

**EFFECT OF DIETARY POLYUNSATURATED FATTY ACID AND RELATED  
NUTRIENTS ON PLASMA LIPIDS, AND SKIN AND HAIR COAT CONDITION  
IN CANINES**

A Thesis

by

SHALEAH LYNNAE HESTER

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2004

Major Subject: Nutrition

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## **ABSTRACT**

Effect of Dietary Polyunsaturated Fatty Acid and Related Nutrients on Plasma Lipids, and Skin and Hair Coat Condition in Canines. (August 2004)

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Chair of Advisory Committee: Dr. John E. Bauer

A study was performed to investigate the effect of diet modifications on skin and hair coat condition in canines. The study included 24 normal adult dogs fed a baseline diet (Ol'Roy®), during an acclimation period of 12 wk (Phase I). Nine female Beagles and 15 male Hound mix-breed dogs were used. For the next 12 wk (Phase II) the dogs were divided into three groups and fed one of three specially formulated diets. They contained similar ingredients and had similar nutrient profiles except for the following differences: Diet A contained lower but adequate amounts of dietary zinc and linoleic acid than diet B. Diet C was similar to B with respect to zinc and linoleic acid but contained more  $\alpha$ -linolenic acid. An evaluation panel conducted skin and hair coat condition scoring on wk 0, 4, 7, and 12 (Phase I) and wk 14, 16, 19, and 24 (Phase II). The panel evaluated the dogs for glossiness, softness, scale, greasiness, and overall condition. Transepidermal water loss (TEWL) and skin hydration (HYDR) assessments were determined on wk 3, 7, and 11 (Phase I) and wk 11, 12, 15, 19, and 23 (Phase II) using a Tewameter® and Corneometer® respectively.

Blood samples were collected on d 0, 5, 8, 16, 28, 56, and 84. Profiles of plasma phospholipid fatty acids were determined at each collection period. Serum zinc concentrations were analyzed on wk 12, 14, and 24. The hypothesis was that a diet containing increased LA, ALA, and zinc concentrations (diet C) would show improvements of skin and hair coat condition in dogs compared to the other diets. All three test diets caused significant improvements compared to Ol'Roy®. Diet B caused more improvement than diet A in both subjective and objective assessments of skin and hair coat. Based on mean values diet B is better to be fed to dogs that need to improve skin hydration and diet C should be fed to dogs that need to decrease TEWL. Diet C not only led to improvements in skin and hair coat condition, but also provided additional benefit by producing less pro-inflammatory conditions in the skin.

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## **CHAPTER I**

### **INTRODUCTION**

The commercial pet foods manufactured today are typically complete, well-balanced, and meet the nutrient profiles set forth by expert panels and regulatory bodies. Thus, severe nutritional deficiencies are uncommon in companion animals. In spite of this phenomenon, pet owners often perceive skin and hair coat condition as an indicator of their animal's optimal nutrition and well-being. Indeed dietary factors, especially essential fatty acids, play an important role in the maintenance of healthy skin and hair coats. Fatty acids are important in providing membrane fluidity and maintaining the cutaneous water permeability barrier, and function as precursors of metabolic pathways (1). They are also significant in the etiology and treatment of certain skin diseases (2). Skin is the largest metabolically active organ in the canine body, and nutritional deficiencies can cause impairment of its quality and function. The sheer magnitude of the skin and hair coat can have a large impact on the nutritional requirements of the animal. Dietary deficiencies of certain individual nutrients can lead to poor skin and coat quality. Specifically, diets that are low in fatty acids and/or zinc have been associated with poor skin and hair coat quality in dogs. These nutrients likely play an important role in the normal biology and physiology of the skin and hair coats in dogs (3).

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This thesis follows the style and format of the Journal of Nutrition.

*Fatty Acids and Skin Health.* The essentiality of dietary fatty acids has been known for many years, and they are important for growth, reproduction, and maintenance of healthy skin in dogs (4). Nonetheless, there has yet to be a fully established description of essential dietary fatty acid requirements for several animal species, including dogs. Due to numerous nutritional and technical problems with laboratory rat diets recommendations have been made by the American Institute of Nutrition (AIN) to include a fat source that contains both linoleic [LA, 18:2(n-6)] and  $\alpha$ -linolenic [ALA, 18:3 (n-3)] fatty acids. AIN was created to identify dietary standards for nutritional studies with laboratory rodents (5).

Vertebrates are able to synthesize fatty acids of the (n-9) series *de novo*. However, the (n-3) and (n-6) fatty acids are not synthesized and required in the diet (6,7). The (n-6) polyunsaturated fatty acids (PUFA) are readily obtained from terrestrial animal and plant seed sources. The (n-3) PUFA are found in marine life sources such as fish, phytoplankton, and plants (8). Leaf tissues of terrestrial plants also contain reasonably high amounts of ALA but these materials are low in total fat and may not constitute major dietary fat sources for omnivorous and/or carnivorous animals (6).

Clinical signs of essential fatty acid (EFA) deficiencies include poor growth, infertility, thin discolored hair coat, scaly skin, sebaceous gland hypertrophy with increased sebum viscosity, increased epidermal turnover rate, increased DNA synthesis in keratinocytes, weak cutaneous blood vessels that

rupture easily, decreased wound healing and increased transepidermal water loss (TEWL) (4,9). Results of animal studies suggest that (n-3) fatty acids are anti-inflammatory and useful in the prevention of chronic diseases such as cancer, stroke, thrombosis, allergy and aging (6,10). The (n-3) fatty acids have increasingly gained popularity in the treatment of pruritic skin conditions, such as atopy, in dogs (11-13). They are thought to work by shifting the availability of eicosanoid precursors towards the production of less inflammatory mediators (13). Evidence for this effect was reported in a study in which dogs with canine pruritic skin disease were supplemented with high dietary amounts of (n-3) marine fish oil ( $66 \text{ mg}^{-1} \text{ kg}$  of body weight per day) over a 6 wk period. At the end of the study, dogs showed significant improvement in pruritus as well as skin and hair coat character (11).

Because fatty acids are a component of the phospholipid portion of cell membranes, dietary intake will affect membrane composition. When incorporated into the diet, (n-3) and (n-6) fatty acids compete for the same metabolic enzyme reactions (i.e.  $\Delta 6$  desaturase) in the body. The competition exists due to the need for chain elongation and desaturation of LA and ALA to 20-carbon eicosanoid precursors. Upon activation of membrane phospholipases, a metabolic cascade is initiated in which the (n-3) and (n-6) phospholipid fatty acids competitively react with enzymes to be incorporated into leukotrienes, prostaglandins and hydroxylated eicosatetraenoic acids (14). When physical or chemical trauma to the cell membrane occurs, (n-6) fatty acids (primarily

Arachadonic Acid, AA) are converted into prostaglandins of the 2 series ( $\text{PGE}_2$ ) and leukotrienes of the 4 series ( $\text{LTB}_4$ ). By contrast, the (n-3) fatty acids, when present, are transformed into 3 series prostaglandins ( $\text{PGE}_3$ ) and 5 series leukotrienes ( $\text{LTB}_5$ ), which are less pro-inflammatory than their corresponding (n-6) fatty acid isomers (6). Therefore, the presence of newly synthesized  $\text{LTB}_5$  would serve to inhibit  $\text{LTB}_4$ -induced neutrophil activation and thus diminish  $\text{LTB}_4$  mediated allergic or inflammatory conditions in skin and other tissues (12,14-18). Consequently specific combinations of (n-3) and (n-6) fatty acids may benefit the health and appearance of pet animals such as the dog by modulating inflammatory conditions in the skin.

Studies investigating the anti-inflammatory response of (n-3) fatty acids and their use in prevention and treatment of chronic diseases has been studied primarily in mice, rats, humans, horses, and in in vitro studies, with only a limited amount of research being conducted in dogs [reviewed in Bauer, Henry et al. (6,19)]. Fatty acid metabolism of dogs is similar in some respects to that of humans. For example, canine peroxisomal fatty acid metabolism is more similar to humans than rodents. Thus, for peroxisomal lipid metabolism, studies in dogs may lead to a better understanding of human lipid metabolism disorders. However, most studies of canine lipid metabolism have not documented precise amounts and types of dietary fatty acids consumed (20). Therefore, definite conclusions with respect to dietary fatty acid consumption and physiologic response have not been possible. Hence, studies designed to more clearly

recognize and determine specific effects of EFA are needed in order to further enhance this area of investigation.

*Zinc and Skin Health.* One objective of the present study was to evaluate the effects of diets enriched in EFA and zinc on the skin and hair coat in dogs. In addition to dietary fatty acids, zinc plays a vital role in regulating many aspects of cellular metabolism, many of which are concerned with the maintenance of healthy skin and hair coat. Zinc is required for the utilization of fatty acids and is a participant in both inflammatory and immune systems (2). It is a cofactor for RNA and DNA polymerases, thus, its presence is also of importance for rapidly dividing cells including those in the epidermis (2). A deficiency of zinc is considered rare in dogs and is usually only seen when the availability of dietary zinc is decreased or genetic or disease problems with zinc absorption are present (21). Common signs of zinc and EFA deficiencies are similar in that both are associated with sebaceous gland hypertrophy leading to a greasy, dull hair coat (4,22). However, zinc deficiencies usually produce a more profound seborrheic condition with a distinct type of keratinization (parakeratotic hyper-keratosis) being visible histopathologically (21).

*Importance of Both Zinc and LA on Skin Health.* Of the studies dealing with zinc and fatty acids, LA appears to be important as it relates to zinc concentrations. Two of the most widely researched nutritional skin problems are those related to zinc and LA. Zinc is essential for the conversion of LA to AA, by activating the  $\Delta 6$  desaturase enzyme, which is then incorporated into cell

membranes or converted into prostaglandins or leukotrienes (3). In a study done by Marsh et al. (3) it was demonstrated in healthy dogs that diets enriched with both LA and zinc showed significant improvement in skin and hair coat condition. The dogs fed increased amounts of both nutrients exhibited less scale, had more luster to their coat, and showed reduced levels of TEWL than a control group that was fed a basal diet containing the minimal amount of LA and zinc recommended by the National Research Council at that time (23). Skin of dogs supplemented with either zinc or LA alone were not improved by these measures (3). These data suggest that an interaction between zinc and LA is required in order to significantly enhance the condition of the skin and hair coat. It was hypothesized that zinc and LA must be supplemented in combination with each other in a healthy dog's diet in order for improvements in skin condition to be evident (3). In the present study we also expected improvements to be further evident in skin and hair coat condition when both EFA (LA and ALA) were supplemented in combination with zinc, primarily due to a sparing effect of ALA on LA conversion.

*Skin Physiology and Assessment.* Mammalian skin is a highly dynamic organ adapting to changes in its environment. It provides structural, sensory, immunologic, and physiologic functions and contributes an essential barrier function against potential environmental insults (24). The epidermis of the skin is comprised of keratinocytes that undergo a highly organized maturation process ultimately leading to desquamation (24). Structural components within

these cells utilize important lipids, necessary to maintain a protective barrier. Together they form the stratum corneum, the outermost layer of the epidermis. Environmental, physical, and nutritional alterations can modify epidermal structure and function. In addition to keeping environmental insults outside the body, the epidermal barrier also functions to keep water and other important metabolites inside the body. In this way, metabolic processes consonant with health can be maintained.

Techniques to characterize the skin's barrier function have included a number of noninvasive methods to measure moisture content and its loss through the skin surface (25). In humans day to day variations of these measurements exist (26) which are also likely to occur in dogs. One of these measurements is the determination of skin hydration (HYDR) using a method known as corneometry (27). This technique determines capacitance of the skin due to its behavior as a dielectric medium and typically assesses 10-20  $\mu\text{m}$  thickness of the stratum corneum. Because it is a measure of the water content of the skin, it is only an indirect measure of barrier function. Nonetheless, it can be related to the extent of hydration under various physiologic conditions either in response to injury, metabolic phenomena, or topical therapies. Skin hydration scores reported for human skin evaluations differ depending upon body site studied. Such variation may also be expected in dogs although little information exists as to normal canine skin hydration scores. Nonetheless, changes in

individual or mean hydration scores in a group of animals may serve as an index of skin health with higher scores typically considered more desirable.

Another method that has received some attention in dogs is assessment of the integrity of the skin barrier to insensible water loss (28,29). This technique is performed by measuring TEWL through the epidermal surface. The TEWL value is a measure of the rate of water lost through the skin in  $\text{g/hr-m}^2$  and is an estimate of skin's ability to retain moisture. It is an index of the extent of possible damage of the skin's water barrier function. Because water loss through the skin normally occurs by passive diffusion through the epidermis, higher TEWL values indicate greater water loss and are consistent with increased damage of the barrier function of stratum corneum such as may occur during irritant exposure, self-excoriation, or atopic dermatitis (30).

Recent work on TEWL and HYDR has been reported in dogs (28,29). One study investigated conditions under which TEWL measurements might be reliably used in Labrador Retrievers (29). It was found that less sample variance occurred when dogs were trained to stand without movement during the entire measurement period. Also, body site variation existed and recent hair clipping was shown to affect the value obtained. No significant effect of age on TEWL was found (2 to 7 y old vs. 8 to 11 y old).

Another study in dogs investigated both TEWL and HYDR measurements using different body sites in 5 healthy Beagle dogs (28). Reproducible results were found with the corneometry measurement (HYDR) but TEWL values were

less consistent. It was concluded that TEWL measurements were not reproducible enough to be compared between different animals, body site, or day of assessment. By contrast, although corneometry values varied among dogs and body location, the findings were reproducible and believed to be reliable for additional investigation.

*Study Objectives.* Although studies in dogs using these techniques (TEWL and HYDR) have been limited, both methods are non-invasive and may prove useful in veterinary clinical dermatology and nutrition. The present study involved evaluating HYDR and TEWL measurements in two different canine groups and to determine whether variability existed. The present study was conducted to also address two primary objectives. The first was to compare a diet adequate in LA and zinc to one that contained enriched amounts of LA and zinc on skin and hair coat condition in dogs. The second objective was to compare a diet rich in LA and zinc with one also enriched with ALA, in addition to LA and zinc, to determine whether additional improvements of skin and hair coat condition might exist. The effect of the diets on plasma phospholipid fatty acids was also evaluated. The hypothesis was that a diet high in a combination of LA, ALA, and zinc will show more improvement in skin and hair coat condition in dogs compared to a diet enriched only in LA and zinc, and that beneficial plasma phospholipid fatty acid profiles would also result.

