Effects of Temperature and Humidity on Diet Stability During Transit to the United Kingdom followed by 18 Months of Storage at IPS

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Background: Many factors can affect the shelf life of a diet including temperature, light, moisture and oxygen. Previous data from a 2011 study found that our diets are nutritionally complete up to 18 months from the date of manufacture when stored under recommended temperature and humidity conditions (<72º/21ºC and ≤ 50% humidity). Another study in 2015 evaluated product stability during trans-Pacific transport via ocean liner from United States to Taiwan followed by storage at our Taiwanese Certified LabDiet® Dealer's facility at 70°F (21°C) and 50% relative humidity. Temperature and humidity levels exceeded our recommended storage conditions during the 50-day transit period by up to 20ºF and 20% humidity. Despite exposure to excessive environmental conditions, diets were found to be nutritionally stable after 24 months from the date of manufacture. Conditions during trans-Atlantic transit and storage in the United Kingdom may vary from those experienced in the Taiwan study. Considering the temperate climate in UK, the warehouse that LabDiet® products are stored in at International Product Supplies (IPS) is not climate controlled. Previous records showed temperatures frequently reach between 20-25°C during the summer months, and occasionally above 25°C (e.g. 26.5°C and 26.1°C on 17&18 June 2017). Relative humidity is often recorded at >80%. Historically, no issues have been reported for LabDiet® products stored under these conditions, however, nutritional data over time had not been extensively evaluated.

Objective: The objective of this study was to evaluate product stability of diets during trans-Atlantic transport followed by storage at our Certified LabDiet® Dealer's warehouse in the United Kingdom for up to 18 months post manufacture date.

Study design: LabDiet® 5002, 5048 and 5L66 were manufactured in July/August and then transported via ocean liner from the United States to the United Kingdom from late September to early October 2018. Diets were stored at IPS's warehouse until February 2020. Schedule details listed below. Data loggers that record time, temperature and humidity were placed within the container to monitor conditions during transport (Figure 1). Temperature and humidity were recorded at the IPS warehouse throughout the study (Table 1).

