Influence of diet on blood parameters in young greyhounds

by

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SYMBOLS AND ABBREVIATIONS

*	= Statistical interaction
PCV	= Packed Cell Volume (%)
RBC	= Red Blood Cell Count ($10^6/mm^3$)
Hb	= Hemoglobin (gm/dl)
WBC	= White Blood Cell Count ($\#/mm^3$)
MCV	= Mean Corpuscular Volume (μ^3)
MCH	= Mean Corpuscular Hemoglobin (10^{-12}gm/ml)
MCHC	= Mean Corpuscular Hemoglobin Concentration (%)
Wt	= Weight (kg)
Ca^{+2}	= Calcium (mg/dl)
Inorg P	= Inorganic Phosphorus (mg/dl)
Glu	= Glucose (mg/dl)
BUN	= Blood Urea Nitrogen (mg/dl)
Crt	= Creatinine (mg/dl)
UA	= Uric Acid (mg/dl)
Trig	= Triglycerides (mg/dl)
Chol	= Cholesterol (mg/dl)
TP	= Total Protein (g/dl)
Alb	= Albumin (g/dl)
Glob	= Globulin (g/dl)
A/G	= Albumin/Globulin ratio
Fe^{+2}	= Ferrous Iron (mcg/dl)
SAP	= Serum Alkaline Phosphatase (IU/l)
LDH	= Lactate Dehydrogenase (IU/l)

SGOT = Serum Glutamic Oxaloacetic Transaminase (IU/l)

SGPT = Serum Glutamic Pyruvic Transaminase (IU/l)

Na^+ = Sodium (meq/l)

K^+ = Potassium (meq/l)

Cl^- = Chloride (meq/l)

T CO_2 = Total Carbon Dioxide (Bicarbonate and Dissolved) (meq/l)

D Bil = Direct Bilirubin (mg/dl)

T Bil = Total Bilirubin (mg/dl)

BUN/Crt = Blood Urea Nitrogen/Creatinine ratio

VLDL = Very Low Density Lipoproteins

A Gap = Anion Gap (meq/l)

Osmo = Osmolality (mosm/l)

Ion Ca^{+2} = Ionic Calcium (mg/dl)

CPK = Creatine Phosphokinase (IU/l)

Amyl = Amylase (Somogyi Units)

SDH[1] = Succinate Dehydrogenase (IU/l)

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[1]Presented as C.D.H. which is assumed to be a typographical error. SDH can be elevated in hepatopathies which may coincide with elevated SGPT.

INTRODUCTION

Greyhounds are a breed of dog whose hematology values during the racing season are sufficiently different from other breeds that the published clinical canine values do not apply. Management of and stresses on greyhounds are also unique. It needs to be established whether these management differences and stresses are responsible for the hematology picture or if greyhounds are indeed physiologically different. Very little has been done in the United States to contribute to our knowledge of this breed. Even though the greyhound industry has been recognized in the United States since 1878 (15), the last ten years has seen a surge in dog racing interest throughout the country. With the rising number of race tracks there will be increasing numbers of trainers, breeders and ultimately dogs. These dogs demand an increased amount of medical attention,and so, the veterinary profession has begun to recognize the unique aspects of the greyhound. In order to properly diagnose disorders and prevent treatment and management errors a thorough knowledge of the greyhound's physiology is essential.

Due to the unique stresses a greyhound is subjected to it may be beneficial to divide the greyhound life into four categories; growing, training, racing and breeding; to determine the normal blood parameters within each period.

In the literature the main interest to date has been the racing stage. Only one report focused on values from the greyhound's first year (34). This growing period should be examined in more detail. If differences exist between greyhound and non-greyhound pups, then perhaps

they can be truly called physiologic differences. If dietary management is responsible for the hematology differences this should also be recognized.

The objectives for this study are to 1) establish a set of normal blood values for growing greyhound pups, and 2) determine the effect of diet on the greyhound pup's blood composition.

LITERATURE REVIEW

Hematology

Greyhound hematology values have been determined for the adult, actively racing animal (20, 28, 34, 45, 47, 58, 74). Unsuccessful attempts have been made to correlate performance with blood parameters (20, 74). Although it is established that greyhounds have a unique hemogram, examination of the greyhound pup blood picture is lacking.

Table 1 is a summary of known greyhound hematology values to date with non-greyhound, basenji, and beagle values included for comparison. From the table it is obvious that adult greyhounds have higher values for PCV, RBC, and Hb than non-greyhounds. In contrast, WBC and TP are lower for greyhounds than other breeds. RBC indices are similar for all dogs. However, Courtice claims that the four highly trained adult greyhounds he tested had a mean corpuscular volume over twice the value he established for mongrels (16). Porter and Canaday reported lower MCHC values and higher MCV values for the greyhound than other authors (58). Exercise can affect PCV. Staaden has studied PCV fluctuations within individual racing greyhounds and reports a resting PCV of 45%, a minimally excited PCV of 55 to 60% and a post-race PCV of 72% (68). He states that maximal PCVs are very consistent within an individual animal and may be more important than resting PCV due to blood viscosity factors. The increased viscosity may result in the higher mean arterial pressure and hypertrophied heart common to the greyhound. Reece has studied PCV changes in rested and excited beagles (60). He obtained a

Table 1. Summary of Greyhound Hematology Values (Non-GH[a] data included for comparison) See SYMBOLS AND ABBREVIATIONS for units Mean ± SD or ( ) range unless otherwise stated

Author	(25)	(63)	(51)	(65)	(34)
Breed	Basenjis	Beagles	Beagles	Non-GH[a]	GH[a]
Age	4-6 mos.	6-12 mos.	6-12 mos.	b	b
PCV	44.00±2.40	43.50±4.00	M[c]44.00±1.30	45.0	51.00±5.33
RBC	6.56±0.46	-	-	6.8 (5.5- 8.5)	7.23±0.695
Hgb	14.40±0.82	14.20±1.50	M[c]14.88±0.39 F 15.47±0.25	15.0 (12.0-18.0)	17.65±1.82
WBC	13.59±1.75	13.20±3.50	-	11.5 (6.0-17.0)	9.50±1.62
MCV	67.20±2.90	-	-	70.0 (60.0-77.0)	71.25±3.75
MCH	21.90±0.90	-	-	22.8 (19.5-24.5)	24.38±1.24
MCHC	32.70±0.60	-	-	34.0 (32.0-36.0)	34.50±1.20
TP	6.60±0.25 (plasma)	5.66±0.44 (not specified)	M[c]5.20±0.67 F 5.00±0.34 (serum)	(6.0- 8.0) (plasma)	5.89±0.85 (serum)

[a]GH = Greyhound (for all following tables).
[b]Age not reported (for all following tables).
[c]M = male; F = female (for all following tables).
[d]Significantly different from males ($P \leq 0.01$).

