prebiotic specifically designed for aquafeeds can increase apparent digestibility of nutrients and energy to subadult goldfish. In addition, the effects of these prebiotics on GI tract microbiota were characterized by DGGE analysis.

MATERIALS AND METHODS

Diet Formulation and Feeding

A total of six diets similar to those used by Burr et al. (2008a) were prepared for evaluation in this study. The control diet was formulated to contain 40% crude protein (CP), exclusively from menhaden fish meal and 10% lipid to provide an estimated available energy of 14.6 kJ/g (Table 1). Five experimental diets were formulated to be similar in proximate composition to the control diet (40% CP), but with approximately 50% of the protein supplied by menhaden fishmeal and 50% provided by solvent-extracted soybean meal. To four of the experimental diets, prebiotics were singularly added at 1% of dry weight in place of cellulose, and the basal diet was not supplemented with any prebiotic. The prebiotics evaluated were GroBiotic®-A, mannanoligosaccharide (MOS), galactooligosaccharide (GOS) and inulin.

Chromic oxide (Cr_2O_3) was the non-digestible marker added to all diets at 1% of dry weight (Gaylord and Gatlin, 1996). Ingredients of the basal diet were mixed in bulk with a commercial food mixer in sufficient quantity for use with all test diets. Fractions of the bulk-mixed basal diet were used for test diet preparation. Diets were pressure pelleted through a 3-mm die using a meat grinder, then air-dried at 25°C and stored at -20 ^{0}C until fed (Burr et al., 2008).

Each diet was fed to duplicate groups of 20 goldfish weighing an average of approximately 15 g each, in a system consisting of 110-liter aquaria. The aquaria were operated as a recirculation system, with the temperature maintained at 25-26°C by conditioning the ambient air. Water was maintained at 3 ppt total dissolved solids by

mixing synthetic sea water and sodium chloride with fresh well water. The recirculating system included a common settling chamber, biological filter and sand filter. Dissolved oxygen was maintained close to air saturation by blowing compressed air through air stones into each tank. A12-h light: 12-h dark cycle was maintained throughout the experiment with fluorescent lighting controlled by timers. Water quality was monitored periodically and maintained within an acceptable range for goldfish (Lochmann et al., 1997).

The six diets were each assigned to two separate aquaria of fish using a completely randomized design to obtain duplicate fecal samples per diet. All fish were conditioned using a commercial diet (Rangen Extr 400, Buhl, ID) for 2 weeks before feeding the experimental diets. Fish were fed to apparent satiation twice daily, morning and evening, 7 days a week. Fish were sampled, without killing them, once a week over the course of 4 weeks to obtain adequate fecal samples for analysis. On the day of fecal collection, 4 h post feeding, fecal material will be collected using the stripping technique (Austreng, 1978; Hajen et al., 1993). All the fish in each tank were physically restrained and pressure was applied to the abdomen to initiate defecation into pans cleaned with 95% ethanol before collection. Fish were returned to their respective tanks and allowed to recover from handling. Samples were collected from all fish in each tank over the course of 4 weeks and pooled as one composite sample per tank (two tanks per diet). The fecal material was dried at 60 ^{0}C for 24 h, grounded with mortar and pestle and placed into sterile bags and stored at -20 ^{0}C until analysis.

Nutrient Analysis of Diet and Fecal Samples

The diets and fecal samples were analyzed for organic matter by drying for 2 h at 120 ºC and then ashed at 550 ºC for 3 h (Association of Official Analytical Chemists, 1990). The dry samples were hydrated and analyzed for lipid content using the chloroform/methanol extraction method (Folch et al., 1957). Crude protein was determined according to the Dumas method(Ebeling, 1968) using a Leco Nitrogen Determinator (Model FP-528; Leco, St Joseph, MI, USA). The organic matter fraction was estimated by difference (100 - % ash). Energy content was determined using an isoperibol bomb calorimeter (Model 6200; Parr Instrument, Boline, IL, USA). Chromic oxide was determined using the method of Furukawa and Tsukahara (1966). Based on those analyses, digestibility coefficients were computed using the following formula: (%) =100 - [100 x (% dietary Cr_2O_3 / % fecal Cr_2O_3) x (% fecal nutrient / % dietary nutrient)] (National Research Council, 1993).

