

Longitudinal Assessment of the Canine Fecal Microbiota in Response to Dietary Hempseed By-Product and Oil: A 90-Day Nutritional Intervention Study

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Supplementary file

Table S1: Component percentages and nutritional composition of hemp diet.

Ingredient	Components (%)		
	Control (FC)	Hempseed byproduct (FB)	Hempseed oil (FO)
Cassava	31.41	30.41	31.41
Soybean meal	10	10	10
Cassava meal	10	-	10
Poultry meal	10	10	10
Meat & Bone meal	15	15	15
Tuna meal	8	8	8
Cellulose fiber	1	1	1
Hemp byproduct	-	11	-
Premix	2.01	2.01	2.01
Poultry fat	4.5	4.5	4.5
Beef tallow	3.5	3.5	2
Marine fish oil	1.5	1.5	1
Hempseed oil	-	-	2
Liquid palatants	2.5	2.5	2.5
Powder palatants	0.5	0.5	0.5
Preservative	0.08	0.08	0.08
TOTAL	100	100	100
Proximate nutritional composition (%)			
Moisture	7.29	6.91	3.48
Protein	26.1	27.26	26.77
Fat	13.35	12.57	16.74
Fiber	5.95	9.06	6.82
Ash	13.55	12.35	13.84

Methodology: Moisture (AOAC official method -2016-930.15), Protein (AOAC official method -2016-2001.11), Fat (AOAC official method -2016-2003.05), Fiber (AOCS approved procedure Ba 6a-05), Ash (AOCS official method -2016-942.05)

Table S2 An assessment of body condition score (BCS), feed intake, and fecal score with results summarized using means and standard deviations (SD).

		FC		FB		FO		<i>p</i> -value
BCS		Mean	SD	Mean	SD	Mean	SD	
(9-score)	Day-0	4.12	0.60	4.50	0.50	4.12	0.78	0.385
	Day-45	4.13	0.60	4.37	0.48	4.25	0.66	
	Day-90	4.25	0.66	4.25	0.43	4.37	0.68	
Feed intake								
(g/day)								
	Day (0-3)	268.54	30.05	262.29	24.98	263.75	26.30	0.066
	Day (44-46)	269.58	30.00	260.42	11.22	267.50	20.69	
	Day (88-90)	272.92	14.29	262.71	27.82	273.75	10.96	
Fecal score								
(7-score)								
	Day (0-3)	2.33	0.56	2.29	0.55	2.38	0.58	0.095
	Day (44-46)	2.04	0.46	2.42	0.50	2.25	0.53	
	Day (88-90)	2.25	0.53	2.42	0.50	2.50	0.51	

BCS was assessed using the 9-point Laflamme scale (1 = emaciated, 4.5 = ideal, 9 = obese). Fecal score was assessed using a 7-point scale (1 = very hard/dry pellets, 2 = hard/dry, 3 = firm/dry, 4 = firm/moist, 5 = soft/moist, 6 = loose/pudding-like, 7 = watery diarrhea). Feed intake is reported in grams per day (g/day).

Table S3 An assessment of blood hematology and chemistry with results summarized using means and standard deviations (SD).

