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Research Article

Diets containing naturally occurring iron or naturally occurring iron plus supplemental ferrous sulfate both maintain normal hematological status in adult dogs

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ABSTRACT

Iron is an essential mineral for dogs due to its roles in physiological processes. The NRC recommends the adequate intake for iron in adult dogs is 0.5 mg/kg of body weight. While iron should be present in high enough concentrations in the diet to maintain normal hematological status and prevent iron deficiency anemia, too much dietary iron can increase lipid oxidation in the food during preparation and storage, subsequently reducing shelf life, palatability, and nutritional content of foods. In general, iron is supplemented to diets to ensure that no dogs are deficient. The primary objective of this study was to investigate the ability of a diet containing either 0 mg/kg or 80 mg/kg supplemental iron to support normal hematological parameters in adult dogs. Twenty-two adult dogs were randomly assigned to one of two treatment diets: Test Diet 1, Experimental maintenance formula with 115 mg/kg endogenous iron + 0 mg/kg supplemental iron; and Test Diet 2, Experimental maintenance formula with 115 mg/kg endogenous iron + 80 mg/kg supplemental iron (ferrous sulfate). Dogs consumed treatment diets for 26 weeks, with clinical assessments (e.g., hemoglobin concentration/total iron binding capacity) assessed throughout the trial (0, 2, 4, and 6 months). Baseline iron related endpoints were not different between diet treatments ($P > 0.50$), except for mean corpuscular hemoglobin concentrations ($P = 0.046$). No differences were found for iron related end points between the two diet treatments ($P>0.05$) at any time point. In some diets no supplemental iron will be needed, based on the diet matrix, whereas others may require some supplemental iron. By reducing iron inclusion in the formulations of canine diets, food stability and shelf life may be improved. This reduction in supplemental iron can also reduce cost of formulation, benefiting food manufacturers, consumers, and adult dogs.

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Introduction

Iron is a dietary, essential trace mineral for dogs, largely due to its role in biochemical activities. Presently, there has been very little research completed regarding iron requirements of adult dogs. Two publications have presented estimates of iron requirements and suggest an iron intake of 1 mg/kg (BW)/day or 26.5 – 27.0 mg/kg diet on a dry matter basis (DMB) is adequate for adult dogs [1, 2]. In growing puppies there have been three key iron-related studies conducted; however, only one was an iron requirement study. Using ferrous sulfate heptahydrate ($FeSO_4 * 7H_2O$), Chausow and Czarnecki-Maulden estimated the iron requirement

of growing puppies to be 81-84 mg/kg diet [3]. In a study of iron source bioavailability, Fly and Czarnecki-Maulden determined diets containing 20 mg/kg iron (ferrous sulfate) did not support normal hemoglobin levels in puppies [4]. Lastly, Fry and Kirk showed a diet containing 90 mg/kg iron (ferrous chloride) helped restore normal hemoglobin in puppies with iron deficiency anemia [5]. However, care should be taken when applying results from studies in puppies to adult dogs as these two life stages differ in their recommended allowance; NRC recommended allowances for puppies and adult dogs are 88 mg/kg and 30 mg/kg of iron on a DMB, respectively [6].

For a healthy population of animals, iron is largely provided by plant and

animal ingredients, but can also be supplemented in organic and inorganic forms such as iron proteinate and ferrous sulfate, respectively. Iron is incorporated into many proteins/enzymes and is involved in oxygen transport and storage [7]. The majority of iron in the body exists in HGB of mature RBCs and erythroid precursors, with smaller amounts in myoglobin, enzymes, and cytochromes [8]. At the core of RBC oxygen transport is the HGB molecule, where heme iron can reversibly bind oxygen for transport [9]. Iron is required in large amounts for erythropoiesis (production of RBCs) and HGB synthesis; inadequate iron intake for RBC production in dogs results in smaller mature RBCs (microcytosis) and low HGB concentration (hypochromasia) [10, 11]. Over time, inadequate iron concentrations in the body will result in iron deficiency anemia. In puppies, feeding an iron deficient diet containing 0 mg/kg of iron on a DM basis induces iron deficiency anemia after 35 days of consumption [5]. The MCV of RBCs and MCHC can be used to assess iron deficiency, and although useful, these measures are insensitive [5]. Therefore, biochemical measures of stored iron have also been used. These include SI, ferritin, and TIBC. It should be noted, however, that these markers can be affected by inflammation, making these measures non-specific [12]. Recent interest has been focused on the sensitivity and specificity of reticulocytes in determining iron deficiency. Reticulocytes are a subpopulation of immature erythrocytes, present during the normal turnover of RBCs, and can represent the current state of erythropoiesis [5]. Since reticulocytes remain in circulation for only a few days, measures of their size and HGB concentrations are more sensitive and specific, making them better indicators of iron deficiency than other currently used assessments [5].

