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The effects on nutrient utilization and stool quality of Beagle dogs fed diets with beet pulp, cellulose, and Miscanthus grass[1,2]

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Abstract

Dogs can benefit from dietary fibers. Traditionally, cellulose (**CE**) and beet pulp (**BP**) have been used by pet food companies as insoluble and soluble fiber sources. Miscanthus grass (**MG**) is a novel fiber ingredient made from *Miscanthus giganteus*, a C4 grass produced for its fiber content, but it has not been evaluated for dogs. The objectives of this study were to determine the effects of different fiber sources on nutrient utilization and stool consistency by dogs. Twelve Beagle dogs were fed 3 dietary treatments varying in their fiber sources (BP, CE, MG). Diets were fed for a 14-d period (9 d adaptation), fecal samples were collected (5 d total fecal collection) and scored. Nutrient digestibility was estimated using total fecal collection (**TFC**). Dogs fed BP diet had softer stools than dogs fed CE and MG (3.15 vs. 3.68 and 3.64, respectively). Wet fecal output was higher for dogs fed CE compared to MG, with dogs fed BP having the lowest values (254.3 g vs. 241.6 g vs. 208.5 g, respectively). Dogs fed CE and MG had lower DM digestibility than dogs fed BP ($P < 0.05$), dogs fed BP had lower CP digestibility compared with dogs fed MG and CE (81.4% vs. 85.5% and 85.8%, respectively). In conclusion, MG could be used as an alternative fiber source to CE.

Key words: beet pulp, cellulose, digestibility, dry dog food, fecal consistency, Miscanthus grass

Introduction

While pet owners may question the addition of fibers to a carnivore (dogs and cats) diet and make the choice to purchase diets dense in nutrients and energy, this may be detrimental to their pets. This hypothesis is supported by the increasing numbers of overweight and obese animals in our homes. In 2007, 52% of dogs and 55% of cats were considered overweight or obese by their veterinarians compared with 56% and 60% in 2017 (APOP, 2017). Clearly, the pet's energy intake is overestimated by the owner, which resulted in an increasing number of overweight and obese animals. Obesity is considered a disease by veterinarians which can negatively impact long-term animal health. For example, it can lead to joint, heart, metabolic, and endocrine issues, along with chronic inflammation (Kealy et al., 2002; German, 2006; Laflamme et al., 2006; German et al., 2009). Thus, weight control is a key factor to improve companion animal quality of life and overall longevity.

Pet food companies produce diets with reduced energy content that are intended for weight loss and management. They decrease calorie density by lowering fat and adding fibrous ingredients.

Unlike starch in the diet, fiber is not digested by the animal's digestive enzymes; thus, it contributes little if any calories. Historically, **CE** has been the standard fiber source in low calorie diets (Burrows et al., 1982; de Godoy et al., 2013; Koppel et al., 2015). The ingredient "cellulose" is produced from trees in the process of making paper pulp. Several studies have shown that CE is poorly fermented (Sunvold et al., 1995a,b), can decrease DM and OM digestibility in dogs (Muir et al., 1996), and increase fecal output (Wichert et al., 2002). In addition, the effects of CE addition are concentration dependent; therefore, higher inclusions in the food will decrease digestibility and increase fecal excretion (Burrows et al., 1982). Despite its benefits for caloric dilution, CE is expensive when compared to other fiber sources. Agricultural industries generate fibrous "wastes" as a result of producing human food ingredients. For example, these would include **BP**, wheat bran, corn fibers, peanut hulls, rice bran, pea fiber, and others. Beet pulp is a prominent fiber used in pet foods. It is generated from the sugar beet industry after sugar is extracted for the sweeteners market. Beet pulp has been evaluated in dog foods and found to be moderately fermentable (Sunvold et al., 1995a,b) and resulted in better DM and organic matter digestibility compared to CE (Howard et al., 2000; Middelbos et al., 2007). However, CP and CFat digestibility declined slightly when BP was added to the diet (Fahey et al., 1990a,b; Muir et al., 1996; Sabchuk et al., 2017). Similar to CE addition, there is a concentration dependent effect of BP addition to the diet. Fahey et al. (1990a) reported a linear decrease in DM, OM, and fat digestibility as the BP content of the diet increased from 0% to 12.5% in 2.5% intervals. However, fiber sources like BP do not reduce the calorie content to the degree CE does. Since their soluble fiber content is much greater than CE, short chain fatty acids (**SCFA**) are produced through fermentation and used as energy by the animal (Hamer et al., 2008; Voet et al., 2016). These agricultural co-products have been successful in the pet food industry. The pet food market is in a constant search for new ingredients and discounts the use of byproducts. One potential fiber ingredient option is **MG** (*Miscanthus giganteus*), which is a C4 grass. Since the fiber of this grass is the intended product, it might be a well-accepted alternative to CE. Miscanthus grass has also been explored for application in cellulosic ethanol production (Adams et al., 2018), construction materials, paper-pulping, and as an absorbent (Visser and Pignatelli, 2001). However, to our knowledge, MG has never been evaluated for a pet food application. It was our hypothesis that MG could be an alternative to CE in dog foods. The objectives of this study were to determine the effects of different fiber sources on nutrient utilization and stool consistency by dogs.