Parameter (Abbreviation; normal range)	Date	FC	FB	FO	P-value
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
Hemoglobin HGB (11.9-18.9 g/dL)	Day 0	15.04 $\pm$ 1.12	13.71 $\pm$ 2.45	13.26 $\pm$ 1.47	0.139
	Day 30	13.53 $\pm$ 1.44	14.09 $\pm$ 1.94	14.03 $\pm$ 1.77	0.778
	Day 90	16.20 $\pm$ 1.50	15.21 $\pm$ 2.01	14.10 $\pm$ 0.82	0.071
Hematocrit HCT (35-57 %)	Day 0	44.53 $\pm$ 3.27	41.03 $\pm$ 6.26	39.68 $\pm$ 4.10	0.130
	Day 30	40.05 $\pm$ 3.80	43.25 $\pm$ 5.47	41.49 $\pm$ 4.39	0.396
	Day 90	47.93 $\pm$ 4.09	46.04 $\pm$ 5.15	41.80 $\pm$ 2.49	0.043
Mean corpuscular volume MCV (66-77fL)	Day 0	63.68 $\pm$ 2.81	64.25 $\pm$ 4.60	65.49 $\pm$ 4.51	0.664
	Day 30	65.53 $\pm$ 4.60	67.13 $\pm$ 5.54	64.03 $\pm$ 3.04	0.405
	Day 90	64.01 $\pm$ 2.56	66.33 $\pm$ 3.94	67.45 $\pm$ 4.27	0.208
Mean corpuscular hemoglobin MCH (21-26.2pg)	Day 0	21.51 $\pm$ 0.93	21.35 $\pm$ 1.17	21.88 $\pm$ 1.53	0.690
	Day 30	22.13 $\pm$ 1.69	21.80 $\pm$ 1.41	21.61 $\pm$ 0.99	0.761
	Day 90	21.63 $\pm$ 0.85	21.86 $\pm$ 1.16	22.77 $\pm$ 1.73	0.240
Mean corpuscular hemoglobin concentration MCHC(21-26.2g/dL)	Day 0	33.79 $\pm$ 0.52	33.29 $\pm$ 1.30	33.41 $\pm$ 1.02	0.589
	Day 30	33.74 $\pm$ 1.06	32.54 $\pm$ 0.79	33.76 $\pm$ 1.16	0.040
	Day 90	33.80 $\pm$ 0.55	33.00 $\pm$ 0.88	33.75 $\pm$ 0.91	0.108
Mean Platelet Volume MPV (8.7-13.2fL)	Day 0	10.63 $\pm$ 1.14	11.53 $\pm$ 1.26	11.71 $\pm$ 1.18	0.176
	Day 30	11.16 $\pm$ 1.16	10.84 $\pm$ 1.44	9.81 $\pm$ 1.40	0.134
	Day 90	9.81 $\pm$ 1.25	10.75 $\pm$ 1.53	10.95 $\pm$ 0.90	0.218
