General Health Effects and Toxicity of a Specific Mixture of Isomalto-oligosaccharides

Donal F. Day, PhD[1*] ¶, Chang-Ho Chung, PhD [2¶], Maren Hegsted, PhD [3¶], M.L. Marceau-Day, PhD [4¶], Kathleen McCutcheon[5¶]

[1] Audubon Sugar Institute, Louisiana State University AgCenter, Saint Gabriel, Louisiana, United States of America

[2] Sejong University, Seoul, Republic of Korea

[3] Department of Human Nutrition, Louisiana State University AgCenter, Baton Rouge, Louisiana, United States of America

[4] CAMD, Louisiana State University, Baton Rouge, Louisiana, United States of America

[5] Department of Human Ecology, Louisiana State University AgCenter and Bioassay Animal Laboratory, Baton Rouge, Louisiana, United States of America

* corresponding author

Email: dday@agcenter.lsu.edu

¶ These authors contributed equally to this work

Abstract

Prebiotics are non-digestible carbohydrate compounds, often referred to as "soluble fiber". Although these carbohydrates are non-nutritive, they can influence the gut microbiome. An isomalto-oligosaccharide (IMO) prebiotic, specifically, a fermented mixture of maltosyl-isomalto-oligosaccharides (MIMO, DP 3-9), was tested for acceptability, toxicity, morphological/histopathological and biochemical changes in laboratory rats that were given three concentrations of MIMO (5, 10, and 20 % w/w and an energy control) added to their feed. Male Sprague Dawley rats were used to evaluate potential adverse effects of this specific IMO. No evidence of adverse effects was noted in any of the rodent test groups given the prebiotic, even when it was presented as a significant percentage of their diet (e.g. 20 %). It appears that this prebiotic mixture was well-tolerated, non-toxic, and may be suitable for use as dietary supplement in other mammals.

Introduction

The microbiome of the human gut is a biofilm comprised of a community of highly diverse bacterial species [1,2] that work together to process food, transfer nutrients, and bolster the immunity of the host to disease [3,4]. Additionally, neurotransmitters regulated by the gut (gut-brain axis), such as serotonin [5] and GABA (γ-aminobutyric acid), in particular [6], may affect the mental state of the host. It is therefore reasonable to hypothesize that a healthy microbiome equates to a healthy host with a healthy mind. The current Western diet has removed many traditional fermented foods because fermenting/pickling/salting are no longer the primary means of food-preservation or food production such as sourdough bread fermentation. These traditionally preserved and fermented foods can be sources of IMOs that act as prebiotics

[7, 8]. Prebiotics are, typically, non-nutritive carbohydrate compounds that can survive in the gastrointestinal tract long enough to travel to the colon, where they can influence growth and/or activity of commensal microorganisms, while, ostensibly, contributing to the well-being of the host. Prebiotics have been demonstrated to alter the distribution and activity of microorganisms that make up the gut microbiome [1]. Modification of the intestinal microbiome is now recognized as a potential key to improving host-health, and among other health benefits, can help control obesity and reduce instances of infectious disease [4]. The gut microbiome is so important to human health that physicians carry out fecal transplants to combat high morbidity in patients in which the colonic flora lacks the beneficial organisms and diversity necessary for good health [9]. In fact, it has been found that sterile mice inoculated with fecal material from obese mice were significantly more likely to become obese than those inoculated with microbiota from slim donors [10, 11]. A similar effect was noted in human twins where obesity was associated with phylum-level changes in the microbiome [12]. A recent review [13] indicates that the efficiency of chemotherapeutic drugs also is linked to the health of the microbiome. Efforts to improve microbiome health have generally been non-targeted, relying on the delivery of packages of "healthful" microorganisms (probiotics), typically *Lactobacillus* and *Bifidobacteria* spp. A more targeted approach is through use of compounds which either selectively enhance healthful indigenous microbial populations (prebiotics) or could be used to improve the viability of a co-administered probiotic formula (synbiotics).

Many complex carbohydrates have been reported to exert a prebiotic effect [14], including lactulose [15], inulin, fructrooligosaccharides (FOS, from chicory, yacon or artichoke) [16] and galactooligosaccharides (GOS, typical in human breast milk) [17]. It has been

suggested that "probiotic microorganisms" possess cell-associated glycosidases, which favor the degradation of prebiotic oligosaccharides [18] producing a selective advantage for these microbes. Common pathogenic species, such as *E. coli,* do not possess the capacity to degrade many oligosaccharides and are, therefore, selectively disadvantaged. Sanz et al., 2005 reported that for glucan prebiotics, particular linkage-types are important. Specifically, α-glucans may act as prebiotics [14]. Those oligosaccharides showing the greatest benefit contain α-(1,2), α-(1,6), α-(1,3) and/or α-(1,4) linkages. The most effective oligosaccharide prebiotics were noted to be smaller than 10 sugar units in terms of molecular weight (Degree of Polymerization [DP <10]), and further, that molecular weight can alter the bacterial preference of the prebiotic [19]

As have been noted in the ancient food supply, particularly in wild sourdough breads [7] and other fermented foods such as kimchi [8], IMOs are non-nutritive glucose chains, similar to, but smaller than starch (α-(1,4) glucan) and/or dextran (α-(1,6) glucan), that may be used as bioactive food additives to modify the human microbiome. When used as dietary supplements, these additives were noted to augment fermentation in the large intestine, increase mineral absorption, and lower blood cholesterol levels [20]. Isomalto-oligosaccharides, terminated with an α-(1,4) linkage have been reported to be effective in controlling *Salmonella*, and have been demonstrated to exert this prebiotic effect on broiler chickens [21].