Materials and Methods

Ingredients and Dietary Treatments

Dietary treatments were made from a similar base ration (90%) and 1 of 3 fiber sources at 10% inclusion (Table 1). The high inclusion of dietary fibers was chosen to simulate a diet used for weight management. Diets were formulated according to AAFCO (2015) nutrient profiles for adult dogs at maintenance and to be isonutritional with the exception of the fiber source contribution (Table 2). The base ration ingredients were sourced as a preblend from a commercial feed mill (Fairview Mills, Seneca, KS). The experimental fiber sources included were MG (Renew Biomass, Springfield, MO), CE and BP (Fairview Mills, Seneca, KS).

To the preblend, fiber sources, chromium sesquioxide, and titanium dioxide were mixed in a paddle mixer (140 kg capacity) for 5 min. Dog foods were produced in a single screw extruder (model E525, Extru-Tech Inc., Seneca, KS). After extrusion, experimental diets were dried to less than 10% moisture in a convection oven. Briefly, 5 kg of extruded kibbles were evenly spread on a perforated cookie sheet. The cookie sheets were placed on racks and then in the oven for a predetermined temperature (115.5 °C) and time (50 min). Then coated with chicken fat and flavor enhancer. Coated diets were stored in plastic bags in a temperature-controlled room (25 °C) for 7 d prior to the start of the feeding trial and nutrient analyses.

Table 1. Ingredient composition of experimental diets, as is basis

Ingredient	Percentage
Fiber source	10.00
Chicken by-product meal, low-ash	29.96
Brewers rice	17.12
Corn	17.12
Wheat	14.55
Corn gluten meal	5.14
Titanium Dioxide	0.40
Chromium Sexquioxide	0.25
Potassium chloride	0.30
Salt	0.50
Choline chloride	0.23
Natural antioxidant	0.17
Vitamin premix[1]	0.15
Trace mineral premix[2]	0.10
Chicken fat[3]	3.00
Flavor enhancer[3]	1.00

[1]Vitamin E Supplement (119,816 IU×kg^{-1}), Niacin Supplement (97,104 mg×kg^{-1}), Calcium Pantothenate (18,279 mg×kg^{-1}), Vitamin A Supplement (25,744,497 IU×kg^{-1}), Thiamin Mononitrate (21,378 mg×kg^{-1}), Pyridoxine Hydrochloride (8,306 mg×kg^{-1}), Riboflavin Supplement (7,079 mg×kg^{-1}), Vitamin D3 Supplement (1,380,000 IU×kg^{-1}), Biotin (105 mg×kg^{-1}), Vitamin B12 Supplement (33 mg×kg^{-1}), Folic Acid (1,080 mg×kg^{-1}), as is basis.
[2]Zinc Sulfate (88,000 mg×kg^{-1}), Ferrous Sulfate (38,910 mg×kg^{-1}), Copper Sulfate (11,234 mg×kg^{-1}), Manganous Oxide (5,842 mg×kg^{-1}), Sodium Selenite (310 mg×kg^{-1}), Calcium Iodate (1,584 mg×kg^{-1}), as is basis.
[3]Added during the coating to the dried kibbles.