Plateletcrit PCT (0.14-0.46%)	Day 0	0.15 $\pm$ 0.06	0.16 $\pm$ 0.09	0.20 $\pm$ 0.10	0.581
	Day 30	0.19 $\pm$ 0.09	0.19 $\pm$ 0.08	0.20 $\pm$ 0.05	0.962
	Day 90	0.21 $\pm$ 0.05	0.16 $\pm$ 0.08	0.17 $\pm$ 0.10	0.435
Reticulocyte percent RET (0-1%)	Day 0	0.46 $\pm$ 0.20	1.24 $\pm$ 1.36	0.72 $\pm$ 0.48	0.197
	Day 30	0.56 $\pm$ 0.48	1.11 $\pm$ 1.73	0.30 $\pm$ 0.14	0.300
	Day 90	0.39 $\pm$ 0.20	0.65 $\pm$ 0.49	0.69 $\pm$ 0.55	0.353
Plasma proteins PP (6-7.5 g/L)	Day 0	7.25 $\pm$ 0.87	7.95 $\pm$ 1.02	7.15 $\pm$ 0.98	0.215
	Day 30	7.65 $\pm$ 1.09	8.13 $\pm$ 1.53	7.03 $\pm$ 0.70	0.187
	Day 90	6.85 $\pm$ 0.60	7.90 $\pm$ 1.43	7.53 $\pm$ 0.99	0.165
Erythrocytes RBC (4.95 - 7.87 10 ⁶ /uL)	Day 0	6.99 $\pm$ 0.37	6.43 $\pm$ 1.18	6.08 $\pm$ 0.74	0.115
	Day 30	6.14 $\pm$ 0.78	6.49 $\pm$ 1.09	6.50 $\pm$ 0.82	0.673
	Day 90	7.49 $\pm$ 0.66	7.00 $\pm$ 1.17	6.22 $\pm$ 0.61	0.046
White blood cell count WBC (5-14.1 10 ³ /uL)	Day 0	11.38 $\pm$ 1.61	10.96 $\pm$ 2.93	13.08 $\pm$ 2.04	0.163
	Day 30	12.88 $\pm$ 1.73	9.98 $\pm$ 3.83	11.77 $\pm$ 2.12	0.124
	Day 90	11.92 $\pm$ 3.31	10.23 $\pm$ 3.76	13.22 $\pm$ 1.22	0.221
Percent Neutrophil count (NEUT %) NEUT (55-75%)	Day 0	54.56 $\pm$ 6.58	56.83 $\pm$ 6.32	55.19 $\pm$ 8.17	0.806
	Day 30	51.95 $\pm$ 6.60	52.53 $\pm$ 4.89	53.15 $\pm$ 6.51	0.925
	Day 90	56.01 $\pm$ 7.95	53.15 $\pm$ 8.84	53.30 $\pm$ 5.34	0.719
Percent Lymphocyte count LYMPH (13-30%)	Day 0	24.80 $\pm$ 8.66	28.13 $\pm$ 8.53	23.85 $\pm$ 8.61	0.588
	Day 30	27.89 $\pm$ 8.99	27.78 $\pm$ 7.63	23.16 $\pm$ 5.72	0.379
	Day 90	23.61 $\pm$ 7.62	28.96 $\pm$ 7.81	28.63 $\pm$ 9.69	0.383

