

# Use of Grain Sorghum as the Primary Cereal Ingredient in Premium Pet Food Products



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## PROJECT OBJECTIVES

The overall objective of this project was to develop nutritionally balanced and highly palatable premium pet food products with grain sorghum as the primary cereal ingredient and carbohydrate source. These premium pet foods will be targeted toward diabetic, obese and geriatric pets where the resistant and low glycemic nature of sorghum starch would be especially attractive, while utilizing a balanced formulation and the special 'dry processing' characteristic of extrusion to provide adequate and bio-available amino acid profile and palatability. The specific objectives of this study include:

1. Development of balanced formulations for extruded dry dog food with two low tannin sorghum varieties as the primary cereal ingredient and a rice-based formulation as the control.
2. Milling of grain sorghum to different particle sizes and its utilization in production of dry expanded dog food kibbles using extrusion processing.
3. Physico-chemical characterization of extruded dog food.
4. In-vitro digestibility studies for extruded dog food.
5. Evaluation of postprandial responses (glucose and insulin), fermentation in the colon (measurement of short chain fatty acids in feces) and in-vivo digestibility of sorghum-based diets in comparison with a standard rice-based diets using laboratory kennel dogs.
6. Palatability testing for sorghum-based pet foods in an industry-setting kennel.

## EXECUTIVE SUMMARY

Findings from this study include the following:

1. Extrusion can be used to process sorghum-based dry expanded dog food products with physico-chemical characteristics similar to products based on conventional rice and corn-based formulations. Sorghum can be an effective alternative to rice or corn as a carbohydrate source in extruded pet foods.
2. When properly processed and coated with fat and palatants, foods composed primarily of red or white sorghum are normally eaten by dogs, and no consumption issues were experienced.
3. High in-vivo digestibility indicated that raw material quality for diets including sorghum and the processing conditions were appropriate, and both red and white sorghum can replace corn in dog food without reducing digestibility of the diet.
4. Special processing considerations (such as fine grinding or high extrusion energy input) are not necessary for sorghum based diets to achieve good digestibility, thus having potential for reduction in the cost of production.
5. Coarse ground sorghum based diets had potential prebiotic effect with beneficial implications on intestinal and general health of the dogs, including production of butyric acid from colonic fermentation. Prebiotic resistant starch can be generated during processing of coarse ground sorghum-based diets without extra costs, at the same level (0.5 to 1.0 percent dry matter basis) as commercial prebiotics.
6. Sorghum-based diets appear to have lower glycemic index as compared to control corn and rice-based diets and require correspondingly a more 'muted' insulin response, which is potentially more beneficial for diabetic dogs.

7. Palatability trials indicated that dogs preferred sorghum (red) based diet as compared to corn based control diet with the former recorded as first choice food for 71 percent of the animals. The sorghum-based diet was also consumed overall in a much larger proportion than the control (75 percent versus 25 percent).