## CHAPTER II

### MATERIALS AND METHODS

*Animals and Design.* The study included 24 normal adult dogs fed a baseline diet, during an acclimation period of 12 wk (Phase I). Nine clinically healthy female Beagles and 15 male Hound mix-breed dogs were used ranging in age from 1.5 y to 6.5 y with a median age of 4 y. Body weights were in the range of 15 to 40 kg. Prior to the start of the study, the dogs were physically examined and blood samples were collected for complete blood counts, serum biochemistry profiles, and serum TSH and T4 concentrations. All dogs in the study were healthy and laboratory tests were within the normal limits.

The dogs were fed a discount store type dry extruded, complete and balanced dog food product during Phase I (Ol'Roy Premium Formula®, Wal-Mart Inc., Bentonville, AR, USA). The dogs were provided feed daily to maintain their adult body weight and given free choice to water. They were also weighed on a weekly basis. For the next 12 wk (Phase II) the dogs were fed one of three specially formulated complete and balanced diets (Diets A, B, C). The diets were formulated using similar ingredients and had similar nutrient profiles except for the following differences: Diet A contained adequate Association of American Feed Control Officials (AAFCO) amounts of dietary zinc and LA (31). Diet B contained increased amounts of LA and zinc. Diet C was similar to Diet B with respect to zinc and LA being held constant, but it also contained increased ALA.

Total dietary fat of all diets was constant at 12 % (as-fed basis). The diets were isocaloric (based on 3800 kcal/kg), and met the AAFCO standards for total protein (amino acid), vitamins, and minerals (31). The quantity of antioxidants present in the diets were comparable and in excess of AAFCO profiles in order to protect the high amounts of polyunsaturated fatty acids included. Diet A contained 120 mg/kg zinc, 11 g/kg LA, and 1.5 g/kg ALA. Diet B had 350 mg/kg zinc, 33 g/kg LA, and 1.5 g/kg ALA. Diet C contained 350 mg/kg zinc, 33 g/kg LA, and 12 g/kg ALA. These amounts are reported on an as-fed basis. The main source of LA in diet B was sunflower seed, and flaxseed was the main source of LA and ALA for diet C. There were also other fat sources in these diets that contributed to the total amounts of LA and ALA present in the diets. All other dietary components were similar. The nutrient analysis, list of main ingredients, fatty acid composition, as well as the LA, ALA, and zinc content of each diet can be found in Appendix A.

*Animal Care and Housing.* All dogs were housed in the Laboratory Animal Resources and Research (LARR) facilities, Texas A&M University. They were individually housed and cared for in kennels according to the American Physiological Society Guidelines for Animal Research and according to guidelines set forth by Texas A&M University Care and Use Committee.

*Acclimation to Discount Store Type Diet and Subjective and Objective Assessments (Phase I).* In Phase I all 24 dogs were acclimated to a complete and balanced discount store-type dog food (Ol'Roy®) for 12 wk. Ol'Roy was

chosen for the acclimation diet in this study because it is currently the most popular selling discount store-type dog food in the United States. On this basis there was an interest in comparing Ol'Roy® to diets higher in fat and fatty acid types (Phase II diets) to determine the differences in effects on skin and hair coat condition. Information pertaining to the contents of the acclimation diet can be found in Appendix A. During Phase I skin and hair coat condition scores were performed by an evaluation panel. This panel consisted of 2 veterinary nutritionists, 2 veterinary dermatologists, and 3 nutrition graduate students. Evaluations were conducted on wks 0, 4, 7, and 12 of the acclimation period. Skin transepidermal water loss and skin hydration assessments were determined on wk 3, 7, and 11 using a Tewameter® and Corneometer® respectively (Courage-Khazaka, Koln, Germany).

*Effect of Diet Modification on Skin and Hair Coat (Phase II).* After the 12 wk acclimation period, the 24 dogs were randomly divided into three groups of eight each (three Beagles and five Hounds per group). The groups were then fed one of three complete and balanced diets (Diet A, B, or C) described above for an additional 12 wk. The three diets used in this phase of the study were manufactured by the project sponsor according to our specifications (Nutro Products, Inc., City of Industry, CA). The Evaluation Panel again conducted additional skin and hair coat condition assessment scores on wk 14, 16, 19, and 24. TEWL and HYDR assessments were also determined on wk 11, 12, 15, 19, and 23 using a Tewameter® and Corneometer® respectively. During the

second phase of the study blood samples were collected and plasma harvested on d 0, 5, 8, 16, 28, 56, and 84 in disodium ethylenediamine tetraacetic acid ( $\text{Na}_2\text{EDTA}$ ) (Sherwood Medical, St. Louis, MO) containing tubes. Profiles of plasma phospholipid fatty acids were determined at each sample collection time. The dogs had their food removed by 5 P.M. the day prior to blood sampling. Blood collection occurred before 10 A.M. on the following morning. At that time, 12 mL of blood was obtained via jugular venipuncture into a blood collection tube containing EDTA as anticoagulant. Blood samples were spun in a low speed centrifuge (International Equipment Company, Needham, MA) for 10-15 min. The plasma samples were then separated and placed in plastic flip top tubes and stored at  $-20^\circ\text{C}$  until further analysis.

*Lipid Extraction.* Plasma samples were subjected to lipid extraction using a modified version of the Folch et al. (32) procedure. A total of 500  $\mu\text{L}$  of each plasma sample was transferred to a 12 mL Teflon-lined screw top glass test tube. To each sample, 9.0 mL of chloroform:methanol (2:1, v/v) with 0.2% glacial acetic acid was added. The samples were then shaken in a Shaker-in-the-Round (Model S-500, Kraft Apparatus Inc., Mineloa, NY) for 20 min at room temperature. After shaking, 2.0 mL of distilled water was added to each sample. They were briefly mixed on a Vortex-Genie Model K-550-G (Scientific Industries Inc., Bohemia, NY) then shaken for another 10 minutes. A Centra-7 centrifuge (International Equipment Company, Needham, MA) was used to spin samples for 20 min at 2800 rpm. The infranates were transferred to clean test tubes, and

5.0 mL of chloroform:methanol:water (3:48:47, v/v/v) was added to wash the samples. The samples were again shaken for 10 min and spun for 15 min. The infranates were transferred, filtered through glass wool into clean test tubes. The glass wool was washed with a small amount of chloroform. Each sample was purged with nitrogen gas and stored in tightly sealed screw-capped glass tubes at -20°C until further analysis as described below.

*Thin Layer Chromatography.* Plasma phospholipid subfractions were obtained by lipid separation using thin layer chromatography (TLC). Lipid samples were dried under nitrogen gas and re-suspended in 300 µL chloroform, 150 µL of which was applied to a 20x20 cm, 250 µm thickness silica gel G coated plate (Fisher Scientific, Suwanee, GA). Prior to use the plates were washed in chloroform:methanol (2:1, v/v), air dried briefly, and activated in a 110°C oven (National Appliance Company Model 5510, Portland, OR) for 1 h. The plates were stored in a sealed plate box until needed, but were stored no longer than one week. The lipid extracts were developed in a filter paper (Whatman International, Ltd., Maidstone, England) lined, covered glass tank. Hexane:ether acetic acid (80:20:1, v/v/v) was used as the mobile phase. The tank was equilibrated for no less than 1 h prior to developing a plate with samples. Plates were loaded with 4 samples, and at least one plate contained a standard lipid mixture (TLC Standard #18-5-A, Nu-Check Prep, Elysian, MN). Plates were then developed in the tank until the solvent front reached 1-2 cm from the top of the plate. Phospholipid subfractions were scraped into clean

teflon-lined screw top test tubes, covered with nitrogen gas, and prepared for transmethylation prior to gas chromatography of the fatty acid methyl esters.

*Methylation of Plasma Phospholipid Fatty Acids.* Following TLC and scraping of phospholipid fractions into clean test tubes, the lipids were transmethyated. To each plasma phospholipid/silica gel G sample, 2 mL of 4% sulfuric acid in methanol was added. Tubes were capped, mixed, and heated in a 90°C water bath (GCA Percision Scientific, Thelco 182, Model 66570, Chicago, IL) for one hour. Tubes were removed from the water bath and allowed to cool. Three (3) mL of hexane was then added to each tube, and the tubes were mixed. The samples were spun in a centrifuge for 20 min at 2500 rpm at room temperature. The supernatants, which contained the fatty acid methyl esters, were transferred, filtering through glass wool, to a clean test tube. The transmethyated samples were purged with nitrogen gas and stored at -20°C for subsequent capillary gas chromatography.

*Gas Chromatography.* The fatty acid methyl esters from above were evaporated to dryness under nitrogen gas, and 40 µL of hexane was used to resuspend the methyl esters. Three microliters of each sample was then injected onto the capillary column (Famewax® fused silica capillary column, 0.25 µm thick, 30 m long, and 0.32 mm ID) of the Hewlett Packard Series II 5890 Gas Chromatograph using a split ratio of 18:1(20). Helium was used as the carrier gas. The temperature began at 175°C held for 8 min, ramped to 230 at 1°C over 10 min and the final oven temperature was 250°C at a rate of 12°C over 10 min.

Phospholipid runs were terminated at 45 min. Hewlett Packard's HP ChemStation software package was used to generate results from the analyses. Authentic fatty acid methyl ester standards (Nu-Check-Prep, Elysian, MN) were used to identify the individual fatty acid peaks by comparing retention times.

*Serum Zinc Analysis.* Serum samples were sent to Associate Regional University Pathologists (ARUP) Laboratories for zinc analysis (Salt Lake City, UT). The samples were collected on wk 12, 16, and 24 of Phase II. Five Hundred (500)  $\mu$ L of each serum sample was transferred to a 12 mL Teflon-lined screw top glass test tube. To each sample 500  $\mu$ L of 15% nitric acid with yttrium (internal standard) was added. The samples were briefly mixed and then 4 mL of distilled water was added. They were then centrifuged for 5 min at 3000 rpm. The samples were analyzed on a five point calibration via Inductively Coupled Mass Spectrometry (ICPMS, ParkinElmer Life and Analytical Sciences, Inc., Boston, MA). The calibration standard concentrations were 0, 12, 60, 120, and 300  $\mu$ g/dL. The isotope of zinc analyzed was 64. A standard curve was obtained and the concentrations of zinc in the unknown samples were calculated from this curve.

*Subjective Assessments (Phase I & Phase II).* During Phase I skin and hair coat evaluations were determined on wk 0, 4, 7, and 12. During Phase II the evaluations were conducted on wk 0, 2, 4, 7, and 12 by the evaluation panel. The scoring system consisted of a five point scale. Prior to the evaluations, the dogs were bathed with DVM Tearless™ shampoo (DVM Pharmaceuticals, Inc.,

Miami, FL) on d -7, 29, and 57. The dogs were bathed indoors and rinsed with regular tap water and then towel dried. The bathing was conducted far enough in advance of the evaluations to minimize any shampoo effect on the condition scores.

Evaluations were performed indoors in a room with fluorescent illumination from above. The evaluations were conducted before noon on the day of scoring. The dogs were individually brought into the room, which was separate from the kennels, to be evaluated by the panel. Prior to beginning the evaluations the panel was instructed as to assessment procedures. One dog from the group was randomly chosen to be the pre-test subject. One of the dermatologists (Christine A. Rees) described a systematic technique to score the dogs' skin and hair coat conditions. This was done to help assure panel members scored the dogs in a similar fashion and to help reduce the amount of variability among them. The evaluators were allowed full contact with the dogs and as much time as needed with each dog to score them. An ordinal scoring system was used with a 1-5 scale (5 best) assigning integer values for each parameter (Subjective Integer Assessment, SIA) (Table II-1). A tick mark scoring system was also simultaneously applied in which a mark was made on an equidistant 1-5 number line enabling the evaluators to generate a set of continuous data (Subjective Tick Mark Assessment, STMA) that could be evaluated for normality and as parametric data compared to the non-parametric

**TABLE II-1**

The Skin and Hair Coat Scoring Was Based on a Five Point Scale (1 Being

Least Desirable and 5 Most Desirable)

**Glossiness**

- 1= Very dull (poorest, no shine at all)
- 2= Moderately dull
- 3= Slightly shiny
- 4= Moderately shiny
- 5= Very shiny (best, a lot of shine)

**Greasiness**

- 1= Very greasy (poorest)
- 2= Moderately greasy
- 3= Mildly greasy
- 4= Minimally greasy
- 5= Not greasy (best)

**Scale**

- 1= Very scaly (poorest)
- 2= Moderately scaly
- 3= Mildly scaly
- 4= Minimally scaly
- 5= No scale (best)

**Softness**

- 1= Very brittle (poorest)
- 2= Moderately brittle
- 3= Slightly soft
- 4= Moderately soft
- 5= Very soft (best)

**Overall Coat Quality**

- 1= Poor (very dull, brittle, dry, scaly, or greasy) (poorest)
- 2= Fair
- 3= Good
- 4= Very Good
- 5= Excellent (very shiny, very soft, no scale, not greasy) (best)

ordinal (SIA) data. Appendix B contains the sample scoring form that was used. The evaluation of Glossiness was conducted by having the evaluators stand at a small distance (approximately 3 m) from the dogs and determine the amount of shine to the coat. When evaluating greasiness the panel would rub their hand down the dogs back then rub their fingers together in order to feel the amount of grease that came off of the dog's coat. The hair coat was brushed back, by hand, in the opposite direction of the way it naturally grew on each dog to determine the amount of scale present. To determine the softness of the hair coat, the evaluators ran their hands over the dog's body in the direction of the hair's natural growth. Lastly, the dogs were given an overall skin and hair coat condition score by each evaluator. The panel members washed and dried their hands between each dog to assure that there was no residue left on their hands from the previous dog.

