# Diet affects nutrient digestibility, hematology, and serum chemistry of senior and weanling dogs[1,2,3]

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**ABSTRACT:** The objective of this experiment was to determine the effects of age and diet on serum chemistry, hematology, and nutrient digestibility in healthy dogs. Twelve senior (11 yr old; six males and six females) and 12 weanling (age = 8 wk old; six males and six females) beagles were randomly assigned to one of two dietary treatments: 1) an animal product-based (APB) diet or 2) a plant product-based (PPB) diet. The APB diet was primarily composed of brewer's rice, chicken by-product meal, and poultry fat, whereas the primary ingredients of the PPB diet included corn, soybean meal, wheat middlings, and meat and bone meal. Dogs remained on experiment for 12 mo. A 4-d total fecal collection was performed to determine apparent macronutrient digestibilities after 3 and 10 mo. Blood samples were collected at baseline and after 3, 6, 9, and 12 mo on study. After 3 mo, dogs fed the APB diet had greater ($P < 0.001$) DM (6 percentage units) and OM (7 percentage units) digestibilities than dogs fed the PPB diet. Senior dogs had greater DM (2.5 percentage units; $P = 0.07$) and OM (3 percentage units; $P < 0.01$) digestibilities than young dogs. Dogs fed the PPB diet had a lower ($P < 0.001$) fecal DM percentage (7.5 percentage units) and greater ($P < 0.001$) fecal output (253 vs. 97 g/d, as-is basis). After 10 mo, age did not affect nutrient digestibility or fecal characteristics. However, the effect of diet after 10 mo was similar to that observed after 3 mo, as dogs fed the PPB diet had a lower ($P < 0.001$) fecal DM percentage (7 percentage units), lower OM (4 percentage units; $P = 0.09$) and fat (6 percentage units; $P < 0.001$) digestibilities, and greater ($P < 0.005$) fecal output (235 vs. 108 g/d, as-is basis). At baseline, most serum metabolites were different between age groups, with weanlings having several metabolite concentrations outside the reference ranges for adult dogs. Blood cholesterol, red blood cells, hemoglobin, hematocrit, creatinine, total protein, albumin, bilirubin, sodium, chloride, and alanine transaminase were present in greater ($P < 0.05$) concentrations in senior dogs, but weanling dogs had greater ($P < 0.05$) concentrations of glucose, platelets, Ca, P, K, and alkaline phosphatase. Over time, blood cholesterol concentrations were affected by age ($P < 0.05$) and diet ($P < 0.01$). Senior dogs had greater ($P < 0.05$) cholesterol concentrations than weanling dogs. Moreover, dogs fed the APB diet had greater ($P < 0.05$) cholesterol concentrations than dogs fed the PPB diet. Overall, although serum metabolite concentrations of weanlings were different from senior dogs at baseline, as weanlings matured into young adults, metabolite concentrations were similar to those of senior dogs. Diet had the largest effects on nutrient digestibilities and fecal characteristics. Canine age and diet must be considered when interpreting experimental and clinical data.

Key Words: Aging, Dog, Nutrient Digestibility, Serum Chemistry

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## Introduction

Because the average life expectancy of companion animals continues to increase, due in part to improve-

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ments in veterinary care and diet quality, identifying physiological changes that alter the nutritional needs of the aged animal are of interest. Although body size is a factor when classifying dog life stage (large breeds are considered to be older at a younger chronological age), most are considered to be "old" once they reach 7 to 10 yr of age (Goldston, 1989; Hayek, 1998). By identifying changes in serum metabolites, hematology, and digestive efficiency of aged dogs, researchers may be able to formulate diets that are more appropriate for this life stage. Conversely, the weanling population also has unique physiological characteristics and nutritional needs. Per unit of BW, growing puppies (<6 mo old) require approximately twice the caloric intake of adult dogs (Case et al., 2000). To account for new muscle and bone growth, greater protein (22 vs. 18%), Ca (1.0 vs. 0.6%), and *P* (0.8 vs. 0.5%) concentrations are recommended for growing puppies than for adults (AAFCO, 2003).

Although "normal" serum metabolite and hematology values have been identified for healthy adult dogs, these guidelines may not be appropriate for evaluating the health of weanling or aged animals. Several groups have reported the effects of age on canine serum chemistry and hematology (Kaspar and Norris, 1977; Fukuda et al., 1989; Lowseth et al., 1990), but few have monitored changes over the first year of life. In addition, the effect of diet on serum metabolites in dogs often has been ignored.

Decreased digestive efficiency has been reported in elderly cats (Taylor et al., 1995) and humans (Pelz et al., 1968); however, limited published evidence exists to support such an effect in dogs. In contrast, Sheffy et al. (1985) reported an increased digestive efficiency in senior dogs, but others have observed no effect of age (Taylor et al., 1995). Little has been published on nutrient digestibility by dogs less than 1 yr of age. Therefore, our objective was to evaluate the effects of diet on serum chemistry, hematology, and apparent nutrient digestibility by young and senior dogs.

**Table 1.** Ingredient and chemical composition of the plant product-based (APB) and animal product-based (PPB) diets fed to weanling and senior dogs[a,b]

| Ingredient | APB, % (as-fed basis) | PPB, % (as-fed basis) |
|---|---|---|
| Corn | — | 45.00 |
| Brewer's rice | 44.23 | — |
| Chicken by-product meal | 32.91 | — |
| Soybean meal | — | 19.96 |
| Poultry fat | 14.99 | 3.97 |
| Wheat middlings | — | 13.20 |
| Meat and bone meal | — | 10.00 |
| Beet pulp | 4.00 | 4.00 |
| Dehydrated egg | 2.20 | 2.20 |
| Sodium chloride | 0.65 | 0.65 |
| Potassium chloride | 0.65 | 0.65 |
| Choline chloride | 0.13 | 0.13 |
| Vitamin premix[c] | 0.12 | 0.12 |
| Mineral premix[c] | 0.12 | 0.12 |
| Analyzed composition | | |
| Dry matter, % | 93.8 | 94.3 |
| | % of DM | |
| Organic matter | 92.8 | 92.3 |
| Ash | 7.2 | 7.7 |
| Crude protein | 28.0 | 25.5 |
| Acid hydrolyzed fat | 22.6 | 11.2 |
| Total dietary fiber | 4.8 | 15.2 |
| Gross energy, kJ/g | 22.51 | 19.87 |

[a]Provided per kilogram of APB diet: choline, 2,654 mg; vitamin A, 15,200 IU; vitamin $D_3$, 900 IU; vitamin E, 62.5 IU; vitamin K, 0.6 mg; thiamin, 13.1 mg; riboflavin, 14.0 mg; pantothenic acid, 25.3 mg; niacin, 70.0 mg; pyridoxine, 13.56 mg; biotin, 0.11 mg; folic acid, 949 μg; vitamin $B_{12}$, 129 μg; manganese (as $MnSO_4$), 19.6 mg; iron (as $FeSO_4$), 253.9 mg; copper (as $CuSO_4$), 17.8 mg; cobalt (as $CoSO_4$), 2.4 mg; zinc (as $ZnSO_4$), 166.9 mg; iodine (as KI), 6.3 mg; and selenium (as $Na_2SeO_3$), 0.32 mg.