Table 1 - Composition (g/100 g dry weight) of each diet

	Diet designation						
	IFN	**Control**	**Basal**	**GroBiotic®-A**	**Mannan-oligosaccharide (MOS)**	**Inulin**	**Galactooligo-saccharide (GOS)**
Select menhaden meal [1]	5-02-009	58.7	27.1	27.1	27.1	27.1	27.1
Soybean meal, dehulled	5-04-612	0.0	35.5	35.5	35.5	35.5	35.5
Dextrin [2]		14.7	18.0	18.0	18.0	18.0	18.0
Menhaden oil [3]	7-08-049	6.3	8.5	8.5	8.5	8.5	8.5
Vitamin premix [4]		3.0	3.0	3.0	3.0	3.0	3.0
Mineral premix [4]		4.0	4.0	4.0	4.0	4.0	4.0
Carboxymethyl cellulose [2]		2.0	2.0	2.0	2.0	2.0	2.0
GroBiotic®-A [5]		0.0	0.0	1.0	0.0	0.0	0.0
MOS [6]		0.0	0.0	0.0	1.0	0.0	0.0
Inulin [7]		0.0	0.0	0.0	0.0	1.0	0.0
GOS [8]		0.0	0.0	0.0	0.0	0.0	1.0
Cellulose [2]		10.3	0.9	0.0	0.0	0.0	0.0
Chromium III oxide [9]		1.0	1.0	0.9	0.9	0.9	0.9
Analyzed composition (% dry weight) [10]							
Protein (%)		48.7	40.3	41.6	42.7	41.6	42.0
Lipid (%)		11.7	10.7	10.0	12.6	7.9	9.6
Gross energy (kJ/g)		19.0	19.1	19.5	19.2	19.5	19.4

[1] Contained 73.8% protein and 10.0% lipid; Omega Protein, Abbeville, AL, USA.
[2] USB, Cleveland, OH, USA.
[3] Omega Protein, Reedville,VA, USA.
[4] Moon and Gatlin (1991).
[5] International Ingredient, St Louis, MO, USA.
[6] Alltech, Nicholasville, KY, USA.
[7] Encore Technologies, Plymouth, MN, USA.
[8] Friesland Foods Domo, Zwolle, the Netherlands.
[9] Sigma-Aldrich, St Louis, MO, USA.
[10] Means of two analyses

Characterization of Gut Microbiota

In order to evaluate potential changes in gut microbiota in response to the dietary prebiotics, fecal samples from goldfish fed the various diets were analyzed for microbial composition. Polymerase chain reaction (PCR) was conducted using the method of Hume et al. (2003). The use of bacteria-specific PCR primers to conserved regions flanking the variable V3 region of 16S rDNA was used. The primers (50 pmol of each primer; primer 2, 5′ -ATTACC GCGGCTGCTGG- 3′; primer 3 with a 40 base pair GC clamp (33) 5′ -CGCCCGCCGCGCGCG GCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′) were mixed with Jump Start Red-Taq Ready Mix (Sigma Chemical Company, St. Louis, MO) according to the manufacturer's instructions, 250 ng of pooled (83 ng/replicate) template DNA from each of the three replicates was added along with 10 µg of bovine serum albumin (BSA) to help stabilize the reaction. The PCR amplifications were performed on a PTC-200 Peltier Thermal Cycler (MJ Research, Waltham, MA). A touchdown PCR program was used to minimize artificial by-products. The program used was as follows: 1) denaturation at 94.9 ºC for 2 min; 2) denaturation at 94.0 ºC for 1 min; 3) annealing at 67 ºC for 45s, -0.5 ºC per cycle; (to minimize formation of artificial products) (Hume et al., 2003); 4) extension at 72 ºC for 2 min; 5) repeat steps 2 to 4 for 17 cycles; 6) denaturation at 94 ºC for 1 min; 7) annealing at 58 ºC for 45 sec; 8) repeat steps 6 to 7 for 12 cycles; 9) extension at 72 ºC for 30 min; 10) 4 ºC final.

Denaturing Gradient Gel Electrophoresis

Denaturing gradient gel electrophoresis (DGGE) was run following the method of

Hume et al. (2003) as modified from Muyzer et al. (1993). The amplicons were separated on 8% polyacrylamide gels [(vol/vol) acrylamide-bisacrylamide ratio of 37.5:1 (Bio-Rad, Richmond, CA)] with a 30% to 60% urea-formamide gradient (100% denaturing 7M urea and 40% formamide) using a Dcode System (Bio-Rad, Hercules, CA). The amplicons were mixed with an equal volume of 2X loading buffer [0.05% (wt/vol) bromophenol blue; 0.05% (wt/vol) xylene cyanol; and 70% (vol/vol) glycerol] and 7 µL was loaded into each sample well (16-well comb). The gels were run at 60 volts for 17 hours in 0.5X Tris-Acetate-EDTA buffer (TAE) (20 mM Tris (pH 7.4); 10 mM sodium acetate; 0.5 Methylenediaminetetraacetic acid (EDTA); Bio-Rad, Hercules, CA) at 59ºC. Gels were stained for 30 min with SYBR® Green I (USA Amersham Life Sciences, Cleveland, OH) diluted 1:10,000. The fragment analysis pattern relatedness was determined with Molecular Analysis Fingerprinting software (v 1.6; Bio-Rad, Hercules, CA). This analysis is based on the Dice similarity coefficient and the unweighted pair group method using arithmetic averages (UPGMA) for clustering. Comparisons between sample band patterns were expressed as a percentage similarity coefficient (%SC).