The most prevalent canine diets on the market are chicken based, with iron concentrations in chicken meat and by-products ranging from 3 - 90 mg/kg [13]. Vegetable and legume ingredients included in canine diets also naturally contain iron. For example, raw split peas contain 48 mg/kg of iron, while raw potatoes, sweet potatoes, and corn contain an average of 8.10 mg/kg, 6.10 mg/kg, and 5.2 mg/kg, respectively [13]. While not all sources of iron are equally available from commonly fed ingredients, these ingredients contribute to the overall iron concentration of the diet. Additionally, many diets are supplemented with iron, resulting in iron concentrations well above AAFCO minimum recommendations of 40 mg/kg on a DMB of iron for adult maintenance [14]. However, high inclusion of iron in diets can have detrimental effects on shelf life, palatability, and nutrient quality. Transition metals such as iron interact readily with oxygen and other free radicals due to their paramagnetic state, facilitating the reduction reaction/oxidation of compounds such as lipids, proteins, and sugars [15]. Lipid oxidation of food results in formation of peroxides, ketones, and aldehydes, reduced nutritional value, and changes in sensory properties of the food (e.g. odor and flavor) [16]. Furthermore, lipid oxidation of food is the main determining factor of shelf life, with oxidation occurring in raw materials as well as during processing, packaging, and storage of food [16]. Therefore, the ability to reduce supplemental iron will deliver a more stable food matrix at a lower cost to the manufacturers.

The purpose of this study was to evaluate differences in nutritional adequacy of diets with no supplemental iron, compared to those with supplemental iron (80 mg/kg, DMB) for adult dogs. Based on the information summarized, we hypothesized that diets containing either 0 mg/kg supplemental iron (total analyzed iron 128 mg/kg) or 80 mg/kg supplemental iron (total analyzed iron 178 mg/kg) would support normal hematological parameters in adult dogs.

Materials and Methods

The conditions set forth by the AAFCO [17] for proving nutritional adequacy were used as a design guideline for this study. This study was approved by the Procter & Gamble Pet Care Animal Care and Utilization Committee (AUP #012-9121).

I Selection of Study Population

Inclusion criteria required that dogs were an adult dog ($\geq$1 year of age), in good health, and willing to consume dry food. Subjects were excluded if they showed any signs of iron deficiency anemia and/or they required any medication that may interfere with iron utilization. During the course of the study, dogs could be removed at the discretion of the attending veterinarian in consultation with the Sponsor Study Team for the following reasons: a medical condition, unrelated to dietary treatment, requiring intervention that would impact the study parameters; hematology results that, in the opinion of the veterinarian with the research team, lead to health risk for the dog; or a dog loses more than 15 % of its initial body weight as described in the AAFCO protocol for nutritional adequacy.

For the primary endpoints of HGB and packed cell volume (HCT), the criteria are defined in the AAFCO maintenance protocol. If the situation occurred that a dog had values for the primary endpoints at any of the time points that fell outside of the defined AAFCO parameters, then the following steps occurred: 1) a confirmatory blood sample was collected and sent to Colorado State University for analysis; 2) the frozen serum sample for that dog was sent immediately to Kansas State for secondary endpoint analysis; 3) the veterinarian did a physical examination; and 4) the clinical, technical, and veterinary leads, met to evaluate the data. If it was determined that indeed the animal showed clinical signs of iron deficiency anemia, then the animal would be immediately removed from the study and medical care administered. In addition, all animals on the same dietary treatment would be removed from the study, hence stopping the entire dietary treatment and ending the study.