[b]Provided per kilogram of PPB diet: choline, 2,457 mg; vitamin A, 16,300 IU; vitamin $D_3$, 900 IU; vitamin E, 74.1 IU; vitamin K, 1.2 mg; thiamin, 14.4 mg; riboflavin, 11.5 mg; pantothenic acid, 23.9 mg; niacin, 79.3 mg; pyridoxine, 15.8 mg; biotin, 0.24 mg; folic acid, 1,024 μg; vitamin $B_{12}$, 33.3 μg; manganese (as $MnSO_4$), 24.0 mg; iron (as $FeSO_4$), 214.6 mg; copper (as $CuSO_4$), 23.1 mg; cobalt (as $CoSO_4$), 2.4 mg; zinc (as $ZnSO_4$), 144.3 mg; iodine (as KI), 24.0 mg; and selenium (as $Na_2SeO_3$), 0.27 mg.

[c]Trouw Nutrition USA, LLC, Highland, IL.

## Materials and Methods

### *Animals and Diets*

Senior (average age = 11.1 ± 0.6 yr; six males and six females) and weanling (8 wk old; six males and six females) beagles (Marshall Farms USA, Inc., North Rose, NY) were used in this experiment. Three of each gender and age were randomly assigned to one of two dietary treatments prepared by Wenger Manufacturing Co. (Sabetha, KS). The animal product-based (**APB**) diet was primarily composed of highly digestible, animal-derived ingredients and formulated to exceed the CP and fat recommendations for growth and reproduction provided by the Association of American Feed Control Officials (**AAFCO**) (AAFCO, 2003; 30% CP and 20% fat; Table 1). The plant product-based (**PPB**) diet was primarily composed of plant-derived ingredients and was formulated to meet CP and fat recommendations for growth and reproduction provided by AAFCO (2003; 22% CP and 8% fat; Table 1). The exception was the inclusion of meat and bone meal (10% of diet). Both diets were formulated to meet all other nutrient requirements for growth and reproduction according to AAFCO (2003). The amount of food initially offered was calculated by using standard equations to determine energy requirements of active adult dogs (ME requirement, kcal = $132 \times BW_{kg}^{0.67}$) and small breed puppies (ME requirement, kcal = $375 \times BW_{kg}^{0.67}$) (Case et al., 2000). The amount of food offered was adjusted to maintain initial BW in seniors and to allow ad libitum access in weanlings throughout the experiment. Because Atwater factors tend to overestimate ME values of most pet foods (Case et al., 2000), modified Atwater factors (3.5, 3.5, and 8.5 kcal/g of protein, carbohydrate, and fat, respectively) were used to estimate ME content of

each diet and to determine the initial food offered. Using the modified Atwater factors, the APB and PPB diets were estimated to contain 4.21 and 3.26 kcal ME/g, respectively. Food refusals were weighed daily and food intake calculated. Senior dogs were weighed at baseline and every 4 wk of the experiment. Young dogs were weighed at baseline, every week during the first month on study, and monthly thereafter to ensure normal growth for this breed. Dogs were housed individually in kennels (1.1 × 0.9 m) in temperature-controlled rooms with a 12-h light:12-h dark cycle at the Edward R. Madigan Laboratory on the University of Illinois campus (Urbana). The University of Illinois Campus Laboratory Animal Care Advisory Committee approved all animal care procedures prior to initiation of the experiment.

### *Sample Collection and Handling*

Blood samples from 12-h fasted dogs were collected via jugular puncture at baseline and after 3, 6, 9, and 12 mo on study. At each collection time, 4 mL were collected into a nonheparinized, evacuated tube for use in determination of serum metabolite profile. Another 3 mL of blood was collected into an evacuated tube containing EDTA to measure red blood cell, hemoglobin, hematocrit, and platelet concentrations. A 4-d total fecal collection was performed for the measurement of apparent macronutrient digestibility after 3 (during the rapid-growth phase of young dogs) and 10 mo (young dogs were considered young adults) on the experiment. During the fecal collection phase, total feces were removed from the floor of the pen, weighed, composited, and frozen at –20 C until laboratory analyses. Diet and fecal samples were dried at 55 C in a forced-air oven. After drying, samples were ground through a 2-mm screen in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ).

### *Chemical Analyses*

Diet and fecal samples were analyzed for DM and ash using AOAC (1984) methods. Organic matter was calculated by subtracting ash concentration from DM. Crude protein was determined from Leco total N values according to AOAC (1995) methods. Total lipid content was determined by acid hydrolysis followed by ether extraction according to AACC (1983) and Budde (1952). Total dietary fiber (**TDF**) concentration was determined according to Prosky et al. (1984, 1992).

Blood concentrations of red blood cells, hemoglobin, hematocrit, and platelets were determined using a Cell-Dyn 3500 hematology analyzer (Abbott Laboratories, Abbott Park, IL). Serum metabolite concentrations were determined using a Hitachi 911 clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN).

### *Statistical Analyses*

A 2 × 2 × 2 factorial arrangement of treatments (age, diet, and gender) in a completely randomized design was utilized in this experiment. Nutrient digestibility data were analyzed using the PROC GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Data generated from blood samples were analyzed at baseline and over time (differences from baseline) using the PROC GLM and PROC MIXED procedures of SAS, respectively. Because variance changed over time, the autoregressive heterogeneous variances model was determined to be the most appropriate and was used for the analyses over time. A probability of $P < 0.05$ was accepted as being statistically significant, and $P < 0.10$ was indicative of trends.

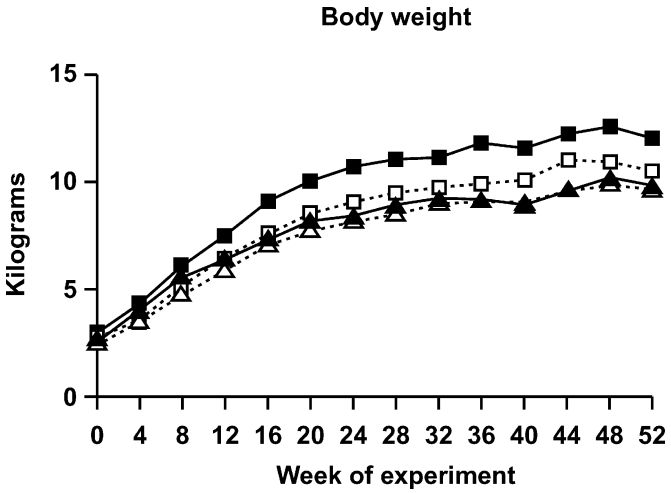


**Figure 1**. Growth data for weanling male dogs consuming a plant product-based diet (–■–), weanling male dogs consuming an animal product-based (APB) diet (- - □ - -), weanling female dogs consuming a plant product-based (PPB) diet (–▲–), and weanling female dogs consuming an animal product-based diet (- - △ - -) (n = 3/treatment). Males grew at a faster ($P = 0.006$; pooled SEM = 0.41) rate than females. Dogs on the PPB diet tended to grow at a faster ($P = 0.06$) rate than dogs fed the APB diet.