*Objective Assessments (Phase I & II).* Measurements for the amount of TEWL in the skin and skin HYDR of the dogs were made using equipment designed for this purpose. Hair was clipped (4 x 6 cm) from the left inguinal region of each dog one week preceding the measurements to minimize any effects of recent hair clipping. Previous studies have reported that hair can interfere with the reported measurements (29). Thus, clipping was done to assure that hair did not affect the readings and to give time for any possible abrasions on the skin to heal. Because hair grows sparsely in the inguinal

region, only a minimal amount of clipping was necessary. Both TEWL and HYDR measurements were performed at this site on the day of measurements. The TEWL values were measured using a Courage-Khazaka (Koln, Germany) Tewameter®300. This device employs a hand-held probe and measures water evaporation on the skin surface based on the vapor pressure gradient estimation method of Nilsson (33). The Courage-Khazaka (Koln, Germany) Corneometer®825 was used to measure skin HYDR. This instrument also uses a hand-held probe and measures skin moisture based on the capacitance of the skin due to its properties as a dielectric medium (27). Measurements on the dogs were taken indoors in a controlled temperature room. The instruments are sensitive to movement which has been reported to alter meter readings (29). Therefore, animals were gently restrained, lying in right lateral recumbancy, on a padded floor-mat and given time to relax so that movements were minimized during data collection. The probes were held in place manually with readings taken at 1 s intervals for 20 s, replicating them three times each, and then calculating an average value. Values generally represented an average of at least 10 determinations, after equilibrium was attained. The TEWL probe was used first followed by the HYDR probe. The data was stored electronically using a laptop computer and appropriate software (Courage & Khazaka Electronics, Koln, Germany). Ambient temperature and humidity values were determined by the probes simultaneously with the transepidermal water loss and hydration measurements.

*Evaluation of Subjective Data (Phase I & II).* The subjective scores of the skin and hair coat condition, for Phase I and Phase II, were first evaluated by correlation analysis (i.e. Pearson Correlation Coefficient) on both the SIA and STMA scores separately. This test was conducted using the SPSS statistics program (SPSS Inc., Chicago, IL). To determine if the evaluators correlated they had to have a Pearson Correlation Coefficient of 0.300 or greater and a correlation p-value of  $p < 0.05$ . Values were evaluated in this manner to determine the extent to which the evaluators correlated with one another. If one or more evaluators did not correlate with at least six other evaluators they were eliminated from subsequent analyses on that particular score in order to help control variability among individual evaluators.

The STMA scores were also analyzed using repeated measures ANOVA for main time, breed, and diet effects and interactions (parametric data). Tukey's multiple comparisons test was used at  $p < 0.05$  to identify where significant differences occurred. A one-way ANOVA was used to compare diet effects of the tick mark data at the end of Phase II (wk 24).

The SIA scores were analyzed using Wilcoxin signed rank test comparing the Phase II starting and end points (wk 12 vs. wk 24). Variance and coefficient of variation were calculated on all dogs during the acclimation period to determine the extent of variability between and among all dogs and between the breeds. Kruskal-Wallis one way non-parametric AOV testing was used to compare main diet effects at the end of Phase II (wk 24). Results of both the

parametric and non-parametric subjective score analyses were compared for consistency of significant findings.

*Evaluation of Objective Data (Phase I & II).* Using the Phase I data, TEWL and HYDR scores were statistically analyzed by repeated measures ANOVA for main time effects. Tukey's multiple comparisons was performed at  $p < 0.05$  or better as indicated, where appropriate, using the *Statistix 7.0®* program (Analytical Software, Tallahassee, FL). Sample variances within and between dogs at each time point were compared using one-way ANOVA at  $p < 0.05$ . The Phase II data for TEWL and HYDR were statistically analyzed by repeated measures ANOVA for main breed and time effects with Tukey's multiple comparisons performed at  $p < 0.05$  or better as indicated, where appropriate. One-way ANOVA was used to analyze TEWL and HYDR diet effects at each individual time period (wk 11, 19, and 23).

*Evaluation of Plasma Phospholipid Fatty Acids Data.* Plasma total phospholipid fatty acids were evaluated using repeated measures ANOVA for main time and diet effects on samples collected during Phase II. A one way ANOVA was also used on each individual time period. Tukey's multiple comparison test ( $p < 0.05$ ) was then used to ascertain where significant differences existed between the diets and time periods. Descriptive statistics were also calculated on the condition scores, transepidermal water loss, skin hydration measurements, and plasma phospholipid fatty acids.

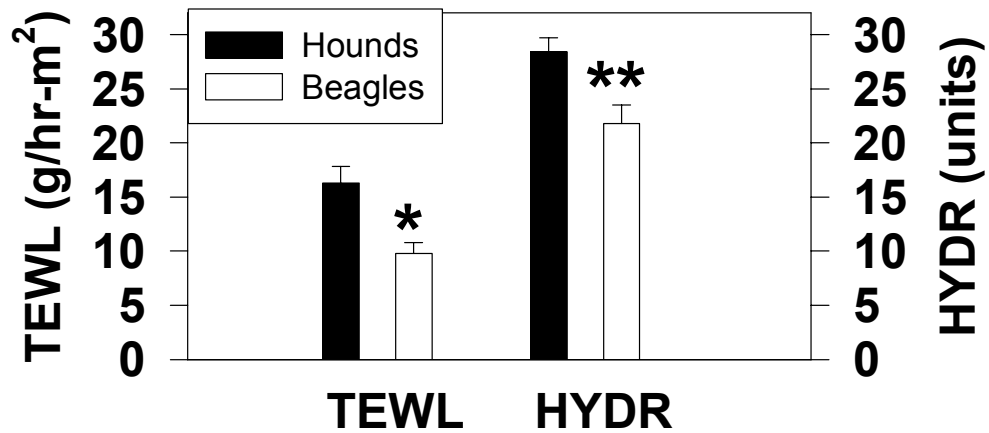
**CHAPTER III**  
**EVALUATION OF CORNEOMETRY (SKIN HYDRATION) AND**  
**TRANSEPIDERMAL WATER LOSS MEASUREMENTS IN FEMALE**  
**BEAGLES AND MALE HOUND-CROSSBRED DOGS FED THE**  
**ACCLIMATION DIET\***

*Analysis of Variance of Transepidermal Water Loss and Skin Hydration Measurements.* Measurements were taken indoors within a temperature range of 20-24°C and relative humidity ranging from 39 to 51%. Linear regression analyses within these ranges were found to have no statistically significant effect on either TEWL or HYDR values. The individual dog mean TEWL values ranged from 2.8 to 50.7 g/hr-m<sup>2</sup> and mean HYDR values ranged from 9.0 to 57.5 units.

A repeated measures ANOVA model with TEWL and HYDR as dependent variables was analyzed resulting in a significant difference between the groups ( $p < 0.001$ ) for each measurement (Figure III-1). A statistically significant time effect was observed for HYDR with mean values at wk 3 greater than those obtained at either wk 7 or 11 (Figure III-2). The TEWL measurements were not significantly different over time.

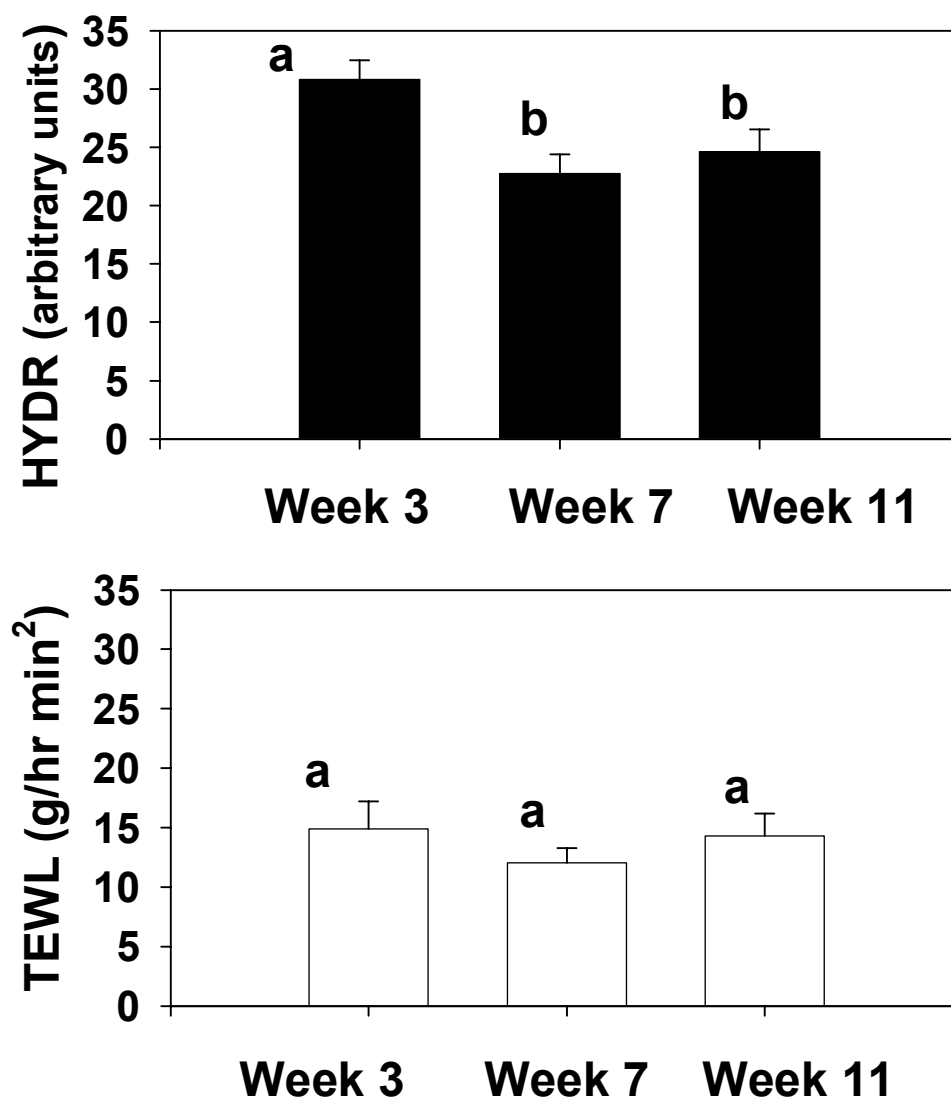
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\*Note: Parts of this chapter have been submitted as an expanded abstract to the Journal of Nutrition, Supplement: Presented at the Waltham International Science Symposium; Nature, Nurture, and the Case for Nutrition; Bangkok, Thailand, October 2003. (In press, 2004)



**FIGURE III- 1** Comparison of mean ( $\pm$  S.E.M.) transepidermal water loss (TEWL) and skin hydration (HYDR) values between male Hounds and female Beagles.

\* TEWL significantly different between breeds ( $p < 0.001$ ) and \*\* HYDR significantly different between breeds ( $p < 0.001$ ) by repeated measures ANOVA followed by Tukey's multiple comparisons over all three measurement times (9 Beagles and 15 Hounds at each time,  $p < 0.001$ ).



**FIGURE III-2** Effect of feeding duration on mean skin hydration (HYDR, filled bars) and transepidermal water loss (TEWL, open bars) values ( $\pm$  S.E.M.).

Note: Letters not in common for a given variable are significantly different by repeated measures ANOVA followed by Tukey's multiple comparisons (9 female Beagles and 15 male Hounds at each time,  $p < 0.01$ ).

*Variability of TEWL Determinations.* Because significant differences between the two groups were observed by ANOVA, variance and % coefficients of variation (%CV) for female Beagles and male Hounds were calculated separately and compared (Table III-1). Within-animal variance for the Beagles was nominally lower but not statistically significant compared to the Hounds and no time effects were observed. The % CV values were also not different between groups or over time. Thus, the data were combined and mean overall within-animal variance and %CV among all dogs were calculated ( $8.9 \text{ g/hr}\cdot\text{m}^2$  and 18% respectively, Table III-1). Between-animal TEWL variation was considerably greater than within-animal measures and statistically significant differences were found with time (Table III-2). A statistically significant lower variance was observed in the Beagles compared to the Hounds but mean Beagle % CV values were not different between the two groups. In spite of the variance difference between the two groups, an overall mean variance was calculated so that a descriptive appreciation of the overall variance of the TEWL measurement among dogs could be determined.

*Variability of HYDR Determinations.* The average overall within-animal variance and %CV for HYDR values among all dogs were  $11.7 \text{ g/hr}\cdot\text{m}^2$  and 13% and no statistical differences were observed between the groups for either of these variables (Table III-1). Between-animals, both variances and % CVs were markedly higher than within-animals. A statistically significant time effect ( $p < 0.05$ ) of sample variances was observed when wk 3 data were compared to

**TABLE III-1**

Average Within-Dog Variability of Transepidermal Water Loss (TEWL) and Skin Hydration (HYDR) Determinations for All Dogs and Beagle and Hound Breeds<sup>1</sup>

Sample Time	All Dogs (n=24)		Beagles (n=9)		Hounds(n=15)	
	Variance (g/hr·m <sup>2</sup> )	% CV	Variance (g/hr·m <sup>2</sup> )	% CV	Variance (g/hr·m <sup>2</sup> )	% CV
<b>TEWL</b>						
Week 3	9.7 ± 3.0	18 ± 2	9.0 ± 2.8	21 ± 4	10.1 ± 2.3	16 ± 2
Week 7	9.8 ± 2.3	21 ± 2	3.5 ± 1.1	24 ± 5	13.2 ± 4.4	20 ± 2
Week 11	7.1 ± 2.1	16 ± 2	3.4 ± 1.3	19 ± 3	8.8 ± 3.1	14 ± 1.8
<b>Average TEWL</b>	<b>8.9 ± 3.1</b>	<b>18 ± 2</b>	<b>5.3 ± 1.9</b>	<b>21 ± 4</b>	<b>10.7 ± 3.6</b>	<b>17 ± 2</b>
<b>HYDR</b>						
Week 3	13.6 ± 2.5	11 ± 1	10.4 ± 3.2	11 ± 1	15.3 ± 3.4	10 ± 1
Week 7	10.5 ± 2.0	14 ± 2	5.9 ± 1.2	14 ± 2	12.9 ± 2.8	15 ± 3
Week 11	11.0 ± 2.3	13 ± 1	5.0 ± 1.9	11 ± 2	14.6 ± 2.5	13 ± 1
<b>Average HYDR</b>	<b>11.7 ± 2.8</b>	<b>13 ± 1</b>	<b>6.1 ± 2.2</b>	<b>12 ± 2</b>	<b>14.3 ± 3.1</b>	<b>13 ± 2</b>