In an effort to understand the safety of a fermentation-produced IMO ("fermentation IMO") for potential use in mammalian systems, this study was undertaken as a safety and preliminary screen for efficacy of this compound. The specific isomalto-oligosaccharides evaluated in this study were produced by a strain of *Leuconostoc* that normally produces highly

branched polymers [22], by using an acceptor molecule (maltose) to limit the molecular weight of the exopolysaccharide produced during sucrose fermentation. The IMOs tested were branched polymers between DP 3 and DP 9 in size, and very similar, but of superior purity, and higher mass-average molecular weight (the majority of molecules comprising DP4 to DP6) compared to enzymatically manufactured IMO-based food-additive ("manufactured IMO"). Such manufactured IMO has been recognized by the Food and Drug Administration (FDA) as Generally Recognized As Safe (GRAS), and is being sold in consumer food products [23], where it may be included in relatively large amounts, e.g. up to 30 g/day [24].

Although other manufactured IMO-based prebiotics have been tested [25], none are representative of the purity, molecular weight, and linkage type of the tested fermentation IMO, and few have been tried *in-vivo*, in rats [26], humans [27, 28], or both [29]. Because it has been noted that both linkage type and molecular weight can have a significant, and often unknown effect on prebiotic selectivity, and because some manufactured prebiotics have been noted to cause potentially negative side effects including bloat and flatulence [30], the aim of this study is to test this specific fermentation IMO product for adverse effects and to do so within a dosing range that should guarantee we would observe them, if they were to occur.

IMO synthesized via fermentation using *Leuconostoc* spp. bacteria, in the range of DP 3-9 (MWD 760-900 Da) with linear α-(1,6) backbones, α-(1,3) branched side chains, and terminal α-(1,4) glycosidic linkages (maltose molecule) were tested. This study provides a simple evaluation for beneficial or detrimental effects of this IMO in young growing rodents, at doses of 5%, 10% and 20% of diet. These high doses of IMO (up to 20% of diet) were chosen to

represent supra-physiologic doses of IMO compared with the average human diet. The average American consumes about 3800 kcal/day [31] of which between 14 and 19 grams is dietary fiber [32]. The human dose of IMO that would constitute 5%, 10% and 20% of diet would be about 60, 119, 238 grams IMO/day respectively. Thus the rat 5%, 10% and 20% IMO diets had fiber 360%, 720% and 1440% higher than the average American total daily fiber intake.

Materials and Methods

This project utilized male Sprague Dawley rats for initial testing of key physical and biochemical markers.

Dietary Composition

Characterization of the IMO used in this study was previously performed by Chung, as part of his doctoral dissertation at the Louisiana State University [33] and further details are given in US patents 7,291,607 [34], 7,772,212 [35]. A typical compositional analysis of the product is given in Table 1 [36]. Oligosaccharides were speciated via HPAEC-PAD (high pressure anion exchange chromatography with pulsed amperometric electrochemical detection, Carbopac PA-100 column, acetate gradient at constant pH > 12.7, ThermoDionex ICS-5000+ system) and quantitated vs. an L-arabinose internal standard. Organic acids and glycerol were determined via HPLC-RID (high pressure liquid chromatography-refractive index detection, BioRad Aminex HPX-87H column, isocratic 0.008N H_2SO_4, Agilent 1100 system) vs. external standards.

Table 1. Compositional analysis of a typical IMO ingredient produced by fermentation.

(*) The majority of molecules comprising DP4 to DP6

DP 1, % brix	**0.02**
DP 2, % brix	**0.37**
MIMO DP 3-9, % brix*	**94.03**
Mannitol, % brix	**0.05**
Acetate, Na, % brix	**5.53**
M_w, Da:	**900.26**
Total	**100**
Purity, %:	**94.03**

The powdered diets were based on a control diet using the recommended AIN-93G rodent diet [37] and were made to contain 5 %, 10 % , and 20 % IMO (w/w), respectively, which replaced the same percentage of sucrose for the 5% and 10% groups, and an additional amount of starch was replaced for the 20% group (see Table 2). The rats were fed *ad libitum*. Feed intake was measured 3 times per week and weights were collected at the beginning and end of the study.

Table 2. Diet Composition by Group. All contain 7% fat.

INGREDIENTS	Control (AIN-93G)					5% IMO			10% IMO			20% IMO		
	kJ/g	kcal/g	g	kJ	kcal	g	kJ	kcal	g	kJ	kcal	g	kJ	kcal
100% AMYLOPECTIN	14.7	3.5	530.7	7777.1	1857.5	530.7	7777.1	1857.5	530.7	7777.1	1857.5	430.7	6311.7	1507.5
IMO														
58.8% solid	6.3	1.5	0.0	0.0	0.0	50.0	314.0	75.0	100.0	628.1	150.0	200.0	1256.1	300.0
41.2% water						35.0			70.0			140.0		
SUCROSE	16.7	4.0	100.0	1674.8	400.0	50.0	837.4	200.0	0.0	0.0	0.0	0.0	0.0	0.0
CASEIN	15.0	3.6	200.0	2997.9	716.0	200.0	2997.9	716.0	200.0	2997.9	716.0	200.0	2997.9	716.0
SOYBEAN OIL	35.4	8.5	70.0	2476.6	591.5	70.0	2476.6	591.5	70.0	2476.6	591.5	70.0	2476.6	591.5
CELLULOSE			50.0	0.0	0.0	50.0	0.0	0.0	50.0	0.0	0.0	50.0	0.0	0.0
MINERAL MIX	3.7	0.9	35.0	129.0	30.8	35.0	129.0	30.8	35.0	129.0	30.8	35.0	129.0	30.8
VITAMIN MIX	16.2	3.9	10.0	162.0	38.7	10.0	162.0	38.7	10.0	162.0	38.7	10.0	162.0	38.7
CHOLINE CLORIDE			1.3	0.0	0.0	1.3	0.0	0.0	1.3	0.0	0.0	1.3	0.0	0.0
L - CYSTINE	16.7	4.0	3.0	50.2	12.0	3.0	50.2	12.0	3.0	50.2	12.0	3.0	50.2	12.0
			Dry Mass	kJ/g	kcal/g	Dry Mass	kJ/g	kcal/g	Dry Mass	kJ/g	kcal/g	Dry Mass	kJ/g	kcal/g
			1000.0	15.3	3.6	1000.0	14.7	3.5	1000.0	14.2	3.4	1000.0	13.4	3.2