## Results and Discussion

### *Growth*

Growth data were collected to monitor normal growth of weanling animals over the course of the experiment. As expected, males grew at a faster ($P = 0.006$) rate than did females, regardless of diet (Figure 1). In addition, dogs consuming the PPB diet tended to grow at a faster ($P = 0.06$) rate than did dogs fed the APB diet. Although statistical differences in growth due to diet were detected, all weanlings were healthy throughout the experiment and grew at a rate that was expected for this breed. Because both diets were balanced to meet all nutrient recommendations and were fed ad libitum, differences in growth were not expected. Although a statistical difference due to diet was detected, it is of little biological significance in this case. In fact, this

**Table 2.** Baseline serum metabolites and hematology of weanling and senior dogs

| Item[a] | Reference range[b] | Senior dog | Weanling dog | Pooled SEM |
|---|---|---|---|---|
| Cholesterol, mg/dL** | 109 to 315 | 208.25 | 140.08 | 9.60 |
| Fasting glucose, mg/dL** | 65 to 127 | 92.75 | 130.83 | 5.94 |
| Red blood cells, $10^6/\mu L$** | 5.5 to 8.5 | 6.26 | 5.27 | 0.13 |
| Hemoglobin, g/dL** | 12 to 18 | 14.54 | 11.69 | 0.33 |
| Hematocrit, %** | 37 to 52 | 40.83 | 35.63 | 0.96 |
| Platelets, $10^3/\mu L$* | 200 to 900 | 383.42 | 516.25 | 27.28 |
| Creatinine, mg/dL** | 0.5 to 1.6 | 0.47 | 0.26 | 0.03 |
| Blood urea nitrogen, mg/dL | 7.0 to 31.0 | 12.68 | 13.46 | 0.77 |
| Total protein, g/dL** | 5.4 to 8.0 | 6.99 | 4.68 | 0.12 |
| Albumin, g/dL** | 2.1 to 4.3 | 3.37 | 3.02 | 0.05 |
| Bilirubin, mg/dL* | 0.08 to 0.5 | 0.18 | 0.12 | 0.01 |
| Calcium, mg/dL** | 7.9 to 11.5 | 10.50 | 11.66 | 0.09 |
| Phosphorus, mg/dL** | 2.4 to 6.5 | 3.73 | 8.95 | 0.22 |
| Sodium, mEq/L* | 141 to 161 | 145.08 | 142.75 | 0.58 |
| Potassium, mEq/L** | 3.9 to 5.7 | 4.41 | 5.53 | 0.08 |
| Chloride, mEq/L* | 104 to 125 | 106.83 | 103.75 | 0.62 |
| Alanine transaminase, U/L* | 17 to 87 | 40.42 | 30.33 | 3.04 |
| Alkaline phosphatase, U/L** | 12 to 110 | 56.08 | 271.67 | 20.77 |
| c-Alkaline phosphatase, U/L | 0 to 40 | 11.08 | 9.01 | 1.43 |
| $\gamma$-Glutamyltranspeptidase, U/L | 1 to 11 | 5.00 | 3.83 | 0.55 |

[a]Significant effect of age: *$P < 0.05$; **$P < 0.001$.
[b]Reference ranges used at the Laboratory of Veterinary Diagnostic Medicine, University of Illinois.

difference was likely due to genotype rather than nutritional regimen. Because weanlings were randomly assigned to treatment, two "large-framed" pups (1 male, 1 female) were allotted to the PPB diet. Because expected adult size of these pups was not known at baseline and because baseline BW was not different between groups, random assignment appeared to be the most appropriate. With the low number of animals per group, however, this allotment was enough to create the appearance that the PPB diet resulted in enhanced growth.

### *Serum Chemistry and Hematology*

As previous reports have demonstrated (Kaspar and Norris, 1977; Fukuda et al., 1989; Lowseth et al., 1990; Strasser et al., 1993), most baseline serum chemistry and hematology measurements were affected by age (Table 2). The effect of age was primarily due to metabolite concentrations of weanlings. This finding is in agreement with Andersen and Schalm (1970), who reported that it takes up to 12 mo for hematological values and serum metabolite concentrations to be similar to those of adult dogs. With the exception of creatinine concentrations that were slightly lower than normal, all baseline measurements taken from senior dogs were within the reference range for dogs (Laboratory of Veterinary Diagnostic Medicine, University of Illinois, Urbana). However, baseline concentrations of fasting glucose, Ca, P, and alkaline phosphatase (**ALP**) from weanling puppies were above the normal range for dogs. Three isozymes of ALP are known to exist in canine serum, including bone ALP (**BALP**), liver ALP, and corticosteroid-induced ALP (**CALP**) (Hoffman and Dorner, 1975). Analysis of serum ALP is an established diagnostic assessment tool of bone and liver diseases (Saini and Saini, 1978). Although early methods had difficulty differentiating these isozymes, methods with high accuracy, such as polyacrylamide gel disk electrophoresis, are now available (Itoh et al., 2002). Because isozyme profiles will vary depending on the diseases (or normal growth), differentiation of individual ALP isozymes is very important for diagnostic purposes. High concentrations of ALP and P in weanlings of the current experiment are not surprising, as they are known to be high in young dogs due to normal bone growth (Kaspar and Norris, 1977; Koscinczuk et al., 1989). Total ALP concentrations of young dogs measured in the current experiment (100 to 300 U/L during first 6 mo) are similar to those of young dogs of other experiments (175 U/L, Syakalima et al., 1997; 187 U/L, Itoh et al., 2002). Because of rapid bone growth, concentrations of Ca also are often elevated slightly in young growing dogs (Hedhammar, 1996). Although stress hormones were not measured in this experiment, the high fasting blood glucose concentrations observed in the weanling population may have been due to the stress induced from the act of sampling blood. Stress-induced hyperglycemia has been demonstrated in humans (Sherwin et al., 1980) and often occurs in cats (Plier et al., 1998).

Baseline red blood cell (**RBC**), hemoglobin, hematocrit, creatinine, and total protein concentrations in weanling puppies were lower than what is considered "normal" in dogs. Strasser et al. (1993) reported similar findings, with lower hemoglobin and hematocrit concentrations ($P < 0.05$) in young (0.5 to 5 yr old) vs. senior (6 to 13.5 yr old) dogs. Moreover, Vajdovich et al. (1997) reported lower ($P < 0.05$) hemoglobin (13.0 vs. 14.9 g/

dL) and total protein (54.5 vs. 60.0 g/L) concentrations in young (<1 yr old) vs. old (>9 yr old) dogs. Red blood cell (5.9 vs. 6.3 cells, $10^6/\mu L$) and creatinine (48.8 vs. 60.2 μmol/L) concentrations were numerically, but not statistically, lower in young vs. old dogs in that experiment (Vajdovich et al., 1997). Low RBC and hemoglobin concentrations are common in young dogs because RBC lifespan is shorter in young dogs and young RBC contain less hemoglobin than old RBC (Bush, 1991). Creatinine, a by-product of normal muscle metabolism, is often present in low concentrations in the young simply because of low muscle mass (Wassner et al., 1977). Because several protein-containing metabolites were present in low concentrations in the weanling population, it was not surprising that total protein concentrations also were low.

Several constituents within the reference range for dogs were different between the age groups at baseline. Similar to results reported by Sheffy et al. (1985), blood cholesterol concentrations were greater in senior dogs compared with young dogs (Figure 2). Although age has been shown to have an effect on blood cholesterol in several species, it has not always been a significant finding in canine experiments (Lowseth et al., 1990; Barrie et al., 1993). Discrepancies between studies may be due to several factors, including genetic background and diet, body condition score, and age of dogs tested.