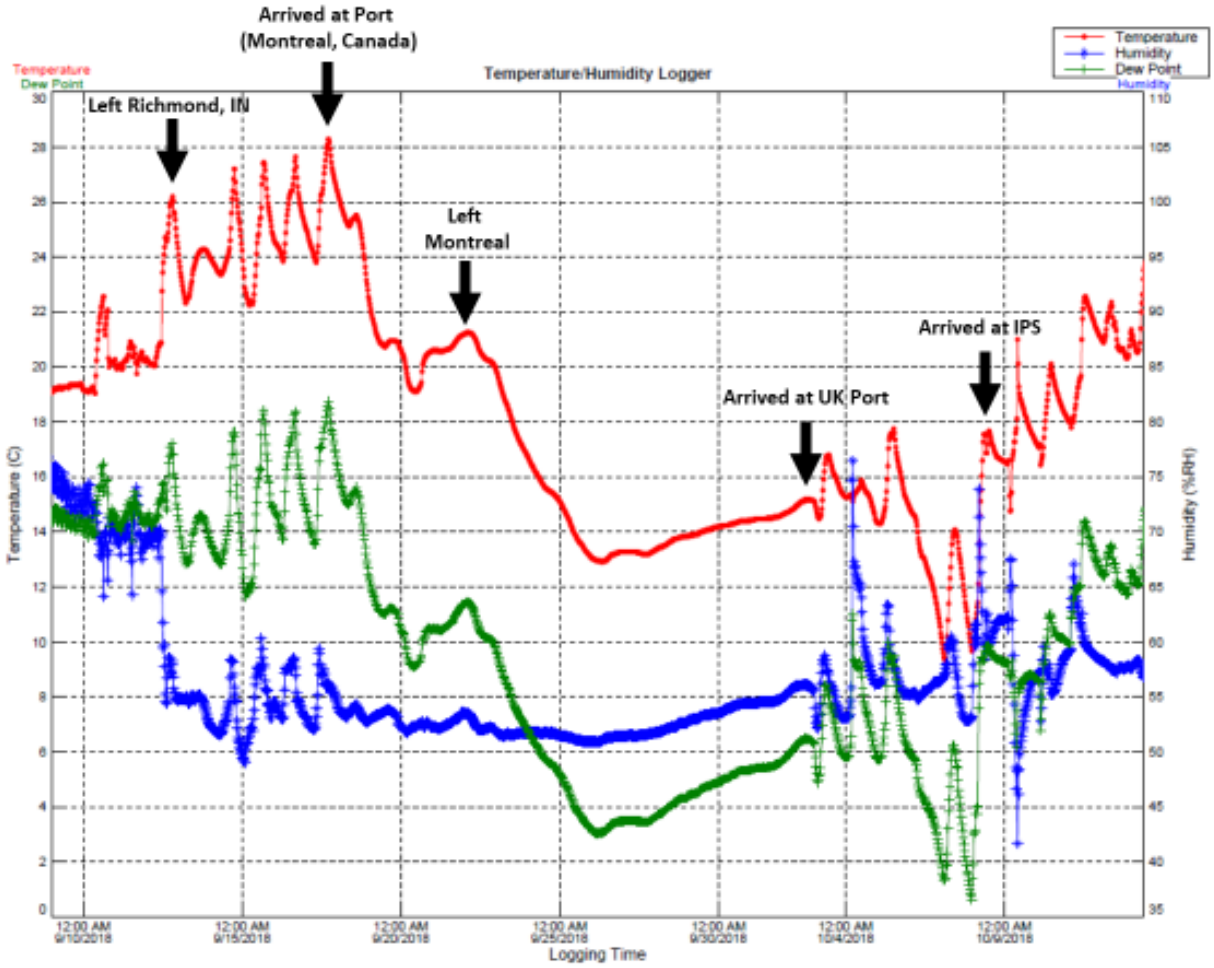
Schedule:
Jul 23: LabDiet® 5L66 manufactured
Aug 2: LabDiet® 5002 manufactured
Aug 17: LabDiet® 5048 manufactured
Sept. 12: All products left Richmond, IN, USA
Sept. 17: Arrived at port in Montreal, Quebec, Canada
Sept. 20: Loaded onto vessel
Sept. 22: Departed Montreal
Oct. 2: Arrived in Southampton, England, UK
Oct 7: Delivered to IPS facility

A sample was taken from each diet for nutritional analysis post manufacturing and samples were sent to Covance Laboratories/Eurofins in Madison, WI for nutritional analysis. Following arrival

at IPS, additional samples were taken at 3 month intervals and sent to Eurofins, UK in Wolverhampton, England, All three products were assayed for the following: moisture, protein, fat, fiber, ash, calcium, phosphorus, selenium, vitamins A, D, E, B1 (thiamin) and B6 (pyridoxine). LabDiet® 5048 was also assayed for vitamin C. Analytical results are reported in Tables 2, 3, and 4.

Results:

Figure 1.



The recommended storage conditions for laboratory animal diets are <21ºC and 50% or less relative humidity. Transport from the manufacturing facility in Richmond, IN to arrival at IPS took a total of 25 days. LabDiet® products were exposed to the highest temperatures (22-28ºC), during the 10-day continental transport from Richmond, IN to port in Montreal, Quebec. It took an additional 10 days to cross the Atlantic Ocean in which the ambient temperature dropped from 20ºC to as low as 13ºC. The temperatures upon arrival to the UK varied between 14-22ºC. Humidity exceeded the recommendation conditions of <50% throughout the journey.