(20)	(74)	(47)	(45)	(58)
GH[a]	GH[a]	GH[a]	GH[a]	GH[a]
1-5 yrs.	2-5 yrs.	2-5 yrs.	1.5-5 yrs.	retired[b]
57.29±4.02	59 (56-63)	- (55-65)	54.00±4.0	M[c] 52.00±6.90 F 54.00±5.30
8.42±0.77	8.4	-	7.28±0.53	M[c] 6.48±0.68 F 6.77±0.71
19.32±1.38	19.3 (18.1-6.82)	- (19.0-21.5)	20.50±1.40	M[c] 13.70±1.90 F 16.40±2.6[d]
7.14±1.74	5.31 (3.80-6.82)	- (3.5- 6.5)	9.63±2.39	M[c] 8.90±3.00 F 8.10±2.59
68.09±2.90	72 (64-80)	- (65-78)	-	M[c] 80.00±8.80 F 79.70±6.70
22.60±1.88	23 (20-26)	- (21-26)	-	M[c] 21.50±2.70 F 24.10±2.60
33.19±2.02	33	-	-	M[c] 26.30±4.50 F 30.70±4.30[d]
5.20±6.70 (serum)	5.8 (5.4-6.2) (not specified)	(4.8-6.5) (plasma)	6.20±0.07 (serum)	M[c] 5.80±0.60 F 5.80±0.7 (serum)

mean of 53.26% for pre-fed excited beagles as compared to control animals of 41.86% PCVs were not obtained following maximum exercise.

The puppy hemogram has been studied in other purebred groups. Andersen and Gee reported that beagle blood values decreased during the first month then steadily increased until about seven months of age (2). Sex differences were not observed in their immature beagles.

Blood Chemistry

Serum chemistry evaluation of greyhounds is less complete than hematology studies. Several researchers have examined select biochemistry values in adult greyhounds with emphasis on the electrolytes (23, 24, 34, 47, 58, 74-76). Lording reports that greyhound blood chemistry values are significantly different from non-greyhound values in four areas (47). TP, Ca^{+2}, and Chol values are lower while bicarbonate values are higher. TP and Ca^{+2} values are lower in greyhound puppies compared to greyhound adults. In addition, SAP, LDH, and Inorg P values are increased in greyhound puppies compared to adults. Crt values may also be higher in greyhound puppies fed a "high cooked meat diet." Additional literature which specifically examined biochemistry values in greyhound pups is non-existent. Heneghan, however, did include growing greyhounds in his study, but the actual ages were not reported (34). Table 2 is a summary of known greyhound blood chemistry values. Beagle data are included for comparison.

McKelvie did a study to determine age changes in serum chemistries of beagle pups (49). Serum samples were collected at 4, 8, and 12 months. Chol, T Bil, and Cl^- did not change. TP, BUN, SGOT, and Amyl

Table 2a. Summary of Greyhound Serum Chemistry Values (Beagle values included for comparison) See SYMBOLS AND ABBREVIATIONS for units. Mean ± SD or ( ) range unless otherwise stated

Author	(39)	(49)	(51)	(34)	(74)	(75)
Breed	Beagles	Beagles	Beagles	GH[a]	GH[a]	GH[a]
Age	13 mos.	4 mos.-2 yrs.	b	growing[b]	b	b
Ca^{+2}	-	9.90± 1.60	-	10.54± 1.40	-	-
Inorg P	-	5.40± 1.80	-	5.78± 2.04	-	-
Glu	99	99.00±22.00	-	49.12±12.33	-	-
BUN	M[c]12.3 F 13.7	15.60± 7.10	12.2±4.0	16.64± 6.18	-	-
Crt	-	0.81± 0.39	-	-	-	-
UA	-	0.67± 0.42	-	-	-	-
Trig	-	-	-	-	-	-
Chol	170.00	218.00±65.00	-	147.40±42.50	-	-
TP	6.20	6.70± 1.10	-	5.89± 0.85	(5.4-6.2)	-
Alb	3.70	3.40± 0.40	-	3.28± 0.41	-	-
Glob	-	-	-	2.59± 0.90	-	-
A/G	-	1.04± 0.12	-	-	-	-
Fe^{+2}	342.00	-	-	1.20± 0.20 ppm	-	-
SAP	-	3.25± 2.17 Bodansky Units	-	(19.50-24.5)	-	-
LDH	-	-	-	-	-	-

(76)	(47)	(23)	(24)	(58)
GH[a]	GH[a]	GH[a]	GH[a]	GH[a]
b	b	experimental[b]	b	retired[b]
-	(1.90-2.60) mmol/l	10.87±0.81	-	M[c] 9.8 ± 0.8 F 9.9 ± 0.7
-	(0.87-2.10) mmol/l	4.31±1.60	-	-
(4.4-5.0) mmol/l	(3.30-6.70) mmol/l	-	-	M[c]101.00±11.3 F 100.00±13.1
-	(3.60-10.40) mmol/l	-	-	-
-	(0.06-0.17) mmol/l	-	-	-
-	(0.00-0.12) mmol/l	-	-	-
-	-	-	-	-
(112-158)	(2.80-6.20) mmol/l	-	-	-
-	(48-65) g/l	-	-	-
-	(23-34) g/l	-	-	-
-	(21-32) g/l	-	-	-
-	-	-	-	-
-	-	-	-	-
(10-120)	-	36.04±16.73	-	M[c] 7.00± 2.1 F 9.30± 3.0 King-Armstrong U
-	(50-400)	-	-	-

Table 2b. Summary of Greyhound Serum Chemistry Values (Beagle values included for comparison) See SYMBOLS AND ABBREVIATIONS for units. Mean ± SD or ( ) range unless otherwise stated

Author	(39)	(49)	(51)	(34)	(74)
Breed	Beagles	Beagles	Beagles	GH[a]	GH[a]
Age	13 mos.	4 mos.-2 yrs.	[b]	growing[b]	[b]
SGOT	18	3.40± 1.49 SFU/dl	-	-	-
SGPT	21	27.20±14.90 SFU/dl	-	-	(33-67)
Na^+	-	182.00±14.00	147.30±3.10	140.40±17.30	(149-153) mmol/l
K^+	-	4.70± 0.50	4.73±0.28	5.16±0.80	(3.9-5.8) mmol/l
Cl^-	-	107.00± 9.00	110.00±2.40	-	-
T CO_2	-	-	24.10±2.40	-	-
D Bil	-	-	-	-	-
T Bil	-	0.19± 0.20	-	-	-
BUN/Crt	-	-	-	-	-
VLDL	-	-	-	-	-
A Gap	-	-	-	-	-
Osmo	-	-	-	-	-
Ion Ca^+	-	-	-	-	-
CPK	49	-	-	-	(41-77)
Amyl	-	614.00±110 Somogyi Units	-	-	-

(75) GH[a] b	(76) GH[a] b	(47) GH[a] b	(23) GH[a] experimental[b]	(24) GH[a] b	(58) GH[a] retired[b]
-	-	(10-80)	-	-	M[c] 46±32.7 F 48±28.70 Karmen U
-	-	(5-80)	-	25.04±14.50 SFU	-
147 mmol/l	-	(138-158) mmol/l	351.45±29.85 mg/dl	-	M[c] 152±5.4 F 152±4.7
4.4 mmol/l	-	(3.8-5.8) mmol/l	5.24±0.46	-	M[c] 4.4±0.3 F 4.6±0.4
113.00 mmol/l	-	(100-115) mmol/l	107.49±9.92	-	M[c] 116±4.5 F 113±3.0
25 mmol/l	-	(22-28) mmol/l bicarb	-	-	M[c] 24±2.5 F 25±2.5
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
13 mmol/l	-	-	-	-	-
-	-	-	-	-	-
-	-	-	-	-	-
-	-	(50-400)	-	-	-
-	-	-	-	-	-

increased with age while Alb, UA, Glu, Inorg P, SGPT, and SAP decreased with age. Andersen and Elvehjem state that Inorg P values are higher in growing dogs in general (4).