Although several components were not within the reference range for weanlings at baseline, 3-mo glucose, RBC, hemoglobin, hematocrit, and 6-mo creatinine, total protein, and P were within the reference range (Figures 2 to 5). The exception was ALP, which did not fall within the reference range until after dogs had been on experiment 9 mo. Serum ALP has been reported to be very high in young dogs and to decrease slowly with age as bone growth slows (Kaspar and Norris, 1977; Van Hoof et al., 1990; Itoh et al., 2002).

Blood cholesterol was one of only a few serum metabolites that was affected by diet over time, with dogs consuming the APB diet having greater ($P = 0.003$) blood cholesterol concentrations than dogs consuming the PPB diet (Figure 2a). These results agree with Julian et al. (1984), who observed greater ($P < 0.05$) blood cholesterol concentrations in dogs consuming high concentrations of cholesterol and/or saturated fat. Increased intake of soluble, viscous fibers has been shown to decrease cholesterol concentrations in some animal experiments. For example, guar gum supplementation (7.5%) has been shown to decrease ($P < 0.05$) blood cholesterol concentrations due to increased ($P < 0.05$) bile acid flux and fecal excretion in rats (Moundras et al., 1997). Blood cholesterol concentrations in dogs consuming the PPB diet were much lower than for the dogs fed the APB diet throughout the experiment, but did not decrease from baseline. Although TDF concentrations were much higher in the PPB diet (15%) vs. the APB diet (5%), it likely did not contain enough soluble, viscous fiber to decrease blood cholesterol concentrations. Dietary fiber (high) and saturated fat (low) concentrations of the PPB diet were likely responsible for the lack of increased cholesterol concentrations that were observed in dogs fed the APB diet.

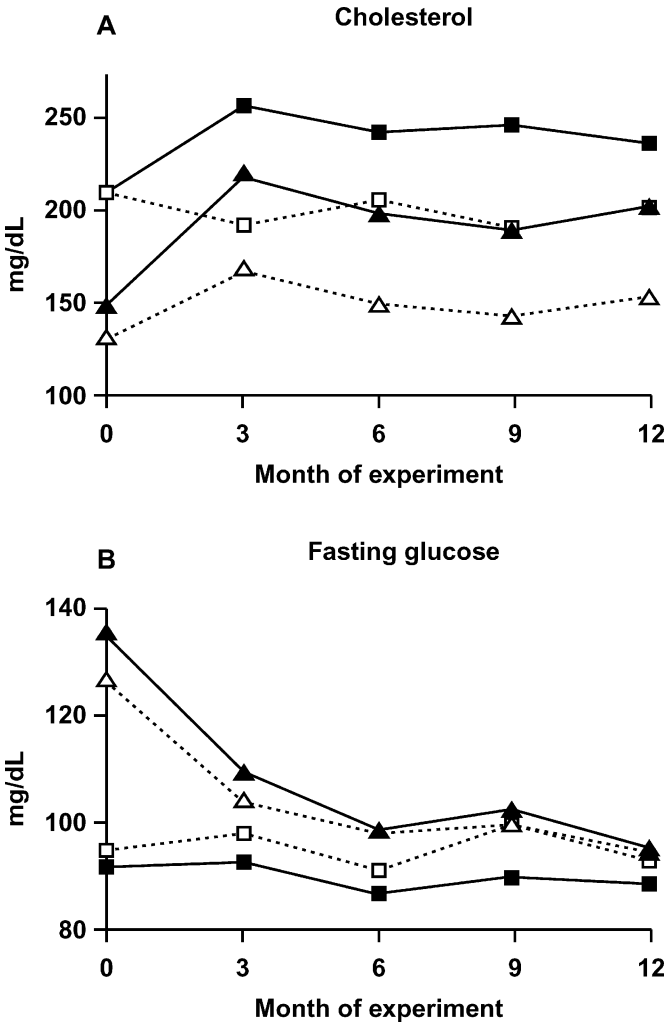


**Figure 2**. Blood cholesterol (a) and fasting blood glucose (b) concentrations of senior dogs consuming an animal product-based diet (–■–), senior dogs consuming a plant product-based diet (- - □ - -), weanling dogs consuming an animal product-based diet (–▲–), and weanling dogs consuming a plant product-based diet (- - △ - -) at baseline and after 3, 6, 9, and 12 mo on experiment (n = 6/treatment). Over time, cholesterol concentrations were affected by diet ($P = 0.003$) and age ($P = 0.03$; pooled SEM = 17.76), whereas fasting blood glucose was affected by age only ($P = 0.003$; pooled SEM = 5.49).

Diet had a minor effect on serum metabolites, but age affected concentrations of most metabolites measured. However, the significant effect of age was primarily due to the changes observed in the young dog population (concentrations approached those of senior dogs) as they matured into young adults. Most serum metabolite concentrations of the young adults (weanling dogs at 12 mo) were similar to those of senior dogs. In general, serum metabolite and hematology measurements in senior dogs remained fairly stable and within the refer-