II Study Design

This was a randomized, parallel design, 6-month study conducted at the P&G Pet Health and Nutrition Center (Lewisburg, Ohio). Dogs were provided with ad libitum water and pair housed within treatment group. Dogs had access to indoor and outdoor runs with indoor runs maintained on a 12 h light and dark cycle, in addition to natural light. Kennels were equipped with raised canvas beds, toys, and heated flooring. Outdoor runs provided enrichment with toys and play yard equipment. All dogs received 40 min of supervised group exercise and socialization, within their treatment group, in a separate fenced yard daily.

Twenty-two adult dogs, representing a mixture of breeds (small, medium, large), (3.6 $\pm$ 2 y, 16.4 $\pm$ 9.1 kg) were enrolled in the study to ensure a minimum of 8 per treatment group completing the study to meet AAFCO recommendations for feeding studies. Breed and gender of dogs were similar between the diet groups: Treatment group 1: female n = 5,

male n = 6, with Bichon Frise n = 1, Brittany n = 3, Dachshund n = 2, German SH Pointer n = 1, Golden Retriever n = 2, Standard Poodle n = 1, Toy Poodle n = 1, Treatment group 2: female n = 5, male n = 6, with Bichon Frise n = 0, Brittany n = 3, Dachshund n = 1, German SH Pointer n = 2, Golden Retriever n = 2, Standard Poodle n = 2, Toy Poodle n = 1. Eligible dogs were randomly assigned to 1 of the following 2 diet groups (~11 dogs/diet group) in a randomized blocked design balanced by breed, age, and gender: Test Diet 1: Experimental Maintenance Formula with 115 mg/kg endogenous iron + 0 mg/kg supplemental iron (ferrous sulfate; total analyzed iron 128 mg/kg), Test Diet 2: Experimental Maintenance Formula with 115 mg/kg endogenous iron + 80 mg/kg supplemental iron (ferrous sulfate; total analyzed iron 178 mg/kg) (Table 1).

Table 1: Nutrient composition of test diets 1 and 2

Nutrient	Test Diet 1[1]	Test Diet 2[1]
DM, g/kg	906.1	913.1
Crude Protein, g/kg	259.6	264.1
Crude Fat, g/kg	166.1	169.8
Ash, g/kg	67.8	67.9
Crude Fiber, g/kg	23.7	24.9
Iron, ppm	128	178

[1]Treatment Diet 1 = Experimental maintenance formula + 0 mg/kg supplemental iron; Treatment Diet 2 = Experimental maintenance formula + 80 mg/kg supplemental iron

The diets differed only in the level and/or source of iron. Veterinary personnel were blinded to the diets assigned to each dog. Diets were provided in plain brown 40-pound bags with colored labels and unique identification codes. All dogs underwent a 117-day washout period on an adult diet containing 0 mg/kg supplemental iron (test diet 1). All dogs assigned to the first treatment continued on test diet 1. The study included a 26-week feeding period in which all dogs were fed their randomly assigned study diet. All diets were identical in terms of nutrient content and made from identical ingredients, with the exception of the concentration of iron included in each diet. All diets utilized contained commercial ingredients/nutrients that are permitted by AAFCO and were considered complete and balanced. Diets were fed as the sole source of nutrition (no treats or other products were permitted) for 26 weeks. Each diet was provided twice daily in kibble form and contained in bags clearly marked with a color label and "Bill of Materials" code corresponding to the test formula included. Dogs were fed to maintain ideal body condition based on caloric intake.

III Sample Collection & Analysis

At baseline assessment (month 0), physical examination, inclusion/exclusion criteria, and randomization were conducted. Whole blood was collected using a vacutainer and jugular sampling at months 0, 2, 4 and 6. Serum samples for determination of select parameters of iron status were collected at months 0, 2, 4 and 6. Samples were analyzed at external clinical laboratories at the Department of Clinical Pathology at Colorado State University and Kansas State University's Veterinary Diagnostic Laboratory (Table 2). For each dog, the month 2, 4, and 6 HGB and HCT values were assessed against the criteria specified by AAFCO [14] for PCV % (individual < 36, mean < 42) and HGB g/dL (individual < 12, mean < 14). Additionally, month 6 average HGB and HCT for each diet were assessed against the criteria specified by

AAFCO for feeding trials.