Table 2. Nutrient composition of experimental diets

Composition	MG[1]	CE[1]	BP[1]
Dry matter	94.30	95.39	95.19
Crude protein[2]	31.02	29.09	29.89
Crude fat[2]	9.00	7.55	7.89
Ash[2]	6.10	5.99	6.84
Crude fiber[2]	6.01	8.24	3.74
Total dietary fiber[2]	19.97	20.47	17.59

[1]MG: Miscanthus grass diet, CE: cellulose diet, and BP: beet pulp diet.
[2]Dry matter basis.

Feeding Trial and Sample Collection

The animal experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC protocol number 3645). Beagle dogs (12, from 2 to 4 yr old) were individually housed in metabolic cages (1.20 × 1.83 m) in a room with controlled temperature (23 °C) and fresh water available ad libitum throughout the duration of the study. Dogs were fed twice daily (8:00 and 16:30) and allowed 30 min to eat at each meal. Initial food allowance was calculated according to the NRC (2006) for adult dogs at maintenance (132 × $BW^{0.75}$) and food metabolizable energy was estimated according to modified Atwater values. Food intake was adjusted to ensure that the dogs would maintain body weight throughout the duration of the experiment. For periods 2 and 3, the amount of food provided was based on the amount of energy provided to each animal in the first experimental period. A subsample of each diet was collected weekly for chemical analysis.

Dogs were adapted to the test diet for 9 d and the following 5 d feces were collected. Dog body weight and body condition score (Laflamme, 1997) were recorded prior to, and at the end of each collection period. During the collection period, feces were collected twice daily, and defecation frequency was recorded. Feces were scored according to the following: 1 (liquid diarrhea) to 5 (hard pellets) with 3.5 considered ideal fecal score (Carciofi et al., 2008). After collection, fecal samples were frozen. A subsample of each experimental diet was collected weekly and then composited for further analyses.

Chemical Analysis

All sample analyses were performed in duplicates, with the exception of the TDF and insoluble fiber analyses that were performed in triplicates. If the variation between the duplicates and among the triplicates was higher than 5%, the analysis was repeated. At the end of each collection period, fecal samples were thawed and placed in aluminum pans and dried to touch in a convection oven at 55 °C for 48 h. Food and fecal samples were ground (Retsch ZM200, Germany) to pass a 1-mm screen and analyzed for moisture (AOAC, 1990; AOAC 930.15), CP (AOAC 990.03), fat by acid hydrolysis (CFat, AOAC 954.02), ash (AOAC 942.05), GE by bomb calorimetry (bomb calorimeter model 1351, Parr Instrument Company, Moline, IL), and total dietary fiber (**TDF**, Prosky et al., 1985). In addition, the dog foods were analyzed for crude fiber (CFiber, AOAC 962.09). Fiber sources were analyzed for neutral detergent fiber (Van Soest and Wine, 1967), ADF and acid detergent lignin (Van Soest, 1963), TDF, and insoluble and soluble fibers (Prosky et al., 1988).

Digestibility Estimation

Nutrient digestibility was estimated using total fecal collection (**TFC**) using the equation bellow:

$$TFC = ((\%ND * FI) - (\%NF * FO)) / ((\%ND * FI))$$

wherein: %ND is the percent nutrient in the diet, FI is the food intake in g, %NF is the nutrient in the feces, and FO is the fecal output in g.

Experimental Design and Statistical Analysis

This experiment was performed as a replicated 3×3 Latin Square design, wherein dog was the column factor, period the row factor, and diet was the treatment. Data was analyzed using statistical software via the general linear model procedure for mixed models (GLMMIX procedure in SAS; v. 9.4). The square, period, and dog nested within square were considered as random factors. Fisher's least square means were considered different at alpha of 5% and trends were considered when the *P*-value ranged from 0.05 to 0.10. The mean fecal score was considered different than 3.5 when the *P*-value was smaller than 0.05.

Results and Discussion

Fiber sources addition in dietary treatments were at 10% rather than an iso-TDF basis (Table 1). The thought behind the same inclusion was to have a high fiber content and a similar base line from the other ingredients of the diet. Thus, our theory was that the results reported would be due to the fiber source alone rather than differences from shifting the contribution from the other ingredients in the diet. Additionally, since weight management and weigh loss pet foods are usually high in fiber, we tried to simulate such diets by adding all tested fibers at 10% of the formula. Nutrient composition among diets was similar (Table 2). As targeted in the production protocol, the moisture for all diets was lower than the targeted 10%. Small variations among the dietary nutrient compositions were partially a result of the fiber sources and will be detailed further.