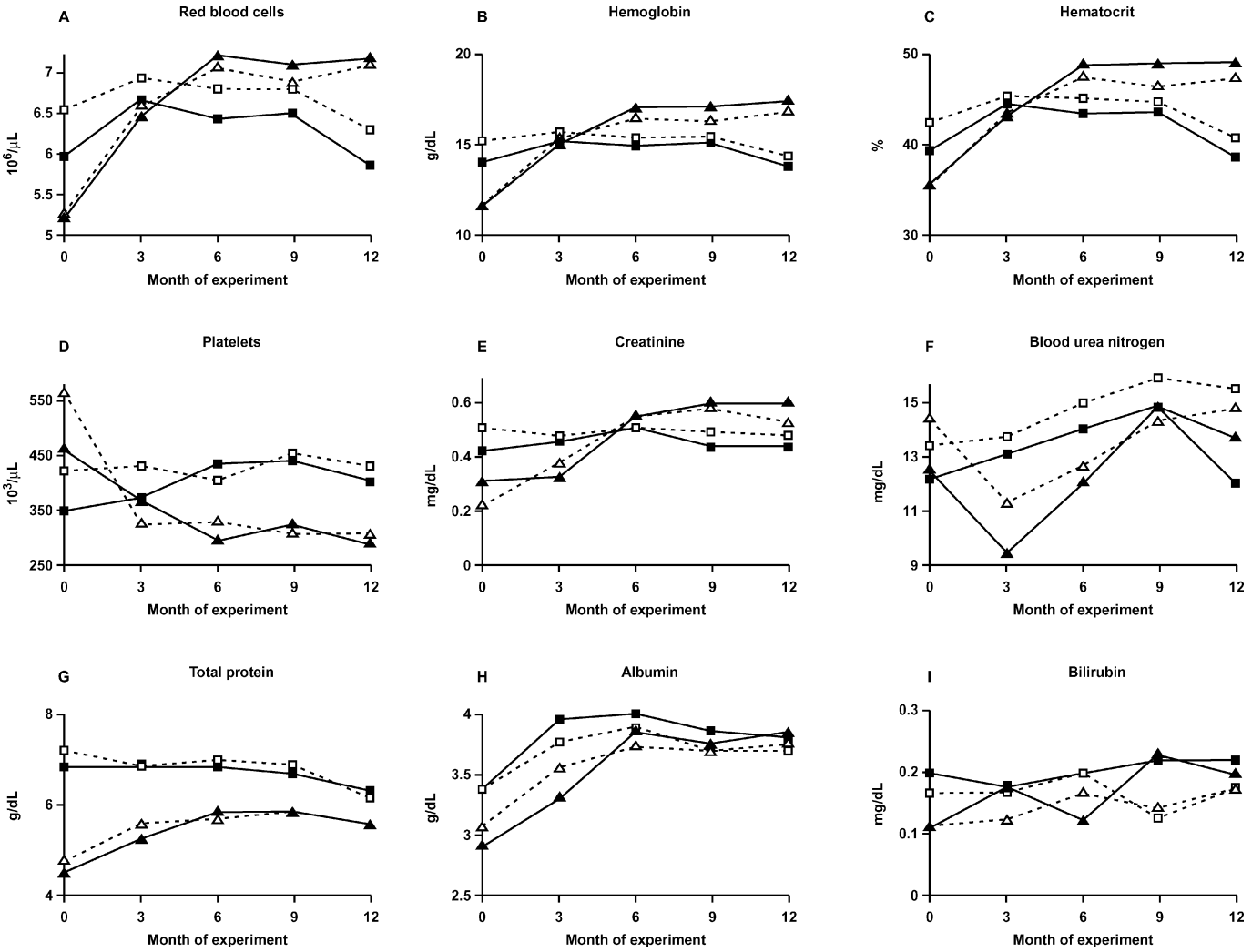


**Figure 3**. Red blood cell (a), hemoglobin (b), hematocrit (c), platelet (d), creatinine (e), blood urea nitrogen (f), total protein (g), albumin (h), and bilirubin (i) concentrations of senior dogs consuming an animal product-based diet (–■–), senior dogs consuming a plant product-based diet (- - □ - -), weanling dogs consuming an animal product-based diet (–▲–), and weanling dogs consuming a plant product-based diet (- - △ - -) at baseline and after 3, 6, 9, and 12 mo on experiment (n = 6/treatment). Over time, age had a significant effect on concentrations (difference from baseline) of red blood cells ($P < 0.001$; pooled SEM = 0.26), hemoglobin ($P < 0.001$; pooled SEM = 0.63), creatinine ($P < 0.001$; pooled SEM = 0.04), total protein ($P < 0.001$; pooled SEM = 0.14), platelets ($P < 0.001$; pooled SEM = 43.58), albumin ($P < 0.001$; pooled SEM = 0.09), and bilirubin ($P < 0.05$; pooled SEM = 0.03). Age also affected hematocrit percent ($P < 0.001$; pooled SEM = 1.83) and tended to influence blood urea nitrogen concentrations ($P = 0.10$; pooled SEM = 1.06). Albumin concentrations also were affected by diet ($P < 0.01$).

ence range throughout the experiment. Exceptions included alanine transaminase (**ALT**), ALP, and CALP, which increased over time in senior dogs (Figure 5). Alanine transaminase concentrations were greater ($P < 0.05$) at baseline in senior dogs and continued to increase over time (near or above the reference range) compared with young dogs. Alanine transaminase is virtually liver specific, but is also present in muscle, kidneys, and erythrocytes (Fraser et al., 1991). Although elevated serum levels of ALT are often signs of hepatic injury or disease, these levels must be interpreted in conjunction with other clinical signs.

Alkaline phosphatase and CALP, the isoenzyme unique to dogs, were influenced by both age and diet over time. High ALP concentrations (most likely due to high BALP) of young dogs approached those of senior dogs as dogs matured, a relationship observed in other experiments (Syakalima et al., 1997; Itoh et al., 2002). As ALP concentrations of senior dogs consuming the PPB diet remained stable, the ALP of senior dogs consuming the APB diet continued to increase over time. The increase observed in total ALP concentrations in senior dogs consuming the APB diet appeared to be primarily due to the increase in CALP, which dramatically increased over time and ended up out of the reference range after 12 mo on experiment. Other researchers also have reported greater serum CALP levels in old dogs compared to young dogs and middle age dogs

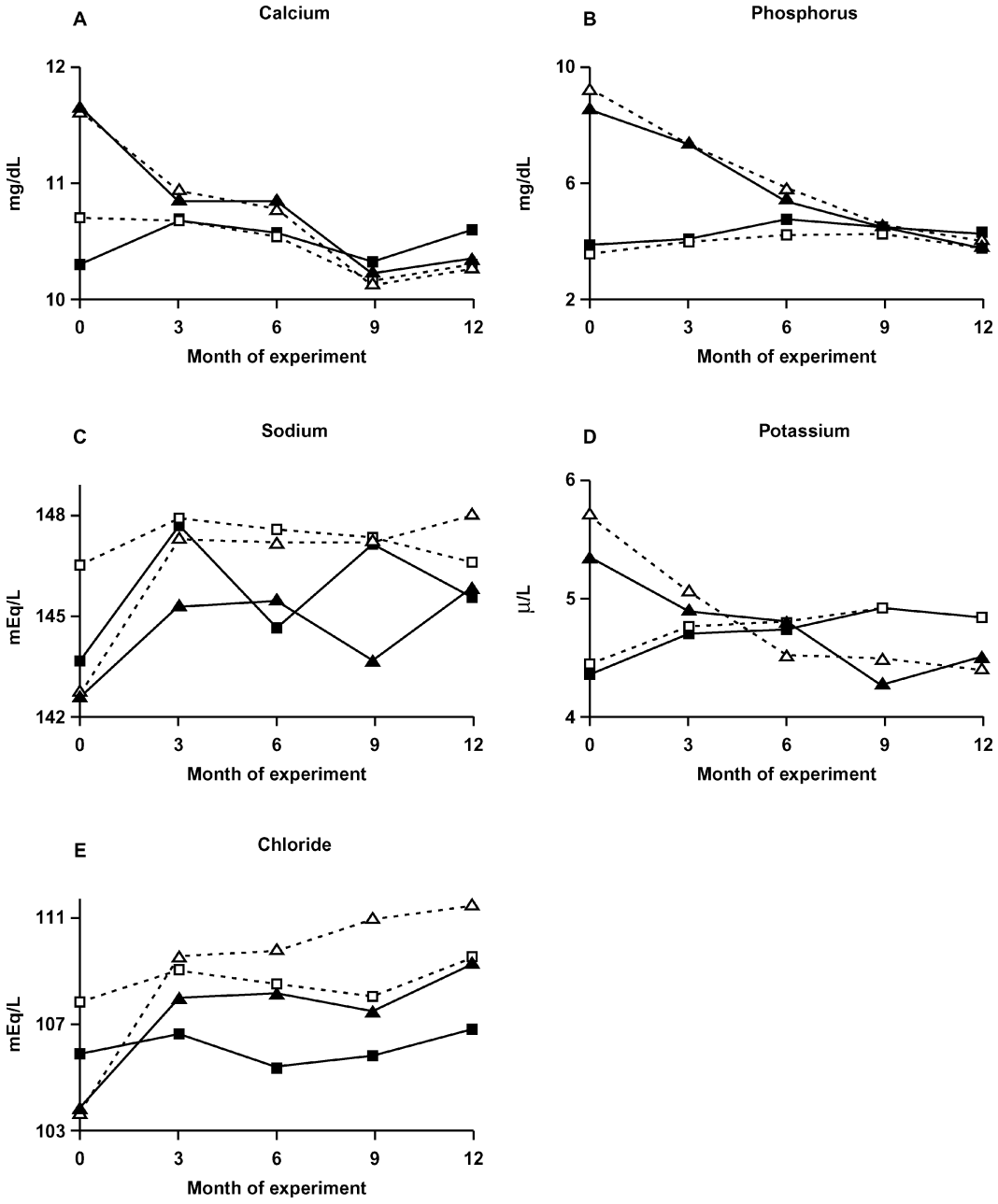


**Figure 4**. Blood Ca (a), P (b), Na (c), K (d), and Cl (e) concentrations of senior dogs consuming an animal product-based diet (–■–), senior dogs consuming a plant product-based diet (- - □ - -), weanling dogs consuming an animal product-based diet (–▲–), and weanling dogs consuming a plant product-based diet (- - △ - -) at baseline and after 3, 6, 9, and 12 mo on experiment (n = 6/treatment). Over time, age affected ($P < 0.001$) concentrations (difference from baseline) of Ca (pooled SEM = 0.18), P (pooled SEM = 0.29), K (pooled SEM = 0.14), and Cl (pooled SEM = 1.08). Blood Ca concentrations also were affected by diet ($P < 0.05$). Pooled SEM of Na = 1.20.