For each dog, the percent change in body weight from baseline at months 2, 4, and 6 was calculated and assessed against the 15 % weight loss criteria specified by study protocol. Additionally, month 6 average weight loss for each diet was assessed against the 10 % weight loss criteria specified by AAFCO.

IV Statistical Analysis

Repeated measures analysis of covariance (RM ANCOVA) was used to compare the laboratory data from the two diet groups in SAS (v. 9.3, Cary, North Carolina). The RM ANCOVA model included fixed effects of diet, time, and diet x time interaction. In addition, the baseline value of the response variable was used as a covariate. The individual dog was considered a random effect. The laboratory measurements were regressed against iron intake on both an absolute scale (mg/day) and on a body weight basis (mg/kg $BW^{0.75}$).

Results

I Exclusions

A 5-year-old, female Brittany, was excluded from the bioassay (RCM23147/ 11326906) due to inconsistencies in food amounts consumed, which led to an overall weight loss of greater than 15 % of total body weight. The dog had a history of inconsistent feeding behaviors, at times eating all of her food and other times leaving orts. The etiology for this behavior is not known. There was no evidence that the dog's tendency to consume or reject test diets differed from her behavior during previous feeding experiences when fed other diets. All other dogs completed the study. This removal from the study, resulted in an n value of 10 rather than 11 for treatment 1.

II Demographic and Baseline Clinical Assessments

Breed and gender of dogs were similar between the diet groups (P >0.05). The mean age (P = 0.76), body condition score (P = 0.34), body weight (P = 0.42), and food intake (P = 0.41) values were not different between diet groups. The mean HCT (P = 0.62) and HGB (P = 0.43) measurements were similar between diet groups at baseline readings (Table 3). Aside from MCHC (P<0.05), means for other iron-related endpoints were not different between diet groups at baseline (P>0.05) (Table 3).

III Primary Clinical Assessments

Dietary treatment means and individual dog values were greater than the AAFCO specified criteria (Table 4) at all time points (Table 5). All dogs had HGB and HCT values ≥ 12 g/dL and ≥ 36% respectively, at month 0. The HCT values for 4 dogs at baseline, 5 dogs at month 2, and 1 dog at months 4 and 6 were higher than the reference range of 55%. Hemoglobin values for two dogs were above the reference range of 20 g/dL. Dietary treatment means for HCT and HGB were not different at months 2, 4, and 6 (P >0.05) (Table 5). Dietary treatment means for other iron-related endpoints were similar between groups at months 2, 4, and 6 (P >0.05) (Table 5).

Table 2: Clinical Laboratory Assessments of Blood Samples[1]

Parameter[1]	P&G Pet Care Analytical Laboratory	Department of Clinical Pathology at Colorado State University	Kansas State Vet Diagnostic Lab
Primary Clinical Assessments			
Hematology			
Complete Blood Count			
PCV (HCT)		X	
HGB		X	
CHr		X	
MCHC		X	
MCV		X	
MCVr		X	
RBC		X	
WBC		X	
Platelets		X	
Reticulocytes		X	
RDW		X	
Iron			X
Ferritin			X
TIBC			X
Secondary Clinical Assessments			
Differential White Blood Count			
Neutrophils		X	
Lymphocytes		X	
Monocytes		X	
Eosinophils		X	
Basophils		X	
Leucocytes		X	
Serum Chemistry			
A/G Ratio	X		
Albumin (G/dl)	X		
ALKP (U/L)	X		
ALT (U/L)	X		
AST (U/L)	X		
B/C Ratio	X		
BUN (Mg/dl)	X		
Calcium (Mg/dl)	X		
Chloride (Mmol/L)	X		
Cholesterol (Mg/dl)	X		
CK (U/L)	X		
Creatinine (Mg/dl)	X		
GGT (U/L)	X		
Globulin (G/dl)	X		
Glucose (Mg/dl)	X		
LDH (U/L)	X		
Magnesium (Meq/L)	X		
Phosphorus (Mg/dl)	X		
Potassium (Mmol/L)	X		
Sodium (Mmol/L)	X		
T Bili (Mg/dl)	X		
T Protein (G/dL)	X		
Triglycerides (Mg/dl)	X		
Uric Acid (Mg/dl)	X		