## **OBJECTIVES 1-3: FORMULATION, MILLING, EXTRUSION AND PHYSICO-CHEMICAL CHARACTERIZATION**

Research was focused on the physico-chemical properties and in-vitro digestibility of sorghum-based dog food produced using pilot-scale single screw extrusion, and the role of particle size and thermal/mechanical energy ratios during processing. Premium dog food diets based on two types of sorghum (white and red) were formulated and milled to three particle sizes (PS = 0.5, 0.8 and 1.6 mm). Control, iso-nutritional diets based on corn and rice were also formulated and milled to 0.8mm particle size. All treatments were extruded at constant processing conditions to produce dry expanded kibbles. In a separate experiment, the two sorghum-based '0.8mm PS' diets were also processed at three specific thermal energy: specific mechanical energy ratios (STE: SME), using different combinations of screw speed and preconditioning temperature (high STE at 300RPM/ 94-95°C, medium at 400RPM/ 82-85°C and low at 500/ 72-73°C). Water and steam flow in the preconditioner was varied to achieve the desired pre-conditioner temperature. The mean in-barrel moisture was 26-27 percent (wb). Diets based on red sorghum required higher SME input and also exhibited higher starch gelatinization and expansion as compared to those based on white sorghum, corn and rice. This pointed toward fundamental differences in processability due to composition of the base cereal ingredient. As particle size increased, SME decreased for both white and red sorghum diets due to less available surface area for hydration, heat penetration and viscosity development. For the same reasons, gelatinization level decreased for both sorghum types with increase in particle size (86-91 percent, 77-88 percent and 66-86 percent for 0.5, 0.8 and 1.6 mm PS, respectively). The resistant starch content of the red and white sorghum diets (2.13-2.56 percent and 1.51-1.97 percent, respectively) was relatively higher than that of the corn and rice diets (1.29 percent and 1.09 percent, respectively). Expansion also decreased with particle size in the case of white sorghum, but did not vary substantially in the case of red sorghum. Specific peak crushing force data ranged from 1.0-2.0 kgf/g, and trends indicated that both expansion and degree of gelatinization impacted the compressive strength of the dried expanded kibbles. In-vitro digestibility of red sorghum based diets was slightly higher (79-83 percent) than white sorghum (74-84 percent) and decreased with an increase in particle size. In-vitro digestibility of 0.8mm PS sorghum-based diets (83-84 percent) were comparable to control diets based on corn and rice (83-85 percent). STE: SME ratios did not have substantial impact on starch gelatinization, although higher STE led to increased expansion and lower mechanical strength. Overall results indicated that extrusion can be used to process sorghum-based dry expanded dog food products with physico-chemical characteristic similar to products based on conventional rice and corn based formulations. Sorghum can be an effective alternative to rice or corn as a carbohydrate source in extruded pet foods.

## **OBJECTIVES 4-5: DIGESTIBILITY AND OTHER IN-VIVO STUDIES**

Dogs were fed the experimental diets (sorghum-based diets versus control-based on corn/rice) in an in-vivo study at UNESP Brazil, which included evaluation of food intake, digestibility, fecal formation, quality and colonic fermentation, and postprandial blood glucose and insulin response analyses. Results for experimental studies on effect of particle size 0.5-1.6 mm and effect of extrusion thermal energy input are detailed in data tables provided at the end (Tables 1-6) and also described below. Some important conclusions include:

1. When properly processed and coated with fat and palatants, foods composed primarily of red or white sorghum are normally eaten by dogs and no consumption issues were experienced
2. High in-vivo digestibility indicated that raw material quality for diets including sorghum and the processing conditions were appropriate, and both red and white sorghum can replace corn in dog food without reducing digestibility of the diet.
3. Special processing considerations, such as fine grinding or high extrusion energy input, are not necessary for sorghum-based diets to achieve good digestibility, thus having potential for reduction in the cost of production.
4. Coarse ground sorghum-based diets had potential prebiotic effects with beneficial implications on intestinal and general health of the dogs
5. Sorghum-based diets appear to have lower glycemic index as compared to control corn and rice based diets and require correspondingly a more 'muted' insulin response, which is potentially more beneficial for diabetic dogs.

**Food intake:** No differences were found in food intake for any of the treatments ( $p > 0.05$ ). That meant that when fed the proper amount of food to keep health body weight, dogs were able to eat everything and no consumption issues were observed for sorghum-based foods. Sorghum inclusion was high (around 50 percent of the food), verifying that when properly processed and coated with fat and palatants, foods composed primarily of red or white sorghum are normally eaten by kennel dogs.

**Total tract apparent digestibility:** No differences on dry matter (total food digestibility), organic matter, protein and energy digestibilities was observed for the diets ( $p < 0.05$ ). The experimental diets had high digestibilities, with mean values of 83.0-84.9 percent for dry matter, 85.3-86.8 percent for organic matter, 86.4-87.6 percent for crude protein, 99.9 percent for starch and 87.9-88.5 percent for gross energy. Rice is generally considered a cereal with superior digestibility, however this was not confirmed for the brown rice used in the present study. It is important to consider that rice has less fiber than sorghum, and due this the rice-based food was formulated with more beet pulp to achieve similar nutrient composition. Not many published studies correct the fiber level in this manner for better comparison of cereal ingredients, which might account for some of the differences commonly observed. No effects of raw material particle size on nutrient digestibility was observed ( $p < 0.05$ ). This is interesting, as in the studied particle size range (0.5-1.6 mm fine grinding) is not a necessary considerations for sorghum-based diets. Digestibility of organic matter and gross energy was lower for the control corn diet processed with medium extrusion energy input when compared to red sorghum extruded with low energy ( $p < 0.05$ ); however no differences were observed among the other treatments consisting of white and red sorghum-based diets processed with low or high extrusion energy input. Overall in-vivo digestibility results point to high digestibility of sorghum-based dog food diets, which implies that the processing conditions and raw material quality for diets including sorghum were appropriate. Also, special processing, such as fine grinding or high extrusion energy input, is not necessary for sorghum-based diets to achieve good digestibility, thus having potential for reduction in the cost of production.