(Syakalima et al., 1997; Itoh et al., 2002). In one report, the proportion of CALP in young (6 d to 1 yr old; 12% of total ALP) and middle-aged (1 to 7 yr old; 11% of total ALP) dogs was much lower than that of old (>7 yr old; 27% of total ALP) dogs (Syakalima et al., 1997). Itoh et al. (2002) also reported a disparity in CALP proportion among age groups, with young (<1 yr old), middle-aged (1 to 7 yr old), and old dogs (>7 yr old) having 1.5, 2.5, and 25.6% of total ALP as the CALP isoenzyme. Although the general appearance of the senior dog population did not decline throughout the experiment, the increased enzyme concentrations (e.g., ALT, CALP) suggest damage to or abnormal metabolic activity in the liver or, in the case of CALP, increased production of stress hormones by the body.

Although cortisol was not measured in the current experiment, concentrations of CALP suggest changes in its secretion over the course of the experiment in old dogs fed the APB diet. Greater serum concentrations of cortisol, the primary glucocorticoid secreted by the

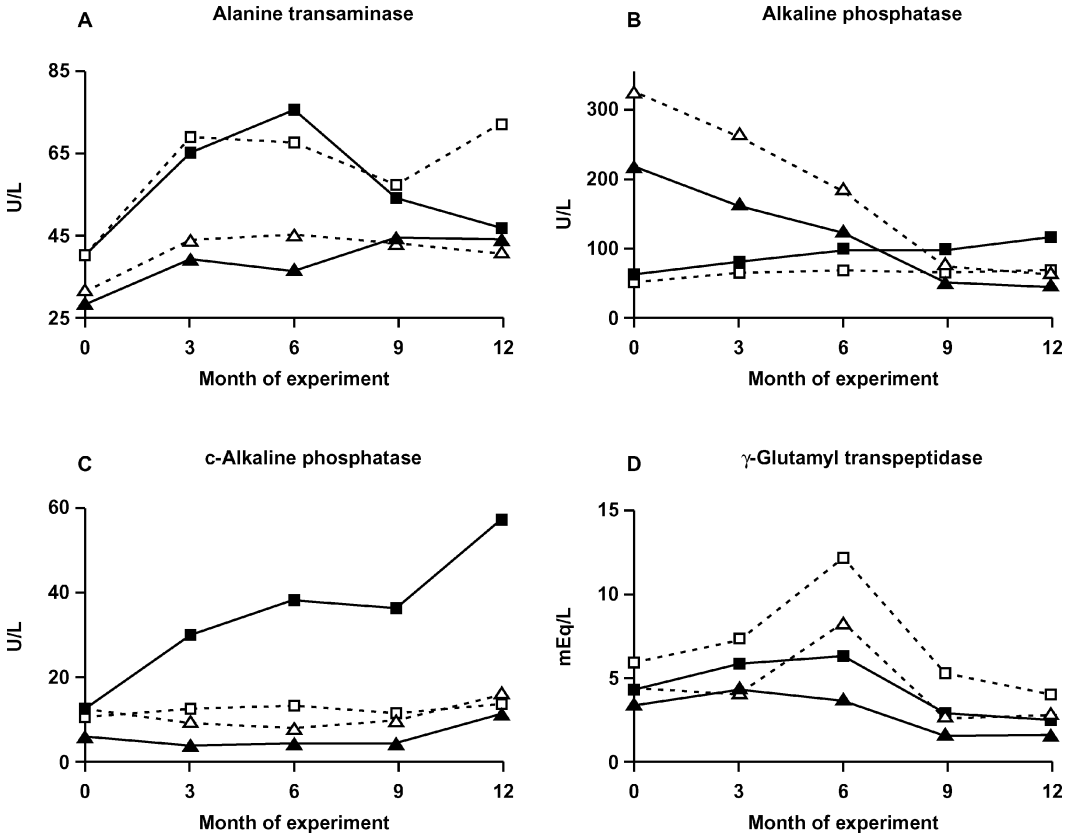


**Figure 5**. Blood alanine transaminase (ALT) (a), alkaline phosphatase (ALP) (b), c-alkaline phosphatase (c-ALP) (c), and γ-glutamyl transpeptidase (GGT) (d) concentrations of senior dogs consuming an animal product-based diet (–■–), senior dogs consuming a plant product-based diet (- - □ - -), weanling dogs consuming an animal product-based diet (–▲–), and weanling dogs consuming a plant product-based diet (- - △- - ) at baseline and after 3, 6, 9, and 12 mo on experiment (n = 6/treatment). Over time, ALT was affected by age ($P < 0.05$; pooled SEM = 8.72), whereas ALP was affected by both age ($P < 0.001$) and diet ($P < 0.05$; pooled SEM = 20.43). An interaction effect between diet and age was observed for c-ALP ($P < 0.05$, pooled SEM = 4.83) concentrations over time. Pooled SEM for GGT = 1.32.

adrenal cortex of dogs (Bush, 1953), have been reported in old dogs (Palazzolo and Quadri, 1987; Gonzalez and Quadri, 1988). Several nonpathological factors may affect blood cortisol concentrations, including gender (Kemppainen et al, 1984), reproductive state (Feldman and Nelson, 1987), breed/size (Reimers et al., 1990), and stress. Environmental stressors, such as confinement (Friend et al., 1985) and low or high ambient temperature (Reid, 1962; Christison and Johnson, 1972), have been shown to influence blood cortisol levels in livestock species. However, because all dogs in the current experiment were housed in a temperature-controlled facility in pens of equal size, these potential stressors were constant across treatments and were unlikely factors for cortisol changes. Finally, metabolic diseases, such as diabetes mellitus, have also been shown to result in greater CALP concentrations (Saini et al., 1978; Eckersall and Nash, 1983; Oluju et al., 1984). Although feeding high levels of dietary fat are known to cause glucose intolerance and insulin resistance in rodents (Pedersen et al., 1991; Axen et al., 2003), diet did not affect fasting blood glucose concentrations in the current experiment. Although several factors are known to affect blood cortisol concentrations, it is unknown which of these may have led to greater cortisol and consequently CALP concentrations in old dogs fed the APB diet in this experiment.