[1] P&G = Procter and Gamble; PCV = Packed cell count; HCT = hematocrit; HGB = hemoglobin; CHr = reticulocyte hemoglobin content; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MCVr = reticulocyte mean corpuscular volume; RBC = red blood cell; WBC = white blood cell; RDW = red cell distribution width; TIBC = total iron binding capacity; A/G = albumin/globulin; ALKP = alkaline phosphatase; ALT = alanine amino transferase; AST = aspartate transaminase; BUN = blood urea nitrogen; B/C = Bun to creatinine ratio; CK = creatine kinase; GGT = gamma-

glutamyl transferase; LDH = lactate dehydrogenase; T Bili = total bilirubin; T protein = total protein

Table 3: Baseline Clinical Assessments for Complete Blood Count Iron Related Endpoints[1]

Parameter[1]	Treatment Diet 1[1,2] n = 10	Treatment Diet 2[1,2] n = 11	P-value	Low Reference Value	High Reference Value
HCT	51.8 ± 5.6	50.7 ± 4.2	0.6169	40	55
HGB	17.8 ± 2.1	17.1 ± 1.4	0.4359	13	20
CHr	25.8 ± 1.1	25.5 ± 0.8	0.4797	22.3	27.9
Ferritin	236.6 ± 55.0	275.5 ± 127.6	0.3731	80	800
Iron	225.4 ± 34.9	183.7 ± 60.7	0.0724	88	238
MCHC	34.3 ± 0.6	33.8 ± 0.3	0.0460	33	36
MCV	73.0 ± 3.1	72.7 ± 1.7	0.8321	62	73
MCVr	86.4 ± 3.5	86.2 ± 3.5	0.9256	77.8	100.2
RBC	7.1 ± 1.0	7.0 ± 0.6	0.6866	5.5	8.5
TIBC	362.7 ± 26.4	342.5 ± 32.4	0.1353	246	450
Transferrin Saturation	62.3 ± 9.7	53.0 ± 15.2	0.1145	25	52

[1]Treatment Diet 1 = Experimental maintenance formula + 0 mg/kg supplemental iron; Treatment Diet 2 = Experimental maintenance formula + 80 mg/kg supplemental iron; HCT = hematocrit; HGB = hemoglobin; CH_r = reticulocyte hemoglobin content; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MCV_r = reticulocyte mean corpuscular volume; RBC = red blood cell; TIBC = total iron binding capacity;

[2] Expressed as mean ± standard deviation

IV Secondary Clinical Assessments

Blood serum and differential white blood cell chemistry values at all time points were not different between diet treatments (P >0.10) and were within reference range for all parameters. Food intake (g/kg BW), body condition scores, and monthly mean stool scores were not different between diet treatments (P >0.05).
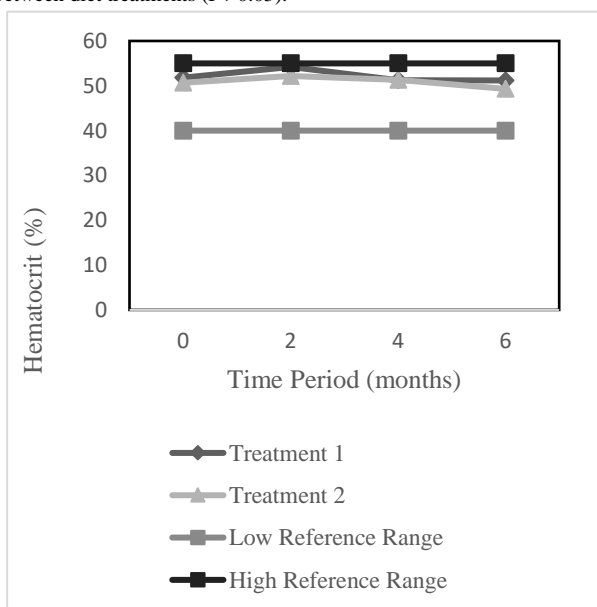