Test Animals

Male Sprague Dawley rats were used to evaluate potential adverse effects of this specific isomalto-oligosaccharide (IMO). Young (6 -8 week old) growing rats were used, because they are more sensitive to adverse dietary conditions. Five to ten rats per treatment group were chosen to permit sufficient data from necropsy and analytical blood tests to evaluate adverse or potential beneficial effects of IMO in their diets. The trial was 6 weeks in length. Upon arrival, all of the rats were weighed and fed the control powdered diet for a one week acclimatization period after which they were reweighed. The rats were singly housed in metabolic cages to allow measurement of food intake, food spillage by the animals and collection of both urine and excrement. They were stratified by weight and then randomly assigned to diet treatment groups. Physical observations, weight changes and food intake patterns were used to aid in monitoring growth and health.

Ethics Statement

This pilot rat-feeding study abided by the Policy for the Care and Use of Animals of Louisiana State University, and that of the LSU Agricultural Center. This project was also in accordance with the NIH "Guide for the Care and Use of Laboratory Animals", and the Louisiana State University or the LSU Agricultural Center Animal Welfare Assurance on file with the U.S. Public Health Service. All experiments were approved by the Louisiana State University School of Veterinary Medicine Institutional Animal Care and Use Committee (IACUC).

Necropsy, Clinical Chemistry and Hematology

At the end of the treatment period, the rats were transported individually in shoe boxes

with micro-isolator lids to the nutrition lab. They were anesthetized with isoflurane gas. While still under anesthesia, they were euthanized via cardiac puncture. Up to 10 cc of blood was recovered by cardiac puncture resulting in termination. If initial attempt at cardiac puncture led to unsuccessful aspiration, the needle was withdrawn, the tip was adjusted, caudad or cephalad, and reintroduced, until successful aspiration was accomplished. This was necessary in 8 animals (see legend to Table 3). The blood samples and carcasses were sent to the LSU Veterinary School for necropsy that encompassed basic gross anatomy, basic pathology, full chemistry panel, and complete blood count with machine differential.

Statistical Analyses

Comparisons were made using ANOVA where P is (T<=t) and the set differential for significance was $P<0.05$. Results are presented as means +/- standard error of the mean (SEM). Using a one-factor analysis of variance (ANOVA), dose groups were compared with respect to the following parameters: dietary intake in grams and kilocalories, weight change, feed and energy efficiency, gross anatomy changes, blood chemistry, and complete blood count values.

Results

Neither IMO-induced mortality nor clinical signs of toxicity were observed in any of the dose groups (5%, 10% and 20%) during the 6 week study period. There was no significant difference between the test groups and control in terms of total weight of feed consumed, nor in terms of energy intake (Table 3 and Fig. 1). On average, each rat consumed between 876 and 1138 grams of feed over a 6 week trial and gained on average between 72 and 142 grams weight per rat over the course of the experiment. Rats are known to be finicky eaters and there were no signs of displeasure with any of the test feed formulations.

Fig. 1. Food vs. IMO Consumed vs. Weight Change (10x). Mean +/- SEM.