Table 1: Highest recorded temperature and humidity during storage at IPS

Month	Temp, °C	Humidity, %
October 2018	21.2	61
November 2018	14.6	71
December 2018	13.1	67
January 2019	11.6	72
February 2019	14.7	75
March 2019	15.5	77
April 2019	18.0	63
May 2019	20.4	63
June 2019	21.8	78
July 2019	30.6	71
August 2019	26.0	74
September 2019	21.0	77
October 2019	17.2	73
November 2019	14.5	83
December 2019	12.2	79
January 2020	12.3	82
February 2020	12.4	68

Diets were stored at IPS for 17 months from Oct. 2018 to Feb. 2020. Ambient warehouse temperature was below 21ºC for 15 of the 17 months with two exceptions, once on Oct. 10, 2018 (21.2ºC) and the other on Jun. 28, 2019 (21.8ºC). July and August (2019) experienced hotter temperatures which is typical. Similar to previous years, temperatures frequently reached between 20-25°C during these months, and occasionally above 25°C (e.g. 25.9-30.6°C from Jul. 23-26th and 26ºC on Aug. 27th). Humidity exceeded 50% during storage, 70% of the time. The highest humidity values recorded were 81, 83, 83% on Nov. 11, 25 and 26th (2019) and 81, 82% on Jan. 14 and 22 (2020), respectively. Otherwise humidity recordings were below 80%.

Table 2: Nutrient stability of LabDiet® 5002, Certified Rodent Diet

Nutrient	Nutr. Req.[1]	Spec[2]	Initial[3,4]	3mo	6mo	9mo	12mo	15mo	18mo
Moisture, %	N/A	<12.00	9.04	8.84	9.18	8.84	8.37	8.82	9.03
Crude Protein, %	15.0-18.0	20.70	21.80	21.30	22.20	22.30	21.80	21.50	22.80
Ash, %	N/A	5.70	6.13	6.00	6.00	6.20	6.20	6.30	6.10
Fat, %	5.0	5.00	6.00	6.00	4.50	5.80	5.30	3.50	4.80
Crude Fiber, %	N/A	4.60	4.65	4.40	4.40	4.20	4.50	5.20	4.70
Ca, %	0.5	0.80	0.80	0.88	0.74	0.86	0.87	0.72	0.91
P, %	0.3	0.59	0.57	0.60	0.60	0.59	0.58	0.55	0.60
Se, ppm	0.2	0.34	0.24	0.26	0.28	0.26	0.28	0.29	0.27
Vitamin A, IU/g	2.4	15.00	14.00	12.90	9.22	10.90	9.46	8.60	9.20
Thiamin, ppm	4.0-6.0	15.00	8.70	12.20	11.80	11.70	10.90	11.20	11.30
Pyridoxine, ppm	1.0-4.0	6.00	5.41	3.91	3.87	3.56	3.97	3.60	4.68
Vitamin D, IU/g	1.0	2.60	N/A	2.19	2.32	1.80	2.16	2.11	1.88
Vitamin E, IU/kg	32.00	65.00	80.40	77.90	71.67	72.20	70.30	69.88	62.10

[1] Nutrient requirements of mice and rats according to Nutrient Requirements of Laboratory Animals (NRC), 1995

[2] Calculated nutrient composition on spec sheet

[3] Initial values were from samples taken immediately post-manufacturing. These values will differ from the calculated values on the respective diet spec sheets as they account for loss that occurred during manufacturing as well as potential assay variation.

[4] Initial samples were assayed by Covance Laboratories/Eurofins (Madison, WI)