Figure 1: Hematocrit percentage in relation to primary clinical assessment time periods. Tables 3 and 5 contain standard deviations for all hematocrit measures
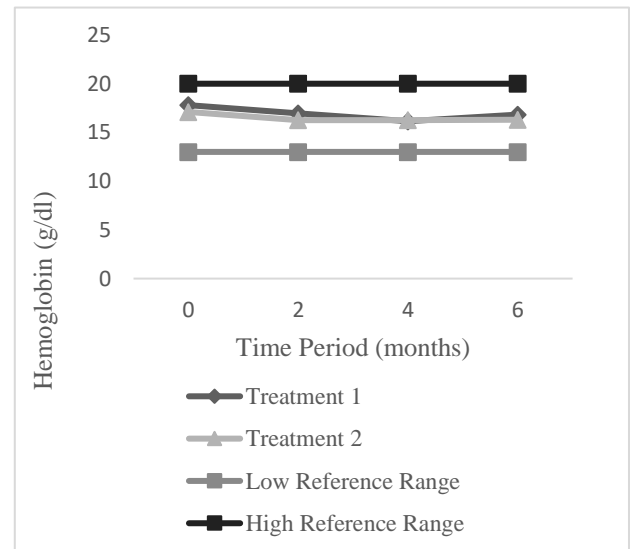

Figure 2: Hemoglobin (g/dL) in relation to primary clinical assessment time periods. Tables 3 and 5 contain standard deviations for all hemoglobin measures

Table 4: AAFCO (2018) Specified Diet Failure Criteria for Blood Cell Parameters

Parameter	Individual	Mean
Packed Cell Volume (Hematocrit) (%)	< 36	< 42
Hemoglobin (g/dL)	< 12	< 14

Table 5: Primary Clinical Assessments for Complete Blood Count Iron Related Endpoints[1]

Parameter[1]	Time Period	Treatment Diet 1[1,2] n = 10	Treatment Diet 2[1,2] n = 11	Diet Comparison P-value	Low Reference Value	High Reference Value
HCT (%)	Month 2	54.21 ± 1.10	52.19 ± 0.91	0.163	40	55
	Month 4	51.23 ± 1.01	51.33 ± 0.77	0.938		
	Month 6	51.19 ± 0.88	49.31 ± 1.06	0.187		
	Overall	52.21 ± 0.74	50.95 ± 0.59	0.203		
HGB (g/dL)	Month 2	16.95 ± 0.34	16.26 ± 0.31	0.141	13	20
	Month 4	16.15 ± 0.34	16.26 ± 0.27	0.811		
	Month 6	16.83 ± 0.27	16.29 ± 0.37	0.262		
	Overall	16.64 ± 0.25	16.27 ± 0.24	0.306		
CHr (pg)	Month 2	25.50 ± 0.15	25.33 ± 0.18	0.481	22.3	27.9
	Month 4	25.23 ± 0.22	25.27 ± 0.14	0.866		
	Month 6	25.35 ± 0.17	25.15 ± 0.18	0.426		
	Overall	25.36 ± 0.13	25.25 ± 0.13	0.566		
Ferritin (ng/mL)	Month 2	286.65 ± 31.48	223.23 ± 19.78	0.096	80	800
	Month 4	246.45 ± 24.36	229.68 ± 16.84	0.579		
	Month 6	275.75 ± 30.56	245.14 ± 21.19	0.420		
	Overall	269.62 ± 23.69	232.68 ± 15.10	0.209		
Iron (ug/dL)	Month 2	183.31 ± 18.01	210.54 ± 16.83	0.279	88	238
	Month 4	212.01 ± 14.00	174.63 ± 14.01	0.079		
	Month 6	208.41 ± 19.70	176.99 ± 15.59	0.228		
	Overall	201.24 ± 13.34	187.39 ± 10.79	0.448		
MCHC (g/dL)	Month 2	31.23 ± 0.13	31.17 ± 0.19	0.800	33	36
	Month 4	31.48 ± 0.15	31.84 ± 0.15	0.099		
	Month 6	32.86 ± 0.15	33.05 ± 0.12	0.330		
	Overall	31.85 ± 0.09	32.02 ± 0.13	0.320		
MCV (fl)	Month 2	76.06 ± 0.25	75.92 ± 0.26	0.697	62	73
	Month 4	75.08 ± 0.33	75.20 ± 0.27	0.784		
	Month 6	72.81 ± 0.27	72.43 ± 0.24	0.288		
	Overall	74.65 ± 0.23	74.51 ± 0.20	0.662		
MCVr (fl)	Month 2	89.23 ± 0.64	87.93 ± 0.87	0.238	77.8	100.2
	Month 4	86.85 ± 1.08	86.32 ± 0.84	0.696		
	Month 6	83.68 ± 0.77	82.81 ± 0.70	0.401		
	Overall	86.59 ± 0.51	85.68 ± 0.59	0.262		
RBC (106/mL)	Month 2	7.13 ± 0.16	6.89 ± 0.14	0.269	5.5	8.5
	Month 4	6.80 ± 0.14	6.84 ± 0.11	0.819		
	Month 6	7.03 ± 0.12	6.83 ± 0.16	0.336		
	Overall	6.99 ± 0.11	6.86 ± 0.10	0.398		
RDW (%)	Month 2	12.36 ± 0.09	12.28 ± 0.09	0.523	12	15
	Month 4	12.42 ± 0.09	12.31 ± 0.10	0.451		
	Month 6	12.64 ± 0.09	12.59 ± 0.11	0.764		
	Overall	12.47 ± 0.07	12.39 ± 0.08	0.493		
TIBC (ug/dL)	Month 2	339.05 ± 9.55	345.05 ± 9.29	0.664	246	450
	Month 4	348.75 ± 10.31	354.86 ± 7.70	0.641		
	Month 6	363.95 ± 10.97	358.05 ± 11.88	0.726		
	Overall	350.58 ± 8.61	352.65 ± 8.70	0.872		
Transferrin Saturation (%)	Month 2	53.87 ± 5.16	61.93 ± 5.57	0.309	25	52
	Month 4	60.84 ± 4.42	49.24 ± 4.54	0.088		
	Month 6	56.81 ± 5.36	49.16 ± 4.52	0.290		
	Overall	57.17 ± 3.56	53.44 ± 3.67	0.497		