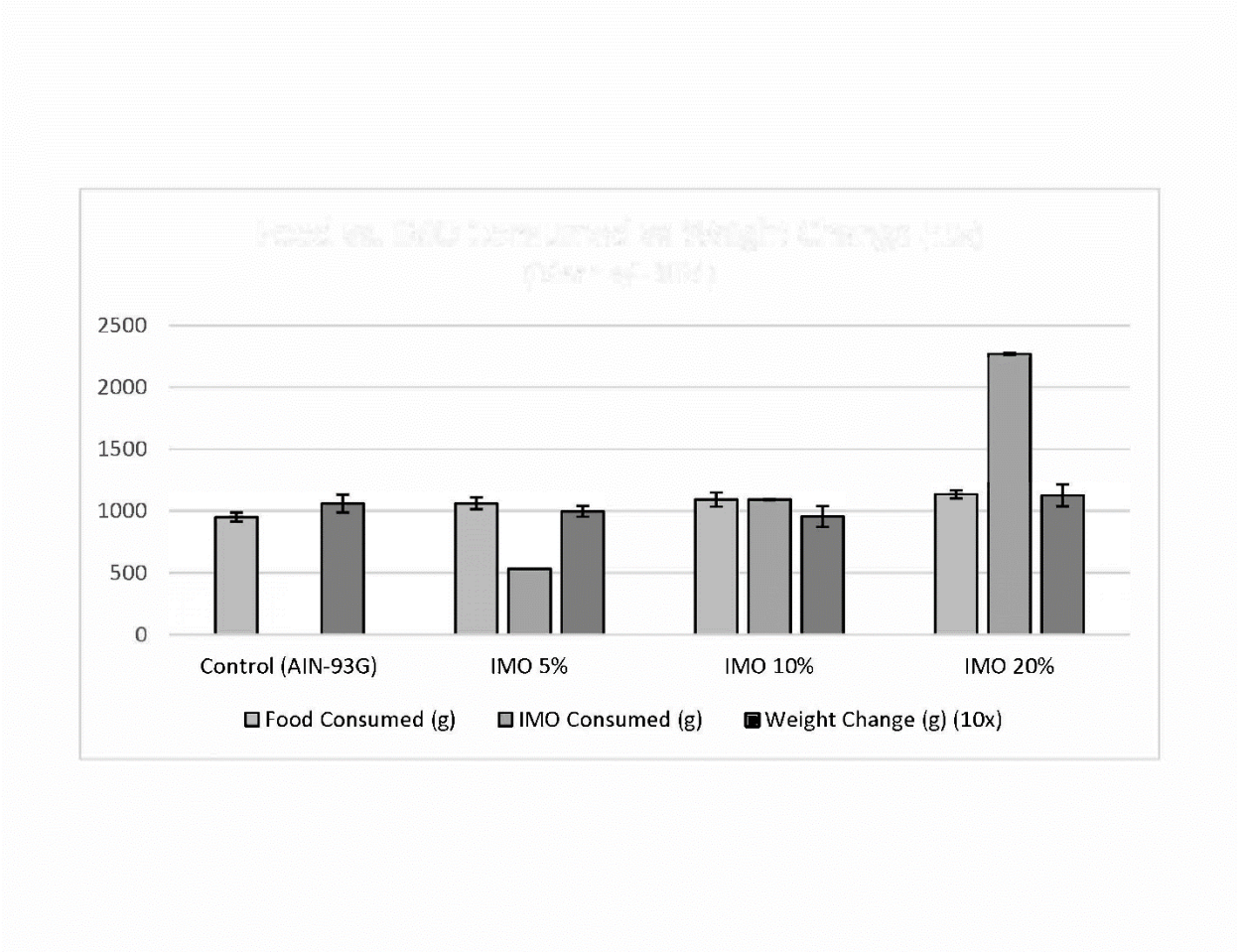

Table 3. Feed Intake and Weight Results. Mean +/- SEM.

	Control (AIN-93G)			**IMO 5%**			**IMO 10%**			**IMO 20%**		
	(n = 5)			**(n = 6)**			**(n = 5)**			**(n = 6)**		
Start Weight (g)	267	+/-	3.7	273	+/-	5.7	273	+/-	10.5	287	+/-	9.5
Final Weight (g)	373	+/-	8.4	373	+/-	9.2	369	+/-	16.9	399	+/-	16.7
Feed Intake (g)	951	+/-	36.6	1062	+/-	48.0	1091	+/-	57.1	1135	+/-	33.0
Weight Change (g)	106	+/-	7.2	100	+/-	4.4	96	+/-	8.4	113	+/-	8.8
Disemboweled Weight (g)	356	+/-	7.3	357	+/-	8.9	352	+/-	16.4	381	+/-	15.6

There was no significant difference in weight gain between any of the test and control groups over the course of the trial (Table 3 and Fig. 2). Feed efficiency calculations, a measure of dietary utilization, also showed no significant difference between any of the test and control groups over the course of the trial (Table 4 and Fig. 3).

Fig. 2. Initial vs End Weight. Mean +/- SEM.