**Fecal production, quality and colonic fermentation:** No differences were observed between diets for fecal score, moisture content and production (as-is and on dry matter basis) ( $p < 0.05$ ). This is compatible with the no significant differences observed for digestibility as described above. The feces produced were very close to ideal on the adopted score system (score of 4), well-formed and shaped, and not too dry. Fecal pH, on the other hand, differed with lower pH for the feces of dogs fed the coarse grind red and white sorghum based diets ( $p < 0.05$ ) as compared to the rice and corn based control diets (Table 3). The lower pH is supported by data for short chain fatty acids,

which indicate higher concentration of fermentation products (total fatty acids, and propionate and butyrate in particular) in dogs fed the coarse sorghum diets. This indicates that part of the sorghum carbohydrates were fermented in the colon, acting as a prebiotic with potential beneficial implications on intestinal and general health of the dogs. Thermal energy input during extrusion did not have a significant effect ( $p>0.05$ ) on short or branched fatty acid concentration (Table 4).

**Postprandial glucose and insulin responses:** Postprandial blood glucose concentration data are summarized in Table 5. The time to reach peak glucose concentration after feeding was higher for sorghum-based experimental diets (160-245 min) as compared to the corn and rice-based control diets (140-160 min). The time to peak increased with the particle size of the grain sorghum used in the diets, and the differences with the control diets were significant ( $p<0.05$ ) in the case of diets formulated with coarser grain. Red sorghum-based diets had lower maximum blood glucose concentration (96-97 mg/dL) as compared to white sorghum (100-104 mg/dL) and control (102-103 mg/dL), and also lowest average increase in glucose concentration (3-4 mg/dL), although these differences were not statistically significant ( $p>0.05$ ). By definition, consumption of high glycemic index (GI) foods results in higher and more rapid increases in blood glucose concentrations than the consumption of low GI foods. The data from the current study suggest that sorghum-based diets are potentially lower in GI as compared to control corn and rice based diets, with a more pronounced effect observed with diets based on coarser ground grain and red sorghum. This needs further investigation and confirmation, but the interactions between 'tightly bound' sorghum proteins in the endosperm and starch granules might be one reason for the lower GI.

Postprandial insulin response data are summarized in Table 6. In general, sorghum-based diets and especially red sorghum (256-316 pmol/L) had lower insulin peak concentrations than corn and rice-based control diets (348-435 pmol/L). One exception was coarsest ground (1.6 mm) white sorghum, which seemed more an experimental anomaly than part of any trend. Also, time to reach peak insulin response was much higher for all sorghum based diets (162-228 min) as compared to control diets (120 min). The peak concentration and response time for insulin corresponded well with blood glucose data, and confirmed that sorghum-based diets are potentially more beneficial for diabetic dogs. In healthy animals, stored insulin is released when the body detects glucose as part of normal phase one insulin response. This allows consumption of food without much rise in blood glucose levels. However in animals with diabetes, who have little or no stored insulin, glucose can spike after meals as it takes time to produce new insulin, called the phase two response. That's why sorghum-based diets with lower GI are have the potential to be more beneficial for diabetic dogs.