Egan's study compared experimental greyhounds to field greyhounds in training, to field mongrels with unknown diet and exercise history, and to selected purebred dogs in the field also with unknown diet and exercise history (23). The influence of diet on canine blood values has been proposed (28, 34, 43, 49) and the effect of exercise intensity has been established (28, 45). Therefore, a direct comparison between the dog groups in Egan's study needs to be re-evaluated. Porter and Canaday's study requires equal scrutiny. Their study compared well-conditioned greyhounds with unknown diets to non-conditioned mongrels with unknown diets (58).

Diet

The typical greyhound diet is a uniform primarily raw muscle meat diet (21, 41, 45). Some authorities recommend cooked beef (18) or cooked turkey as the protein source (21). Vegetables, potatoes, kibble and tripe are among some additives.

The reasons for using raw meat are numerous. Tradition documents the use of meat (15). Greyhounds were originally used to "chase down" game for royal hunters. In addition to being carnivores, they were thought to benefit from the "taste of blood" as an enticer to catch prey. Individual trainer successes and/or superstitions have prolonged this use of meat.

Meat has a high biologic value since its amino acid balance represents an almost ideal protein source (47). The protein content of raw beef is about 14.9% (62). The National Research Council states that the canine protein requirement depends on the metabolizable energy of the diet (53). For growing pups, a diet which has 20% fat should also supply 25% protein. If the fat content is 30% the protein content should be 28.9%. Adult dogs require 6.5% casein or protein equivalent on a dry matter basis.

Davis advocates feeding meat to combat dehydration since meat has a high water content (18). Several researchers acknowledge that greyhounds are typically poor drinkers, maintaining themselves at a slightly dehydrated state (18, 32, 41). Dehydration and hemoconcentration may indeed be a breed difference since racing greyhounds fed meat will not usually exceed a fluid intake of 1.2 liters daily (18). Data to substantiate what is normal water consumption for greyhounds on different diets could not be found, however. Blood osmolality data on greyhounds could not be found in the literature, either. A study by Blythe and Hansen showed that greyhounds are quite susceptible to dehydration stress (11). The longer the period that greyhounds were restricted from water before racing, the more the signs of clinical dehydration and reduced performance were present. It is known, also, that the glomerular filtration rate in the dog can increase about 100 per cent four to five hours after ingesting a raw beef meal (56). How this fluid alteration affects hydration and hence blood parameters needs to be determined.

Hematinics, such as oral iron elixers, injectable iron dextrins or adult human dose mineral tablets, are commonly given to racing dogs in Australia (18, 41). Hematinic use is based on the belief that the extra iron will increase the hemoglobin level, number of RBCs, and hence performance (41) but this correlation does not exist in humans (5). These hematinics are usually administered in addition to the dietary meat and mineral and vitamin supplements. Iron tonics must be withdrawn 72 hours before racing (18) and can cause hepatic and pancreatic inflammation (55). Parenteral iron can produce injection site pain in addition to liver damage (41). Pemberton claims high doses of iron tonics cause serum iron levels above 122 μg/dl with elevated SGPT and SDH (55).

Kronfeld has done extensive work on nutrition of racing sled dogs. In one study, he has proposed a high meat protein/high fat diet for endurance athletes in Alaska and has correlated this diet positively with performance and RBC indices (43). Adkins and Kronfeld showed a significantly higher PCV, Hb, and RBC count for racing sled dogs on a 28 percent protein diet compared to a 23 percent protein diet (1). All subjects succumbed to a Parvovirus outbreak during the race which would overshadow any conclusions from these results. Kronfeld reported a significant increase in BUN of sled dogs when switched from a diet of 18 ounces horsemeat with 8 ounces dry food to 53 ounces of a commercial canned chicken/by-products diet (42). This diet change coincided with the maximal exercise period. The BUN returned to normal when the amount of canned chicken fed was reduced to 27 ounces in conjunction with a lowered activity level. Exercise intensity was most likely contribut-

ing to this change in BUN also. Kronfeld's data cannot be directly correlated to greyhounds. Sled dogs are endurance animals working in a cold environment. They require 2-4 times the maintenance energy requirement (43) and derive 70-90% of their energy from plasma free fatty acids (54). Greyhounds are sprinters needing only 10-20% more energy than maintenance requires (48) and relying on glucose oxidation from carbohydrates for energy (17, 46).

Most greyhound dietary regimens are based on success of individual trainers and dogs. Nutritional status was not considered in the greyhound hematology or serum chemistry studies cited. Lassen et al. did, however, state all diets in his study were largely meat (45). Egan used a meat-mix in his study (23).

Andersen and Gee collected multiple blood samples from beagles fed a diet containing 65.7% dry compound, 34.3% fresh meat and by-products with vitamin supplements (2). Their reported hematology values were 20-25% higher than adult canine values previously published. Heneghan who studied hematology in 420 greyhounds divided into age groups stressed the need for further work on how nutritional status affects hematology of the greyhound (34).

It has been stated by numerous authors that blood values from the greyhound are different than non-greyhound dogs. The effect of conditioning in these dogs has been examined within the greyhound breed and as an explanation for differences between the greyhound and other breeds. Diet has rarely been a concern for altered blood values. It is the purpose of this paper to determine if raw meat in the diet of grey-

hounds alters hematologic and biochemical values which would clinically separate the greyhound from other breeds. This paper will also provide a summary of blood parameters for the greyhound puppy from 2-6 months of age.

MATERIALS AND METHODS

Four litters of greyhound puppies were used in the study. The 33 puppies on loan to the project included 21 females and 12 males. All owners of the puppies requested their return at six months of age. One litter was whelped indoors at the Veterinary Medical Complex. Two litters arrived at 8-10 weeks of age. The fourth litter arrived at 11 weeks of age until the end of the study at 6 months.

Housing included 10 ft. by 75 ft. runs lined with pea gravel. Quonset huts were provided for shelter in addition to a 5 ft. by 10 ft. cover for shade. Pups per pen ranged from 2 to 5 depending on individual aggressiveness. Each litter was randomly divided into two groups. One group was fed 100% Purina Hi-Pro® dog food weighing 141 gm/cup. The second group was given a 50:50 by volume mixture of raw beef and Purina Hi-Pro®. The raw meat weighed 205 gm/cup. On a weight basis this ratio converted to a 59% meat:41% dry diet. All litters were fed ad lib. We assumed maximum consumption when a small amount (1-2 cups) of food remained. Once no food was left the daily allotment was increased until again a small amount remained.

Body weights were determined prior to blood sampling every two weeks. Daily food intake per litter was recorded for each diet group on a total cups per day consumed basis. Kilocalories consumed per kilogram body weight were calculated at 3, 4, 5, and 6 months of age. Weighted averages from each litter were used to determine overall averages for dry and meat fed groups. These values were then plotted against average

age in days at the sampling periods. Average daily gains were determined from these graphs.

Every two weeks a 2 ml blood sample was collected in EDTA from each pup, alternating jugular veins. Samples were refrigerated. The analyses began within 2 hours. PCVs were determined in duplicate using the microhematocrit method. Unheparinized capillary tubes were centrifuged for 5 minutes on an International Microhematocrit Centrifuge at 11,500 rpms (13,000 x G)[1]. Percentages were read on an Adam's Microhematocrit Reader[2].

