## MEAT POWDER POPS: MICROBIOLOGY AND PALATABILITY OF FREEZE-DRIED HORSE MEAT USED IN FROZEN ENRICHMENT

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#### Abstract

Acquiring blood or meat juice to make meat-flavored ice pops can be difficult. An alternative is to make meat powder by freeze-drying readily available raw meat. Freeze-drying is often used for food preservation where the removal of moisture from food products inhibits microbial growth. Horse meat was processed and swabbed for microbial analysis (total plate count, *E. coli*, Salmonella, and Listeria) through multiple stages of the freeze-drying process and after two weeks of storage at room temperature or in a freezer. The process of freeze-drying reduced the microbial growth of the initial wet meat from  $1.27 \times 10^7$  cfu to  $1.87 \times 10^5$  cfu, within satisfactory pathogen limits, after freeze drying. Meat powder pops were made by mixing 1 T of meat powder in 80z of water and then freezing. Frozen ice treats made from meat juice or meat powder were offered to 2.1 African Lion, 0.5 African Painted Dog, 2.0 Bobcat, 1.1 Grey Fox, 1.1 Jaguar, 1.1 Spotted Hyena, and 1.1 Sumatran Tiger to test palatability.

#### Introduction

In animal nutrition certain food products can be difficult to procure. One such example is blood pops for use as enrichment or hydration. At Zoo Miami, animal blood is difficult to purchase and store so substitute pops are made using meat juice that has leached from diet muscle meat or commercial ground meat products while thawing under refrigeration. This meat juice is an aqueous solution that contains water and protein and is tinted red from myoglobin (Huff-Lonergan, 2002). Meat juice is diluted approximately 1:4 v:v with water to make frozen meat juice pops which are used for enrichment, particularly in the summer months. They are also used as a form of hydration and to encourage consumption for carnivores under medical care. As an alternative to meat juice, Zoo Miami used its freeze-dryer to dry horse meat so that it could be powdered and then reconstituted to make a frozen meat powder pop.

Wet food products such as meat are subject to the potential for high microbial growth (Rahman *et al.*, 2005). Handling of the meat during thawing and processing can deteriorate the meat's quality (Dave & Ghaly, 2011) and increase microbiological loads (Sperber, 2009). Freeze-drying is often used as a food preservation method to extend the shelf life of foods by removing water to prevent spoilage. The freeze-drying process puts material under frozen pressure, below its triple point, where it is then heated to cause the ice (water) in the material to sublimate to vapor (Rahman *et al.*, 2005).

This study evaluated the bacterial load of freeze-dried raw horse meat, processed into powder and stored for two weeks. Meat powder pops were also tested for palatability on a wide range of carnivores.

#### **Materials & Methods**

Meat juice was collected daily at the Zoo Miami Nutrition Center (Zoo Miami, Florida 33177) during the process of thawing Nebraska Premium Feline (supplemented ground horse meat, Central Nebraska Packing, Inc., North Platte, Nebraska 69101) and Milliken Small Carnivore (supplemented ground horse meat & beef, Milliken Meat Products Ltd., Ontario L3R 0B4 Canada). Meat juice pops were made immediately wherein two ounces of meat juice was mixed with 14 ounces of water and frozen. Horse meat (Equine Sirloin; Central Nebraska Packing Inc, North Platte, Nebraska 69103) was selected for making meat powder pops. Frozen horse meat was sampled to determine the dry matter content from an independent lab (Zooquarius, Ithaca, New York 14850).

Frozen equine sirloin (FROZEN) was swabbed for microbiology and then placed under refrigeration at 35°F for 18 hours. The goal was to thaw the meat minimally so that it could be cut into pieces approximately the size of a sticky note and 0.5 cm thick. Ten slices (~30g each) were placed on each of four freeze-dryer trays and swabbed for microbiology (WET) and weighed immediately before entering the freeze-dryer (Harvest Right Freeze Dryer with oil-free pump, software version5.0.24, Harvest Right, Salt Lake City, Utah 84116). The freeze-dryer was pre-cooled to 32°F. Freeze-drying occurs automatically beginning with vacuum-freezing, then drying and final dry phases. The final dry cycle runs overnight and can take 2-15 hours.

Once removed from the freeze dryer, the meat was visually inspected to confirm external dryness. Random pieces were broken in half to check interior dryness. Dried meat was reweighed to get a post-dry weight for comparison to the dry matter analysis from the external lab and to determine remaining water weight. The dried horse meat was then placed in a food processor and ground thoroughly into powder. Post-processing microbiology swabs were taken of the powder (DRY). The powder was divided equally into two sealed containers. Container one (2WK ROOM) was left at a controlled room temperature of 70-75°F, and container two (2WK FREEZER) was placed in a freezer at -10°F for two weeks. After two weeks, both were swabbed for microbiology.

For each microbiology sample, three microbiology swabs were run over multiple areas of the meat or powder and then submitted to Midwest Laboratories (Omaha, Nebraska 68144) for test package FS2: Food Screen plus Listeria (total plate count, *E. coil*, Total Coliforms, Staphylococcus, Yeast, Mold, Salmonella, and Listeria).