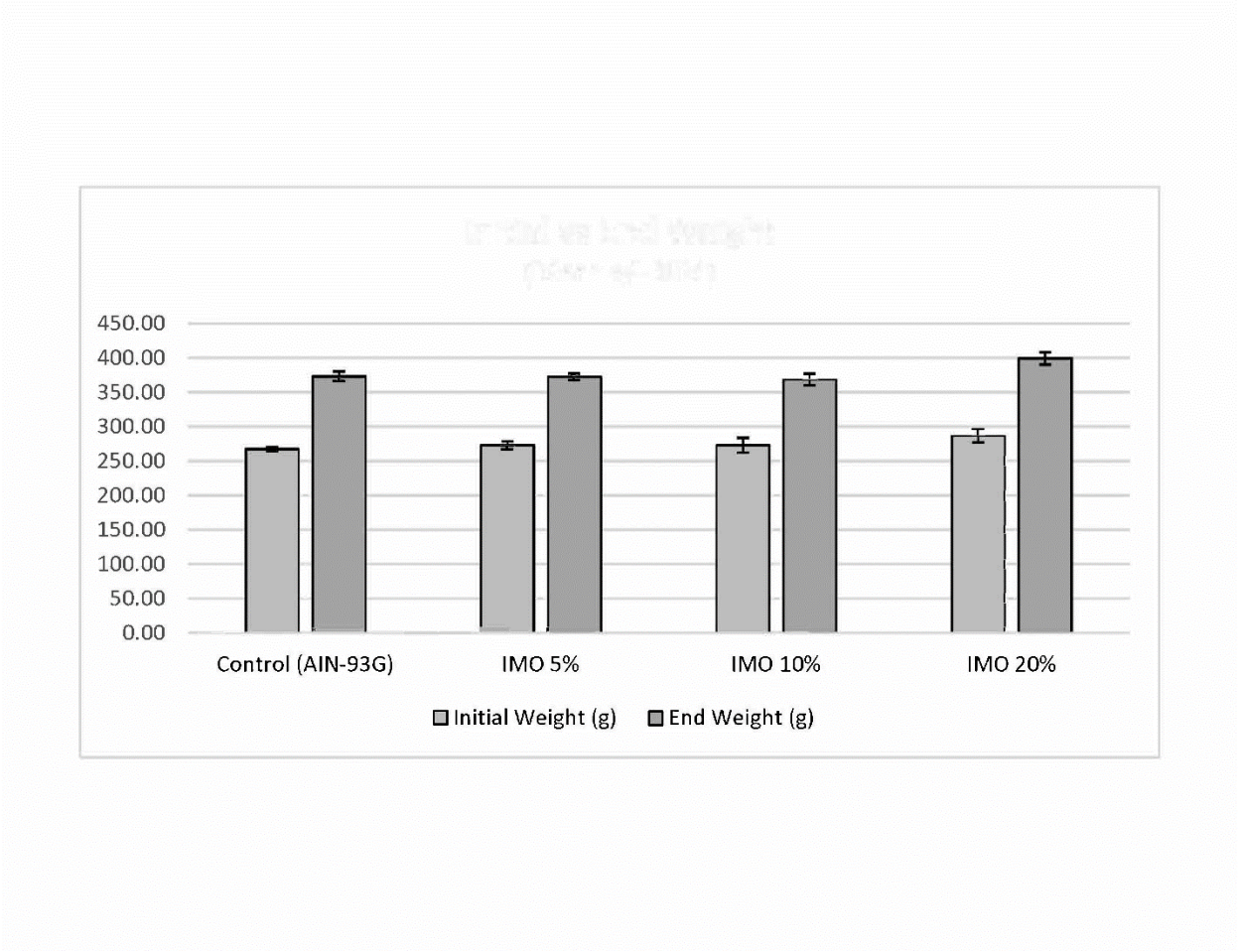

Fig. 3. Feed Efficiency vs Energy Efficiency. Mean +/- SEM.

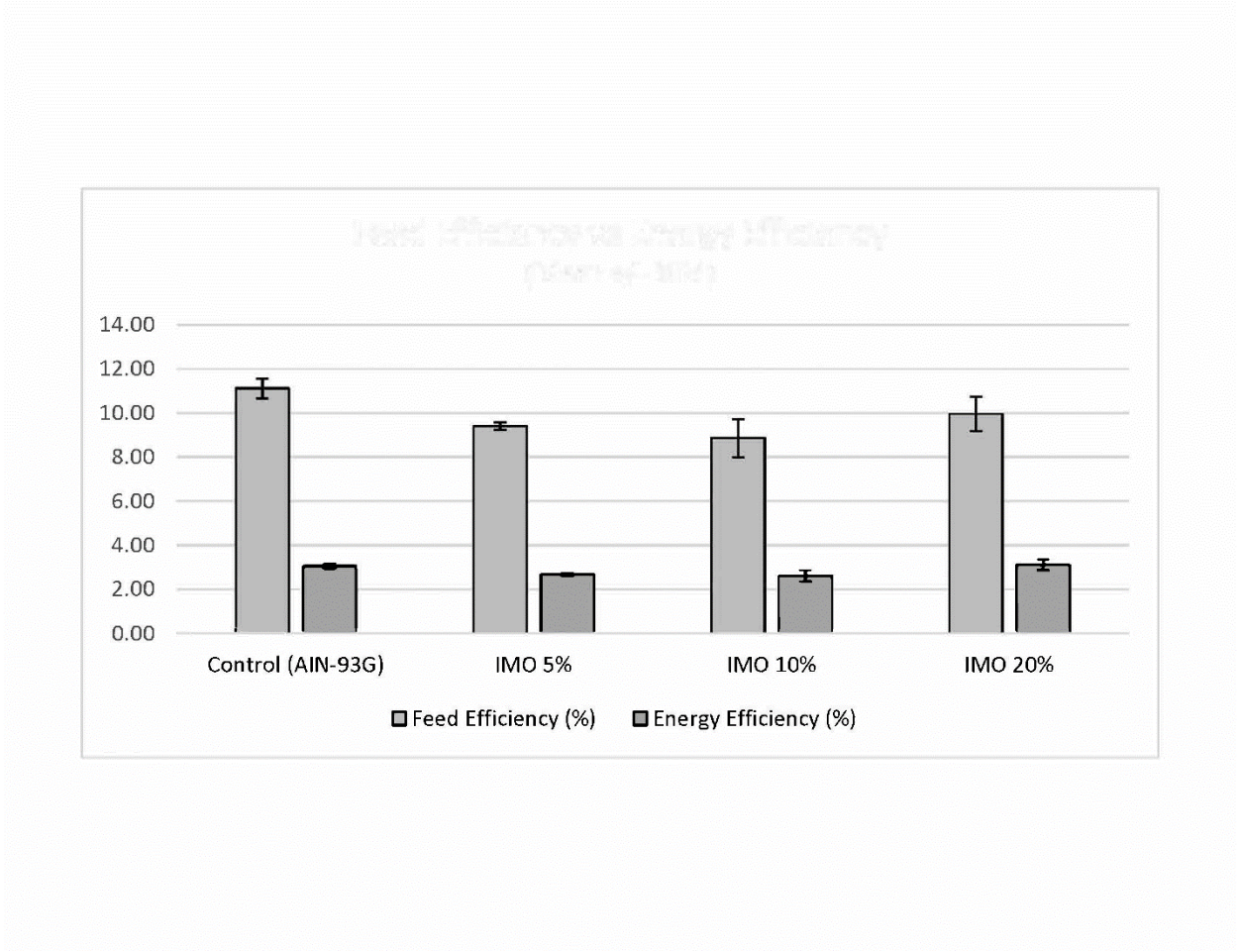

Table 4. Feed Efficiency. Mean +/- SEM.