## OBJECTIVES 6: PALATABILITY

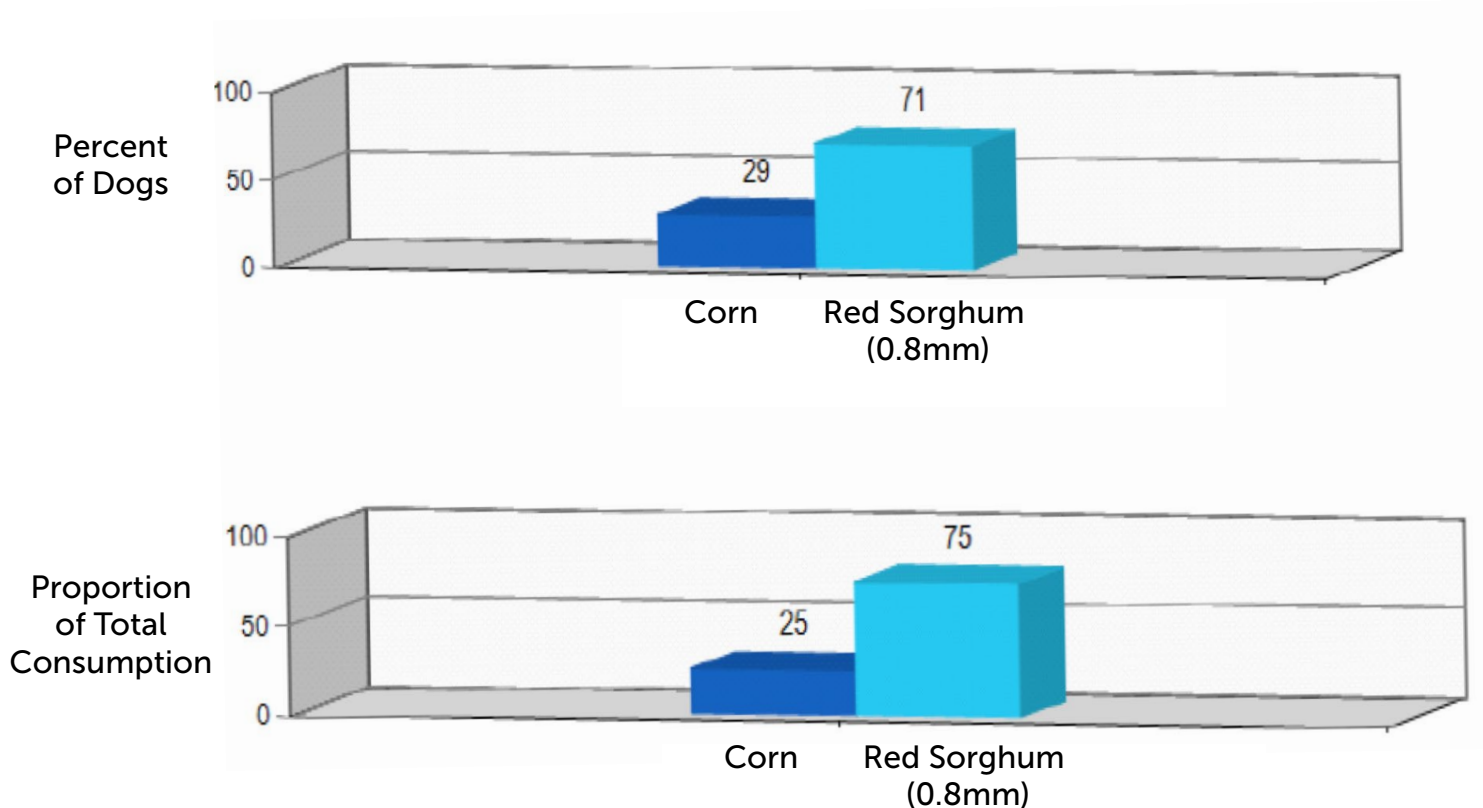
A separate group of dogs were used for palatability trials at Panelis. These trials were conducted with selected treatments (corn control versus red sorghum; white sorghum 1.6mm particle size diet versus 0.8mm particle size diet) using 36 adult dogs (both male and female, of various races). Results are summarized in Figures 1 and 2 below. As shown in Figure 1, dogs preferred sorghum (red) based diets as compared to corn-based control diets with the former recorded as first choice food for 71 percent of the animals. The sorghum-based diet was also consumed overall in much larger proportion than the control (75 percent versus 25 percent). These results were statistically significant ( $p<0.5$ ). Particle size of ground sorghum (0.8mm versus 1.6mm) did not have a significant effect on first choice or proportion of consumption. This further validates the finding based on in vivo digestibility tests that no special processing considerations (such as fine grinding) are necessary for sorghum-based diets, thus having potential for reduction in the cost of food production.