<sup>1</sup> Mean ± SEM. For these comparisons, variances were derived from three mean values each of which were derived from 10 measurements of either TEWL or HYDR. There were no statistically significant time or breed effect in this data (p < 0.05)

**TABLE III-2**

Average Between-Dog Variability of Transepidermal Water Loss (TEWL) and Skin Hydration (HYDR) Determinations for All Dogs and Beagle and Hound Breeds<sup>1</sup>

Sample Time	All Dogs (n=24)		Beagles (n=9)		Hounds (n=15)	
	Variance (g/hr·m <sup>2</sup> )	% CV	Variance (g/hr·m <sup>2</sup> )	% CV	Variance (g/hr·m <sup>2</sup> )	% CV
<b>TEWL</b>						
Week 3	118.9 ± 6.1 <sup>a</sup>	73 ± 1 <sup>a</sup>	49.5 ± 9.1 <sup>a,2</sup>	57 ± 4	158.8 ± 9.7 <sup>a,2</sup>	77 ± 1 <sup>a</sup>
Week 7	37.9 ± 1.1 <sup>b</sup>	51 ± 1 <sup>b</sup>	7.4 ± 1.7 <sup>b,2</sup>	37 ± 3	36.3 ± 6.3 <sup>b,2</sup>	41 ± 1 <sup>b</sup>
Week 11	89.7 ± 4.2 <sup>a</sup>	64 ± 1 <sup>b</sup>	18.2 ± 2.3 <sup>a,b,2</sup>	44 ± 4	110.3 ± 6.2 <sup>b,2</sup>	59 ± 2 <sup>c</sup>
<b>Average TEWL</b>	<b>82.2 ± 3.8</b>	<b>63 ± 1</b>	<b>25.0 ± 4.4<sup>2</sup></b>	<b>46 ± 4</b>	<b>101.8 ± 7.4<sup>2</sup></b>	<b>59 ± 1</b>
<b>HYDR</b>						
Week 3	61.0 ± 4.2	25 ± 1 <sup>a</sup>	98.1 ± 13.5 <sup>a</sup>	35 ± 2	42.9 ± 6.2	21 ± 1 <sup>a</sup>
Week 7	64.4 ± 2.6	35 ± 1 <sup>b</sup>	25.8 ± 1.6 <sup>b</sup>	27 ± 1	73.6 ± 3.7	34 ± 1 <sup>b</sup>
Week 11	91.1 ± 1.7	39 ± 1 <sup>b</sup>	26.2 ± 1.9 <sup>b</sup>	28 ± 2	94.8 ± 4.5	34 ± 1 <sup>c</sup>
<b>Average HYDR</b>	<b>72.2 ± 2.8</b>	<b>33 ± 1</b>	<b>49.7 ± 5.7</b>	<b>30 ± 2</b>	<b>70.4 ± 4.8</b>	<b>30 ± 2</b>

- 1 Mean ± SEM. For these comparisons, variances were derived from three mean values each of which were derived from 10 measurements of either TEWL or HYDR. Letters not in common in a given column for either TEWL or HYDR are significantly different (p < 0.05).
- 2 TEWL variances significantly different between female Beagles and male Hounds at all time points (p < 0.05).

the other measurement times for Beagles but not for the Hounds. However, differences in sample variance between the groups were not statistically significant. A statistically significant decrease was seen in the % CV at wk 3 compared to the other times in the Hounds but generally these values approximated 30% in both groups at all time periods measured (Table III-2).

*Discussion.* Non-invasive techniques for the objective assessment of skin condition are of considerable interest in veterinary clinical dermatopathology and dermatological research. Although TEWL and HYDR measurements have been more commonly used in assessing human skin barrier function, their potential application to veterinary medicine is worthy of evaluation. Systematic investigation of these techniques has not been performed in veterinary species and specific protocols for their use have not been established. Also, questions regarding variability, age, body location, gender, and breed effects have yet to be completely answered.

In the present study, a highly significant difference ( $p < 0.001$ ) between female Beagles and male Hounds was observed for both TEWL and HYDR measurements. Data from both groups was combined in spite of this effect in an effort to extend the observations among dogs in general, and because age, breed, and gender were not blocked in the design. When these tests were conducted on the combined data, statistical significance was still found even though a difference between the groups existed. By combining the groups the results will give a more general representation among all dogs, and if significant

differences are found the data may be useful when making diet recommendations for the dog population. Combination of the groups was also done to increase the sample size per diet ( $n=8$ , 3 female Beagles and 5 male Hounds). Since there were only 3 Beagles per group, it was felt that this was not a large enough number to properly represent the breed.

Unlike an earlier study (29), animals were not specifically trained to stand quietly during the objective measurement period. Nonetheless, variability of the TEWL measurements were similar to that earlier study. It was found that allowing the dogs to lie on a padded floor combined with gentle manual restraint provided minimal movements and allowed measurements to be obtained in triplicate, at 20 s intervals each. Body site was not varied in this study and hair clipping of the inguinal region was performed one week prior to any measurement.

A statistically significant time effect was also observed in HYDR with increases seen at wk 3 but not thereafter. It should be noted that the animals used in this study had been fed different diets prior to their group assignments. A uniformly defined commercial diet was then fed beginning at wk 0 for the entire TEWL and HYDR measurement period. Consequently, the wk 3 HYDR values may have been a reflection of the previous diets the animals had been fed. It was concluded the future studies designed to determine effects of dietary modification on HYDR should expect to feed dogs for more than 3 wk prior to reassessment. The lack of difference between wk 7 and 11 data supports the

existence of a new metabolic skin steady state during this time period. It is unknown whether such a steady state would be present at 4, 5 or 6 wk because evaluations were not performed between 3 and 7 wk in this study. The observed time effect for HYDR measurements is consistent with the concept that some period of time is needed to achieve a metabolic steady-state in skin as a function of dietary modification.

Considerable between-dog variability existed for both TEWL and HYDR measurements. Nonetheless, clear differences between the two groups evaluated in this study were found. However, the study was not designed to control for gender, breed, or age differences within the two groups. Finally, within-dog variations were less marked under the conditions employed than between-dog variations. It appears that both techniques are clinically useful especially where pre- and post-treatment comparison of individual dogs are performed or in studies where each animal serves as its own control.

Finally, corneometry (HYDR) measurements appear more reliable and easier to perform than TEWL. However, in view of new information on the variability of TEWL and HYDR measurements, it appears that both can serve as reasonably stable personal characteristics with which to investigate treatment effects of diet or medications on canine skin condition.

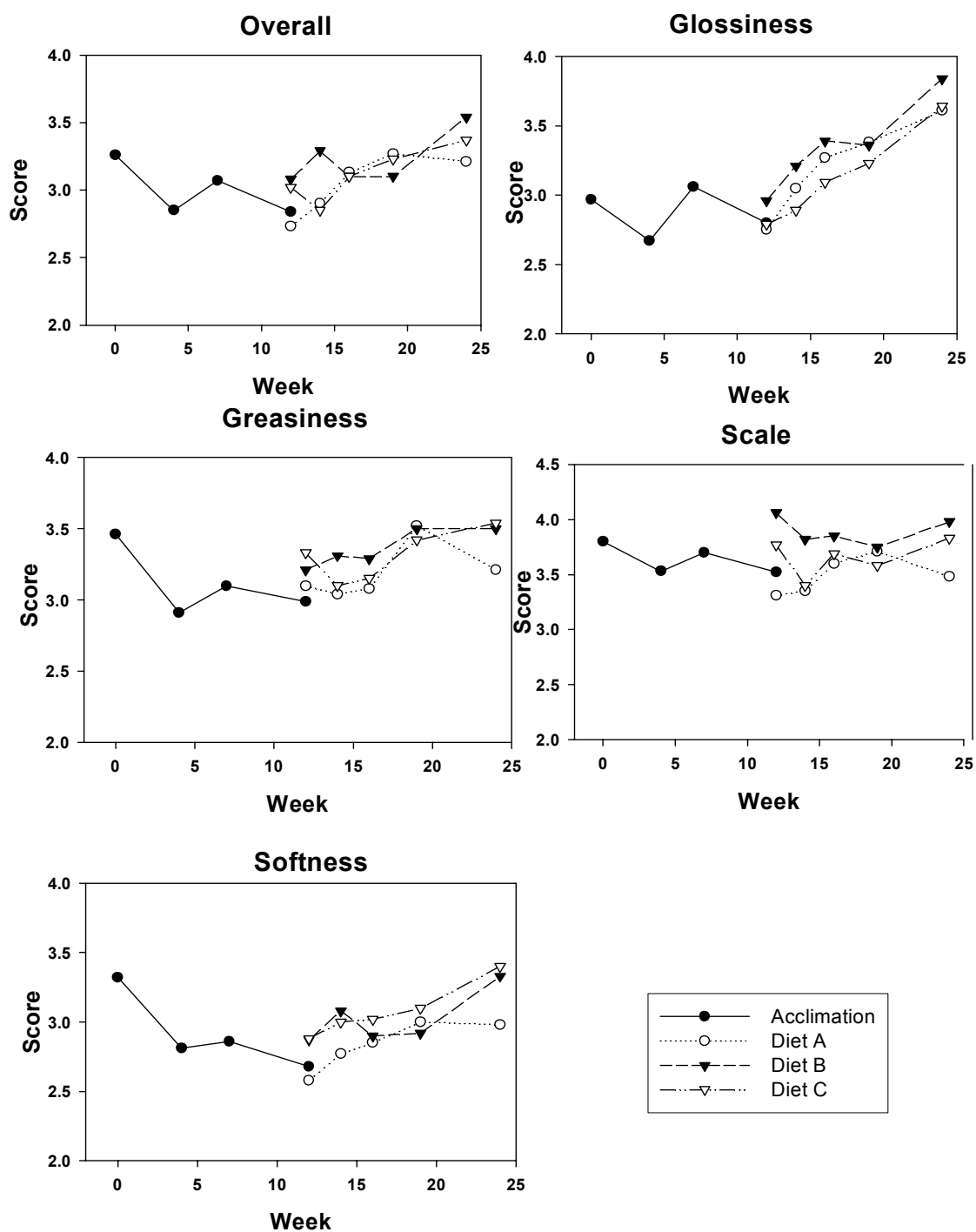
**CHAPTER IV**

**EFFECT OF POLYUNSATURATED FATTY ACIDS AND RELATED  
NUTRIENTS ON CANINE SKIN AND HAIR COAT CONDITION, PLASMA  
LIPIDS, SKIN TEWL, AND SKIN HYDR (PHASE II)**

*Subjective Evaluations.* The hair coat condition scores were initially evaluated by calculating Pearson Correlation Coefficients which revealed the extent to which the evaluators correlated. To determine if the evaluators correlated they had to have a Pearson Correlation Coefficient of 0.300 or greater and a correlation p-value of  $p < 0.05$ . Evaluator data that did not correlate with data from at least six other evaluators were removed from that particular score prior to further statistical analysis.

*Subjective Evaluations – SIA.* The mean values, over time, of dogs fed each of the 3 diets compared to the discount store dog food generally demonstrated improvement for all parameters (Figure IV-1). When wk 24 non-parametric data were compared with wk 12, statistically significant effects were observed overall ( $p < 0.02$ ) and for glossiness ( $p < 0.001$ ) and softness ( $p < 0.01$ ) for each diet studied. It was found that greasiness and scale were not significantly different among the three diets fed (Table IV-1).

*Subjective Evaluations – STMA.* By comparison, when the tick mark scores were analyzed significant improvements for all parameters again were



**FIGURE IV-1** Comparison of Phase I (Acclimation) and Phase II (Diets A, B, C) mean values for Subjective Integer Assessments over time.

**TABLE IV-1**

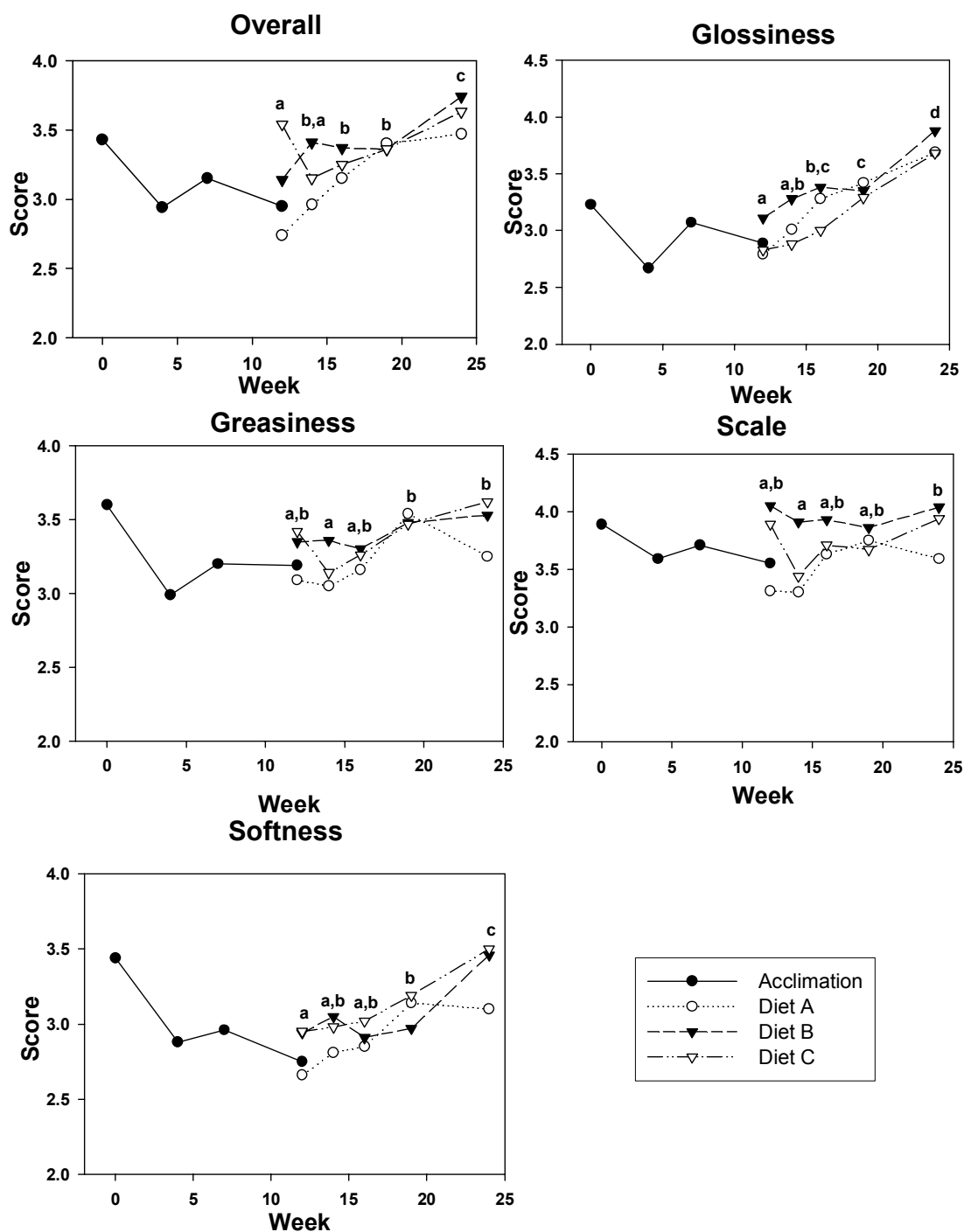
Wilcoxin Signed Rank Test, P-values for Subjective Assessment Integer Scores.