Hemoglobin was measured using the Unopette® microcollection system for cyanmethemoglobin determination[3]. The premeasured diluent contained a 4.98 ml volume of a USP purified water solution containing 0.05% citric acid, 0.02% potassium ferricyanide, 0.005% potassium cyanide, 10% ethylene glycol and 0.1% tris amino methane. A Bausch and Lomb spectrophotometer 70[4] was used to read absorbance at 540 nm. The standard curve was determined using Fisher Diagnostics Cyanmethemoglobin Standard Set 251[5].

Erythrocytes were enumerated using the Unopette® system[6] and hemacytometer. The diluent measured 1.99 mls and contained a solution of 0.01% sodium azide and 0.55% sodium chloride.

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[1]International Equipment Co., Needham, MA.
[2]Clay-Adams, Inc., NY.
[3]Becton-Dickinson and Company, Rutherford, NJ.
[4]Bausch & Lomb, Pasadena, CA.
[5]Fisher Diagnostics, Division Fisher Scientific Company, Orangeburg, NY 10962.
[6]Becton-Dickinson and Company, Rutherford, NJ.

Leukocytes were suspended in 1.98 ml in a Unopette® diluent containing a mixture of 2.86% glacial acetic acid in purified water[7]. A hemacytometer was used to determine counts.

Red blood cell indices were calculated from PCV, RBC, and Hb values collected every two weeks. Determinations included mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Formulas for determining these indices are:

$$\text{MCV} = \frac{\text{PCV (ml/dl)}}{\text{RBC } (10^6/\text{mm}^3)} \times 10 = \mu\text{m}^3 \text{ or fl}$$

$$\text{MCH} = \frac{\text{Hb (g/dl)}}{\text{RBC } (10^6/\text{mm}^3)} \times 10 = \mu\mu\text{g or pg}$$

$$\text{MCHC} = \frac{\text{Hb g/100 mls)}}{\text{PCV (ml/dl)}} \times 100 = \% \text{ or g/dl}$$

Since PCV, RBC count, and hemoglobin values are used to determine the indices, errors in the former may magnify errors in the indices.

A 10 ml jugular blood sample was collected from all pups at monthly intervals. This blood was allowed to clot for at least one hour then centrifuged in an International Clinical Centrifuge® [8] at 3,400 rpm (radius 12.7 cm) for 20 minutes. Serum was pipetted into plastic test

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[7] Becton-Dickinson and Company, Rutherford, NJ.
[8] International Equipment Co., Needham, MA.

tubes, then frozen. Upsher Laboratories, Inc.[9] ran biochemical analyses for 33 parameters.

The diet samples were analyzed for protein, fat, nitrogen free extract, moisture, ash, calcium, phosphorus, crude fiber and total iron at a local chemical laboratory[10].

Statistics

Least-Squares Means (LSM) were used for interpretation of data since this experiment had uneven sex distribution and some missing data points. Therefore, the treatment means have been adjusted for the influence of the unbalanced sexes. Standard errors are given. The uniformity of these standard errors is contributed to the assumption of homogeneous variance upon which LSM are based. Significance levels are set at $P \leq 0.05$.

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[9]Upsher Laboratories, Inc., Kansas City, MO.
[10]Woodsen-Tenent Laboratories, Des Moines, IA.

RESULTS

All parameters were analyzed for sex, diet, and age differences as well as all interactions. Four age categories were used: A1 = 81 to 100 days, A2 = 108 to 129 days, A3 = 136 to 156 days, and A4 = 164 to 184 days. These categories represented two averaged consecutive CBCs two weeks apart. There was one serum sample per age category. Diets were either a 60:40 weight ratio of raw beef to Purina Hi-Pro® dry dog food or a 100% Purina Hi-Pro® diet. An F-test with $P \leq 0.05$ was used to test significance. Least squared means (LSM) were used to obtain averages. The LSM technique accounted for the uneven sex distribution effects. The standard errors of the least squared means are by definition uniform.

Hematology

WBC values were identical to non-greyhound values and showed no age, sex, or diet effects or interactions (Appendix, Table A1).

Age

Hematology values included PCV, RBC, Hb, and WBC. PCV, RBC, and Hb showed significant age differences disregarding diet or sex of puppies (Table 3). All three parameters increased significantly with increasing age.

Diet*age interaction

PCV, RBC, and Hb values were also significant for a diet*age interaction (Table 4). These three parameters were lower for the meat diet

Table 3. Hematology Variables Significant for Age Effects
Means are LSM (± SEM)

Parameter	<----------AVERAGE AGE (days)---------->			
	91.00	118.00	146.00	175.00
	n=40	n=66	n=66	n=66
PCV (%)	36.82 (.44)a[1]	38.63 (.33)b	41.06 (.33)c	46.11 (.33)d
RBC (10^6/mm^3)	6.10 (.13)a	6.56 (.10)b	7.07 (.09)c	8.12 (.10)d
Hb (g/dl)	11.78 (.18)a	12.94 (.13)b	13.72 (.13)c	16.06 (.13)d
MCHC (g/dl)	32.61 (.26)a	33.46 (.19)b	33.42 (.19)c	34.82 (.19)d

[1]Letters compare significance within rows. All values with different letters are significantly different ($P \leq 0.05$). (For all subsequent tables unless otherwise stated).

Table 4. Hematology Variables Significant for Diet*Age Interaction
Means are LSM (± SEM)

Parameter	Diet	<----------AVERAGE AGE (days)---------->			
		91	118	146	175
		meat n=20 dry n=20	meat n=34 dry n=32	meat n=34 dry n=32	meat n=34 dry n=32
PCV (%)	Meat	35.80(.64)a	38.01(.47)b	41.48(.46)c	47.19(.47)d*[1]
	Dry	36.24(.61)a	39.28(.46)b	40.63(.46)c	45.04(.46)d
RBC (10^6/mm^3)	Meat	6.12(.18)a	6.42(.14)a*	7.30(.13)b*	8.36(.14)c*
	Dry	6.09(.18)a	6.70(.13)b	6.85(.13)c	7.88(.13)d
Hb (g/dl)	Meat	11.68(.26)a	12.62(.19)b*	13.80(.19)c	16.41(.19)d*
	Dry	11.88(.25)a	13.25(.19)b	13.64(.19)b	15.70(.19)c

[1]All values with asterisks are significantly different ($P \leq 0.05$) within columns. (For all subsequent tables unless otherwise stated).

at 118 mean days then became higher for the last two sampling periods at 146 and 175 mean days. All three parameters for the meat-mix diet were significantly higher ($P \leq 0.05$) than the dry diet values at the final sample.

Indices

Erythrocyte indices were not significantly different between diet groups or sexes. MCHC however, did show a significantly higher value at the final sampling period when compared to the first sampling period.

Serum Chemistries

Age effects

Table 5 summarizes the blood chemistries with significant age effects. Responses to increasing age were varied.

Sex effects

Significant sex effects were observed in Wt and BUN, BUN/Crt, and Chol parameters (Table 6). Females had higher values for BUN and BUN/Crt while males had higher values for Chol and Wt.

Diet effects

Diet effects were significant for SAP, Chol, and D Bil (Table 7). Chol values for the meat-mix diet group were higher than the dry diet group values. SAP measurements showed the reverse pattern with the dry diet group value higher than the meat-mix diet group values. D Bil was significantly higher for the meat group.