Parameter (Abbreviation; normal range)	Date	FC	FB	FO	P-value
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
Percent Monocyte count MONO (2-10%)	Day 0	5.14 $\pm$ 1.72	5.36 $\pm$ 1.99	4.86 $\pm$ 0.93	0.826
	Day 30	5.11 $\pm$ 0.45	6.81 $\pm$ 3.00	5.64 $\pm$ 1.33	0.215
	Day 90	5.23 $\pm$ 0.99	6.14 $\pm$ 2.73	3.97 $\pm$ 1.52	0.141
Percent Eosinophil count EO (0-9%)	Day 0	15.30 $\pm$ 5.91	9.43 $\pm$ 5.46	15.89 $\pm$ 7.59	0.106
	Day 30	14.93 $\pm$ 5.75	12.31 $\pm$ 5.15	17.91 $\pm$ 7.54	0.222
	Day 90	15.05 $\pm$ 5.36	11.14 $\pm$ 3.80	13.80 $\pm$ 5.75	0.299
Percent Basophil count BASO (0-1%)	Day 0	0.20 $\pm$ 0.12	0.26 $\pm$ 0.18	0.21 $\pm$ 0.06	0.614
	Day 30	0.14 $\pm$ 0.05	0.66 $\pm$ 1.04	0.16 $\pm$ 0.08	0.219
	Day 90	0.10 $\pm$ 0.08	0.61 $\pm$ 0.65	0.13 $\pm$ 0.08	0.035
Blood urea nitrogen BUN (8-28 mg%)	Day 0	18.63 $\pm$ 2.67	16.75 $\pm$ 3.41	21.00 $\pm$ 8.52	0.323
	Day 30	13.75 $\pm$ 3.49	15.25 $\pm$ 3.41	12.50 $\pm$ 2.07	0.222
	Day 90	14.75 $\pm$ 2.71	17.63 $\pm$ 3.96	16.50 $\pm$ 2.66	0.225
Creatinine CRE (0.50-1.70 mg%)	Day 0	0.97 $\pm$ 0.15	1.07 $\pm$ 0.20	1.03 $\pm$ 0.17	0.852
	Day 30	0.98 $\pm$ 0.10	1.13 $\pm$ 0.29	0.96 $\pm$ 0.19	0.687
	Day 90	1.00 $\pm$ 0.13	1.07 $\pm$ 0.21	0.98 $\pm$ 0.10	0.906
Cholesterol CHOL (136-392 mg/dL)	Day 0	167.50 $\pm$ 13.72	151.38 $\pm$ 36.77	152.88 $\pm$ 28.00	0.454
	Day 30	145.00 $\pm$ 20.23	136.88 $\pm$ 26.84	148.38 $\pm$ 17.55	0.567
	Day 90	137.88 $\pm$ 13.95	140.50 $\pm$ 21.75	148.33 $\pm$ 17.03	0.554
Triglycerides TRIG (23-102 mg/dL)	Day 0	53.13 $\pm$ 11.05	47.50 $\pm$ 7.71	49.25 $\pm$ 16.02	0.641
	Day 30	49.25 $\pm$ 8.92	45.50 $\pm$ 10.78	51.13 $\pm$ 14.38	0.065
	Day 90	42.75 $\pm$ 12.86	40.50 $\pm$ 9.21	43.67 $\pm$ 5.20	0.826
Total protein (TP) TP (5.4-7.5 g/dL)	Day 0	6.65 $\pm$ 0.79	7.20 $\pm$ 1.08	6.55 $\pm$ 0.80	0.316
	Day 30	6.70 $\pm$ 0.81	7.40 $\pm$ 1.28	6.45 $\pm$ 0.76	0.158
	Day 90	6.19 $\pm$ 0.48	7.36 $\pm$ 1.18	7.10 $\pm$ 0.79	0.037
Albumin ALB (2.3-3.1g/dL)	Day 0	2.90 $\pm$ 0.27	2.80 $\pm$ 0.59	2.79 $\pm$ 0.30	0.840
	Day 30	2.80 $\pm$ 0.44	2.81 $\pm$ 0.52	2.74 $\pm$ 0.28	0.932
	Day 90	2.91 $\pm$ 0.22	2.95 $\pm$ 0.50	2.85 $\pm$ 0.23	0.872
Aspartate aminotransferase AST (13-15 U/L)	Day 0	35.88 $\pm$ 7.70	34.63 $\pm$ 14.18	31.63 $\pm$ 9.32	0.723
	Day 30	35.13 $\pm$ 19.42	32.63 $\pm$ 12.45	33.13 $\pm$ 6.31	0.929
	Day 90	32.13 $\pm$ 4.73	30.88 $\pm$ 12.79	35.17 $\pm$ 15.22	0.781
Alanine aminotransferase ALT (10-109 U/L)	Day 0	41.75 $\pm$ 33.71	22.00 $\pm$ 9.20	42.00 $\pm$ 51.90	0.459
	Day 30	53.88 $\pm$ 84.43	18.63 $\pm$ 7.23	31.38 $\pm$ 12.78	0.371
	Day 90	33.63 $\pm$ 15.41	19.13 $\pm$ 8.10	61.17 $\pm$ 90.97	0.286
Alkaline phosphatase (ALP) ALP (8-76 U/L)	Day 0	23.38 $\pm$ 10.64	44.13 $\pm$ 57.66	28.50 $\pm$ 8.47	0.463
	Day 30	24.75 $\pm$ 13.45	34.63 $\pm$ 43.66	19.13 $\pm$ 9.49	0.518
	Day 90	18.88 $\pm$ 10.76	26.13 $\pm$ 26.57	21.17 $\pm$ 9.00	0.719