Comparisons Between wk 12 and 24 of Phase II Feeding Period

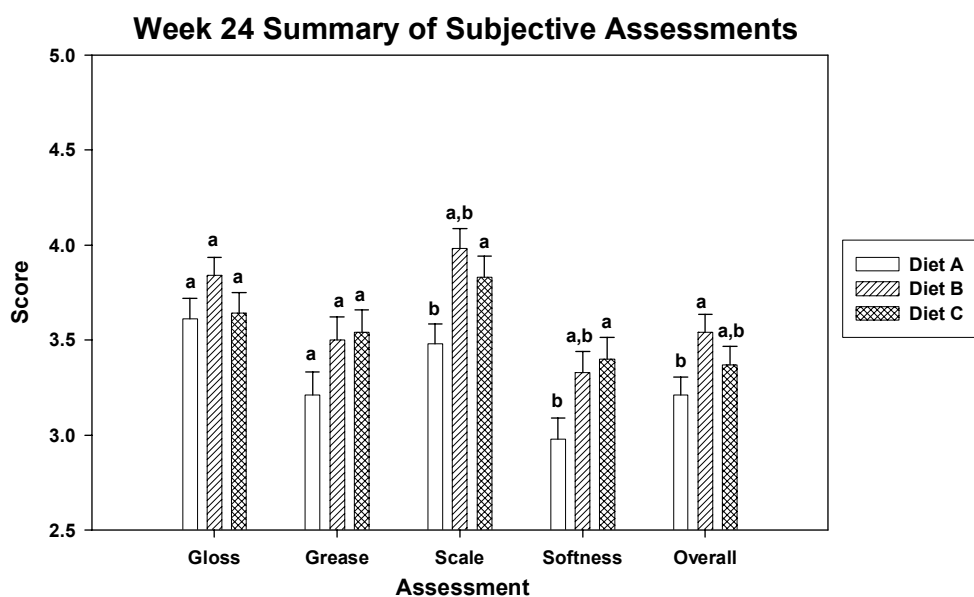
	Gloss	Grease	Scale	Softness	Overall
Diet A	< 0.001	0.517	0.334	0.012	0.002
Diet B	< 0.001	0.082	0.494	0.002	0.002
Diet C	< 0.001	0.254	0.667	0.002	0.016

found, over time, in all diet groups (Figure IV-2). Repeated measures ANOVA revealed a main time effect for overall score, glossiness, and softness all of which were significant at  $p < 0.05$ . Once again, scale and greasiness scores were not significantly different over time during Phase II. It should be noted that during the acclimation period (Phase I, wks 0 through 12) it appeared that a metabolic steady state for skin evaluations occurred beginning with wk 7 and continuing through wk 12. This same phenomenon appeared to exist during Phase II, whereby acclimation was apparent beginning at wk 19 until the end of the experimental period (wk 24). Because of this finding, a Kruskal-Wallis one way non-parametric AOV was performed on the SIA and a one-way ANOVA was performed using STMA data each at wk 24. In these instances, no significant diet differences were found for glossiness or greasiness. However, statistically significant overall scores were seen for the SIA data indicating improvement when diet B was fed compared to diet A. This difference was not significant for the STMA data, although, the relative numeric values improved similarly. When STMA data was analyzed, improvements with diet B compared to diet A were found for both scale and softness scores. By contrast the SIA data resulted in a significant difference between diets C and A when scale and softness were analyzed (Figure IV-3).

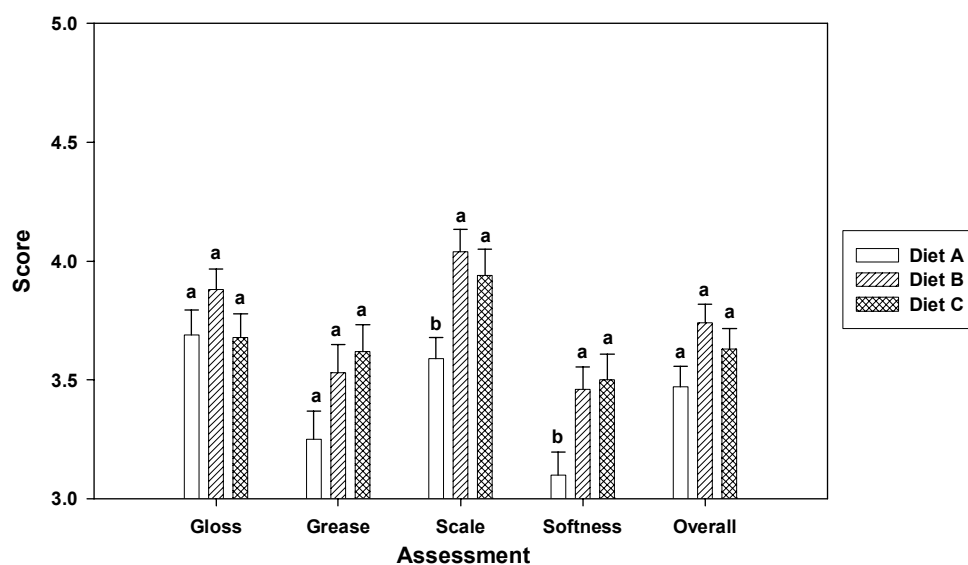
Although not all assessments were statistically significant (wk 24), diets B and C had a tendency to show more consistent numeric improvements for all



**FIGURE IV-2** Comparison of Phase I (Acclimation) and Phase II (Diets A, B, C) mean values for Subjective Tick Mark Assessments over time. Letters not in common for each graph indicate significant differences over time for all diets combined by Repeated Measures ANOVA ( $p < 0.05$ ).



Skin and Hair coat condition scores (Integer) at end of Phase II feeding period (Week 24). Letters not in common for score category are significantly different at  $p < 0.05$ . Results are derived from Kruskal-Wallis One-way Nonparametric AOV test. Error bars indicate standard error of the mean.



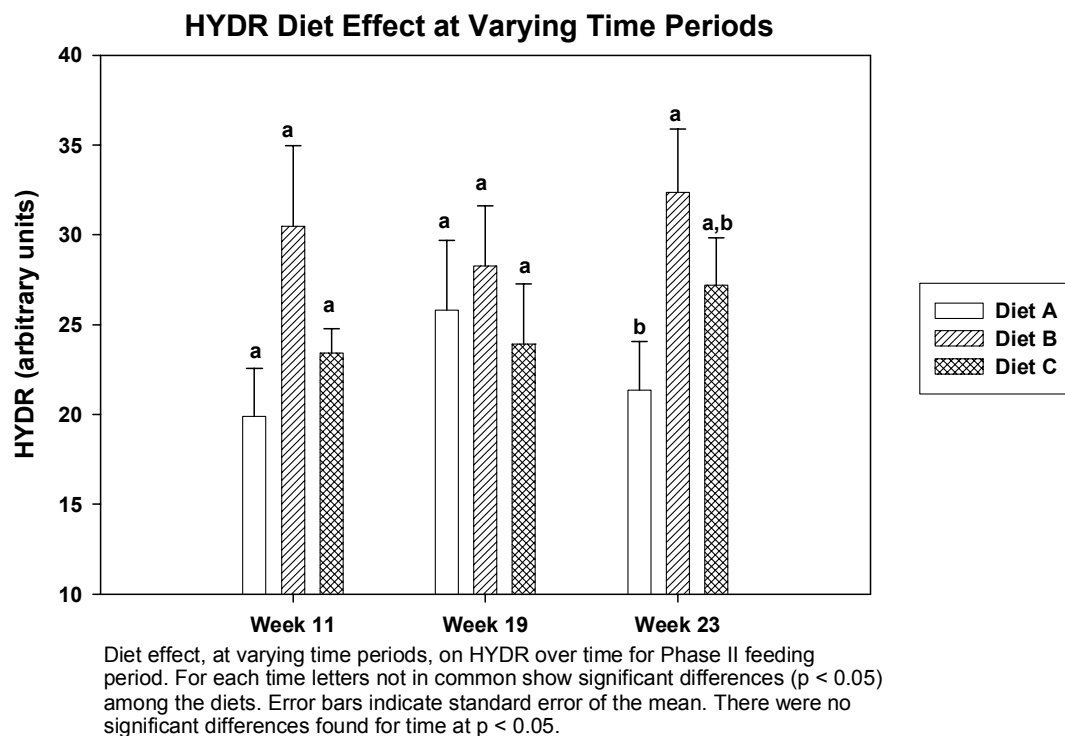
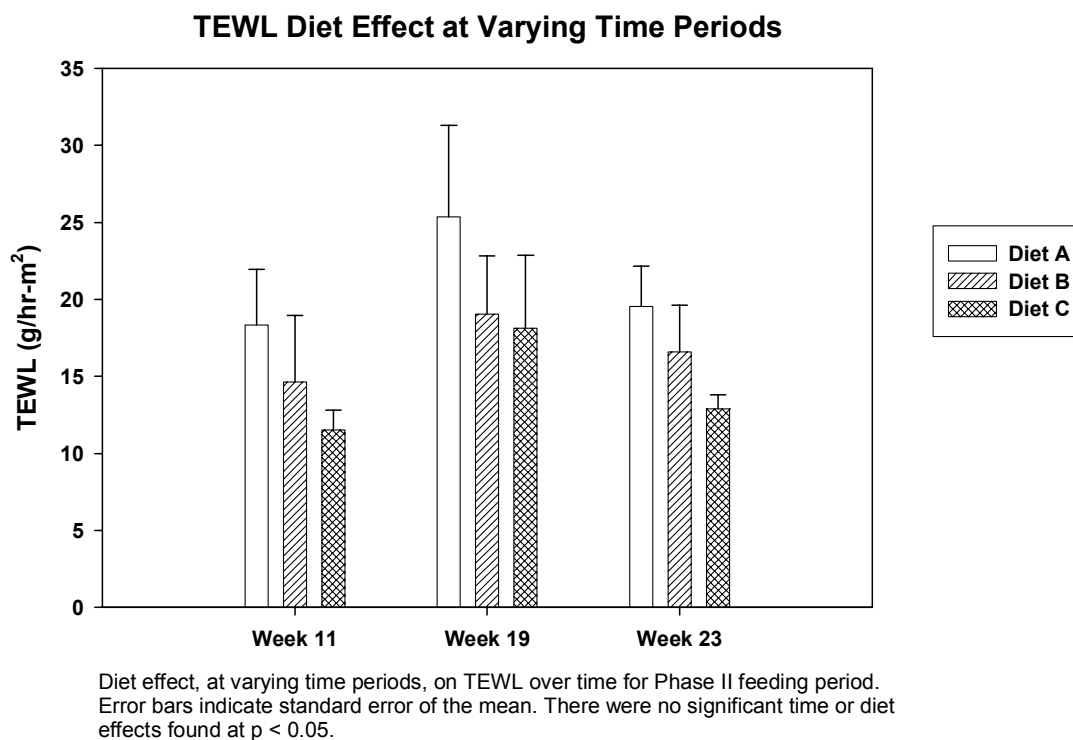
Skin and Hair coat condition scores (Tick Mark) at end of Phase II feeding period (Week 24). Letters not in common for a score category are significantly different at  $p < 0.05$ . Results derived for Repeated Measures ANOVA. Error bars indicate standard error of the mean.

**FIGURE IV-3** Skin and hair coat condition scores (integer and tick mark) at end of Phase II (WK 24).

parameters compared to diet A (STMA and SIA). When the numeric values for dogs fed diet B were compared to diet C modest improvements for the parameters overall score, glossiness, and scale were found. Diet C compared to diets A and B, although not statistically significant, also showed more numeric improvements for greasiness and softness.

*Objective Assessments- Diet Effects on Transepidermal Water Loss and Skin Hydration.* When effects were compared at each Phase II time period (wk 11, 19, and 23) by one-way ANOVA, statistically significant diet effects were found for HYDR but not TEWL (Figure IV-4). Also, there was no significant time effect found for TEWL at  $p < 0.05$  although there appeared to be a numeric tendency for Diet C to have less water loss. There was no significant time effect in skin hydration for any of the diets. At wk 23 Diet B fed dogs showed significantly more skin HYDR than Diet A.

The paired-T test revealed no significant differences between wk 11 and 23 for each diet when both groups were combined. Although, when the female Beagles and male Hounds were evaluated separately for these parameters some significant differences were found. The Beagles fed diet B showed a significant increase ( $p < 0.05$ ) in HYDR. The Hounds fed diet C presented a significant decrease in TEWL and a significant increase in HYDR.



**FIGURE IV- 4** TEWL and HYDR diet effects at varying time periods.

*Plasma Phospholipid Polyunsaturated Fatty Acids Effects by Diet.* The dogs fed diet A showed no significant differences in plasma phospholipids LA, ALA, or docosapentaenoic acid (DPA, 22:5n-3) over time ( $p < 0.05$ ) (Appendix A). Also, the amount of AA on d 16 of the study was significantly different than on d 28, 56, and 84. The amount of AA present increased up to d 16 ( $24.1\% \pm 3.0$ ) then gradually continued to decrease ( $18.9\% \pm 1.8$  at d 84). Day 0 was significantly different than all other times except days 5 and 8 for eicosapentanoic acid (EPA, 20:5n-3), where a continuous numeric increase was seen throughout Phase II up to d 56. Docosahexaenoic acid (DHA, 22:6n-3) was significantly different on d 0 compared to d 8 through 84. Numerical increases in DHA were seen throughout the entire feeding period and d 84 had the highest relative amount.