	Control (AIN-93G) (n = 5)			IMO 5% (n = 6)			IMO 10% (n = 5)			IMO 20% (n = 6)		
Feed Efficiency (g/g x 100)	11.1	+/-	0.5	9.4	+/-	0.2	8.9	+/-	0.9	10.0	+/-	0.8
Energy Efficiency (kcal/g x 100)	3.0	+/-	0.1	2.7	+/-	0.0	2.6	+/-	0.3	3.1	+/-	0.2

Clinical chemistry results showed no significant differences between the IMO groups and controls for any of the parameters monitored (Table 5). In several animals CK, AST and ALT levels were greatly elevated. Given that cardiac puncture technique can affect CK/AST/ALT results (multiple attempts at cardiac puncture can cause additional cardiac and liver damage), values for CK above 500, with corresponding elevations in AST/ALT, were likely reflective of additional needle damage to cardiac and hepatic tissue from multiple attempts at cardiac puncture. These spurious values were removed from consideration prior to calculations and statistical analysis with ANOVA (see legend for Table 5).

Table 5. Clinical Chemistry Results. Mean +/- SEM. (*) Denotes animals that had CK values above 500 U/L, 10 times the reported rat normal value of 50 U/L with simultaneous elevations in ALT and AST. Such high CK levels were felt to be spurious and reflective of excess needle damage to cardiac and hepatic tissue due to variation in cardiac puncture technique. These values were therefore removed from consideration prior to calculations and statistical analysis with ANOVA.

	Control (AIN-93G) (n = 5)			IMO 5% (n = 6)			IMO 10% (n = 5)			IMO 20% (n = 6)		
GLUCOSE (mg/dL)	223.0	+/-	14.8	216.2	+/-	16.1	195.6	+/-	15.2	227.0	+/-	19.3
AST (SGOT)* (u/L)	81.5	+/-	6.1	90.3	+/-	11.6	93.0	+/-	7.4	77.0	+/-	9.3
ALT(SGPT)* (u/L)	36.3	+/-	2.8	47.5	+/-	9.9	49.7	+/-	10.4	35.0	+/-	8.1
ALK PHOS (IU/L)	183.4	+/-	13.6	163.2	+/-	11.2	190.6	+/-	15.2	149.3	+/-	18.7
CK (CPK)* (u/L)	238.0	+/-	51.2	264.8	+/-	78.1	292.0	+/-	34.5	231.7	+/-	53.1
Tot Bili (mg/dL)	0.2	+/-	0.0	0.2	+/-	0.0	0.2	+/-	0.0	0.2	+/-	0.0
Tot PRO (gm/dL)	6.1	+/-	0.1	6.1	+/-	0.1	6.2	+/-	0.1	6.2	+/-	0.1
Alb (g/dL)	3.0	+/-	0.1	3.1	+/-	0.1	3.1	+/-	0.1	3.0	+/-	0.0
Glob (g/dL)	3.1	+/-	0.1	3.0	+/-	0.1	3.1	+/-	0.1	3.1	+/-	0.1
Choles (mg/dL)	82.8	+/-	4.6	78.7	+/-	6.0	91.2	+/-	5.9	98.2	+/-	4.9
BUN (mg/dL)	18.0	+/-	0.5	18.2	+/-	1.3	18.6	+/-	0.5	17.5	+/-	0.5
Cr (mg/dL)	0.3	+/-	0.0	0.5	+/-	0.2	0.3	+/-	0.0	0.3	+/-	0.0
Ca (mg/dL)	10.2	+/-	0.1	10.0	+/-	0.1	10.2	+/-	0.2	10.1	+/-	0.1
P (mg/dL)	5.6	+/-	0.5	5.7	+/-	0.3	5.9	+/-	0.5	6.2	+/-	0.5
Na (mmol/L)	144.4	+/-	2.0	143.3	+/-	0.8	143.2	+/-	0.4	142.3	+/-	0.5
K (mmol/L)	5.6	+/-	0.3	5.6	+/-	0.3	5.8	+/-	0.7	6.0	+/-	0.6
Cl (mmol/L)	101.8	+/-	0.4	102.3	+/-	0.4	102.4	+/-	0.6	102.5	+/-	0.5
TCO2 (mmol/L)	24.5	+/-	1.6	24.7	+/-	0.4	25.9	+/-	0.4	24.8	+/-	0.7
AGAP (mmol/L)	23.7	+/-	3.8	21.9	+/-	0.8	20.8	+/-	1.0	21.0	+/-	1.0

Hematology results showed no significant differences between the IMO groups and control in any of the parameters monitored (Table 6).

Table 6. Hematology Results. Mean +/- SEM.