The CFiber and TDF content were lower for BP compared with MG. The CE diet had the highest CFiber and TDF contents. Crude fiber is measured by boiling the sample in a weak acid followed by boiling in a weak alkali (AOCS, 2017; AOCS Ba 6a-05 method). Due to this digestion, most of the soluble fibers and a portion of the insoluble fibers are removed from the sample. Thus, the dietary fiber content of the sample is underestimated; but it is the standard used on the label by the pet food industry (AAFCO, 2015). While BP has lower CFiber and TDF contents than MG and CE, the soluble fiber concentration is about 3 times higher for BP compared to MG and 10 times higher compared to CE (Table 3).

The fiber profile of MG is more similar to CE than BP. This is a function of the raw materials and how MG is produced. Miscanthus grass is made from the dry canes of *Miscanthus giganteus*. The leaves are separated from the canes as the plant dries in early winter and the plant enters a dormant state. When the field dried canes are harvested, they are ground to produce a fibrous ingredient. Thus, there is an increase in the structural fiber content in the raw material, since the canes have higher concentration of CE than the leaves (Milic et al., 2011). This differs from CE which is chemically derived by the wood pulping process (Dahl, 1884). In this method, wood chips are delignified and other insoluble and soluble fibers and lignin are solubilized and removed. Thus, the CE is concentrated and results in a higher insoluble fiber content when compared to MG (Table 3). The fiber profile of both BP and CE were similar to previous reports (Sunvold et al., 1995a; Jimenez-Moreno et al., 2013).

Food intake was similar among dietary treatments (average 236 g) and no refusals were observed throughout the duration of the feeding trial. Additionally, dog body weight and body condition score were maintained during the experimental procedure (average 10.56 kg and 5.21, respectively for body weight and body condition score; Table 4). Defecation frequency (average 2.96) was not affected by the type of fiber ingredient added to the diet. Fecal scores were similar for dogs fed MG and CE; however, dogs fed BP had softer stools. Wet fecal output was higher for dogs fed CE compared with dogs fed MG, with dogs fed BP having the

Table 3. Fiber fractions of dietary fiber source

Composition[1], %	Miscanthus grass	Cellulose	Beet pulp
DM	95.00	95.30	92.53
Crude fiber	45.20	72.70	18.70
ADF	53.70	80.60	24.30
NDF	73.80	88.40	31.60
Acid detergent lignin	13.00	0.70	5.90
Total dietary fiber	85.50	97.80	57.70
Insoluble fiber	78.60	95.30	33.30
Soluble fiber	6.90	2.50	24.40

[1]As is basis.

least amount of feces. Fecal DM followed the same trend as wet fecal output (Table 4). As proposed previously, BP is a moderately fermentable fiber source (Muir et al., 1996); therefore, a portion of the fiber is fermented and utilized by the microorganisms in the colon, while some of the fiber is poorly fermented or nonfermentable (33.3% insoluble fiber vs. 24.4% soluble fiber, Table 3). The fermentation of the fiber produces SCFA, and gasses (e.g., hydrogen, carbon dioxide, and hydrogen sulfide), which are either absorbed by the animal or expelled through flatulence (Yamka et al., 2006) and (or) in the breath (Felix et al., 2013). Due to the microbial utilization of the fibers for fermentation, less organic material is excreted in the feces. Conversely, CE and MG diets had a higher concentration of insoluble fibers. This type of fiber is known to be nonfermentable (Sunvold et al., 1995a,b). Thus, the more undigested and unfermented material is excreted by the dogs.