Table S4 Analysis of PERMANOVA (Bray-Curtis)

Term	Df	Sum of SQS	R ²	Statistic	<i>P</i> -value	Pairwise of DAY_collect at <i>P</i> = 0.001		
DAY_collect	2	1.5407	0.1299	6.4148	0.001	Comparison	P.value	P.adjust
GROUP_treatment	2	0.2265	0.01909	0.9429	0.461	0 vs 30	0.001	0.003
Residual	84	10.0875	0.85047			0 vs 90	0.001	0.003
Total	88	11.8612	1			30 vs 90	0.014	0.028

Table S5 The most abundant genera (> 0.1% relative abundance of all reads) in healthy dogs fed hemp diet

Taxonomy	Dietary treatment			SEM	P-value		
	FC	FB	FO		Diet	Day	Diet*Day
<i>Blautia</i>	23.8	24.7	23.2	2.43	0.90	0.04	0.01
<i>Peptoclostridium</i>	16.8	15.4	15.7	1.74	0.82	0.04	0.51
<i>Collinsella</i>	11.8	15.8	12.2	1.61	0.12	0.03	0.05
<i>HT002</i>	6.6	6.4	4.0	2.12	0.59	0.67	0.32
<i>Ligilactobacillus</i>	5.7	6.4	5.0	1.66	0.82	<0.01	0.95
<i>Romboutsia</i>	3.7	3.6	1.9	0.95	0.31	0.24	0.86
<i>Turicibacter</i>	2.0	2.6	2.8	0.69	0.66	0.12	0.90
<i>Holdemanella</i>	2.7	1.9	1.8	0.35	0.17	0.04	0.79
<i>Streptococcus</i>	1.8	2.6	2.1	0.81	0.77	0.01	0.51
<i>Clostridium_sensu_stricto_1</i>	1.9	0.7	1.1	0.37	0.07	0.22	0.92
<i>Slackia</i>	1.1	1.1	1.0	0.15	0.66	0.05	0.07
<i>Catenibacterium</i>	1.6	0.9	0.5	0.50	0.31	0.00	0.58
<i>Allobaculum</i>	0.9	0.9	0.6	0.19	0.44	0.48	0.13
<i>Faecalibacterium</i>	1.0	0.3	1.0	0.27	0.10	<0.01	0.01
<i>Peptococcus</i>	0.5	0.5	0.5	0.10	0.98	<0.01	0.82
<i>[Ruminococcus]_gnavus_group</i>	0.7	0.4	0.8	0.20	0.44	0.02	0.70
<i>Sellimonas</i>	0.7	0.5	0.6	0.08	0.17	0.30	0.67
<i>Lactobacillus</i>	1.4	0.2	0.1	0.71	0.34	0.42	0.48

Values are least-squares means of relative abundance $\pm$ standard error of the mean (SEM). Significant difference at p -value ≤ 0.05 .

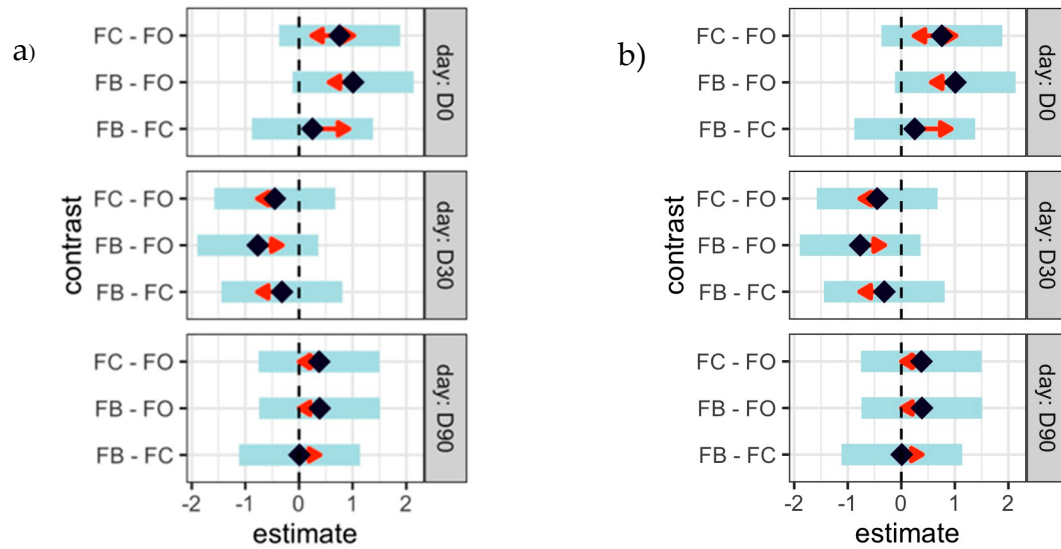


Figure S1 pairwise contrast plots for the three diets (FB, FC, FO) across three timepoints (D0, D30, D90).

a) FDR method (adjust = "fdr"; b) Tukey method (adjust = "tukey")

No statistically significant differences were found. Across all timepoints, pairwise comparisons between the FB, FC, and FO diets revealed no statistically significant differences, as all 95% adjusted confidence intervals overlapped with zero. The most notable trend occurred at Day 0 between the FB and FO diets ($p=0.0888$), but this difference did not reach significance after applying the Tukey adjustment. By Day 90, the mean estimates for all dietary groups converged, showing nearly identical values and high p -values (up to $p=0.9998$) for the comparisons