Statistical Analysis

Apparent digestibility coefficients for organic matter, protein, carbohydrate and lipid in the control and experimental diets were subjected to analysis of variance according to a completely randomized design using the Statistical Analysis System (SAS Institute, 1985). Statistical significance was set at $P < 0.05$, and Duncan's multiple-range test was used for comparison of treatment means.

RESULTS

The apparent digestibility coefficient (ADC) values for crude protein obtained in this study ranged from 62.9% to 83.2%, with the fish-meal control diet having the highest value and the MOS-supplemented diet having the lowest value (Table 2). All prebiotic-supplemented diets had similar crude protein digestibility values compared with basal diet; whereas, the diet supplemented with MOS showed a significantly lower apparent digestibility.

Total lipid digestibility coefficients ranged from 84.6% for the control diet to 69.3% for the diet supplemented with MOS (Table 2). Diets supplemented with MOS, GroBiotic®-A and inulin had significantly lower ADC values compared to the basal diet.

Organic matter digestibility values ranged from 73.4% to 48.8%, with the MOS-supplemented diet having the lowest value, which was significantly different than all the other diets (Table 2). Once again the fishmeal control diet had a similar organic matter ADC as the soybean- meal- basal diet.

The carbohydrate ADC values range from 52.4% for the diet supplemented with GOS to 31.3% for the MOS-supplemented diet (Table 2). All diets had statistically similar ADC values for carbohydrate except the diets supplemented with MOS and GOS.

DGGE analysis (Figure 1) demonstrated that the microbial community was not significantly altered by any of the prebiotics evaluated. The similarity coefficients for each treatment ranged from 92% to 98.5 %.

Table 2 - Percent apparent digestibility coefficient (ADC) values for goldfish fed a fishmeal (FM) control diet or soybean- meal-based diets either unsupplemented (Basal) or supplemented with GroBiotic®-A, inulin, galactooligosaccharide (GOS) or mannanoligosaccharide (MOS) at 1% by weight*

Diet	Protein ADC	Lipid ADC	Organic matter ADC	CHO ADC†
FM control	83.17^{A}	84.62^{AB}	73.41^{A}	38.49^{AB}
Basal	79.24^{A}	87.32^{A}	70.46^{A}	47.14^{AB}
GroBiotic®-A	77.30^{A}	73.35^{D}	67.12^{A}	46.02^{AB}
Inulin	78.94^{A}	78.44^{C}	68.07^{A}	43.13^{AB}
GOS	79.03^{A}	83.20^{B}	71.57^{A}	52.37^{A}
MOS	62.88^{B}	69.29^{E}	48.77^{B}	31.34^{B}
P-value	0.0074	0.0001	0.0266	0.2587
pooled SE^	2.25	0.91	3.73	5.57

*Mean of two replicates. Values within columns and with a common superscript letter do not differ significantly (P>0.05)