No significant differences over time were found for any of the PUFA in dogs fed Diet B, and the following only describes the numeric changes found. On d 0 the amount of LA was slightly lower compared to d 84. The amount of ALA remained constant throughout the entire testing phase. There were increased amounts of AA up to d 16 ( $25.1\% \pm 2.8$ ) then it slightly decreased and remained constant ( $23.2\% \pm 1.9$  at d 84). The level of EPA varied throughout the feeding period, resulting in no change when comparing d 0 to 84.

The dogs supplemented with diet C had no significant differences over time for LA. Day 56 was significantly different then the other days for ALA. An increase was observed at d 56 and slightly dropped at d 84. The amount of AA

was significantly different from the others at d 16 (excluding d 0), upon which an increase was observed ( $25.3\% \pm 2.7$ ). This increase was not constant and proceeded to drop through d 84 ( $19.9\% \pm 1.7$ ). EPA was significantly lower at d 0 compared to 56 and 84. The amount slowly increased to d 56 then remained constant. The amount of DPA was significantly lower on y 0 compared to 16-84, as well as d 5 compared to 84. DPA almost doubled by d 16 (which was significantly different than 0-8), and then proceeded to slightly decrease and become steady. Days 8 and 16 were significantly different for DHA. At d 16, a slight increase was evident and then returned to a fairly constant amount. It should be noted that for most of the plasma phospholipid PUFA in all three diet groups, a numeric increase or decrease was observed at d 16.

*Plasma Phospholipid Polyunsaturated Fatty Acid Effects Between Diets Over Time.* Prior to the Phase II diet period, the dogs had been acclimated to the same diet for 12 wk. The serum phospholipid fatty acid results for d 0 reflect the Phase I basal diet (Ol'Roy®) (Figure IV-5). The only significant differences observed on d 0 for serum phospholipid fatty acids were between diets B and C for AA ( $p < 0.05$ ). Diet B contained a slightly higher amount ( $23.7\% \pm 1.4$ ) compared to the other two diets (A,  $22.9\% \pm 1.2$  and C,  $21.7\% \pm 1.2$ )

A consistent numeric increase in all three diets was observed for plasma phospholipid LA throughout the course of the feeding period. Diet C began with the highest amount at d 0 and remained slightly higher through d 84. The only

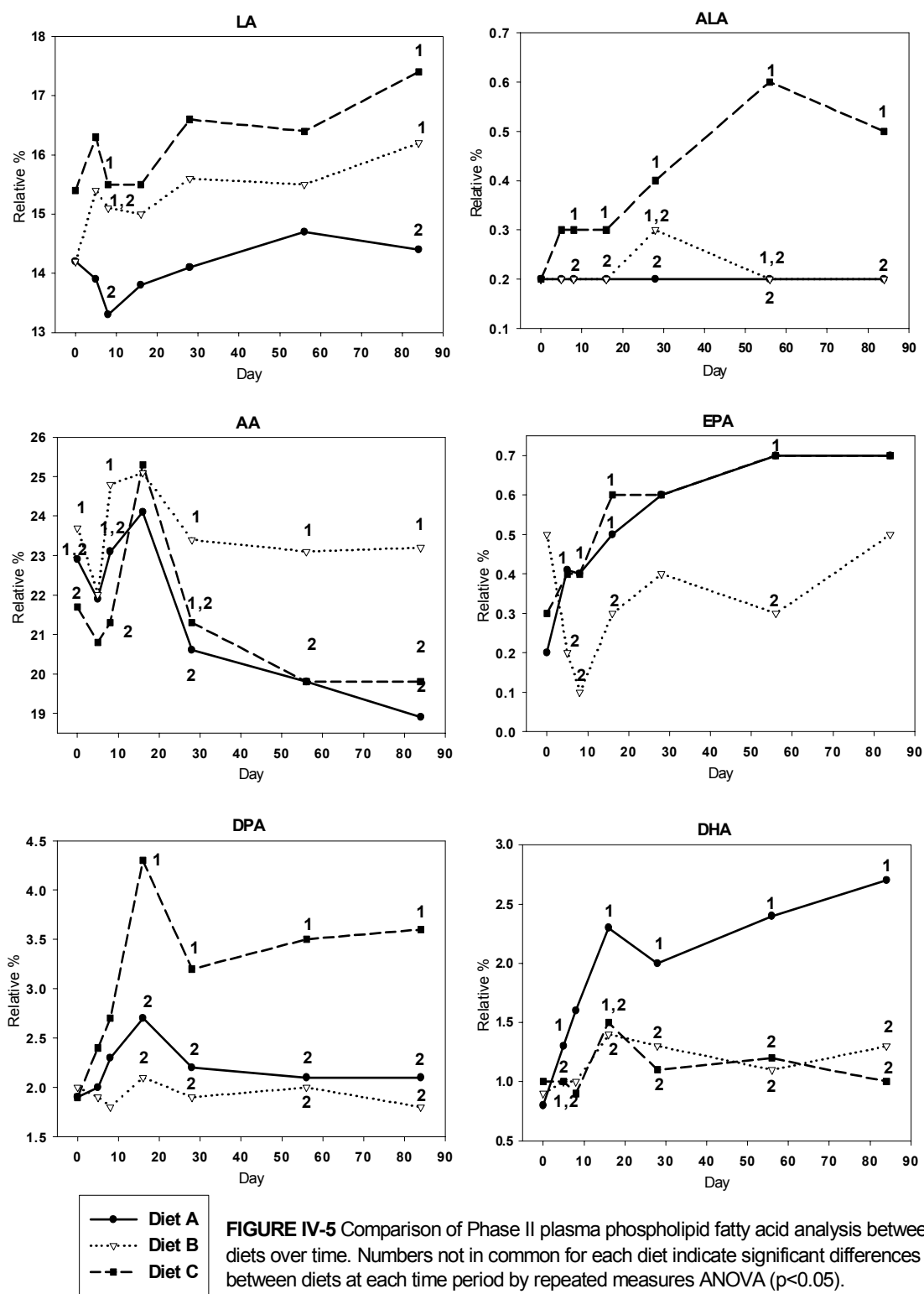
significant differences between LA in the diets were observed at d 8 (diet C higher than diet A) and 84 (diet A was lower than diets B and C).

Numerically, all diets increased in the amount of AA up to d 16. Then diets A and C proceeded to decrease in their amounts. Diet B had little change and was significantly higher on d 84 compared to the other diets. Although not significantly different, diet A had slightly less AA than C at d 84.

Increases in the amount of ALA were observed early in the dogs fed diet C and continued throughout the entire feeding period. Diet C was significantly higher than diet B on d 8, 16, and 84 and diet A on d 8, 16, 28, 56, and 84. The amount of ALA in diets A and B remained constant throughout the entire Phase II feeding period.

Significant differences between the diets were evident early in Phase II for EPA. To start the Phase diet B had the highest amount followed by C and A, but this observation quickly changed. At d 5 diet B was lower than A and C and remained lower through the entire phase, although the differences were only significant on d 5, 8, 16, and 56.

DPA, the chain elongation product of EPA, significantly increased in diet C compared to diets A and B. The increase was evident beginning at d 16 which continued to d 84. The amount of DPA in dogs fed diet C doubled



on d 16 ( $4.3\% \pm 1.0$ ) compared to d 0 and continued to be higher compared to A and B. There were no significant changes in DPA content over time for diets A and B.

The amount of serum phospholipid DHA in diet A was significantly higher on d 16 compared to Diet B. The amount would remain significantly higher than diets B and C beginning at d 28. When comparing day 0 to 84, diet A showed the largest increase.

*Dietary Effects of Zinc Between the Diets.* There were no significant differences found between the diets by repeated measures ANOVA for serum zinc concentration at  $p < 0.05$  (Table IV-2). Diets B and C had approximately 350 ppm zinc while Diet A was approximately 50 ppm. The serum zinc may not reflect the total amount of zinc present in the body.

**TABLE IV-2**

Serum Zinc Concentrations (mcg/dL) During Phase II

<b>Diet</b>	<b>Week 12</b>	<b>Week 16</b>	<b>Week 24</b>
A	$88.6 \pm 2.8$	$83.5 \pm 12.8$	$87.8 \pm 12.9$
B	$89.0 \pm 9.9$	$87.0 \pm 14.3$	$87.9 \pm 12.5$
C	$87.0 \pm 11.0$	$85.6 \pm 11.8$	$81.6 \pm 12.3$

Values are presented as the average  $\pm$  S.D.

No statistically significant differences were found by repeated measures ANOVA,  $p < 0.05$ .

*Discussion.* Current perceptions are that a large number of pet owners and those who show dogs professionally base part of the performance of a diet on the condition of their animal's skin and hair coat. Thus pet food manufacturers work to assure that the diets they produce are able to provide visible improvements in this attribute. Dietary PUFA are of interest because of the metabolic potential of varying the types and amounts of fatty acids in diets in an effort to support skin and coat quality. However, objective assessments of skin and hair coat of dogs are not well established and variability of the responses to such measures as TEWL and HYDR confounds attempts to study fatty acid effects in skin and hair coat condition. This study was designed to evaluate the effect of different amounts of PUFA and zinc on canine skin and hair coat condition, and to gain a better understanding of TEWL and HYDR measurements in dogs.

It should be noted that some of the results in the present study may not have been statistically significant due to the variability between the dogs based on the TEWL and HYDR measurements. However, both objective and subjective assessments of skin and hair coat condition were made and all data taken together appear to suggest some consistent conclusions to be made. Therefore, it is assumed that variability could have possibly had some impact on the statistical significance of other tests in the study. That is why the numeric changes were also noted when evaluating some of the data.

One additional consideration for measurements taken at the skin surface is the potential interference of the hair coat itself. The presence of hair could cause the probes to have indirect contact with the skin therefore leading to additional variability of the measurements. In this study hair was shaved from the site one week prior to testing in order to reduce the amount of variation it might cause. Post-shaving Watson et al. (29) reported that it took 48 h for TEWL to return to normal levels. Shaving could lead to abrasions in the skin which causes damage to the skin's proper barrier function and would allow for increased levels of TEWL. It is assumed that in the present study, shaving was conducted far enough in advance to allow time for any injury to the skin to heal and no visible signs of irritation or abrasions were noted when measurements were performed.

The body site where the TEWL and HYDR measurements were taken was not varied in this study and age, breed, and gender effects were not controlled. Therefore, it can not be determined from the present findings whether age, breed, gender, or body site might have an effect on these measurements.

Dogs fed diet B statistically had more skin hydration at wk 23; therefore, this diet may be more appropriate to be fed to dogs that need to improve skin hydration. Although not significant, dogs fed diet C had less water loss than diets A and B. Thus, dogs that have problems with high levels of TEWL might be supplemented with a diet similar to diet C. Obviously, additional studies

designed to specifically address these possibilities will be needed and these conclusions can only be regarded as speculative at the present.

There were no significant time effects found for TEWL or HYDR for any of the diets used in Phase II. In the subjective assessments (SIA and STMA) improvements over time were significantly observed for overall, glossiness, and softness for all 3 diets. It appears, from the Phase I and Phase II subjective and objective assessments and the plasma phospholipid analyses that a metabolic steady state relating to changes in skin requires approximately 7 wk to occur. Campbell & Dorn (34) conducted a 12 wk study with sunflower oil supplementation and found that it takes 6 wk for a metabolic steady state to be achieved. The male Beagles in their study presented serum and cutaneous changes in PUFA beginning at 3 wk. However, it is still unknown whether such a steady state would have been achieved earlier than 7 wk in the present study. The reason for this is that observations were not made between 3 and 7 wk. Nonetheless, results from Campbell & Dorn's (34) study appear to support our findings. Future studies designed to determine dietary modification effects on skin condition should feed dogs for at least 3 wk, and 6-7 wk does not appear to be unreasonable. In addition, it is apparent that 12 wk is a reliable amount of time to determine dietary effects in skin and hair coat condition and plasma phospholipids.

The hair coat condition scores were initially evaluated by Pearson Correlation Coefficient to determine the extent to which the evaluators' scores

correlated. To determine if they correlated they had to have a Pearson Correlation Coefficient of 0.300 or greater and a correlation p-value of  $p < 0.05$ . The standards were set at these values because this appeared to be a clear point within which correlation existed between the evaluators. This value also appeared to be a natural breaking point with respect to the correlation coefficient results.

At wk 24 of the subjective assessments, diets B and C had a tendency to show more numeric improvements for all parameters compared to diet A. Although not statistically different, diet B compared to diet C showed slightly more improvement for overall score, glossiness, and scale. Diet B was enriched with LA (sunflower oil), which has been shown to improve the condition of skin and hair coat in other studies (1,12). Evidence to support this theory was presented in a study in which dogs with chronic seborrhea were supplemented with sunflower oil for sixty days (1). At the start of the study the dogs had increased concentrations of oleic acid (18:1 n-9) and decreased concentrations of LA in their skin compared to normal dogs. LA in the epidermis is carried by ceramide 1 and has a vital role in the cutaneous barrier to water loss (9,35,36). Oleic acid provides fluidity to membranes but is not effective in preventing water loss through the cells (37). Animals that have EFA deficiencies will have a substitution of oleic acid and other fatty acids for LA in their epidermal membranes. Although the seborrheic dogs were not EFA deficient, there were decreased amounts of serum LA present. There were also elevated amounts of

AA found in the skin. Dietary supplementation with fatty acids resulted in clinical improvement, decreased amounts of AA, and increased amounts of LA (1). The present study, using normal dogs, did show a numeric increase in plasma phospholipid LA and significant improvements in skin and coat condition in dogs fed diet B. However, we did not see a significant decrease over time in plasma phospholipid AA in dogs fed diet B.