	Control (AIN-93G) (n = 5)			IMO 5% (n = 6)			IMO 10% (n = 5)			IMO 20% (n = 6)		
RBC (103/uL)	7.9	+/-	0.2	7.9	+/-	0.1	7.8	+/-	0.2	7.6	+/-	0.2
Hgb (g/dL)	13.9	+/-	0.3	14.1	+/-	0.2	14.3	+/-	0.3	13.6	+/-	0.3
Hct (%)	40.3	+/-	1.2	41.0	+/-	0.4	41.4	+/-	1.2	39.6	+/-	1.0
RDW (%)	11.7	+/-	0.1	11.4	+/-	0.2	11.6	+/-	0.3	11.6	+/-	0.2
MCV(fL)	51.0	+/-	0.3	52.0	+/-	0.6	53.0	+/-	0.3	52.0	+/-	0.7
MCH (pg)	17.7	+/-	0.2	18.0	+/-	0.3	18.3	+/-	0.1	17.9	+/-	0.3
MCHC (g/dL)	34.7	+/-	0.3	34.5	+/-	0.3	34.6	+/-	0.5	34.4	+/-	0.3
Plat (103/uL)	622.4	+/-	124.3	687.3	+/-	133.4	324.7	+/-	303.0	753.7	+/-	165.3
MPV (fL)	6.2	+/-	0.3	7.1	+/-	0.3	4.6	+/-	1.1	6.4	+/-	0.5
PlasPRO (g/dL)	6.0	+/-	0.2	5.8	+/-	0.1	5.7	+/-	0.2	6.0	+/-	0.2
PCV (%)	39.8	+/-	1.4	41.0	+/-	0.6	40.7	+/-	0.9	40.3	+/-	0.6
WBC (103/uL)	6.9	+/-	0.5	5.8	+/-	0.8	4.8	+/-	0.6	6.7	+/-	1.3
Neutr (%)	11.0	+/-	1.7	14.5	+/-	2.4	10.7	+/-	0.9	22.3	+/-	7.7
Neutr (103/uL)	0.7	+/-	6.1	0.8	+/-	11.6	0.5	+/-	7.4	1.9	+/-	9.3
Lymph (%)	80.0	+/-	2.8	80.2	+/-	9.9	80.7	+/-	10.4	72.3	+/-	8.1
Lymph (103/uL)	5.8	+/-	13.6	4.7	+/-	11.2	3.9	+/-	15.2	4.5	+/-	18.7
Mono (%)	3.4	+/-	51.2	3.8	+/-	78.1	4.7	+/-	34.5	2.3	+/-	53.1
Mono (103/uL)	0.2	+/-	0.0	0.2	+/-	0.0	0.2	+/-	0.0	0.2	+/-	0.0
Eosin (%)	2.2	+/-	0.1	1.2	+/-	0.1	4.0	+/-	0.1	3.0	+/-	0.1
Eosin (103/uL)	0.2	+/-	0.1	0.1	+/-	0.1	0.2	+/-	0.1	0.2	+/-	0.0

Necropsy results (Table 7) showed no significant differences between the IMO groups and the controls in any of the parameters monitored with the exception of cecal measures in the 20% IMO diet. Specifically, for the 20% IMO diet, full and empty cecal weights and cecal contents, were all significantly different compared with control ($p<0.05$) (Fig. 4). Given that IMO is a mostly non-digestible fiber that is selectively fermented by healthful bacteria, the cecal changes were consistent with the expected fermentation, with greater cecal changes seen at higher IMO diets.

Fig. 4. Cecal Weight Changes. Mean +/- SEM. (*) Denotes for IMO 20% that all cecal weights were statistically significantly increased compared to control.