## REFERENCES

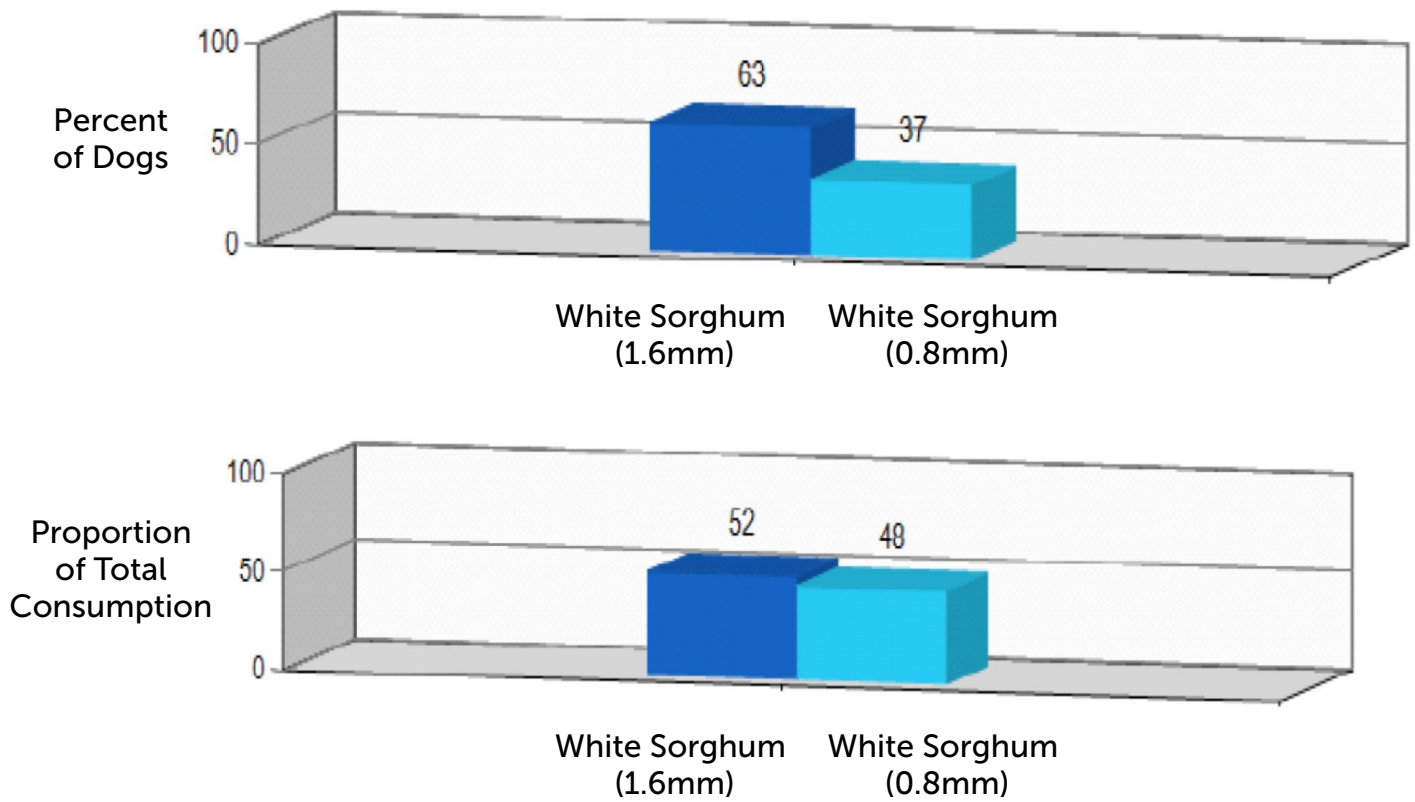
1. <http://lpi.oregonstate.edu/mic/food-beverages/glycemic-index-glycemic-load>
2. <https://www.diabetesselfmanagement.com/blog/learning-about-the-insulin-response/>

## PUBLICATIONS/ PRESENTATIONS

1. Putarov, T., Sa, F.C., Joseph, M., Carciofi, A.C., and Alavi, S. 2014. Sorghum-based extruded pet food: Impact of particle size and process conditions on physico-chemical attributes. AACC International Annual Meeting, October 5 - 8, Providence, RI. Oral Presentation.
2. Putarov, T., Sa, F.C., Joseph, M., Carciofi, A.C., and Alavi, S. 2016. Sorghum in pet food sustainability, processing, nutrition and palatability. Petfood Innovation Workshop & K-State Pet Food Experience, September 13-15, Manhattan, KS. Oral Presentation.
3. Three manuscripts under preparation for publication in peer-reviewed journals.