Table 3: Nutrient stability of LabDiet® 5048, Certified Primate Diet

Nutrient	Nutr. Req.[1]	Spec[2]	Initial[3,4]	3mo	6mo	9mo	12mo	15mo	18mo
Moisture, %	N/A	<12.00	9.47	8.15	8.18	8.26	8.11	8.78	8.17
Crude Protein, %	15.0-22.0	25.80	27.30	26.80	26.60	26.70	26.50	26.20	26.60
Ash, %	N/A	6.50	6.92	7.00	7.00	7.00	6.90	7.10	6.80
Fat, %	N/A	6.60	5.60	5.50	4.40	5.30	4.80	4.70	5.50
Crude Fiber, %	N/A	4.10	4.43	3.80	4.40	4.20	3.70	4.20	3.50
Ca, %	0.8	1.15	1.26	1.33	1.29	1.28	1.37	1.28	1.42
P, %	0.6	0.67	0.72	0.66	0.69	0.69	0.74	0.71	0.72
Se, ppm	0.3	0.37	0.20	0.22	0.17	0.19	0.21	0.22	0.20
Vitamin A, IU/g	8.0	43.00	12.00	28.50	26.40	25.50	28.30	26.90	24.20
Thiamin, ppm	3.0	15.00	9.90	12.20	10.90	11.80	11.40	11.00	11.40
Pyridoxine, ppm	4.0	14.00	12.50	9.53	8.42	9.01	8.66	8.93	8.55
Vitamin D, IU/g	2.5	6.70	N/A	5.60	4.88	6.32	4.76	5.16	4.88
Vitamin E, IU/kg	50.0-100.0	110.00	N/A	105.70	88.67	99.11	106.20	99.88	100.00
Vitamin C, mg/g	0.2	0.75	0.92	1.11	N/A	N/A	1.11	1.06	1.06

[1] Nutrient requirements of non-human primates according to Nutrient Requirements of Nonhuman Primates (NRC), 2003
[2] Calculated nutrient composition on spec sheet
[3] Initial values were from samples taken immediately post-manufacturing. These values will differ from the calculated values on the respective diet spec sheets as they account for loss that occurred during manufacturing as well as potential assay variation.
[4] Initial samples were assayed by Covance Laboratories/Eurofins (Madison, WI)

Table 4: Nutrient Stability of LabDiet® 5L66, Certified High-Density Canine Diet

Nutrient	Nutr. Req.[1]	Spec[2]	Initial[3,4]	3mo	6mo	9mo	12mo	15mo	18mo
Moisture, %	N/A	12.00	6.35	7.10	7.15	7.04	6.38	6.98	6.61
Crude Protein, %	8.0-18.0	27.10	27.80	27.60	27.90	27.90	28.60	27.60	27.40
Ash, %	N/A	7.30	7.14	7.10	7.10	7.20	7.20	7.20	7.10
Fat, %	4.0-8.0	17.50	18.80	17.40	15.60	17.70	17.50	18.20	19.00
Crude Fiber, %	N/A	2.70	2.31	2.40	2.90	2.20	2.60	2.80	1.80
Ca, %	0.2-0.8	1.30	1.54	1.68	1.53	1.49	1.49	1.56	1.53
P, %	0.3-1.0	0.85	0.90	0.94	0.90	0.88	0.94	0.92	0.95
Se, ppm	0.21	0.45	0.30	0.09	0.31	0.33	0.31	0.32	0.32
Vitamin A, IU/g	4.0	26.00	18.70	20.60	19.90	19.70	18.60	17.70	20.50
Thiamin, ppm	2.1	14.00	9.50	14.30	14.40	14.10	14.60	13.70	13.80
Pyridoxine, ppm	1.5	16.00	14.50	11.20	12.10	11.20	10.90	11.00	11.00
Vitamin D, IU/g	0.6	4.00	N/A	3.28	3.73	3.70	3.47	3.48	3.41
Vitamin E, IU/kg	30.0	200.00	175.00	185.00	180.00	177.78	175.60	178.88	182.00

[1] Nutrient requirements of puppies and adult dogs according to Nutrient Requirements of Dogs and Cats (NRC), 2006
[2] Calculated nutrient composition on spec sheet
[3] Initial values were from samples taken immediately post-manufacturing. These values will differ from the calculated values on the respective diet spec sheets as they account for loss that occurred during manufacturing as well as potential assay variation.
[4] Initial samples were assayed by Covance Laboratories/ Eurofins (Madison, WI)