[1]Treatment Diet 1 = Experimental maintenance formula + 0 mg/kg supplemental iron; Treatment Diet 2 = Experimental maintenance formula + 80 mg/kg supplemental iron; HCT = hematocrit; HGB = hemoglobin; CH_r = reticulocyte hemoglobin content; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MCV_r = reticulocyte mean corpuscular volume; RBC = red blood cell; TIBC = total iron binding capacity; RDW = red cell distribution width. [2]Expressed as mean ± standard deviation

Diets containing naturally occurring iron or naturally occurring iron plus supplemental ferrous sulfate both maintain normal hematological status in adult dogs

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Discussion

This study demonstrates that diets without supplemental iron, but exceeding the recommended minimum by AAFCO, result in no dietary iron deficiency signs in multiple breeds of adult dogs fed to weight maintenance. This conclusion is supported by the fact that HGB values for all dogs of both diet treatments at all time points were within the laboratory reference range of 13 – 20 g/dL and all values were above the AAFCO specified criteria. While there is a trend for dogs fed the un-supplemented treatment to have lower HGB at all time points compared with the baseline (Figure 1, 2), no individuals were below reference value at any time point. Had either diet been deficient in iron content, HGB concentration and RBC would decline.

Two-thirds of the body's iron is found in circulating erythrocytes, with other minor iron stores in myoglobin, enzymes, and other proteins [8]. HGB concentration, which is the protein responsible of oxygen transport in RBCs, in combination with other hematological/biochemical indicators, is a useful means of determining the severity of anemia [9, 10]. Iron is an integral part of HGB, and HGB makes up over 95% of the protein in red cells. Thus, a gradual decline in the body's iron content due to a loss of HGB and iron stores leads to iron deficiency anemia [10]. For the current study, any occurrence of anemia would be expected to be chronic due to prolonged theoretical inadequacy of the diet. HCT and RDW for study dogs were within laboratory reference ranges (Table 5), supporting the conclusion that no iron deficiency was present. HCT, or PCV, is the percent of volume of erythrocytes in the blood. Severity of anemia is based on hematocrit/hemoglobin levels in the blood, often through the evaluation of MCV and MCHC [18]. RDW, another measure of anemia, can be used to assess iron deficiency anemia in conjunction with MCV. When compared against different types of anemia, nutritional deficiencies (e.g., iron or folate) resulting in anemia also result in high RDW. Chronic iron deficient anemia will typically manifest as hypochromic (pale) blood and microcytic erythrocytes with high RDW [18, 19]. MHCH values for individual dogs were lower than expected, and decreased throughout the study compared to baseline values, with half the dogs from both treatment groups within the reference range and half below. An explanation for these results lies in the possibility that sample storage-related changes may have occurred since there was no relation to dietary treatment. Red cells tend to swell with storage, leading to an increase in the MCV and decrease of MCHC. This phenomenon occurs rapidly, typically within 24 hours of collection, especially if the blood samples are not kept cool until analysis [20]. Therefore, although there were values that were outside the reference range, none were considered to be clinically relevant. In the future, studies involving MCV and MCHC parameters should confirm that storage time and condition prior to analysis affect these values to support the conclusion that these results are not relevant. While clinics were provided with protocols for proper sample collection, they were not required to report their sample collection technique before analysis. Adding this step to future studies could provide support for this explanation.