Serum albumin has been shown to decrease as dogs age (Lowseth et al., 1990; Hayek, 1998). However, this trend was not observed in the current experiment (Figure 3). Advancing age also has been associated with decreased concentrations of creatinine, Ca, RBC, hemoglobin, and hematocrit, and increased concentrations of Na, K, and platelets (Fukuda et al., 1989; Hayek, 1998). Many of these trends were observed in the current experiment, as RBC, hemoglobin, and hematocrit concentrations changed dramatically over time in young dogs and were present in greater ($P < 0.001$) concentrations than in senior dogs at the end of 12 mo (Figure 3). Low creatinine concentrations in senior dogs may be due to a lack of exercise and decreased muscle mass (Fukuda et al., 1989). Platelet count remained fairly stable in the senior dog population, but concentrations in young dogs continually decreased over time and were lower ($P < 0.001$) than for senior dogs after 12 mo.

**Table 3.** Food intake, fecal characteristics, and total-tract macronutrient digestibilities by weanling and senior dogs after 3 mo on experiment

| | Animal product-based diet | | Plant product-based diet | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item[a] | Senior dog | Weanling dog | Senior dog | Weanling dog | SEM | Age | Diet | Age × diet |
| Food intake (DMB), g/d | 199.1 | 150.4 | 250.4 | 225.6 | 26.03 | 0.17 | 0.03 | 0.65 |
| Caloric intake, kJ/d | 4,482 | 3,386 | 4,977 | 4,483 | 553.3 | 0.17 | 0.17 | 0.59 |
| Fecal output (as-is basis), g/d | 98.0 | 95.2 | 254.1 | 253.0 | 29.46 | 0.95 | 0.001 | 0.98 |
| Fecal output (DMB), g/d | 30.5 | 28.0 | 58.1 | 55.6 | 5.62 | 0.67 | 0.001 | 1.00 |
| Fecal DM, % | 31.6 | 29.5 | 23.6 | 22.4 | 0.83 | 0.07 | 0.001 | 0.55 |
| Fecal output (g/d as-is basis)/ food intake (g/d DMB) | 0.51 | 0.64 | 0.98 | 1.11 | 0.05 | 0.02 | 0.001 | 0.96 |
| DM digestibility, % | 83.9 | 81.1 | 77.2 | 75.3 | 1.22 | 0.07 | 0.001 | 0.74 |
| OM digestibility, % | 87.3 | 83.8 | 80.0 | 77.1 | 1.07 | 0.01 | 0.001 | 0.74 |
| CP digestibility, % | 77.7 | 72.3 | 72.5 | 76.7 | 3.56 | 0.87 | 0.91 | 0.19 |
| Fat digestibility, % | 95.5 | 94.8 | 88.7 | 87.2 | 0.46 | 0.02 | 0.001 | 0.41 |

[a]DMB = DM basis.

Dogs have been shown to develop diabetes at almost any age, but diabetes is most common from 7 to 9 yr of age (Nelson, 1995). Mattheeuws et al. (1984) reported that as dogs increased in age and obesity, regulation of plasma glucose and insulin worsened, which is also similar to results of human studies (Glass et al., 1981). Other reports have also suggested dysregulation of glucose metabolism in geriatric dogs (Sheffy et al., 1985; Mosier, 1989). The link between old age and diabetes is strong, and increased fasting blood glucose concentration is a likely an early sign of diabetes or other metabolic disease (e.g., hyperadrenocorticism). However, in healthy populations of old dogs, fasting blood glucose concentrations are not different from those of healthy young adults (Fukuda et al., 1989; Lowseth et al., 1990). In the current experiment, fasting blood glucose concentrations of healthy senior dogs were not different from that of young adults (weanling population at 12 mo).

Overall, it seems that serum metabolite concentrations are not markedly influenced by diet composition as long as all nutrient requirements are met. In contrast, age has a major impact on most serum metabolites, with weanling puppies having levels different than healthy adults. As growth slowed, however, most metabolites approached reference values for healthy adults. For the most part, elderly dogs have the ability to regulate serum metabolite concentrations, similar to that of young adults, when dietary requirements are met.

### *Nutrient Digestibility*

Food intake (g/d) was greater ($P < 0.05$) for dogs consuming the PPB diet after 3 mo on experiment; however, daily caloric intake (kJ/d) was similar among dogs consuming the APB and PPB diets during this time period (Table 3). As expected, DM and OM digestibilities were greater ($P < 0.001$) for dogs fed the APB diet. The major influence on OM digestibility was that of fat, as it was digested to a greater extent by dogs consuming the APB vs. the PPB diet ($P < 0.001$). Dry matter ($P = 0.07$), OM ($P = 0.01$), and fat ($P = 0.02$) digestibilities also were greater for senior dogs after 3 mo on test. Correspondingly, dogs fed the PPB diet had a lower ($P < 0.001$) fecal DM percent and greater ($P < 0.001$) fecal output (when expressed either on an as-is or DM basis) and fecal output (g/d, as-is basis):food intake (g/d, DM basis) ratio.

Although a significant effect of age was no longer detectable, most effects of diet observed after 3 mo also occurred after 10 mo on experiment (Table 4). Dogs consuming the PPB diet had higher food intake (g/d; $P = 0.001$), caloric intake (kJ/d; $P = 0.02$), fecal output (g/d, as-is and DM basis; $P < 0.001$), and fecal output:food intake ratio ($P = 0.005$), and lower ($P < 0.001$) fecal DM percent than dogs consuming the APB diet. Apparent DM digestibility percent was not different between dogs consuming the two diets. However, a trend for increased ($P = 0.09$) OM digestibility by dogs consuming the APB diet was observed. Greater ($P < 0.001$) amounts of fat were digested by dogs consuming the APB vs. the PPB diet. Crude protein digestibility was greater ($P = 0.005$) by dogs consuming the PPB diet.

Sheffy et al. (1985) also reported lower apparent digestibilities of protein (76.1 vs. 78.6) and fat (80.3 vs. 83.4) for young vs. senior dogs. The senior age group tested by Sheffy et al. (1985) was similar in age to those used in the current experiment (10 to 12 yr old), but their young population was 1 yr old. Therefore, our results do not correlate exactly with those of Sheffy et al. (1985), as we observed differences due to age after 3 mo on experiment (when the young dogs were approximately 5 mo old), but not after 10 mo (young dogs were approximately 1 yr old). Several explanations may be offered for the decreased nutrient digestibility by young dogs observed during mo 3 of our experiment, but only a few recently published experiments have closely examined the physical and functional characteristics of the gastrointestinal tract of young puppies (Buddington et al., 2003; Buddington and Malo, 2003; Paulsen et al., 2003). Lower total-tract nutrient digestibility by young dogs may be due to less absorptive capacity,