**Figure 1.** Palatability results (corn based control diet versus red sorghum 0.8mm grind based diet) – first choice of food (percent of dogs) and proportion of total consumption. Differences are statistically significant ( $P>0.05$ ).



**Figure 2.** Palatability results (white sorghum 1.6mm grind based diet versus 0.8mm grind based diet) – first choice of food (percent of dogs) and proportion of total consumption. Differences are NOT statistically significant ( $P>0.05$ ).

**Table 1.** Nutrient intake, apparent total tract digestibility, and faecal characteristics of dogs receiving the experimental diets based on different cereal sources – effect of particle size

Item	Experimental diets							SEM
	Corn	Rice	RS0.8	RS1.6	WS0.5	WS0.8	WS1.6	
Intake, g*kg of BW <sup>-1</sup> *d <sup>-1</sup>								
Dry matter	13.34	12.29	12.28	12.54	12.36	12.46	12.25	0.283
Organic matter	12.45	11.31	11.42	11.55	11.50	11.56	11.34	0.264
Crude protein	4.43	4.24	4.04	4.40	4.02	4.00	4.16	0.097
Apparent digestibility, %								
DM	82.89	81.47	84.89	82.60	83.25	83.14	82.59	0.373
OM	85.17	84.36	87.18	84.76	86.06	85.57	84.46	0.325
CP	86.78	84.60	88.09	86.54	86.59	86.28	85.89	0.309
Starch	99.7	99.8	99.7	99.7	99.9	99.8	99.7	0.298
Acid-Hydrolyzed fat	89.9	90.3	90.1	89.8	91.1	90.5	90.7	0.432
Gross Energy	86.67	86.22	88.45	86.47	87.60	87.34	86.10	0.287
ME, kcal/Kg	4,242 <sup>b</sup>	4,298 <sup>ab</sup>	4,375 <sup>ab</sup>	4,392 <sup>ab</sup>	4,402 <sup>ab</sup>	4,420 <sup>a</sup>	4,309 <sup>ab</sup>	16.07
Fecal characteristics								
Fecal Score	3.94	4.01	4.00	3.94	4.03	3.96	3.85	0.020
Fecal DM, %	34.66	34.84	34.66	31.63	35.12	33.11	30.04	0.508
Fecal pH	6.39 <sup>a</sup>	6.51 <sup>a</sup>	6.53 <sup>a</sup>	5.97 <sup>b</sup>	6.52 <sup>a</sup>	6.35 <sup>a</sup>	5.95 <sup>b</sup>	0.046
Feces, g*kg of BW <sup>-1</sup> *d <sup>-1</sup> (as-is)	7.22	7.09	5.79	7.40	6.39	6.89	7.64	0.234
Feces, g*kg of BW <sup>-1</sup> *d <sup>-1</sup> (dry)	2.48	2.45	2.00	2.33	2.22	2.28	2.28	0.067

<sup>a,b</sup> Means in the same row not sharing common superscript letters differ (P < 0.05).

n= 6 animals per diet;

**Table 2.** Nutrient intake, apparent total tract digestibility, and faecal characteristics of dogs receiving the experimental diets based on different cereal sources – effect of thermal energy ratio (high or HI versus low or LO).

Item	Experimental diets					SEM	p Value
	CC-MD	RSHI	RSLO	WSHI	WSLO		
Intake, g*kg of BW <sup>-1</sup> *d <sup>-1</sup>							
Dry matter	11.5	11.8	11.8	11.6	11.7	0.08	0.6665
Organic matter	10.8	11.0	11.0	10.8	10.9	0.08	0.7533
Crude protein	3.8	3.9	3.9	3.7	3.8	0.06	0.2936
Starch	4.9	5.1	5.0	4.8	4.8	0.05	0.3144
Total tract apparent nutrient digestibility, %							
Dry matter	83.9	85.6	86.4	84.1	84.6	0.37	0.1540
Organic matter	85.1 <sup>b</sup>	87.8 <sup>ab</sup>	88.2 <sup>a</sup>	86.3 <sup>ab</sup>	86.8 <sup>ab</sup>	0.36	0.0378
Crude protein	86.5	88.4	88.8	86.7	87.6	0.47	0.0892
Starch	99.9	99.9	99.9	99.9	99.9	0.32	0.3190
Gross Energy	86.8 <sup>b</sup>	89.2 <sup>ab</sup>	89.9 <sup>a</sup>	88.0 <sup>ab</sup>	88.4 <sup>ab</sup>	0.01	0.0138
Fecal characteristics							
Score	4.0	4.0	3.7	3.9	4.0	0.06	0.5120
DM, %	31.8	35.7	33.8	31.6	32.7	0.54	0.0803
pH	5.8	5.3	6.4	6.2	6.3	0.21	0.4844
g*kg <sup>-1</sup> *d <sup>-1</sup> (as-is)	6.5	5.2	5.2	6.3	6.0	0.19	0.0824
g*kg <sup>-1</sup> *d <sup>-1</sup> (dry)	2.0	1.9	1.7	2.0	2.0	0.04	0.1865

