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TECHNICAL NOTE

PATHOLOGY/BIOLOGY

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Quantitative Assessment of the Percent Fat in Domestic Animal Bone Marrow*

ABSTRACT: Measurement of the amount of fat in femoral bone marrow can provide a quantitative assessment of the nutritional status of an individual animal. An analytical method is presented for quantitating the percent fat in bone marrow from three domestic species: bovine, canine, and equine. In this procedure, fat is extracted from bone marrow using pentane, and the percent fat recovered is determined gravimetrically. Based on analyses from adult animals (normal body condition scores), the average percentage of fat in the bone marrow was >80%. In cases in which animals have been diagnosed as emaciated or exhibit serous atrophy of fat (body scores of 1 or 2), the femoral bone marrow fat was less than 20%. In domestic animals, bone marrow fat analysis can be a useful, quantitative measure that, when used in conjunction with all other data available, can support a diagnosis of starvation or malnutrition.

KEYWORDS: forensic science, forensic pathology, bone marrow, fat, emaciation, femur, malnutrition, starvation, quantitative

Making a definitive diagnosis of starvation in animals is difficult because there are few quantitative measures of malnutrition/starvation available at postmortem examination. Currently, body condition, lack of body fat, and serous atrophy of fat are used to describe undernourishment. However, these measures are relatively subjective. Development of an analytical method to quantitate the percentage of fat in the bone marrow of emaciated animals is needed in order to assist pathologists in making a starvation/malnutrition diagnosis, especially in animal neglect cases.

The long-term effect of a lengthy and continuous deprivation of nutrients can be defined as starvation (1). Starvation can occur in an animal that is eating, but is unable to digest, absorb, and/or utilize a sufficient quantity of nutrients (2). In addition to lack of food or insufficient nutritional intake, malnutrition can be attributed to injuries, bad teeth, parasitism, neoplasia, toxins, or infectious disease (3). Environmental extremes can cause additional stress in outdoor animals increasing their energy demands (2).

The femur has been used as a standard when evaluating bone marrow fat content because it is readily obtained, has abundant marrow content, an abundant blood supply, and has been described as one of the later fat sources to be utilized (4). In the majority of these studies, a solvent extraction method for the measurement of bone marrow fat was generally found to provide the most consistent results when compared with other methods such as air-drying or compression (5–7).

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The bone marrow of a normal healthy animal is solid, white, and waxy because of the high fat content (8). In an advanced state of starvation, the bone marrow is gelatinous, glistening, red to yellow, and wet to the touch because of the high water content (8). Studies on the use of bone marrow fat analysis have been reported in wildlife; however, there are no published reports of using percent bone marrow fat for diagnosis of starvation in domestic animals and livestock. When malnutrition or starvation in domestic animals occurs, there can be legal ramifications related to poor management and neglect. Diagnostically, there are no quantitative measures of starvation available at postmortem examination. Therefore, a quantitative analytical method that can be used to support a postmortem diagnosis of starvation is warranted.

Materials and Methods

An analytical method for the extraction and quantitation of femoral bone marrow fat has been developed and validated. A sample of pure pork lard (Armour®; ConAgra Foods, Omaha, NE) was used as a control in verifying the method. Using this method, a limited database of normal values in adult domestic species (bovine, canine, and equine) has been established for use in diagnosing cases of starvation. Femurs from normal adult animals were obtained from a meat-processing facility, the local humane society, and from livestock, which were presented for necropsy or disposal. Body condition of each animal was documented as adequate, abundant, or good body fat stores. Therefore, no animals were experimentally manipulated for this study. Femurs were collected from the carcass, and the soft tissue was removed from the exterior of the bone. Femurs from large animals were cut longitudinally using a band saw, and the bone marrow from one-half of the cut femur was removed. For small femurs, the epiphyses were cut off, and all of the bone marrow was removed from the diaphysis. The bone marrow was mixed to homogeneity prior to sampling for analysis.