Table 5. Parameters with Significant Age Effects
Means are LSM (± SEM)

	<---------- AVERAGE AGE (days) ---------->			
	91	118	146	175
Parameter	n=24	n=33	n=33	n=33
BUN (mg/dl)	15.15 (.89)a[1]	18.14 (.72)bcd	18.62 (.72)bcd	17.96 (.72)bcd
Crt (mg/dl)	.67 (.04)a	.18 (.03)b	1.00 (.03)c	1.18 (.03)d
UA (mg/dl)	.32 (.06)ab	.41 (.05)abd	.56 (.05)cd	.50 (.05)bcd
Alb (g/dl)	2.99 (.04)a	3.15 (.03)b	3.24 (.03)c	3.42 (.03)d
SGOT (IU/l)	42.71 (8.06)a	67.19 (6.53)bcd	63.98 (6.53)bcd	72.80 (6.53)bcd
CPK (IU/l)	274.78 (19.45)acd	325.93 (15.60)b	265.70 (16.01)acd	262.67 (15.60)acd
T Bil (mg/dl)	.07 (.01)ad	.10 (.01)bcd	.11 (.01)bcd	.09 (.01)abcd
Ca^{+2} (mg/d	11.93 (.12)ad	11.52 (.10)bcd	11.56 (.10)bcd	11.66 (.10)abcd
Inorg P (mg/dl	9.54 (.21)ab	9.21 (.17)ab	8.58 (.17)cd	8.15 (.17)cd
K^{+} (meq/l)	5.88 (.12)ab	5.81 (.09)ab	5.44 (.09)cd	5.43 (.09)cd
Cl^{-} (meq/l)	116.46 (.075)abcd	115.59(.061)ab	118.38 (.061)acd	118.41 (.061)acd
Fe^{+2} (mcg/dl)	127.86 (10.71)ab	148.97 (8.67)ab	105.72 (8.67)cd	122.88 (8.67)acd
Ion Ca^{+2} (mg/dl)	6.94 (.05)a	6.56 (.04)b	6.44 (.04)c	6.31 (.04)d
Total CO_2 (meq/l)	22.79 (.42)ab	22.53 (.34)ab	20.19 (.34)cd	19.98 (.34)cd
LDH (IU/l)	281.54 (17.54)ad	332.93 (14.13)b	222.69 (14.13)c	279.02 (14.13)ad
SAP (IU/l)	381.03 (7.27)a	266.65 (5.89)b	225.23 (5.89)cd	218.50 (5.89)cd
Wt (kg)	20.18 (.56)a	30.57 (.40)b	38.85 (.40)c	45.41 (.41)d
VLDL (mg/dl)	13.26 (1.27)abcd	14.95 (1.03)abc	16.02 (1.03)abc	11.36 (1.03)ad
Trig (mg/dl)	66.38 (6.35)abcd	74.65 (5.14)abc	79.64 (5.14)abc	56.84 (5.14)ad
Glu (mg/dl)	132.88 (2.29)a	115.96 (1.85)bd	109.23 (1.85)c	119.96 (1.85)bd
TP (g/dl)	4.63 (.09)ab	4.78 (.07)ab	5.03 (.07)c	5.36 (.07)d
Chol (mg/dl)	188.04 (5.69)ac	210.64 (4.61)bd	197.54 (4.61)acd	209.52 (4.61)bcd
Glob (g/dl)	1.64 (.07)abc	1.63 (.05)ab	1.78 (.05)ac	1.94 (.05)d

[1]Individual letters represent interactions as follows: a = value at 91 days compared to all others; b = value at 118 days compared to all others; c = value at 146 days compared to all others; d = value at 175 days compared to all others. Values are significantly different within each letter if letter is absent ($P \leq 0.05$).

Table 6. Serum Chemistries With Significant Sex Effects
Means are LSM (± SEM)

	MALE (n=47)	FEMALE (n=76)
BUN (mg/dl)	16.10 (.64)a	18.84 (.49)b
Weight (kg)	35.86 (.36)a	31.65 (.28)b
BUN/Crt	18.97 (.75)a	21.10 (.56)b
Chol (mg/dl)	211.68 (4.06)a	191.18 (3.10)b

Table 7. Serum Chemistries with Significant Diet Effects
Means are LSM (± SEM)

Parameters	Meat n=63	Dry n=60
SAP (IU/l)	256.27 (4.84)a	289.44 (4.36)b
Chol (mg/dl)	221.02 (3.83)a	181.84 (3.44)b
D Bil (mg/dl)	0.025 (.007)a	0.004 (.008)b

Diet*age interaction

There were several significant interactions among the serum chemistries. Chol, Trig and VLDL showed a significant diet*age interaction (Table 8). Chol values were much higher for the meat group than the dry group. The meat-mix group's Chol levels also increased with each age while the dry group showed an irregular pattern. The dry group final value was lower than the initial.

Trig and VLDL patterns were identical to each other due to the calculation procedure for determining VLDL. The protocol of the medical laboratory used in this study to analyze serum samples was to divide

Trig values by five to determine VLDL values.[1] Therefore patterns in Trig predetermine VLDL response. The meat-mix puppies had significantly higher values at 146 days. Final samples for both diet groups was similar and lower than the first sample.

Table 8. Serum Chemistries with Significant Diet*Age Interactions Means are LSM (± SEM)

		<----------AVERAGE AGE (days)---------->			
		91	118	146	175
Parameters	Diet	meat n=12 dry n=12	meat n=17 dry n=16	meat n=17 dry n=16	meat n=17 dry n=16
Chol (mg/dl)	Meat	191.84(8.25) a[1]	224.77(6.52) b*	227.95(6.52) c*	239.54(6.52) d*
	Dry	184.24(7.84) a	196.50(6.53) b	167.13(6.53) c	179.50(6.53) d
Trig (mg/dl)	Meat	65.33(9.21) abd	68.24(7.27) abd	101.47(7.27) c*	56.30(7.27) abd
	Dry	67.42(8.75) abcd	81.06(7.28) ab	57.81(7.28) abc	57.38(7.28) acd
VLDL (mg/dl)	Meat	13.12(1.83) abd	13.64(1.45) abd	20.35(1.45) c*	11.29(1.45) abd
	Dry	13.40(1.74) abcd	16.25(1.45) ab	11.69(1.45) acd	11.44(1.45) acd

[1]Individual letters represent interactions as follows: a = value at 90 days compared to all others; b = value at 118 days compared to all others; c = value at 146 days compared to all others; d = value at 175 days compared to all others. Values are significantly different within each letter if letter is absent ($P \leq 0.05$).

[1]Upsher Laboratories, Inc., Kansas City, MO.

Diet*sex interaction

A diet*sex interaction existed for Glu, SGPT, Trig, and VLDL (Table 9). Females fed the dry diet had significantly higher ($P \leq 0.05$) Glu values than females fed the meat-mix. The dry fed females Glu values were also significantly higher ($P \leq 0.05$) than dry fed males. The males' Glu values were not significantly different between diet groups.

SGPT values also showed contrasting sex effects within diet but they were opposite to the Glu pattern. Females fed the meat-mix diet had higher values than females fed dry food. Males fed dry food had higher values than males fed the meat-mix. Within diet the females fed the meat-mix had significantly higher SGPT values than males fed the meat-mix and males fed all dry food had significantly higher values than females on all dry food. Trig and VLDL had similar patterns as SGPT and were identical to each other due to the aforementioned method of VLDL calculation. Again, females on the meat-mix diet had higher values for Trig and VLDL than females on the dry food diet, while males on dry food had higher values than males on the meat-mix. Within the meat-mix diet females had higher Trig and VLDL values than males. Within the dry food diet males had higher Trig and VLDL values than females.