Additionally, as fermentation takes place, complex molecular structures are being metabolized into smaller molecules by the colonic microbiome. With an increase in the SCFA production, there can also be an increase in water in the lumen. As undigested materials (e.g., fibers) are fermented to the other more soluble molecules (SCFA, lactic acid, carbon dioxide, hydrogen gas, ammonia), an osmotic pull is created towards the lumen (Felix et al., 2013). Additionally, several of these substances are acids that at the luminal pH are ionized. Therefore, the luminal pH decreases over time due the transformation of soluble indigestible food components into SCFA, lactic acid, and carbon dioxide (Biagi et al., 2010; Felix et al., 2013). As a response to the drop in pH, the colon may secrete more water (with bicarbonate) into the lumen to increase the pH and decrease any possible chemical irritation. Thus, an increase in fecal moisture is likely a reaction of the dog's colon to a combination of these 2 factors (osmotic pull and drop in pH). While this hypothesis still needs to be confirmed, an increase in fecal water and decrease in fecal pH due to microbial activity was reported by different researchers (Fahey et al., 1992; Guevara et al., 2008; Biagi et al., 2010; Felix et al., 2013; Panasevich et al., 2013; Silva et al., 2016). For example, Guevara et al. (2008) fed dogs diets containing BP, and different types of corn fibers. These authors reported a decrease in fecal DM when BP (moderately fermentable fiber) was added to the diet compared to the tested corn fibers. While the TDF content of these fiber sources were similar, the soluble fiber content of the corn fibers was much lower than the BP (Guevara et al., 2008), thus supporting the hypothesis that the increase in soluble fibers in the colonic lumen may shift the water movement and decrease fecal DM. In addition to this shift in water movement in the colonic lumen, fiber sources have different water holding capacities that could also contribute to decrease water absorption and fecal DM.

Nutrient and energy digestibility were estimated by TFC (Table 5). Dogs fed BP diet had higher DM and OM digestibilities compared with dogs fed MG, and dogs fed CE had the lowest DM and OM digestibilities (Table 5). Gross energy digestibility was higher for dogs fed BP than MG and CE (85.2% vs. 82.3% and 81.8%, respectively). These results could be explained by the higher content of fermentable fibers in BP compared to the other 2 test fibers. As these fibers are fermented in the colon, more energy is absorbed by the animal (SCFA, lactic acid) and converted into gasses (carbon dioxide, hydrogen gas, methane, etc.); thus, less energy is eliminated as fecal material. The digestibilities of DM, OM, and GE are higher for fiber sources that have more fermentable fiber content. Silvio et al. (2000) fed dogs experimental diets varying in the proportion of insoluble and soluble fiber by changing the inclusions of CE and pectin and then measured digestibility at the ileum and total tract. They reported a decrease in fecal DM percentage as pectin content of the diet increased at the expense of CE, supporting the hypothesis that fermentation of soluble fibers could increase fecal water content. Yet, ileal DM digestibility was not affected by the insoluble to soluble fiber ratio of the diet. However, total tract DM digestibility increased as dietary CE was replaced by pectin. This is a good example that the fibers sources are responsible for changes in the DM, OM, GE, CP, CFat, and TDF total tract digestibility. Similar results were also reported by Cole et al. (1999) and Middelbos et al. (2007). The increase in apparent total tract digestibility is a result of the concentration of the products formed through fermentation. For example, Cutrignelli et al. (2009) used German Shepherd and Neapolitan Mastiff fecal inoculum and reported lower concentrations of acetate, propionate, and butyrate for pure CE compared to BP. In that experiment, only 2.5% of organic matter disappeared for the pure CE, while for BP it was 46.81%. Additionally, the maximum rate of fermentation of pure CE was about 10 times lower compared to BP. Similarly, in this experiment, both CE and MG had a higher concentration of insoluble (nonfermentable) fibers, thus dog fecal output was higher (Table 4) and DM, OM, and GE digestibilities were lower (Table 5). Conversely, CP and CFat digestibility were higher for dogs fed MG and CE than BP. Total dietary fiber digestibility was higher for dogs fed the BP diet compared with dogs fed the MG diet, and animals fed CE diet had the lowest TDF digestibility (63.0% vs. 46.1% vs. 37.5%, respectively; Table 5). The fermentation of the soluble fibers from the BP may have 2 outcomes: increased fermentation end products and microbial mass. Thus, as fermentation increases, more microorganisms are excreted by the animal and an underestimation of true digestibility is expected when fermentable fibers are present in the diet. Similarly, Muir et al.