**Table 4.** Food intake, fecal characteristics, and total-tract macronutrient digestibilities by weanling and senior dogs after 10 mo on experiment

| | Animal product-based diet | | Plant product-based diet | | | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| Item[a] | Senior dog | Weanling dog | Senior dog | Weanling dog | SEM | Age | Diet | Age × diet |
| Food intake (DMB), g/d | 183.5 | 148.6 | 235.2 | 237.7 | 18.63 | 0.40 | 0.001 | 0.33 |
| Caloric intake, kJ/d | 4,130 | 3,346 | 4,675 | 4,724 | 389.6 | 0.36 | 0.02 | 0.30 |
| Fecal output (as-is basis), g/d | 122.1 | 93.2 | 231.1 | 239.0 | 17.90 | 0.57 | 0.001 | 0.32 |
| Fecal output (DMB), g/d | 36.3 | 28.4 | 54.0 | 54.8 | 3.43 | 0.32 | 0.001 | 0.22 |
| Fecal DM, % | 29.7 | 30.7 | 23.7 | 23.2 | 0.69 | 0.76 | 0.001 | 0.28 |
| Fecal output (g/d as-is basis)/ food intake (g/d DMB) | 0.67 | 0.70 | 0.98 | 1.00 | 0.09 | 0.79 | 0.005 | 0.97 |
| DM digestibility, % | 80.1 | 78.9 | 76.8 | 76.8 | 2.43 | 0.80 | 0.28 | 0.80 |
| OM digestibility, % | 84.0 | 82.8 | 79.3 | 79.9 | 2.12 | 0.88 | 0.09 | 0.69 |
| CP digestibility, % | 72.3 | 74.5 | 80.8 | 81.2 | 2.34 | 0.58 | 0.005 | 0.71 |
| Fat digestibility, % | 93.6 | 94.0 | 87.5 | 87.9 | 0.72 | 0.61 | 0.001 | 0.98 |

[a]DMB = DM basis.

lower production of pancreatic and intestinal enzymes, or differences in bacterial populations in the large bowel. Small intestinal length, weight, and surface area have been shown to be much higher in puppies than adult dogs when adjusted for BW; however, total absorptive area is greater in adults due to larger body size (Paulsen et al., 2003). Activities of gastric, pancreatic, and intestinal brush-border membrane enzymes have been shown to change during postnatal development in dogs (Buddington et al., 2003), but it is unknown whether these changes may have resulted in the lower nutrient digestibility by young dogs observed in the current experiment. Finally, because microbial populations have been shown to alter total-tract nutrient digestibility, it is possible that the changes in gut microbial ecology that occur as dogs age may be responsible for the decreased apparent digestibilities observed in young dogs in the current experiment (Buddington, 2003).

Differences in apparent nutrient digestibility due to diet were not surprising and may be the result of several factors, one of which is the inclusion of soybean meal (**SBM**) as a protein source in the PPB diet. Due to the presence of natural nondigestible oligosaccharides (raffinose, 14 mg/g; stachyose, 52 mg/g; Grieshop et al., 2001), SBM often leads to greater fecal production, looser stools, and lower apparent nutrient digestibility when fed to dogs (Bednar et al., 2000; Clapper et al., 2001). However, when researchers measure ileal digestibility to determine what is digested by the animal without the confounding effect of the microbiota present in the large bowel, decreased digestibility as a result of SBM inclusion in the diet often is not detected.

Similar to the increases in total-tract CP digestibility by dogs fed the PPB diet after 10 mo on the current experiment, Zuo et al. (1996) reported increases in both ileal and total-tract CP digestibility by dogs fed SBM-containing diets vs. those consuming poultry meal. Similarly, Clapper et al. (2001) reported that soy-containing diets had greater ($P < 0.01$) ileal and total-tract CP digestibility than did poultry meal-containing diets. Food intake was not affected by diet in that study, but soy-containing diets resulted in greater ($P < 0.01$) fecal output (g/d as-is basis) and higher ($P < 0.01$) fecal scores (looser stools) than was the case for dogs consuming a poultry meal-based diet (Clapper et al., 2001). Bednar et al. (2000) also reported greater ($P < 0.05$) fecal output and fecal scores for dogs fed SBM-containing diets compared with those fed diets containing poultry by-product meal or poultry meal. Ileal digestibility was not influenced by diet in that experiment, but SBM resulted in decreased total tract DM and OM digestibilities as compared with poultry-containing diets (Bednar et al., 2000). In agreement with the results of the current experiment, Yamka et al. (2003) observed a linear decrease ($P < 0.001$) in ileal and total tract DM digestibility as SBM increased in dog diets. These researchers also observed a linear decrease ($P < 0.002$) in ileal, but not total tract, CP digestibility. The discrepancies that exist among these experiments may have been due to the amount (Zuo et al., 1996; 19 or 37% SBM; Bednar et al., 2000; 30% SBM; Clapper et al., 2001; 34 to 44% soy-containing ingredient; Yamka et al., 2003; 15 to 46% SBM; current experiment, 20% SBM) or type (SBM, soy flour, soy protein isolate) of soy-containing ingredient included in the diet and/or the composition of the diet with which it was compared (e.g., poultry meal, poultry by-product meal, meat and bone meal).

Another explanation for the lower apparent digestibility and increased fecal output by dogs consuming the PPB diet was the difference in TDF concentration (4.8 vs. 15.2%) between the diets. Increases in fecal weight from fiber consumption may be due to increased excretion of undigested plant matter (e.g., nonfermentable fibers) or increases in fecal bacteria and lipid excretion (e.g., viscous, fermentable fibers) (Chen et al., 1998). As stated previously, some of the TDF originated from SBM (contains approximately 6% TDF) in the form of nondigestible oligosaccharides. However, corn (contains approximately 9% TDF) and wheat midds (contains approximately 43% TDF) were also primary contributors of fiber in the PPB diet (Grieshop et al.,

2001). Numerous experiments have documented decreased nutrient digestibility and increased fecal bulk with diets containing TDF concentrations similar to those of the PPB diet used in the current experiment (Fahey et al., 1990; 1992; Kienzle et al., 1998). Diets containing approximately 12.5% TDF resulted in decreased ($P < 0.05$) total-tract DM and OM digestibility, increased ($P < 0.05$) wet fecal weight, and decreased ($P < 0.05$) fecal DM percent compared with the control diet (6% TDF; Fahey et al., 1990). Similarly, diets containing approximately 11% TDF resulted in decreased ($P < 0.05$) total-tract DM and OM digestibility, increased ($P < 0.05$) amount of wet feces excreted (g/d), and decreased ($P < 0.05$) fecal DM percent compared with the control diet (approximately 6% TDF; Fahey et al., 1992).

Much of the influence on apparent digestibility in the current experiment was on the lipid fraction, as fat digestibility was approximately 6 percentage units lower in dogs fed the PPB vs. the APB diets, regardless of age. Dietary fiber has been shown to alter lipid absorption and bile acid metabolism and reduce bile acid reabsorption either directly or indirectly via short-chain fatty acid production (Eastwood, 1992). The most direct means of decreasing fat or cholesterol digestibility is by decreasing its absorption in the small intestine by binding polysaccharide structures and/or by being physically hindered and protected from pancreatic and biliary secretions (Eastwood, 1992; Kim, 2000). Similar binding mechanisms may lead to increased bile acid excretion as well. Another potential mechanism of decreasing fat absorption may be by decreasing pancreatic lipase production. Stock-Damgé et al. (1983) observed increased ($P < 0.05$) total pancreatic secretion in dogs after 4 wk of wheat bran supplementation (5 g/d), but reported a decreased ($P < 0.05$) lipase concentration.

## Implications

Weanling and senior dogs have unique physiological characteristics and therefore have unique nutritional needs. Because weanlings often have serum metabolite profiles that do not fit within the reference ranges for healthy adults, researchers must be aware of these differences when analyzing these data. Although the type of dietary constituents included in dog diets has large effects on nutrient digestibility and fecal characteristics, most serum metabolites were unchanged in healthy animals. Provided that all nutrient requirements are met, the composition of diet seems to have minor effects on serum metabolite concentrations in weanling and senior dogs.

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