Sex*age interaction

Wt and BUN/Crt showed a significant sex*age interaction (Table 10). Males weighed more than females and both sexes increased Wt with age. BUN/Crt values for males showed a pattern that peaked at 118 days then decreased steadily at 146 and 175 days. Female BUN/Crt values decreased with increasing age.

Table 9. Serum Chemistries with Significant Diet*Sex Interactions
Means are LSM (± SEM)

Parameters	Diet	MALE meat n=17 dry n=30	FEMALE meat n=46 dry n=30
Glu (mg/dl)	Meat	121.83 (2.60)a	112.34 (1.56)b*
	Dry	118.98 (1.94)a	124.89 (1.94)b
SGPT (IU/l)	Meat	53.10 (12.70)a	86.22 (7.63)b*
	Dry	76.14 (9.47)a	46.30 (9.65)b
Trig (mg/dl)	Meat	65.70 (7.28)a	79.97 (4.34)a*
	Dry	72.81 (5.39)a	59.02 (5.39)a
VLDL (mg/dl)	Meat	13.15 (1.45)a	16.04 (0.86)a*
	Dry	14.57 (1.07)a	11.82 (1.07)a

Table 10. Parameters with Significant Sex*Age Interactions
Means are LSM (± SEM)

		<----------AVERAGE AGE (days)---------->			
		91	118	146	175
Parameters	Sex	Male n=8 Female n=16	Male n=13 Female n=20	Male n=13 Female n=20	Male n=13 Female n=20
Wt (kg)	Male	20.38(.91) a	32.19(.62) b*	41.58(.62) c*	49.28(.64) d*
	Female	19.97(.65) a	28.95(.51) b	36.12(.50) c	41.54(.50) d
BUN/Crt	Male	18.81(1.80) acd*[1]	22.24(1.32) bc	19.33(1.32) abc	15.49(1.32) ad
	Female	25.96(1.22) ab	23.48(1.06) ab	19.52(1.06) c	15.44(1.06) d

[1]Individual letters represent interactions as follows: a = value at 90 days compared to all others; b = value at 118 days compared to all others; c = value at 146 days compared to all others; d = value at 175 days compared to all others. Values are significantly different within each letter if letter is absent ($P \leq 0.05$).

Average Daily Gain

There was no difference between diets for average daily gain (ADG). ADG decreased with increasing age for both diet groups (Table 11). The meat group showed a steady decline with the final ADG approximately 75% of the initial calculation. The ADG for the dry diet group decreased to approximately 50% of its initial value. The greatest decline in gain occurred between 5 and 6 months. One litter had ADG values considerably less than the other three litters during this time. A hookworm infection (Ancylostoma caninum) may have been the cause for decreased gain in the dry group.

Diet Analysis

The diets were analyzed by an analytical chemical laboratory[2] and results are shown in Table 12.

Dietary Iron

Total dietary iron content for the meat-mix diet was 86 ppm. Dietary iron content for the 100% dry diet was 250 ppm. Ferric vs. ferrous iron quantity was not available.

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[2]Woodsen-Tenent Laboratories, Des Moines, IA.

Table 11. Average Daily Gain (lb/day)
Means (± SD)

	<----------AGE---------->		
	3-4 mo	4-5 mo	5-6 mo
Diet	meat n=17 dry n=16	meat n=17 dry n=16	meat n=17 dry n=16
Meat	0.340 (0.051)a	0.281 (0.032)a	0.214 (0.028)b
Dry	0.325 (0.050)a	0.301 (0.035)a	0.168 (0.026)b

Table 12. Diet Analysis

	Diet	
Component	60% Meat:40% Dry (%)	100% Dry (%)
Moisture	52.21	8.67
Fat	11.93	11.37
Protein	21.09	27.79
Crude Fiber	1.6	2.2
Ash	2.39	6.11
Nitrogen Free Extract	10.78	43.86
Phosphorus	0.32	0.86
Calcium	0.46	1.27

DISCUSSION

Hematology - Correlation with Serum Iron and Total Protein

The hematology results (PCV, RBC, and Hb) showed similar age and diet*age effects. There was a steady increase in the three parameters among all the greyhound pups with advancing age. Beagle pups showed a steady increase in these three parameters from one month to eight months of age (3). The increased erythrocyte numbers in the beagle had been attributed to ingesting food other than milk. Although the authors did not suggest a mechanism, it seems likely that the solid food the pups were exposed to had a higher iron content than their dam's milk. In addition, milk forms an iron-protein complex (35) which may make the iron in milk less available for erythropoiesis.

Animals have their highest water content as newborns (13, 44). As age and body weight increase the total blood volume increases but the blood volume per kilogram body weight declines. As age in pigs increased from birth to 6 weeks the extracellular fluid volume (ml/kg body weight) steadily decreased while the intracellular fluid volume (ml/kg body weight) increased (44). This increase in intracellular fluid volume is due to a massive increase in number of cells. Therefore, normal increases in RBC, PCV, and Hb result as age increases.

RBC, PCV, and Hb values were used to calculate the erythrocyte indices. No differences in MCV and MCH were observed between diet groups or sex. Only MCHC showed a significant positive age effect.

At the final blood sample, the meat diet pups' PCV, Hb, and RBC values were significantly higher ($P \leq 0.05$) than the dry diet pups' values. This may indicate a cumulative effect of the meat diet on hematology parameters. Either the pups fed raw meat produced more RBCs or additional fluid shifts are occurring causing reduction in extracellular volume. Further sampling with increasing age may elucidate this.

Whether iron ingested from raw beef blood could have a hematinic effect and thus cause the increased hematology values was evaluated in this study. Supplemental iron, either oral or parenteral, has been known to increase RBC numbers, PCV, Hb, MCV, MCH, and serum iron concentration in iron deficient/neonatal calves (14, 61), iron deficient lambs (16), iron deficient pigs (40, 44, 48) and suckling beagles (78). Weight gain has been documented to increase with iron supplementation also (44, 48, 61, 64, 70). The greyhounds in this study were not iron deficient. Serum iron values of 100-300 μg/dl are considered normal in domestic animals (33). Serum iron values for the greyhound pups were not significantly different between diet groups even though the total dietary iron levels between diet groups was significantly different. However, the dry diet had approximately three times as much total iron content as the meat mix diet.

The NRC requirements for iron in the puppy are 1.32 mg dietary iron/kg body weight per day which insures an absorbable iron level of 0.66 mg iron/kg body weight per day (53). Excess iron many times above this requirement is excreted unmetabolized. Both diets in this study exceeded the requirement for iron.

Iron is generally more available in meats than in cereal products (6). Heme iron from meat is primarily ferrous iron whereas iron stored in plants is generally in the ferric form. Iron must be in the ferrous (Fe^{+2}) form to be absorbed from the gut, but this absorption can decrease when body stores are adequate (6, 7, 38). Inorganic iron (Fe^{+3}) can be converted to the ferrous, absorbable form by interaction with hydrochloric acid in the stomach and ascorbic acid (Vitamin C) in the duodenum (66). A report by Sherman et al. states that soybean iron is more available than iron in dry beef skeletal muscle (67). In addition, he claims that beef skeletal muscle could not produce complete Hb regeneration in anemic rats when fed at a level to provide maintenance iron (0.3 mg total iron). Iron availability data in the rat can be used as a guide for dogs (52). In lieu of the similar serum iron values between diet groups and the lower dietary iron level in the meat-mix diet it cannot be stated that the meat was serving as a hematinic due to its iron content.