<sup>a,b</sup> Means in the same row not sharing common superscript letters differ (P < 0.05).

n= 6 animals per diet;

**Table 3.** Short-chain and branched fatty acids of dogs fed the experimental diets (mean and standard error, DM basis) - effect of particle size.

Item	Experimental Diets							SEM	P value
	Corn	Rice	RS0.8	RS1.6	WS0.5	WS0.8	WS1.6		
Short-chain fatty acid, mmol/g of DM									
Acetic acid	319.2	312.6	309.4	341.9	238.3	335.7	359.0	11.3	0.1081
Propionic acid	161.6 <sup>ab</sup>	137.3 <sup>b</sup>	157.3 <sup>ab</sup>	222.9 <sup>a</sup>	124.1 <sup>b</sup>	164.1 <sup>ab</sup>	211.2 <sup>a</sup>	7.4	0.0003
Butyric acid	59.5 <sup>b</sup>	57.1 <sup>b</sup>	59.8 <sup>b</sup>	73.5 <sup>ab</sup>	53.7 <sup>b</sup>	61.1 <sup>ab</sup>	84.8 <sup>a</sup>	2.5	0.0044
Total SCFA	540.3 <sup>ab</sup>	507.0 <sup>ab</sup>	526.4 <sup>ab</sup>	638.3 <sup>a</sup>	416.1 <sup>b</sup>	560.8 <sup>ab</sup>	655.0 <sup>a</sup>	19.5	0.0116
Branched-chain fatty acids, mmol/g of DM									
Isobutyric acid	6.6	5.5	7.5	4.9	5.3	5.6	5.0	0.3	0.2881
Valeric acid	0.4 <sup>b</sup>	0.6 <sup>b</sup>	0.7 <sup>b</sup>	2.5 <sup>a</sup>	0.6 <sup>b</sup>	0.7 <sup>b</sup>	2.4 <sup>a</sup>	0.1	0.0001
Isovaleric acid	10.8	7.7	10.9	8.6	9.2	8.1	8.0	0.5	0.2950
Total BCFA	17.8	13.8	19.1	16.0	15.1	14.3	15.4	0.7	0.4839
Total volatile fatty acid, mmol/g of DM	558.1 <sup>ab</sup>	520.8 <sup>ab</sup>	545.5 <sup>ab</sup>	654.3 <sup>a</sup>	431.2 <sup>b</sup>	575.1 <sup>ab</sup>	670.3 <sup>a</sup>	19.7	0.0129

<sup>a,b</sup> Means in the same row not sharing common superscript letters differ (P < 0.05).

n= 6 animals per diet;

**Table 4.** Short-chain and branched fatty acids of dogs fed the experimental diets (mean and standard error, DM basis) - effect of thermal energy ratio (high or HI versus low or LO).