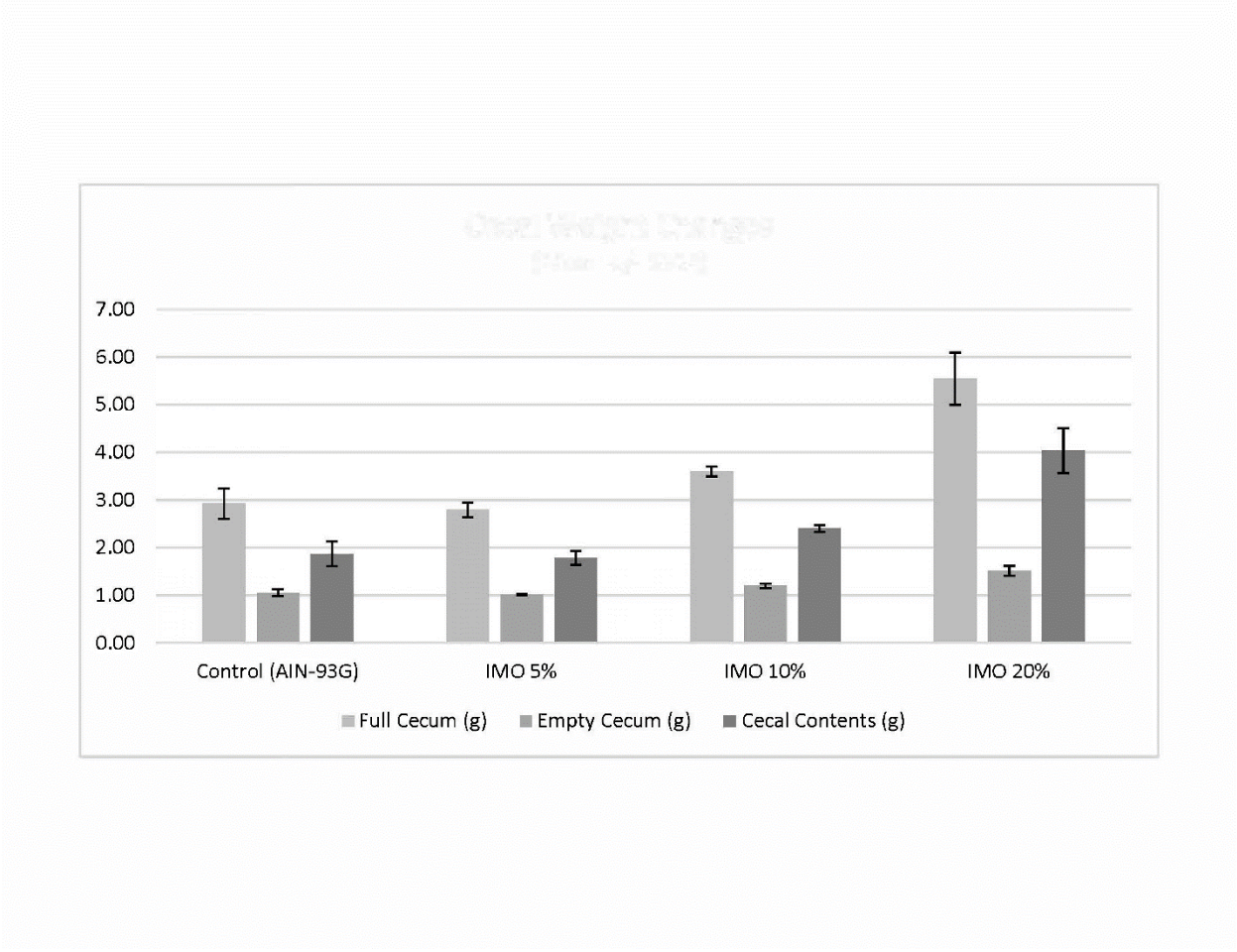

Table 7. Necropsy Results. Mean +/- SEM. (*) Denotes for IMO 20% that all cecal weights were statistically significantly increased compared to control.

	Control (AIN-93G) (n = 5)			IMO 5% (n = 6)			IMO 10% (n = 5)			IMO 20%* (n = 6)		
Heart	1.3	+/-	0.08	1.3	+/-	0.06	1.2	+/-	0.08	1.4	+/-	0.07
Spleen	0.7	+/-	0.06	0.7	+/-	0.03	0.7	+/-	0.04	0.7	+/-	0.05
Kidney	2.4	+/-	0.04	2.3	+/-	0.07	2.2	+/-	0.10	2.5	+/-	0.08
Liver	11.8	+/-	0.36	11.3	+/-	0.35	11.2	+/-	0.54	12.0	+/-	0.59
Lungs	1.3	+/-	0.05	1.2	+/-	0.10	1.4	+/-	0.04	1.3	+/-	0.11
Cecum (full)*	2.9	+/-	0.32	2.8	+/-	0.15	3.6	+/-	0.10	5.5*	+/-	0.55
Cecum (empty)*	1.1	+/-	0.07	1.0	+/-	0.02	1.2	+/-	0.05	1.5*	+/-	0.10
Cecum (contents)*	1.9	+/-	0.26	1.8	+/-	0.15	2.4	+/-	0.07	4.0*	+/-	0.47
GI Tract	17.6	+/-	1.28	15.2	+/-	0.48	16.1	+/-	0.72	18.7	+/-	1.35
Total Fat	9.6	+/-	0.77	9.1	+/-	0.60	9.6	+/-	0.79	10.7	+/-	1.10

Discussion

A literature survey of IMO yielded eleven *in-vivo* studies with rats or mice, using IMO produced via enzymic means (dextranase or dextransucrase with a variety of substrates including starch, dextran, and donor-acceptor systems with sucrose), where no adverse effects were found (one short term study on protein digestibility, two on dental caries with IMO in the animal's drinking water, one short term study of cyclic nigerosylnigerose where IMO was used as control, none related to the proposed research). Eight of these rat studies involved the use of IMO that were not representative of the material tested in this work. Specifically, five of them used a product called "IMO-900" manufactured by Baolingbao Biology, Co. Ltd. [37, 38, 39, 40, 41]. IMO-900 is a product made from starch with (relative to the product tested in this work), a low M_w distribution of 437.4 Da (DP 2.7) and was, with the exception of panose, primarily straight α-(1,6) glucan, not MIMO. Iwaya, et al. appeared to be looking at similar molecules, but the M_w distribution of S and L-IMO were on the low (DP 3.3; 534.6 Da) and high (DP 8.4; 1360.8 Da) ends of the continuum [41] and gave no quantitative information that could be used to derive purity.

Bouhnik, Y. et al performed a small (8 subjects per group), short, controlled study of the bifidogenic potential of several nondigestible carbohydrates, including enzymatic IMO. In that study no adverse effects were observed in any group, and the IMO group did not show a significant increase in *bifidobacteria* [42]. Conversely, Wang, S. et al performed a controlled study of fermented milk, supplemented with probiotics and enzymatic IMO at high levels, on mice and humans, and showed significant increases in *bifidobacteria* and *lactobacilli,* decreases

in fecal *enterobacilli,* and no adverse effects [43]. Similarly, Thitarum, *et al* performed a controlled study of fermented IMO supplemented diets (1%, 2% and 4% of diet) for young broiler chickens previously challenged with 10^8 cfu of *Salmonella enterica* ser. t*yphimurium*, and showed a significant 2-log reduction in the level of cecal *S. typhimurium* for the inoculated birds in the 1% IMO group, and no adverse effects for any group [44].

Conclusion

The specific fermentation IMO tested appears to be safe when ingested by rats at a wide range of levels that would all be substantially higher than would be expected for prebiotic supplementation of a normal human diet. No negative effects were observed in rats studied in terms of clinical chemistry, hematology or necropsy results. The test groups did not differ significantly from those on the control diet.

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