A couple of things must be considered when interpreting the analytical values. First, initial samples were analyzed at a different laboratory than subsequent samples (3-18mo). Variation between laboratories may contribute to differences seen between the initial and subsequent data points. We have noted variation between initial and 3-month data in previous studies. Unfortunately having the samples assayed at the same laboratory is not feasible. The largest differences observed were between initial and 3-month samples with the initial sample testing lower in multiple nutrients for the LabDiet® 5048 and 5L66. Comparing the remaining (3 through 18-month) data, very little changes were noted suggesting minimal nutrient loss

occurred. The second thing to consider is analytical variation for the nutrients being assayed. For example, the Association of American Feed Control Officials Incorporated (AAFCO) states the analytical variation for vitamin A is 30%. Analytical variation explains why some samples show increases in the value above that of analysis taken at an earlier date when, realistically, it is not possible for nutrients to increase in the diet during storage.

As expected, macronutrients (protein, fat, fiber) and minerals (calcium, phosphorus, selenium) remain stable throughout transit and long-term storage. Vitamins are the most susceptible of all nutrients to various environmental conditions. There is an extensive amount of data that shows temperature and humidity are the primary factors in nutrient degradation and the level of degradation can vary with each vitamin.

Vitamins A, E, C, thiamin and pyridoxine were initially chosen for evaluation during this study. Vitamin D was not on the original assay list but was later added, thus, initial data was not collected post manufacturing. Overall, the average vitamin loss during storage from 3 to 18 months was minimal (<12%) except for vitamin A in the 5002 which experienced a loss of 27% (Table 5). Despite such a loss, the final concentration at 18 months exceeded the nutrient requirements of rodents by almost 4x. Similar vitamin A losses were reported in the 2011 study. An average of 12 and 25.9% vitamin A loss noted in LabDiet® 5053 and 5015, respectively, from start to finish over an 18-month study. Greater losses were reported in the Taiwan study (2015) where vitamin A loss averaged 46.8% and 69.5% in LabDiet® 5053 and 5058, respectively, over 24 months. Despite the observed losses across studies, all diets continued to meet and safely exceed the animal's requirements.

Table 5. Average % Vitamin Loss During Storage (3-18mo) at IPS

	5002	5048	5L66
Vitamin A	27	8	6
Thiamin	7	7	1
Pyridoxine	0	9	0
Vitamin D	6	7	0
Vitamin E	11	7	3
Vitamin C	N/A	3	N/A

Very little (0-0.5%) vitamin E loss during storage was observed in the 2011 study compared to 0-22% in the 2015 Taiwan study. In the current study, Vitamin E losses were observed and varied from 3-11% across diets. After 18 months of storage, all three diets continued to meet the species vitamin E requirements.

Vitamin D was not evaluated in either 2011 or 2015 studies. A study that looked at vitamin retention in extruded pet food monthly for 12 months reported retention levels for vitamin D3 at 12 months of storage at 30-45% depending on the source of the vitamin (beadlet versus spray dried), and average loss per month of 4-12% (BASF, 2000). Vitamin D3 loss in the current study was not as severe, even at 18 months from the date of manufacture. At the end of the study, vitamin D3 levels in the 5002, 5048, 5L66 were 2x, 2x and 5.5x the nutrient requirement for rodents, non-human primates and canines, respectively.

As for the B vitamins, pyridoxine was found to be relatively stable which is consistent with data for both rodent and non-human primate diets evaluated in 2011. Thiamin is known to be one of the more unstable vitamins. It has the highest level of degradation compared to all other vitamins during autoclaving (up to 80%). In 2000, BASF reported 45% thiamin retention in extruded pet food 12 months post manufacturing with an average loss of 4% per month. Thiamin loss varied across the four diets from 0-13.4% in the 2011 study but 0% loss was reported for all three diets evaluated in the Taiwan (2015) study. The current study observed minimal losses as well (7% max) with all three diets continuing to safely exceed species thiamin requirements by 2.5-6.5x at 18 months post manufacture.