TABLE 1—*Validation data–recovery of fat from simulated samples.*

Sample	Recovered Fat (%)	Moisture Lost (%)	Remaining Dry Matter (%)	Total (%)	% of Theoretical Fat	% of Theoretical Moisture	% of Theoretical Dry Matter
Mixed Rep 1	4.80	88.3	10.3	103	207*	99.7	113
Mixed Rep 2	15.5	75.8	11.3	103	112	101	104
Mixed Rep 3	21.2	71.0	9.40	102	117	96.6	112
Mixed Rep 4	27.9	72.0	3.30	103	175†	96.9	33.7
Mixed Rep 5	52.8	38.6	7.06	98.4	104	97.4	72.1
Mixed Rep 6	75.7	14.1	7.78	97.5	99.7	102	75.9
Mixed Rep 7	2.70	88.6	8.77	100	104	99.0	112
Mixed Rep 8	1.20	90.7	8.25	100	107	99.2	110
Mixed Rep 9	7.10	86.1	8.53	102	123	100	101
Mixed Rep 10	10.3	83.0	7.77	101	109	99.6	110
Average	–	–	–	100	109	99.4	99.6
% RSD	–	–	–	1.70	6.99	1.76	16.4

*Excluded from statistics—possible weighing error.
†Excluded from statistics—analyst error.
RSD, relative standard deviation.

A portion (>0.5 g) of the mixed bone marrow was placed into a preweighed cellulose thimble (Whatman®; Maidstone, England), and the weight was recorded. The sample was dried at approximately 100°C for at least 1 h, cooled to room temperature, and reweighed to determine percent moisture lost. The bone marrow fat was then extracted with pentane (150 mL) using a Soxhlet apparatus (Kimble Chase, Vineland, NJ), which is comprised of an Allihn condenser, a Soxhlet extraction tube, a heating mantle, and a preweighed round bottom flask containing several boiling stones. The extraction was carried out in a chemical fume hood with a reflux rate of approximately 5–6 drops per second. After 1-h (minimum) extraction, the pentane remaining in the Soxhlet extraction tube was carefully transferred to the round bottom flask. The round bottom flask was left in the heating mantle to allow the pentane to evaporate in order to recover the fat. The cellulose thimble (with remaining dry matter) and the round bottom flask containing the fat were dried at approximately 100°C for 30 min to remove residual pentane, cooled to room temperature, and final weights of the thimble and round bottom flask were obtained. The percent dry matter and the percent fat recovered were calculated. The sum of the percent moisture lost, the percent dry matter, and the percent fat totaled approximately 100%.

Results and Discussion

The average percent fat recovered from the lard control sample was 99% (2.65% relative standard deviations [RSD], $n = 3$). Studies were performed simulating various levels of moisture (varying amounts of prepared gelatin), fat (varying amounts of lard), and dry matter (varying amounts of inert boiling stones). The average percent recovery as calculated from the theoretical values was consistent and within established acceptance criteria of ±20% of the theoretical amount of added fat (Table 1). All data means and % RSD were calculated by Microsoft® Office Excel® 2007, Microsoft Corporation, Redmond, WA. Outliers were determined prior to calculating means using Grubbs' Test for Detecting Outliers, http://www.graphpad.com/articles/grubbs.htm.

For all three species, the average percent of fat in the bone marrow of femurs from normal, adult animals was >80%. The percent of bone marrow fat from individual femurs ranged from 63% to 101% (Table 2). Data from the three species were also evaluated for seasonal and gender variations (Tables 3 and 4). With the exception of summer (79%), percent bone marrow fat was greater than 80% across seasons and gender.

TABLE 2—*Average bone marrow fat content in normal adult animals.*

Species	*n*	Mean (%)	RSD (%)	Range (%)
Bovine	19	91	6.73	80–101
Canine	15	82	14.0	65–98
Equine	12	86	11.7	63–99

RSD, relative standard deviation.

TABLE 3—*Average bone marrow fat content in normal adult animals by season.**

Season	*n*	Mean (%)	RSD (%)	Range (%)
Fall	6	92	9.65	80–101
Spring	16	90	5.51	80–98
Summer	17	79	13.19	63–96
Winter	7	92	8.05	79–101

*Data were collected over a 2-year span (April 2005–May 2007).
RSD, relative standard deviation.