†Determined by difference

^Pooled standard error

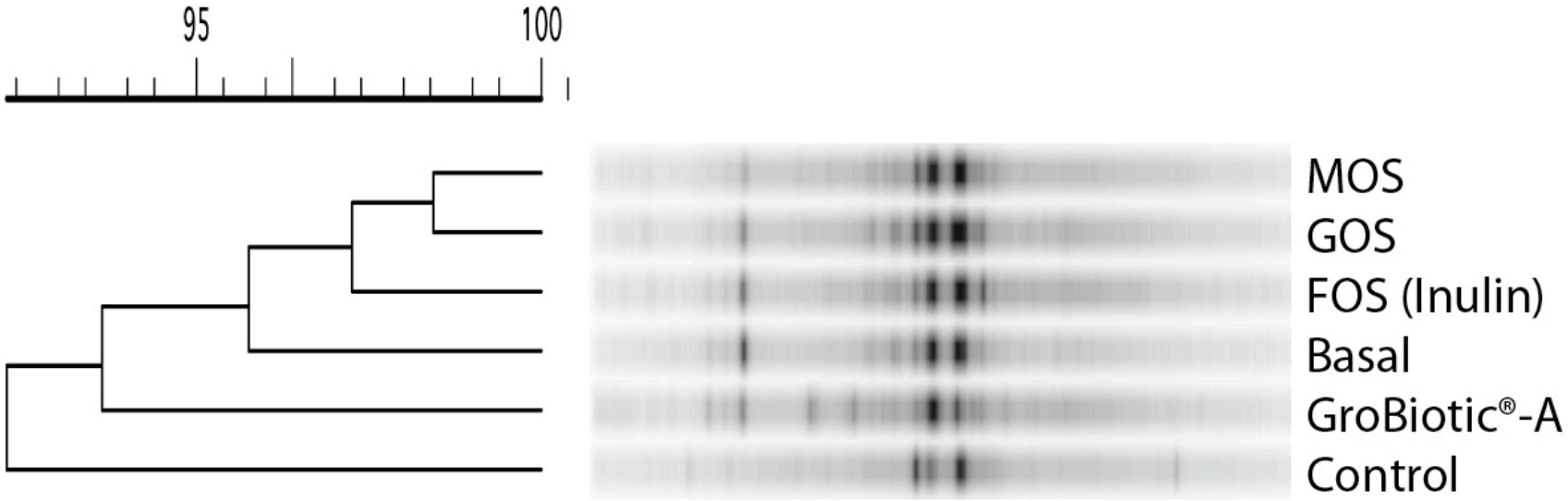

Figure 1 - Denaturing gradient gel electrophoresis of bacterial 16S rDNA amplicons from intestinal contents of goldfish fed a fishmeal control diet or soybean-meal-based diets either unsupplemented (Basal) or supplemented with GroBiotic-A, inulin (FOS), galactooligosaccharide (GOS) or mannanoligosaccharide (MOS) at 1% by weight . The scale to the left of the figure indicates percentage similarity coefficients.

DISCUSSION

Results of this experiment revealed that none of the prebiotics increased the apparent digestibility coefficient (ADC) values of the soybean meal-based diets by goldfish. The fishmeal control diet yielded the highest apparent digestibility coefficients, although for most nutrient groups the soybean-meal-based diets were equally well digested by goldfish, in contrast to previous observations with the carnivorous red drum (Burr et al., 2008b).

A potential error associated with stripping of feces for digestibility determination is the possibility of having undigested matter and intestinal sloughing contaminate the samples, resulting in an underestimation of the ADC. However, if careful stripping is employed from the posterior intestine, experiments with rainbow trout have demonstrated that the ADCs are highly correlated with those obtained by dissection (Austreng, 1978). Collecting excreted feces from the water greatly increases the opportunity for nutrients to leach into the water and provide inflated ADC values. Stripping of feces can be performed after fish are allowed to adapt to a diet and can easily be used immediately after a growth trial, when handling stress is no longer detrimental to the performance of the fish. However, it is difficult to strip feces from small fish, both because of the small amount of fecal material produced, and because the stripping may impose considerable stress on the fish. However, goldfish in the present experiment did not appear to be adversely affected by the stripping procedure and readily consumed feed the day after a stripping episode.

Fish size and species have been reported to affect nutrient digestibility estimates

(Windell et al., 1978; Ferraris et al., 1986; Medale et al., 1991; Olivia-Teles and Rodrigues, 1993; Watanabe et al., 1996). The ability of goldfish to digest plant material is related to their omnivorous natural feeding habits, their long digestive tract even in the absence of an acid stomach.

As a general rule, the intestine of carnivorous species is relatively short and straight with much of the acidic digestion taking place in the stomach. In contrast, herbivores species usually have long intestines, thus increasing the surface area available for absorption and providing more time for nutrient to be digested and absorbed. Relative intestine length (the ratio of intestine length to body length) is usually used to compare fish species, and ranges from 0.5 to 2.4 for carnivores, 0.8 to 5 for omnivores, and 2 to 21 for herbivores (Al-Hussaini, 1947; Kapoor et al., 1975). Intestinal elongation leads to an increase in the area from which nutrients can be absorbed (Stroband et al., 1979; Stroband and Veen, 1981). The absence of a stomach in goldfish is presumably compensated for by a transfer of gastric functions to other parts of the digestive tract, and/or elimination of certain functions (Caceci, 1984). Presumably due to these morphological differences in the digestive tract of goldfish, the ADC values for this specie were higher for the soybean-meal-based diets compared with those of the carnivorous red drum determined in a previous study (Burr et al., 2008b).