For palatability tests, freeze-dried meat powder was reconstituted with water (1 T meat powder per 8oz water). The pop was left to rehydrate for three minutes, stirred and then frozen to make 'meat powder pops.' Some muscle fiber remained apparent. Once frozen, the meat powder pop was swabbed for microbiology (POP).

To test the palpability of the meat powder pop versus meat juice pop, frozen pops were offered to seven carnivore species (2.1 African Lion, 0.5 African Painted Dog, 2.0 Bobcat, 1.1 Grey Fox, 1.1 Jaguar, 1.1 Spotted Hyena, and 1.1 Sumatran Tiger). For each animal, a 16oz meat powder pop and a 16oz meat juice pop were placed 3 feet apart in the enclosure, and then animals were given access. The response was observed for 20 min. Three responses were measured as 1) which item was consumed first, 2) percent consumption – none, partial, complete, and 3) the time from the first interaction to 100 percent consumption.

## Discussion

The FROZEN sample of meat contained a level of pathogens considered satisfactory within the EU (less than 5 x  $10^5$  cfu; CR, 2005) of  $1.97 \times 10^4$  cfu, which is within the suggested microbial limits for the USDA (Kotula, 1970). The WET sample was higher in pathogens than the FROZEN and significantly higher than the acceptable limit at  $1.27 \times 10^7$  cfu. Based on previous microbiological studies of freeze-dried raw animal matter we expected the microbiology testing to indicate that the DRY sample would contain the lowest level of pathogens than all other samples, which was not the case with a result of  $1.87 \times 10^5$  cfu. The level of pathogen in the DRY sample was below the acceptable limit but still higher than the FROZEN sample (Table 1). The 2WK FREEZER sample had a lower level of pathogens than the DRY sample at  $1.04 \times 10^4$ , likely due to temperature storage (Berry et al., 2008). The 2WK ROOM sample was far below the accepted level of pathogens at  $5.7 \times 10^3$ , which was unexpected. The potential of leftover moisture in the horse meat powder could allow pathogen growth at higher temperatures (Rahman et al., 2005), potentially being in a sealed container protected the sample. Rahman et al. (2005) showed a high positive correlation (r = 0.990) between aerobic plate count and residual water content in raw meat samples post-drying as well as lower (P < 0.05) aerobic counts in freeze-dried meat samples,  $3.4 \times 10^4$  cfu/g, when compared to fresh meat  $5.9 \times 10^6$  cfu/g. Similarly freeze-drying reduced aerobic counts of freshly killed mealworms from  $3.8 \times 10^8$  to  $2.9 \times 10^4$  cfu/g, and freshly killed house crickets from 9.3x10<sup>7</sup> to 1.1x10<sup>4</sup> cfu/g (Caparros et al., 2017). Freeze-drying is known to mostly inactivate microorganisms rather than kill them. Once the product is rehydrated, aerobic counts could increase rapidly (Caparros et al., 2017). Two separate microbiology samples of frozen meat powder pops were taken, POP (24-hours frozen) and POP2 (14 days frozen), which had levels of pathogens detected at  $>3.0 \times 10^6$  cfu and  $6.9 \times 10^3$  cfu, respectively (Table 1). POP sample was not diluted by the lab resulting in a less specific result, but it is unclear why the POP sample was higher in pathogens. This could indicate the variability between samples, length of time frozen, or lab analysis.

At Zoo Miami, meat juice pops are notably preferred over beef broth and chicken broth pops. In initial attempts of using meat powder pops, dholes and clouded leopards not consuming normal diet items due to health concerns have consumed the meat powder pops when offered as meat juice pops were not available. We expect the meat powder pops to be well consumed, similar to the meat juice pops.

# Conclusion

Freeze-dried product, similar to frozen product, is a good way to safely store raw meat even at room temperature to maintain low pathogen levels. Freeze-drying increases the possibilities of safely storing food for enrichment or use as a medication vector.

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Table 1. Wherebolology results (cru) of horse meat.								
Sample Name	<b>Aerobic Plate</b>	E. coli	<b>Total Coliforms</b>	Yeast	Mold	S. aureus	Salmonella	Listeria
FROZEN	1.97 x 10 <sup>4</sup>	<10	<10	<10	<10	<10	negative	negative
WET	1.27 x 10 <sup>7</sup>	<10	650	40	<10	<10	negative	negative
DRY	1.87 x 10 <sup>5</sup>	<10	<10	<10	<10	<10	negative	negative
2WK ROOM	5.70 x 10 <sup>3</sup>	<10	<10	<10	<10	<10	negative	negative
2WK FROZEN	$1.04 \text{ x } 10^4$	<10	<10	<10	<10	<10	negative	negative
POP	$>3.00 \times 10^{6}$	<10	<10	<10	<10	<10	negative	negative
POP2	$6.90 \times 10^3$	<10	<10	<10	<10	<10	negative	negative

 Table 1. Microbiology results (cfu) of horse meat.