Circulating iron (SI) is bound to and transported by the protein transferrin. Transferrin is measured indirectly by its TIBC. Percent or transferrin saturation is the ratio of serum iron concentrations to TIBC, with a saturation % of < 20 being indicative of iron deficiency [21].

Decreased SI and ferritin concentrations, normal or elevated TIBC, and low transferrin saturation can be associated with chronic iron deficient anemia [21]. However, these results can be confounded by other factors, such as inflammation, which may influence these values; thus, to be deemed iron deficiency anemia complete blood count must reveal microcytosis or hypochromasia [21]. While there is an assay to assess serum ferritin in dogs, there are no direct reports of its value in determining iron deficiency anemia [21]. All dogs in the present study remained healthy through the study with no signs of inflammation processes occurring. SI concentrations and ferritin values remained within laboratory reference ranges (Table 5) and suggests that sufficient body stores of iron were maintained throughout the study. Most dogs in the study had TSAT above reference ranges at all time points compared to laboratory references (Table 5) and the literature. There was no change in TSAT at month 6 compared to the baseline, and there was no evidence of a treatment specific difference. High TSAT values could point to iron overload, however, TIBC was not low as would be expected in such a case. Therefore, it is unlikely that an iron overload condition was present in the supplemental iron treatment group.

Fry and Kirk found that the magnitude of the differences between values, resulting from feeding an iron deficient diet, at day 0 and subsequent time points (up to 70 to 105 days later) was much greater for reticulocyte indices than for conventional indices [5]. As reticulocytes are in circulation only for 1-2 days, they reflect a more current condition of red blood cell production, and as such, their evaluation provides a more sensitive index of iron status. Fry and Kirk also found that when dogs were considered to have iron deficient anemia (based on study duration), TIBC and ferritin concentrations were still within laboratory reference values, whereas reticulocyte indices were below laboratory reference ranges suggesting iron deficiency anemia [5]. A study by Steinberg and Olver found that low CHr and low MCVr were associated with iron deficiency and were detectible earlier than some other hematological or biochemical indices [22]. Since these reticulocyte indices can be detected earlier than some other indices and CHr and MCVr were within laboratory reference ranges over the course of the study for all dogs, these findings also indicate that iron deficiency was not present.

In conclusion, both dietary treatments supported normal HGB and iron status in adult dogs over the course of the study. Common hematological and biochemical indices were used to assess iron status. Reticulocyte indices, which are now becoming more common practice, were also used to assess iron status and suggested normal iron status. Since no iron deficiency anemia was detected, these data support the nutritional adequacy of reduced supplemental and/or elimination of supplemental iron in diets that meet the minimum iron concentrations designed for adult dogs at maintenance.

Abbreviations

NRC, National research council; DMB, dry matter basis, HGB, hemoglobin; RBC, red blood cells; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; SI, serum iron; TIBC, total iron binding capacity; AAFCO, Association of American Feed Control Officials; HCT/PCV, packed cell volume; RDW, red cell distribution width; SH, short haired; TSAT, transferrin saturation; CHr, reticulocyte hemoglobin content; MCVr, reticulocyte mean corpuscular volume

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