Non-human primates and guinea pigs are the only laboratory animal species that require supplemental vitamin C in their diets. Ascorbic acid in its free form is very unstable and largely contributed to the short shelf life of diets before a more stable form of vitamin C was developed. Supplemental vitamin C is provided by L-ascorbyl-2-polyphosphate which is the most stable form used by feed manufacturers. At 6 and 9 months from the date of manufacture, the LabDiet® 5048 was assayed for ascorbic acid instead of L-ascorbyl-2-polyphoasphate and thus the results were invalid (Table 3). Results at 12, 15 and 18 months show that the vitamin C remained stable throughout the duration of storage at ambient temperatures and relative humidity.

Considering the same vitamin ingredients are used in all diets, the differences in the amount of loss between studies may be explained by differences in environmental conditions the diets were exposed to. Regardless of the losses observed in the current study, nutrient concentrations at 18 months post manufacturing continued to meet and safely exceed the nutrient requirements of the targeted species.

Discussion:

Several conditions can affect product stability with temperature and humidity being the two biggest factors. The time of year for this study was chosen strategically to represent the most extreme environmental conditions the diets would be exposed to during a calendar year. The diets were manufactured in July/August and transported during September which are considered the hottest months of the year and likely has potential for the largest degree of loss.

The IPS product stability study was designed similar to the 2015 Taiwan study. In 2015, Taiwan was chosen as the final shipping point considering diets shipped to Asia via sea had the greatest potential for exposure to more extreme temperatures and humidity outside our storage recommendations. Asia is also the furthest shipping distance and thus the longest transit time compared to any other locations to which LabDiet® ships. It took approximately 50 days from leaving the manufacturing facility to arriving at our dealer's facility in Taiwan. The travel time to IPS took 25 days. Comparing the two studies, temperatures and humidity during transit were more extreme during transit to Taiwan. The maximum temperature the diets were exposed to during transit in the IPS study was 22-28°C (71.6-82.4°F) over a 10-day period before leaving port. In the Taiwan study, maximum temperatures reached over 26.6-32°C (80-90°F) for a 14-day period prior to leaving port. Temperatures across the ocean were similar for both studies,

however, once product arrived at port, ambient temperatures in Taiwan (21-26.6°C/70-80°F) exceeded that of England (14-22°C/57-71.6°F). Humidity was greater than 50% for the duration of transit in both studies, however, it was approximately 10% higher in the Taiwan study.

Once the diets reached our Certified LabDiet® Dealer facility in Taiwan, diets were stored at 21°C/70°F and <50% humidity. Environmental conditions in the IPS warehouse are not controlled due to the temperate climate in the United Kingdom. Temperatures in the IPS warehouse were relatively mild throughout the 17-month storage period with the exception of July and August which frequently exceeded 21°C/70°F with max recording of 30.6°C/87°F. Humidity ranged from 35-83% throughout storage.

The diets evaluated in the Taiwan study experienced a greater level of degradation for some nutrients (vitamins A and E) compared to those in the IPS study. On the other hand, thiamin degradation was higher in the IPS study than Taiwan. Regardless of the degree of loss, all diets evaluated in current and previous studies continue to meet the minimum nutrient requirements of the NRC at the end of the testing period at 18 or 24 months.

The shelf life of LabDiet® products is 9 months from the date of manufacture for non-irradiated diets and 12 months from the date of manufacture for irradiated diets. The purpose of testing beyond the date of our shelf life policy was to show that the diets continue to be nutritionally stable and a sudden nutrient loss after a certain point in time is not to be expected. Vitamin loss is gradual over time suggesting that time also plays a factor in that loss and not just the level of humidity or the temperature. The results of these study show that LabDiet® products are nutritionally sound even when exposed to conditions that deviate beyond ideal conditions.

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