Taken together the data from this study indicates that diets B and C appear to be better diets to improve skin and hair coat condition compared to diet A. Accumulation of AA in the plasma phospholipid results in pro-inflammatory conditions in the skin. The dogs fed diet B were found to have the largest amount of AA at the end of the study periods in plasma phospholipid fatty acids. By contrast plasma phospholipid AA in dogs fed diet C was comparatively lower. While diet B exhibited the most improvements for the subjective assessments diet C was similar in many respects and was not statistically different from diet B. Also, diet C resulted in a favorable fatty acid profile by having increased amounts of plasma phospholipid LA but lower amounts of plasma phospholipid AA (as a pro-inflammatory mediator). Diet C also increased the amount of plasma phospholipid EPA compared to B, and this increase is also associated with less pro-inflammatory conditions. Thus diet C exhibits the potential for a beneficial response in several ways. Therefore, diet C may be a better diet because it not only improves condition scores it also has

a biochemical basis for a less pro-inflammatory state occurring due to injury such as scratches, traumas, or inflammatory skin disorders.

The accumulation of plasma phospholipid LA in diet C compared to diet B may be due to a sparing effect of ALA on LA. LA is very important in the general health and function of the skin. A similar study (12) was conducted in dogs in which a comparison was made between dogs fed diets supplemented with flax seed (source of ALA and LA) or sunflower seed (source of LA). Results suggested that ALA may have inhibited the conversion of LA to its longer chain polyunsaturated metabolites (AA) by competition for the  $\Delta 6$  desaturase enzyme system thereby resulting in a more dramatic accumulation of LA when a diet enriched in ALA (flaxseed) was fed. This could be an explanation for the observed improvements in skin and hair coat scores when supplemented with diets high in ALA. It was speculated in one study that competition between ALA and LA for chain desaturation and elongation may have caused an accumulation and subsequent incorporation of LA in the skin ceramide fractions and hair follicles of the dogs (12). The accumulation of plasma phospholipid LA in diet C compared to B in the present study further supports this possibility.

The ratio of LA:ALA in the diets could help to further explain the competition between these two fatty acids. The ratio of the diets was as follows: Ol'Roy®-20; Diet A-15; Diet B-35; Diet C-4. Even though the ratio in diet C is low, an accumulation of LA is still seen in the plasma phospholipids of the dogs. This can be due not simply to increased amounts of LA in the diet but also

because plasma phospholipid LA may be spared from further conversion by the increased amounts of ALA present. This effect can also be seen to a similar, but lesser, extent in diet A. While the ratio for diet A is greater than diet C, it is nonetheless more than two times lower than diet B. This same sparing effect when diet A was fed may also have occurred.

The amount of total fat in the diets has not been ruled out as a possible cause of improvement in any of the changes seen in this study. The acclimation diet (Ol'Roy®) contained ~23.5% energy as fat and the test diets contained on average ~28.6% which is a 20% difference when compared. Further investigation is needed in order to determine the extent that total fat may have on skin and hair coat condition when given in conjunction with PUFA.

It should also be noted that the plasma phospholipid fatty acid levels in diet C, which contained flax seed, demonstrated an accumulation of DPA. Previous studies in our lab have shown that diets enriched in ALA accumulate DPA in the plasma phospholipid fraction (20). In addition, amounts of other (n-3) fatty acids (i.e. EPA, DPA, and DHA) present in diets per se would also result in the accumulation of these fatty acids in the plasma phospholipids. For that matter, more EPA was present in diet C (0.05% DM) compared to the other diets. This amount could have contributed to plasma phospholipid DPA accumulating in this group of dogs. It is more likely, however, that the increased amounts of ALA in the diet (0.91% DM) would explain the accumulation of DPA in dogs fed

diet C. It is unknown at this time whether plasma phospholipid fatty acid DPA accumulation affects the skin and hair coat condition.

It is also of interest that diet A resulted in a large accumulation of plasma phospholipid DHA. This increase is likely due to a direct effect of DHA in the diet itself, because diet A contained relatively high amounts of DHA (0.06% DM). This finding may also help explain lower amounts of AA in the phospholipid fractions due to substitution of the phospholipids with more (n-3) FA.

There was no variation in the concentration of serum zinc among the diets. Serum zinc concentrations may not reflect tissue amounts and biopsy specimens may be needed in order to find differences in dogs as a function of dietary zinc. Diets B and C each contained approximately 350 ppm zinc while Diet A was approximately 50 ppm. Improvements in skin and hair coat did not appear to be related to serum zinc concentration. It remains to be determined whether tissue contents may be different due to increased dietary amounts.

Rees et al. (12) reported that skin and hair coat condition scores were only improved for a brief period of time when fed LA and ALA enriched diets. Improvements were only sustained for 28 d when supplemented with flax seed and sunflower seed. The present study demonstrated improvements sustained for a longer amount of time. One possible explanation for this difference is the manner in which the flaxseed or sunflower oil was provided in the diet. In the Rees et al. study, the seeds were ground, the base diet was moistened and the ground seeds were then coated on the surface of a dry-type extruded diet (20).

In the present study the ground flax seeds and sunflower oil were incorporated into the fatty acid matrix of the diet and cooked and processed into a dry extruded complete and balanced diet. This may have some effect on bioavailability of diet ingredients between the two studies. Another possible reason for the difference may be the amount of total fat in the diets. The three diets in the present study contained ~28.6% energy as fat versus ~25% in the Rees et al. study which amounts to a 14.4% difference in fat between the diets of these two studies. The higher % of fat may, in part, explain improvements being sustained for a longer period of time in the present study.

It was hypothesized that a diet enriched with LA, ALA and zinc would show more improvements in skin and hair coat condition compared to one enriched only in LA and zinc. While skin and hair coat improvements between diets C and B were similar, diet C (flax seed) appears to have additional benefits compared B or A. Whereas diets B and C both proved to be better diets for improving skin and hair coat conditions compared to diet A, diet C resulted in a more desirable plasma phospholipid fatty acid profile compared to diet B. Thus if an improved (n-3) fatty acid effect in addition to incremental improvements in skin and hair coat condition are desired, then diet C might be preferred. The results of this study have clinical application in regards to treating or improving skin and hair coat condition in dogs. There is also a potential for these results to have further application in dermatological conditions in humans.

## **CHAPTER V**

### **SUMMARY**

This study of diets containing varying amounts of LA and ALA in the canine model provided beneficial information regarding improvements in skin and hair coat condition. The study was divided into two 12 wk periods (Phase I and Phase II). In Phase I the dogs were supplemented with a complete and balanced discount store dog food (Ol'Roy®). The dogs were tested for the amount of transepidermal water loss (TEWL) and skin hydration (HYDR) over time. The amount of within- and between-animal variation was evaluated for these two objective assessments in Phase I. In Phase II the dogs were divided into three equal groups and fed one of three diets: Diet A contained adequate amounts of LA and zinc, Diet B had increased amounts of LA and zinc, and Diet C had the same enriched amounts of LA and zinc as diet B but increased amounts of ALA. It was hypothesized that diet C would show incremented improvements in skin and hair coat condition compared to the other two diets.

Based on statistically significant differences over time for data from both Phase I and Phase II, a feeding period of seven weeks is recommended to acclimate dogs to a diet in order to produce a metabolic steady state environment for skin evaluations. Thus comparisons made after 12 wk are valid for the diets used in this study.

Significant improvements in skin hydration with diet B versus diet A were present at wk 23. TEWL in Phase II showed no significant differences between diets or over time due to high variability, although, there were numeric improvements in diet B and C compared to diet A. Also, there was a numeric tendency for Diet C to have less water loss compared to diets A and B.

A considerable amount of between dog variability was found throughout the study. The amount of variability was tested in Phase I for TEWL and HYDR and existed for both measurements. Due to this finding it is expected that the variability would exist in Phase II as well. Some significant differences were not found throughout Phase II for the various parameters tested and this finding is believed to be attributed to the variability between the dogs.

The TEWL and HYDR measurements are beneficial assessments in assessing the effects diet or medications on canine skin condition. Based on Phase I and Phase II results, the corneometry (HYDR) measurements appear to be more reliable and easier to perform than TEWL. Both of these techniques have clinical application where pre- and post-treatment comparisons in individual dogs are performed.

All three test diets (A, B, and C) showed significant improvements in skin and hair coat condition compared to the discount store dog food (Ol'Roy®). Most notable (statistically significant) were improvements found for all diets for glossiness, softness, and overall score when comparing wk 12 (baseline) to wk 24 (12 wk feeding). Upon comparison of the diets to each other, diet B showed

more improvement by subjective evaluations compared to diet A. Mean values for diet C showed a tendency to be improved compared to diet A, although statistical significance was not always seen. Numerically diet B improved the skin and hair coat condition scores slightly more than diet C.

The amount of plasma zinc present in the dogs fed the three diets did not vary. The observed improvements in skin and hair coat condition do not appear to be related to plasma zinc concentrations. It may be necessary to collect biopsy specimens in order to find differences in dogs as a function of dietary zinc.

In conclusion, diet B (a diet containing increased amounts of LA and zinc) compared to diet A (a diet containing AAFCO adequate amounts of these nutrients) showed improvement in skin and coat condition. Diet B presented moderately more improvement in skin and coat condition scores than diet C, but this finding was not statistically significant. TEWL and HYDR measurements can serve as reasonable stable characteristics with which to investigate treatment effects of diet or medication on canine skin condition. Based on mean values diet B is a better diet for dogs that need to improve skin hydration. By comparison dogs that have problems with high levels of TEWL should be supplemented with a diet similar to diet C. Diet B tended to exhibit improvements in the subjective and objective assessments compared to diet A. This study suggests that diets enriched with LA, ALA, and zinc will not only lead to improvements in skin and hair coat condition, it will also have an additional benefit by producing less pro-inflammatory conditions in the skin. This is shown

in diet C by the increased amounts of plasma phospholipid fatty acids LA and EPA without the accumulation of pro-inflammatory precursors such as AA. If an (n-3) fatty acid effect is expected than the dog needs to be fed diet C.

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## **APPENDIX A**

**TABLE A-I**  
**Fatty Acid Content of Diets**

<b>Fatty Acid</b>	<b>OI'Roy</b>	<b>Diet A</b>	<b>Diet B</b>	<b>Diet C</b>
<b>12:0</b>	0.04	ND	ND	ND
<b>14:0</b>	0.08	0.28	0.13	0.08
<b>14:1</b>	0.02	0.04	0.02	0.01
<b>15:0</b>	0.01	0.05	0.02	0.01
<b>15:1</b>	ND	ND	ND	ND
<b>16:0</b>	2.09	2.95	2.29	1.88
<b>16:1</b>	0.48	0.33	0.43	0.33
<b>17:0</b>	0.03	0.12	0.05	0.04
<b>17:1</b>	0.02	0.07	0.03	0.03
<b>18:0</b>	0.60	1.94	0.97	0.98
<b>18:1 n-9</b>	3.07	4.74	4.35	4.53
<b>18:1 n-7</b>	0.31	0.49	0.31	ND
<b>18:2 n-6</b>	2.22	1.33	3.89	3.70
<b>18:3 n-3</b>	0.11	0.09	0.11	0.91
<b>20:0</b>	0.04	0.10	0.04	0.17
<b>20:1 n-9</b>	0.01	0.02	0.02	0.07
<b>20:2 n-6</b>	0.03	0.01	0.06	0.01
<b>20:4 n-6</b>	0.02	0.02	0.05	0.01
<b>20:5 n-3</b>	0.03	0.02	0.01	0.05
<b>22:0</b>	ND	ND	ND	ND
<b>22:1 n-9</b>	ND	ND	ND	ND
<b>22:5 n-3</b>	0.01	0.02	0.04	0.01
<b>22:6 n-3</b>	0.02	0.06	0.01	0.04
<b>24:0</b>	0.01	0.01	0.02	0.01

Fatty acids reported as % on Dry Matter Basis

ND= not detected

**TABLE A-II**  
**Linoleic Acid, Alpha-linolenic Acid, and Zinc Content of Phase II**  
**Diets (energy basis and g/kg as-is)**

	Zinc	Linoleic Acid		Alpha-Linolenic Acid	
<u>Diet</u>	<u>mg/kg</u>	<u>Energy %</u>	<u>g/kg</u>	<u>Energy %</u>	<u>g/kg</u>
A (tallow)	120	2.43	11	0.22	1.5
B (sunflower)	350	8.77	33	0.22	1.5
C (flaxseed)	350	8.77	33	1.63	12

**TABLE A-III**  
**Nutrient Analysis of Diets**

<b>DIET</b>	<b>Ol'Roy</b>	<b>Diet A</b>	<b>Diet B</b>	<b>Diet C</b>
<b>Moisture</b>	12.0%	6.2%	6.6%	6.2%
<b>Protein</b>	21.0%	22.9%	22.3%	22.9%
<b>Fat</b>	9.0%	13.1%	12.9%	13.3%
<b>Ash</b>	8.2%	8.8%	8.6%	8.8%
<b>Fiber</b>	4.0%	2.1%	2.1%	2.7%
<b>Carbohydrate</b>	45.8%	46.9%	47.5%	46.1%
<b>Calories</b> kcal/100g	325	357.9	356.5	356.9

Amounts shown as % as-is Basis

**TABLE A-IV**  
**Main Ingredients of Diets**

<b>Ol'Roy</b>	<b>Diet A</b>	<b>Diet B</b>	<b>Diet C</b>
Ground Yellow Corn	Lamb Meal	Lamb Meal	Lamb Meal
Meat and Bone Meal	Ground Rice	Ground Rice	Ground Rice
Soybean Meal	Rice Flour	Rice Flour	Rice Flour
Wheat Middling	Rice Bran	Rice Bran	Rice Bran
Animal Fat	<b><i>Beef Tallow</i></b>	<b><i>Sunflower Oil</i></b>	<b><i>Ground Flax Seed</i></b>
Chicken by-product Meal	Natural Flavor	Poultry Fat	Poultry Fat
Brewers Rice	Rice Gluten	Natural Flavor	Natural Flavor
Animal Digest	Dried egg Product	Rice Gluten	Rice Gluten
Salt	Dried Beet Pulp	Dried Egg Product	Dried Egg Product
Calcium Carbonate	Potassium Chloride	Dried Beet Pulp	Dried Beet Pulp
Choline Chloride	L-Lysine	Potassium Chloride	Potassium Chloride
Zinc Sulfate	Dried Kelp	L-Lysine	L-Lysine
Ferrous Sulfate	Salt	Dried Kelp	Dried Kelp
Vitamin E Suppl.	Choline Chloride	Salt	Salt
Zinc Oxide	Zinc Sulfate	Choline Chloride	Choline Chloride
Niacin	Vitamin E Suppl.	Zinc Sulfate	Zinc Sulfate
Copper Sulfate	Taurine	Vitamin E Suppl.	Vitamin E Suppl.
Manganous Oxide	Ferrous Sulfate	Taurine	Taurine
Vitamin A Suppl.	Ascorbic Acid	Ferrous Sulfate	Ferrous Sulfate
Calcium Panthothenate	Biotin	Ascorbic Acid	Ascorbic Acid
Biotin	Copper Proteinate	Biotin	Biotin
Vitamin B12 Suppl.	Niacin	Copper Proteinate	Copper Proteinate
Pyridoxine Hydrochloride	Manganous Oxide	Niacin	Niacin