None of the prebiotics evaluated increased protein digestibility of the soybean-meal-based diets although the values were similar to that of the fishmeal control diet. The fishmeal control diet were very well utilized by goldfish with the protein ADC values similar to those reported for other species such as yellowfin seam bream (Wu et al., 2006), rainbow trout (Glencross et al., 2005; Ogunkoya et al., 2006), Atlantic salmon

(Refstie et al., 2000) and channel catfish (Wilson et al., 1985). The diet supplemented with MOS showed significantly lower apparent digestibility for protein, as well for lipids, organic matter and carbohydrates, although the reason for this was not readily apparent. A possible explanation would be that the MOS interfered negatively with the microbial population in the digestive tract, down-regulating the enzymes involved in nutrient digestion/absorption. Further study is needed to determine the mode of action responsible for this reduction in nutrient digestibility.

Lipid ADC values of the experimental diets containing MOS, GroBiotic®-A and inulin tended to be lower by an average of 5.9% than the basal diet in the present study. The decrease in lipid uptake may have been due to the energy needs of the host being met by catabolism of carbohydrates and protein. Another hypothesis is that the prebiotics influenced the uptake of dietary lipids by down-regulating enzymes involved in lipid digestion, for example lipase. Many fish use lipids efficiently as an energy source, as with most vertebrates. The age and weight of the fish and the total lipid content of the diet may also influence digestibility (Leger, 1985).

Organic matter and carbohydrates ADC values were not significantly increased with inclusion of any of the prebiotics tested and the MOS supplemented diet again had the lowest ADC value. The fishmeal control diet did not have a significantly higher ADC value, which contrasts to the results reported for red drum (Burr et al., 2008b). It has been shown in humans that some intestinal microbial communities can increase the energy made available to the host, possibly through the microbial degradation if indigestible carbohydrates (Turnbaugh et al., 2006). Also, the enhancement of carbohydrate digestibility may be due to the microbial community producing enzymes

that are either lacking or occurring only at low levels in the host.

The intestinal microbiota shows evidence of some selection of certain genera, which can multiply, in the conditions of this environment to form large populations of facultative and obligate anaerobes (Trust et al., 1979). To survive passage through the digestive tract, bacteria must be able to resist low pH, digestive enzymes, the effects of lysozyme and immunoglobulins in the gut mucus, and possibly anaerobic conditions in some regions. The structure of the digestive tract has some influence on whether gut microbiota differ significantly from that of the fish's diet and environment. Amylase activity of the GI tract microbiota from ayu or sweetfish, commom carp, channel catfish, Japanese eel, and tilapia was reported to occur in a higher percentage of the isolated anaerobic bacteria (64.8%) as compared with the aerobes (20%) (Sugita et al., 1997). The greater amylase activity indicated that the anaerobic microbiota of the GI tract might play an important function in the digestive capabilities of the host. Also, cellulase activity was found to occur in the stomachs of many fish species and was apparently due to the production of this enzyme by gut microbiota (Stickney et al., 1974).

Recalcitrant molecules, as found in dietary fiber, could become an energy source with enzymatic assistance from the endogenous microbiota. Anaerobic carboxymethylcellulase-producing bacteria were isolated from the intestinal tract of pinfish, (Luczkovich and Stellwag, 1993). That study suggested the enzymes produced by the microbiota may assist the host in obtaining energy from otherwise indigestible dietary nutrients.

Further studies with GroBiotic®-A are being conducted to characterize the effects of prebiotic-supplemented diets on goldfish production and *Aeromonas* spp resistance.

The results of the present, study, however, indicated that prebiotics did not improve the availability of nutrients in soybean- meal-based diets to the goldfish.

CONCLUSIONS

All of the evaluated prebiotics did not increase nutrient digestibility of the soybean-meal-based experimental diets to goldfish as previously reported for red drum. The fishmeal control diet yielded the highest apparent digestibility coefficients, although similar values were found with the soybean-meal-based experimental diets, which were equally well digested by goldfish. Diversity of microbiota from goldfish fed the various prebiotics also was not affected based on DGGE analysis. Additional research is needed to determine the mechanisms that altered protein, lipid, organic matter, carbohydrate and energy digestibility of the prebiotic-supplemented diets in some fish species but not in others.

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VITA

Name:	Thiago Raggi
Address:	2258 TAMU Department of Wildlife and Fisheries Sciences College Station, TX 77843-2258
Email Address:	thiago_82@tamu.edu
Education:	B.S., Animal Science, Universidade Estadual Paulista, 2006 M.S., Wildlife and Fisheries Sciences, Texas A&M University, 2009