Item	Experimental Diets					SEM	P value
	CC-MD	RSHI	RSLO	WSHI	WSLO		
Short-chain fatty acid, mmol/g of DM							
Acetic acid	367.4	269.6	289.6	327.9	318.4	11.86	0.0786
Propionic acid	223.5 <sup>a</sup>	139.9 <sup>b</sup>	151.5 <sup>b</sup>	150.0 <sup>b</sup>	153.8 <sup>b</sup>	6.94	<.0001
Butyric acid	67.2	53.2	70.9	63.6	70.9	3.44	0.4828
Total SCFA	658.1 <sup>a</sup>	462.8 <sup>b</sup>	512.0 <sup>ab</sup>	541.5 <sup>ab</sup>	543.1 <sup>ab</sup>	19.84	0.0201
Branched-chain fatty acids, mmol/g of DM							
Isobutyric acid	6.3	8.1	7.8	5.7	7.3	0.36	0.1744
Valeric acid	0.63	0.57	0.75	0.50	0.55	0.05	0.2408
Isovaleric acid	9.6	12.0	11.5	8.3	10.8	0.57	0.5889
Total BCFA	16.5	20.7	20.1	14.5	18.6	0.94	0.2076
Total volatile fatty acid, mmol/g of DM	674.6 <sup>a</sup>	483.4 <sup>b</sup>	532.0 <sup>ab</sup>	556.0 <sup>ab</sup>	561.8 <sup>ab</sup>	19.95	0.0278

<sup>a, b</sup> Means in the same row not sharing common superscript letters differ (P < 0.05).

n= 6 animals per diet;

**Table 5.** Blood glucose response (minimum, median and maximum concentration and time for peak concentration) in dogs fed experimental diets – effect of particle size.

Item	Control	RR	RS		WS		
		0.8	0.8	1.6	0.5	0.8	1.6
Minimum (mg/dL)	78.7±1.3	83.0±2.0	77.8±1.9	81.7±2.9	79.1±2.2	79.3±1.6	80.6±1.4
Median (mg/dL)	90.1±1.8	91.8±1.9	87.6±1.9	88.9±2.7	91.9±3.1	90.4±1.6	90.5±1.7
Maximum (mg/dL)	103.5±2.8	102.3±2.1	97.1±1.9	96.0±3.0	104.2±3.2	103.2±2.3	99.8±1.7
Time to peak (min)	160.0±45.6 <sup>b</sup>	140.0±29.7 <sup>b</sup>	185.0±36.7 <sup>ab</sup>	245.0±51.6 <sup>a</sup>	160.0±36.9 <sup>b</sup>	190.0±32.6 <sup>ab</sup>	230.0±28.6 <sup>a</sup>
Average increase (mg/dL)	6.5±2.3	9.1±2.0	4.2±2.3	3.4±1.9	8.6±2.2	6.0±1.8	15.4±1.3
Maximum increase (mg/dL)	19.3±3.9	18.6±2.7	13.5±2.5	10.3±2.4	20.7±2.8	18.5±2.6	16.4±1.7

<sup>a, b</sup> Averages in the rows without a common lowercase letter are significantly different (p <0.05). Comparison valid for the same variable;

**Table 6.** Blood insulin response (minimum, median and maximum concentration and time for peak concentration) in dogs fed experimental diets – effect of particle size.

Item	Control	RR	RS		WS		
		0.8	0.8	1.6	0.5	0.8	1.6
Minimum (pmol/L)	30.5±6.7	37.6±10.6	35.5±7.4	34.9±7.6	32.7±6.0	30.8±3.1	62.4±11.7
Median (pmol/L)	201.9±22.2	128.7±15.0	170.7±14.6	116.9±16.0	177.4±31.6	120.6±11.4	216.5±25.4
Maximum (pmol/L)	435.3±40.2 <sup>ab</sup>	348.1±40.3 <sup>bc</sup>	315.8±37.6 <sup>bc</sup>	255.7±46.6 <sup>c</sup>	406.5±70.7 <sup>ab</sup>	269.3±20.1 <sup>c</sup>	506.9±42.9 <sup>a</sup>
Time to peak (min)	120.0±15.5	120.0±21.9	162±28.6	204.0±55.0	216.0±19.6	228.0±18.3	220.0±25.3
Average increase (pmol/L)	192.3±20.4	83.3±10.2	131.9±13.6	116.9±16.0	152.3±23.0	91.9±8.9	149.2±11.4
Maximum increase (pmol/L)	431.0±25.9 <sup>a</sup>	302.0±39.1 <sup>bc</sup>	276.1±38.6 <sup>c</sup>	206.3±43.0 <sup>c</sup>	412.7±47.6 <sup>ab</sup>	223.8±15.6 <sup>c</sup>	437.1±34.6 <sup>a</sup>

<sup>a, b</sup> Averages in the rows without a common lowercase letter are significantly different (p <0.05). Comparison valid for the same variable;