To elucidate the possibility of a dehydration factor contributing to increased blood parameters, TP and total body water volume need to be analyzed. Total body water was not determined in this study, however, TP was determined. There was a significant increase in serum TP over age for both diet groups in this study. This is in agreement with results from beagle pups sampled at 4, 8, and 12 months which showed steady increases with increasing age (50). At 4 and 8 months of age beagles had higher TP levels than the greyhounds. Low to low-normal total blood protein levels are documented for adult greyhounds (47, 74)

and in greyhound pups (34). Our findings confirm that greyhound puppies have serum protein values lower than non-greyhound puppies. Heneghan speculated that low serum protein may be the compensation for high blood viscosity related to high PCV (34). He did not suggest a mechanism, however. A study by Brown, Dubach and Smith gives support to his theory. Non-greyhound dogs given repeated transfusions had decreases in TP which continued even after transfusions ended and RBC count returned to normal (12). Greyhound puppies do not have elevated PCVs but their low serum protein levels may be due to genetic advantage which evolved to combat an impending viscosity factor.

The meat fed greyhound pups had higher serum TP values, approaching significance ($P \leq 0.06$) for the last two sampling periods than did pups on dry food. The possibility of dietary protein alone causing the differences in serum protein was considered. However, the meat-mix diet contained less protein than the dry diet. The meat-mix diet was 21 percent protein while the dry diet was 28 percent protein.

The minimal protein requirement for a puppy eating a food that provides 4 kcal/g metabolizable energy is 25% (53). The dry diet used was very close to this at 3.89 kcal/g metabolizable energy. The meat-mix diet provided only 2.35 kcal/g metabolizable energy. If all variables in the two diets were equal, amino acid content, digestibility, etc., the meat-mix fed puppies would need to consume about 50 percent more food to get the same protein level as the dry fed group. This was not the case. The dry diet puppies actually consumed more total kilo-

calories which may be attributed to increased dietary fiber common in plant source foods.

The difference in amount consumed between diets may also be attributed to differences in dietary protein quality which can vary greatly. Digestibility and biologic value are two modes of dietary protein comparison. Digestibility is the ratio of food absorbed to the amount ingested. Biologic value (BV) is based on how close a protein's amino acid content is to the dog's estimated amino acid requirement (0-100%) as reported by the NRC (46, 53). It is the amount of protein utilized compared to the amount absorbed. Good quality dry pet foods average from 75-80% protein digestibility (37, 46). It is assumed Purina Hi-Pro®, used in this study, is a good quality dry pet food. Although BV for Purina Hi-Pro® protein was unknown, the BV for its primary protein constituents, corn and soybeans, are 45 and 67, respectively (46). When properly mixed, vegetable proteins can meet protein requirements (53). In comparison, raw beef averages 90% protein digestibility and has a BV of 78 (46). The higher quality dietary protein in beef coupled with the lower protein percentage in the diet may in fact be equal to the lower quality dietary protein that is found in more abundance in the dry food. This is a likely explanation since the average daily gain was virtually the same between diet groups.

Total protein values above normal are usually due to either dehydration or inflammatory disease (22). Dehydration produces a relative hyperproteinemia while inflammation causes an absolute hyperproteinemia from antibody and fibrinogen production. Dietary protein alone can

increase serum protein levels when the animal is in a protein deficient state, but a multi-stage feedback mechanism which includes regulation of protein intake, gastric emptying, transport into cells, protein synthesis and amino acid degradation prevents blood proteins from accumulating above normal once the deficient state is corrected (30). This is true for well balanced protein excesses as well as an excess in individual amino acids. Protein synthesis cannot be stimulated by excess amino acid supply (73) since excess protein is used as energy or stored as fat (46).

Due to the similarity in total dietary protein quantity consumed between the meat-mix group and the all dry food group, it seems doubtful that protein ingestion alone is responsible for the difference observed in total serum protein. In independent beagle studies where one group of researchers used a mix of meat and dry food and the other researchers used all dry food, total serum protein was higher for the dogs fed a meat mix (49, 51). The percentage of meat vs. dry in the mixed diet was not available. Several other blood values were elevated for this same meat-fed group. Among them were Alb, Na^+, and BUN, while K^+ was identical between studies (49). These changes may all be due to dehydration in meat-fed dogs. Further work to establish kidney involvement needs to be done.

Serum Chemistries

Most serum chemistries showed a significant age effect even though within this category the trends were varied.

Cholesterol

Males had significantly higher Chol values than the females. Estrogen decreases Chol levels in humans (26, 31). Although the greyhound data in this study is in agreement with human data it is not known what the circulating estrogen levels of the puppies was between 3 and 6 months old. Several researchers have reported that among non-greyhounds, males have lower Chol than females (39, 59). Another study reported no sex differences for blood Chol levels among beagles (50). Clearly, the effect of sex on Chol values in greyhounds has not been established.

It is probable that diet has a major influence on blood Chol values in the greyhound. The pups on the meat-mix had significantly higher Chol values than the pups on dry food. The fat in red meat has been incriminated in elevating Chol values. Although dogs are believed resistant to acquiring atherosclerosis from dietary fat (31), they are apparently not resistant to hypercholesterolemia. A recent report by Bjovedt states that renal vessel lesions consistent with arteriosclerotic changes may not be uncommon in young greyhounds (8). Although cholesterol clefts were not present in the lesions Bjovedt acknowledged the need to correlate his findings with dietary intake as well as other factors. The meat fat may be responsible for the higher serum Chol values for the meat-mix fed dogs in this study. Animal fat is high in saturated fats associated with high Chol levels (31). The meat-mix diet used in this study had 11.93% fat which is not excessive, however, the

degree of saturated fat versus unsaturated fat may be a contributing factor.

The blood Chol values reported in this study are higher than those previously published for dogs and specifically greyhounds (Tables 5-8). Although specific ages were not reported in any of these previous studies the standard ages of growing, training, racing and breeding greyhounds are quite uniform (21, 27). Growing age includes puppies 0-12 months old. Training/racing age includes dogs 1-5 years old. Breeding age includes dogs over 5 years old.

Taylor and Hauler reported the training greyhound Chol range to be 112-158 mg/dl (76). A recent Oregon study claimed a similar value of 128 $\pm$ 26 mg/dl to be the average Chol value for racing greyhounds (45). Lording found greyhound Chol values ranging from 108-234 mg/dl to be lower than non-greyhounds which ranged from 151-302 mg/dl (47). Serum Chol values for growing greyhounds have been established at 147.4 $\pm$ 42.5 mg/dl by Heneghan (34). He also reported decreased Chol values for racing greyhounds and increased Chol values for breeding greyhounds. The Chol averages for the dry diet group are in agreement with Heneghan's growing greyhound values (34). However, Heneghan did not publish the type of diet that his greyhounds were fed. The fat content of the dry diet in this study was 11.37%. The fat and Chol content of those diets used in studies which evaluate serum Chol in greyhounds is essential in determining the true range.

Laboratory techniques for Chol determination may be responsible for discrepancies in reported serum Chol values, too. Upsher Laboratories[1] which ran blood chemistry analyses for this study, used enzymatic methods. Taylor and Hauler used the Leibermann-Burchard reaction which is a colorimetric determination based on chemical methods (76). Enzymatic methods are deemed more accurate since there is less interference with non-sterol substances that do react in chemical methods (31, 72).