Table 4. Food intake, defecation frequency, fecal score, wet fecal output, and fecal dry matter of dogs fed diets with different fiber sources

Diet	MG[1]	CE[1]	BP[1]	STD[2]	P-value
BW, kg	10.60	10.56	10.53	0.41	0.4483
Body Condition Score	5.19	5.23	5.21	0.37	0.8858
Food Intake, g/d/dog	235.2	234.0	234.6	6.33	0.6529
Defecation Frequency, no/d × dog	2.98	3.03	2.88	0.16	0.6293
Fecal Score [3]	3.64[a]	3.68[a]	3.15[b]	0.06	<0.0001
Wet Fecal Output, g/d × dog	241.6[b]	254.3[a]	208.5[c]	6.44	<0.0001
Fecal Dry Matter, %	38.70[b]	40.94[a]	29.25[c]	0.52	<0.0001

[1]Dietary treatments; MG: Miscanthus grass, BP: beet pulp, CE: cellulose
[2]STD: Standard deviation
[3]Fecal score: 1—liquid diarrhea, to 5—dry hard pellets.
[a-c]Means with unlike superscripts differ ($P < 0.05$).

Table 5. Apparent total tract digestibility of dogs fed diets with varying fiber sources estimated by total fecal collection method

Digestibility, %	MG[1]	BP[1]	CE[1]	STD[2]	P-value
DM	78.2[b]	81.3[a]	77.2[c]	0.37	<0.0001
OM	82.1[b]	86.1[a]	80.8[c]	0.32	<0.0001
GE	82.3[b]	85.2[a]	81.8[b]	0.24	<0.0001
CP	87.9[a]	84.5[b]	87.6[a]	0.24	<0.0001
Crude fat	90.7[a]	88.8[b]	90.9[a]	0.56	0.0099
Total dietary fiber	46.1[b]	63.0[a]	37.5[c]	0.72	<0.0001

[1]Dietary treatments; MG: Miscanthus grass, BP: beet pulp, CE: cellulose.
[2]STD: Standard deviation.
[abc]Means with unlike superscripts differ ($P < 0.05$).

(1996) reported a higher OM digestibility when dogs were fed BP diet compare to CE.

While the fiber composition of the diet has little impact on ileal digestibility, it can impact overall caloric content. Given that most of dogs in the United States are overweight (36.4%) and obese (19.6%; APOP, 2017), weight loss and management using insoluble nonfermentable fiber sources (e.g., MG and CE) could be an alternative to aid weight loss of overweight and obese animals. Even though the fecal output may increase, the goal of decreasing the energy intake would be met and there are no studies evaluating consumer's perception on fecal volume and (or) weight with the fiber content of the diet. Another benefit to the addition of dietary fibers to pet foods is to promote gut fill, improve satiety, and reduce begging and scavenging behaviors (Pappas et al., 1989). Fiber can also be used as a prebiotic. Pet foods targeting gut health gained popularity in recent years. In these diets, soluble fermentable fibers are preferred (e.g., BP). These fiber sources will serve as substrate for the microorganisms in the large intestine and stimulate fermentation. Butyrate, a fermentation end product, is the preferred fuel source for the colonocytes (Bergman, 1990; Topping and Clifton, 2001), and has been considered a potential for prevention of colon cancer in humans (Tungland and Meyer, 2002; Hamer et al., 2008). However, as detailed previously, with an increase in fermentation, luminal water content may increase, and feces could become softer. In this case, MG as an insoluble fiber source provided a good stool quality at a high level in the diet and reduced the energy intake by lowering energy digestibility; thus, it could be used as an alternative fiber source in dog foods.

Conclusion

Despite dogs being carnivores, they can benefit from fiber consumption for either weight management and (or) gut health. In general, dietary fiber decreases DM and OM total tract digestibility. The fiber composition of these 3 different fiber sources affected stool consistency and nutrient utilization differently. Dogs fed BP had softer stools and lower wet fecal weight and higher digestibility coefficients for DM, OM, GE, and TDF, whereas dogs fed CE and MG had harder stools and higher CP and CFat digestibility. Despite the differences in ingredient composition between CE and MG, both fiber sources affected nutrient digestibility and stool quality in a similar fashion. Considering these results, MG could be an alternative fiber source to CE in dry extruded dog foods.

Author Contributions

R.A.D.: Experiment conduction, data and sample collection, sample analysis, statistical analysis, data interpretation, and manuscript preparation. C.G.A.: Experiment design, data interpretation, and manuscript revision.

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