TABLE 4—*Average bone marrow fat content in normal adult animals by gender.*

Gender	*n*	Mean (%)	RSD (%)	Range (%)
Female	27	88	11.6	63–101
Male	13	82	11.1	65–94

RSD, relative standard deviation.

TABLE 5—*Bone marrow fat content in emaciation or neglect cases.*

Species	*n*	Mean (%)	RSD (%)	Range (%)
Bovine	17	8	135	<1–34
Canine	25	17	135	<1–64
Equine	22	9	104	<1–38

RSD, relative standard deviation.

Data on the percent fat in bone marrow from samples submitted for analysis in emaciation or neglect cases are presented in Table 5. In these cases, signs of malnutrition, such as lack of body fat and prominence of ribs and tuber coxae, were noted in the clinical

history and/or at postmortem examination. At necropsy, serous atrophy of fat was noted, particularly in the perirenal region and coronary grooves. The mean percentage of fat was <20% in bovine, canine, and equine. Therefore, the percent of fat in bone marrow from cases of malnutrition was much lower than the values for normal individuals. In legal neglect cases submitted to the Indiana Animal Disease Diagnostic Laboratory (ADDL) in West Lafayette, Indiana, values of <1% were not uncommon. Overall, based on the results from this study, <20% fat, as derived from the largest mean of all species tested, in femoral bone marrow can be indicative of severe malnutrition.

Owing to the nature of cases submitted to ADDL, it became necessary to determine the overall stability of the bone marrow fat in intact femurs that were found months after death. Femurs were harvested from two equines submitted for necropsy (both equines had adequate body fat stores upon postmortem examination); one femur was immediately analyzed to determine the baseline percent fat present (70%) in the bone marrow, while the second femur was placed in a wire cage in a secluded outdoor location where it was exposed to typical West Lafayette, Indiana environmental conditions from April through December. One of the caged femurs was removed and analyzed after 3 months of environmental exposure, while the second was removed after 6 months of exposure. This preliminary long-term study showed that bone marrow fat decreased by approximately 28% after 3 months and approximately 33% after 6 months. While samples for this study were obtained from healthy animals where time of death was established, it is recognized that additional studies would be required to distinguish between bone marrow fat loss because of malnutrition versus loss because of decomposition.

Conclusions

The procedure for extraction of fat from femoral bone marrow can provide a quantitative assessment of the nutritional status of an individual animal. Based on bone marrow fat analyses from adult bovine, canine, and equine, the normal percentage of fat in the bone marrow is >80%. Animals that have been diagnosed as severely emaciated frequently have femur bone marrow fat values of <20%. In domestic animals, where the investigator frequently has access to clinical and/or necropsy data, analysis of bone marrow fat is a useful, quantitative measure that should be used in conjunction with all other data available to support a final diagnosis.

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References

1. Spraycar M, editor. Stedman's medical dictionary. Baltimore, MD: Williams and Wilkins, 1995.
2. Radostits OM, Gay CC, Blood DC, Hinchcliff KW, editors. Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses. New York, NY: W. B. Saunders Company, 2000.
3. Hungerford TG. Diseases of livestock, 9th edn. New York, NY: McGraw-Hill Book Company, 1990.
4. Ballard WB. Bone marrow fat as an indicator of ungulate condition—how good is it? Alces 1995;31:105–9.
5. Greer KR. A compression method indicates fat content of elk (Wapiti) femur marrows. J Wildl Manage 1968;32:747–51.
6. Neiland KA. Weight of dried marrow as indicator of fat in caribou femurs. J Wildl Manage 1970;34:904–7.
7. Verme LJ, Holland JC. Reagent-dry assay of marrow fat in white-tailed deer. J Wildl Manage 1973;37:103–5.
8. Cheatum EL. Bone marrow as an index of malnutrition in deer. NY State Conserv 1949;3:19–22.

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