Hypothyroidism has been correlated with, but is not specific for, hypercholesterolemia (22). Racing greyhounds, though displaying a wide range of serum thyroxine (T_4) values, generally fall within the low to low-normal range of non-greyhounds (10). A relationship between hypoproteinemia and hypercholesterolemia also exists and can be due to a nephrotic syndrome (22). Further research to characterize cholesterol interactions in the greyhound needs to be done.

Blood urea nitrogen

BUN is a measure of kidney function, protein intake, and hydration status, but must be evaluated in conjunction with other hematology and serum chemistry results (22, 59).

BUN was significantly different between sexes (Table 4). Females had higher serum BUN values. Whether estrogen levels were responsible for this increase is speculative. Normal values for growing greyhounds have been published as 16.64 ± 6.18 mg/dl (34). McKelvie reports 17.0 ± 6 mg/dl as the average for beagles pups with no established sex differ-

[1]Upsher Laboratories, Inc., Kansas City, Missouri.

ences (50). The greyhound puppies in this study appear to be within the normal range.

Triglycerides and very low density lipoproteins

Serum Trig levels are helpful in determining the nutritional status of an animal. Trig levels are highest when nutritional intake is adequate and lowest when glucose is unavailable as in starvation (31). VLDLs have a major role in transport and metabolism of plasma lipids; particularly transport of Trig from the liver to extrahepatic tissues. The factor which increases the synthesis of Trig and the secretion of VLDL by the liver is feeding a high carbohydrate diet. Decreased synthesis occurs when there are high levels of circulating free fatty acids from either fasting or uncontrolled diabetes mellitus (7, 26, 32).

Trig and VLDL responses in this study were identical due to the indirect calculation of the VLDL from The Trig values. Within the female greyhounds, Trig values were highest for those on the meat diet. In contrast, Trig values were highest for the males on the dry diet. Both Trig and VLDL showed a significant diet separation at the 146^{th} day. The meat-mix group had higher values at this point. Both serum Trig and serum VLDL values were lowest at the last sample. A hookworm infection during the last month of the study may have contributed to a decreased glucose availability which may have further manifested itself in decreased Trig production. The pups on the dry diet seemed more sensitive to the parasites as their weight gain was the smallest during this period.

Glucose

Glu values were significant for an age effect. The highest value was at the first sampling period and the lowest at the third sampling period. Activity level of the pups may have been responsible for this pattern. The more active periods would coincide with the lowest Glu values.

Glu values were also significant for a diet*sex interaction. Males and females responded oppositely to each other. Males fed the meat-mix had significantly higher values than females fed the meat-mix, but females fed the dry food had significantly higher values than males fed the dry food. Activity or food consumption levels may be contributing to this response. However, Glu and Trig patterns would be expected to be similar since they directly interact to determine energy status. Opposite trends were observed however and cannot be explained.

Serum glutamic pyruvic transaminase

SGPT showed an interesting sex response. Male puppies on dry food had higher SGPT values than male pups on the meat-mix diet. However, female pups on the meat-mix diet had much higher SGPT values than those on the dry food diet. Females had the highest and the lowest SGPT averages. Conflicting data has been reported for the beagle. One study claims that no differences exist in SGPT between sexes (63). An additional study claims higher values for males (39). Egan claims no sex differences exist for greyhound SGPT values though there is a wide range for clinically normal greyhounds (24).

Normal SGPT values for non-greyhound are 4-66 IU/l.[2] In this study, greyhound SGPT values were significantly higher than non-greyhound values (Table 9). Management procedures may also contribute to these higher values. The greyhounds in this study were on a daily heartworm preventive[3] which has been known to cause liver damage in some breeds. Though greyhounds are not listed as a problem breed, perhaps they do react subclinically. It is accepted that elevations in SGPT are not considered clinically significant until a 3-5 fold increase is recorded (22). However, this study cannot conclude that elevated SGPT values are physiologic due to the possible management contributions.

Serum alkaline phosphatase

SAP values were higher for all greyhounds in this study compared to reported puppy normals. In addition, SAP values were significantly higher in the animals fed the dry diet.

Elevated SAP values are normal in young animals undergoing bone formation, though the increase is usually small (22, 23, 36). Lording found normal increases in SAP for greyhound puppies but did not give any values (47). Beagle puppy averages for SAP were listed as 61 IU/l and 62 IU/l for males and females, respectively (59). Adults show lower values. Elevated SAP values with elevated SGPT and/or SDH values indicate hepatocellular disease (9, 22, 23). Additional reasons for high SAP activity include neoplasia, high serum cortisol, and osteoblastic bone disease (22).

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[2]Upsher Laboratory, Kansas City, KS.
[3]Filaribits®-Norden Laboratories.

Explanations for the higher SAP values noted in this study are speculative. Bone growth may be more prolonged - greyhounds are not considered adults until 18 months of age. The increased ratio of bone to soft tissue in greyhounds due to low body fat may produce a relative increase in SAP. Whether the daily heartworm preventive can elevate SAP in greyhounds is not known, therefore this increased value cannot be called physiologic. These increased SAP values do decrease with advancing age as reported with other breeds. A recent study has proposed a correlation between high cortisol, high cholesterol and low thyroxine levels in greyhounds (76). Perhaps underlying endocrine differences in the greyhound are responsible for the increased SAP.

SUMMARY

The greyhound blood composition is not identical to other canine purebreds or to mongrels. In the past, these hematological differences have been attributed primarily to level of training or to genetic make-up. Although these are valid factors, diet has not been considered a factor in contributing to or maintaining any of these blood differences. The data presented show that diet alone can produce differences in the blood components of greyhounds. These dietary differences, though not all explainable, show many interactions between diet, age, and sex. The significantly higher ($P \leq 0.05$) PCV, RBC, and Hb values observed for the meat-mix fed pups indicate either dehydration or a true increase in erythrocyte numbers. TP for the meat-mix pups was approaching a significant elevation ($P \leq 0.06$) compared to the dry diet pups. Although total body water determinations were not evaluated in this study, they would help elucidate whether or not the meat diet was actually causing fluid loss.

Most serum chemistries examined showed age effects, though responses to age were varied. Chol values had significant age effects, as well as sex and diet effects and a diet*age interaction. Chol was significantly lower for females and may be attributed to estrogen levels. Chol was higher for the meat-mix fed pups and increased progressively over age for the meat-mix puppies only. This can be attributed to the saturated fats in raw beef.

Glu and Trig patterns were expected to respond similarly since the level of Glu predetermines the level of Trig. However, the Glu values were inversely related to the Trig values. This relationship is unexplainable, but could be due to individual puppy activity levels and/or sensitivity to a hookworm infection which seemed to affect the female pups fed the dry food the most.

SGPT and SAP values were higher than values published for non-greyhounds. Even though these may be true physiological increases, it cannot be concluded until the effects of administering daily heartworm preventive to greyhounds is known.

In conclusion, diet alone does affect greyhound puppy blood values, although explanations for all responses are not evident. Management factors such as heartworm preventive medication and intestinal parasite outbreaks may complicate these responses. More research to characterize the dietary role in blood parameters and the possible physiological sequelae needs to be conducted.

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APPENDIX

Table A1. Blood Parameters with Non-significant Diet, Age, and Sex Effects and Interactions
Means are LSM (± SEM)

Parameter	175 day average
WBC	11394 ± 759
Na^+	156.04 ± 0.82
A Gap	22.61 ± 0.68
Osmo	325.21 ± 1.70