**TABLE A-V**  
**Comparison of Plasma Phospholipid Analysis Over Time and Between Diets**

Diet	Fatty Acid	Sample Time						
Diet A		Day 0	Day 5	Day 8	Day 16	Day 28	Day 56	Day 84
	16:0	13.9±0.9 <sup>a,b</sup> <sub>1</sub>	14.7± 2.1 <sup>a</sup> <sub>1</sub>	13.9±3.8 <sup>a,b</sup> <sub>1</sub>	11.1±2.8 <sup>b</sup> <sub>1</sub>	14.6±1.2 <sup>a</sup> <sub>1</sub>	14.9±0.8 <sup>a</sup> <sub>1</sub>	14.6±1.2 <sup>a</sup> <sub>1</sub>
	18:0	27.5±1.7 <sup>a</sup> <sub>1</sub>	26.2±1.3 <sup>a</sup> <sub>1</sub>	26.4±1.2 <sup>a</sup> <sub>2</sub>	26.5±1.0 <sup>a</sup> <sub>1</sub>	26.3±1.6 <sup>a</sup> <sub>2</sub>	26.2±1.3 <sup>a</sup> <sub>2</sub>	25.6±1.7 <sup>a</sup> <sub>2</sub>
	18:1n-9	5.9±0.3 <sup>c</sup> <sub>2</sub>	7.5±0.8 <sup>a,b</sup> <sub>1</sub>	6.7±0.8 <sup>a,b,c</sup> <sub>1</sub>	6.3±1.3 <sup>b,c</sup> <sub>1</sub>	7.3±0.7 <sup>a,b</sup> <sub>1</sub>	7.4±1.0 <sup>a,b</sup> <sub>1</sub>	7.8±0.8 <sup>a</sup> <sub>1</sub>
	18:1n-7	3.4±0.5 <sup>a,b</sup> <sub>1</sub>	2.8±0.3 <sup>c</sup> <sub>1</sub>	3.1± 0.2 <sup>b,c</sup> <sub>1</sub>	2.9±0.4 <sup>b,c</sup> <sub>1</sub>	3.2±0.2 <sup>a,b,c</sup> <sub>1</sub>	3.0±0.5 <sup>b,c</sup> <sub>1</sub>	3.6±0.2 <sup>a</sup> <sub>1</sub>
	18:2n-6	14.2±0.9 <sup>a</sup> <sub>1</sub>	13.9±1.3 <sup>a</sup> <sub>1</sub>	13.3±1.2 <sup>a</sup> <sub>2</sub>	13.8± 1.8 <sup>a</sup> <sub>1</sub>	14.1±2.3 <sup>a</sup> <sub>1</sub>	14.7±1.3 <sup>a</sup> <sub>1</sub>	14.4±1.2 <sup>a</sup> <sub>2</sub>
	18:3n-3	0.2±0.1 <sup>a</sup> <sub>1</sub>	0.2±0.04 <sup>a</sup> <sub>1</sub>	0.2±0.04 <sup>a</sup> <sub>2</sub>	0.2±0.1 <sup>a</sup> <sub>2</sub>	0.2±0.1 <sup>a</sup> <sub>2</sub>	0.2±0.03 <sup>a</sup> <sub>2</sub>	0.2±0.1 <sup>a</sup> <sub>2</sub>
	20:4n-6	22.9±1.2 <sup>a,b</sup> <sub>1,2</sub>	21.9±2.1 <sup>a,b,c</sup> <sub>1</sub>	23.1±2.8 <sup>a,b</sup> <sub>1,2</sub>	24.1±3.0 <sup>a</sup> <sub>1</sub>	20.6±1.2 <sup>b,c</sup> <sub>2</sub>	19.8 ±2.2 <sup>b,c</sup> <sub>2</sub>	18.9 ±1.8 <sup>c</sup> <sub>2</sub>
	20:5n-3	0.2±0.1 <sup>c</sup> <sub>1</sub>	0.41±0.1 <sup>b,c</sup> <sub>1</sub>	0.4±0.2 <sup>a,b,c</sup> <sub>1</sub>	0.5±0.1 <sup>a,b</sup> <sub>1</sub>	0.6±0.2 <sup>a,b</sup> <sub>1</sub>	0.7±0.2 <sup>a</sup> <sub>1</sub>	0.7±0.2 <sup>a,b</sup> <sub>1</sub>
	22:5n-3	1.9±0.8 <sup>a</sup> <sub>1</sub>	2.0±0.4 <sup>a</sup> <sub>1</sub>	2.3±0.8 <sup>a</sup> <sub>1</sub>	2.7±0.7 <sup>a</sup> <sub>2</sub>	2.2±0.4 <sup>a</sup> <sub>2</sub>	2.1±0.3 <sup>a</sup> <sub>2</sub>	2.1±0.3 <sup>a</sup> <sub>2</sub>
	22:6n-3	0.8±0.4 <sup>d</sup> <sub>1</sub>	1.3±0.1 <sup>c,d</sup> <sub>1</sub>	1.6±0.6 <sup>b,c</sup> <sub>1</sub>	2.3±0.5 <sup>a,b</sup> <sub>1</sub>	2.0±0.7 <sup>a,b,c</sup> <sub>1</sub>	2.4±0.2 <sup>a</sup> <sub>1</sub>	2.7±0.3 <sup>a</sup> <sub>1</sub>
Diet B	16:0	13.4±0.9 <sup>a</sup> <sub>1</sub>	13.0±1.2 <sup>a,b</sup> <sub>1</sub>	13.0±2.5 <sup>a,b</sup> <sub>1</sub>	10.8±2.7 <sup>b</sup> <sub>1</sub>	12.8±1.0 <sup>a,b</sup> <sub>2</sub>	13.2±1.1 <sup>a,b</sup> <sub>2</sub>	12.6±1.5 <sup>a,b</sup> <sub>2</sub>
	18:0	27.9±1.3 <sup>a</sup> <sub>1</sub>	28.1±2.4 <sup>a</sup> <sub>1</sub>	27.0±1.4 <sup>a</sup> <sub>1,2</sub>	26.5±2.2 <sup>a</sup> <sub>1</sub>	27.2±1.1 <sup>a</sup> <sub>1,2</sub>	28.1±0.7 <sup>a</sup> <sub>1</sub>	26.6±1.9 <sup>a</sup> <sub>1,2</sub>
	18:1n-9	5.8±0.3 <sup>a</sup> <sub>2</sub>	6.0±0.5 <sup>a</sup> <sub>2</sub>	5.8±0.8 <sup>a</sup> <sub>2</sub>	5.7±0.6 <sup>a</sup> <sub>1,2</sub>	5.8±0.8 <sup>a</sup> <sub>2</sub>	5.7±0.5 <sup>a</sup> <sub>1</sub>	5.8±0.8 <sup>a</sup> <sub>2</sub>
	18:1n-7	3.4±0.4 <sup>a</sup> <sub>1</sub>	3.0±0.5 <sup>a</sup> <sub>1</sub>	3.1±0.5 <sup>a</sup> <sub>1</sub>	2.7±0.4 <sup>a</sup> <sub>1</sub>	3.2±0.3 <sup>a</sup> <sub>1</sub>	3.0±0.23 <sup>a</sup> <sub>1</sub>	3.3±0.4 <sup>a</sup> <sub>1,2</sub>
	18:2n-6	14.2±0.9 <sup>a</sup> <sub>1</sub>	15.4±1.8 <sup>a</sup> <sub>1</sub>	15.1±1.6 <sup>a</sup> <sub>1,2</sub>	15.0±1.7 <sup>a</sup> <sub>1</sub>	15.6±2.1 <sup>a</sup> <sub>1</sub>	15.5±2.3 <sup>a</sup> <sub>1</sub>	16.2±0.9 <sup>a</sup> <sub>1</sub>
	18:3n-3	0.2 ±0.1 <sup>a</sup> <sub>1</sub>	0.2±0.1 <sup>a</sup> <sub>1</sub>	0.2±0.1 <sup>a</sup> <sub>2</sub>	0.2±0.1 <sup>a</sup> <sub>2</sub>	0.3±0.1 <sup>a</sup> <sub>1,2</sub>	0.2±0.04 <sup>a</sup> <sub>1,2</sub>	0.2±0.1 <sup>a</sup> <sub>2</sub>
	20:4n-6	23.7±1.4 <sup>a</sup> <sub>1</sub>	22.0±3.1 <sup>a</sup> <sub>1</sub>	24.8±1.4 <sup>a</sup> <sub>1</sub>	25.1±2.8 <sup>a</sup> <sub>1</sub>	23.4±1.8 <sup>a</sup> <sub>1</sub>	23.1±2.8 <sup>a</sup> <sub>1</sub>	23.2±1.9 <sup>a</sup> <sub>1</sub>
	20:5n-3	0.5±0.7 <sup>a</sup> <sub>1</sub>	0.2±0.1 <sup>a</sup> <sub>2</sub>	0.1±0.1 <sup>a</sup> <sub>2</sub>	0.3±0.3 <sup>a</sup> <sub>2</sub>	0.4±0.2 <sup>a</sup> <sub>1</sub>	0.3±0.1 <sup>a</sup> <sub>2</sub>	0.5±0.1 <sup>a</sup> <sub>1</sub>
	22:5n-3	2.0±0.3 <sup>a</sup> <sub>1</sub>	1.9±0.5 <sup>a</sup> <sub>1</sub>	1.8±0.7 <sup>a</sup> <sub>1</sub>	2.1±0.6 <sup>a</sup> <sub>2</sub>	1.9±0.6 <sup>a</sup> <sub>2</sub>	2.0±0.9 <sup>a</sup> <sub>2</sub>	1.8±0.5 <sup>a</sup> <sub>2</sub>
	22:6n-3	0.9±0.5 <sup>a</sup> <sub>1</sub>	1.0±0.3 <sup>a</sup> <sub>1,2</sub>	1.0±0.7 <sup>a</sup> <sub>1</sub>	1.4±0.7 <sup>a</sup> <sub>2</sub>	1.3±0.5 <sup>a</sup> <sub>2</sub>	1.1±0.1 <sup>a</sup> <sub>2</sub>	1.3±0.3 <sup>a</sup> <sub>2</sub>
Diet C	16:0	14.4±0.7 <sup>a</sup> <sub>1</sub>	13.5±1.4 <sup>a</sup> <sub>1</sub>	13.6±2.6 <sup>a</sup> <sub>1</sub>	8.9±1.86 <sup>b</sup> <sub>1</sub>	13.0±0.9 <sup>a</sup> <sub>2</sub>	12.8±0.7 <sup>a</sup> <sub>2</sub>	12.8±0.7 <sup>a</sup> <sub>2</sub>
	18:0	28.1±1.4 <sup>a</sup> <sub>1</sub>	28.3±.7 <sup>a</sup> <sub>1</sub>	28.9±2.7 <sup>a</sup> <sub>1</sub>	27.2±1.0 <sup>a</sup> <sub>1</sub>	28.3±1.0 <sup>a</sup> <sub>1</sub>	27.9±1.8 <sup>a</sup> <sub>1,2</sub>	27.8±1.1 <sup>a</sup> <sub>1</sub>
	18:1n-9	6.5±0.6 <sup>a</sup> <sub>1</sub>	6.2±0.6 <sup>a</sup> <sub>2</sub>	5.7±0.5 <sup>a</sup> <sub>2</sub>	5.1±0.6 <sup>a</sup> <sub>2</sub>	5.4±0.6 <sup>a</sup> <sub>2</sub>	6.1±2.5 <sup>a</sup> <sub>1</sub>	5.4±0.4 <sup>a</sup> <sub>2</sub>
	18:1n-7	3.3±0.5 <sup>a</sup> <sub>1</sub>	3.0±0.6 <sup>a</sup> <sub>1</sub>	3.2±0.4 <sup>a</sup> <sub>1</sub>	2.8±0.4 <sup>a</sup> <sub>1</sub>	3.5±0.7 <sup>a</sup> <sub>1</sub>	3.1±0.7 <sup>a</sup> <sub>1</sub>	3.1±0.5 <sup>a</sup> <sub>2</sub>
	18:2n-6	15.4±2.4 <sup>a</sup> <sub>1</sub>	16.3±2.8 <sup>a</sup> <sub>1</sub>	15.5±1.6 <sup>a</sup> <sub>1</sub>	15.5±1.8 <sup>a</sup> <sub>1</sub>	16.6±1.9 <sup>a</sup> <sub>1</sub>	16.4±2.0 <sup>a</sup> <sub>1</sub>	17.4±1.6 <sup>a</sup> <sub>1</sub>
	18:3n-3	0.2±0.1 <sup>a,b</sup> <sub>1</sub>	0.3±0.1 <sup>a,b</sup> <sub>1</sub>	0.3±0.1 <sup>a,b</sup> <sub>1</sub>	0.3±0.1 <sup>a,b</sup> <sub>1</sub>	0.4±0.1 <sup>a,b</sup> <sub>1</sub>	0.6±0.6 <sup>a</sup> <sub>1</sub>	0.5±0.1 <sup>a,b</sup> <sub>1</sub>
	20:4n-6	21.7±1.2 <sup>a,b</sup> <sub>2</sub>	20.8±3.3 <sup>b</sup> <sub>1</sub>	21.3±2.0 <sup>b</sup> <sub>2</sub>	25.3±2.7 <sup>a</sup> <sub>1</sub>	21.3±2.1 <sup>b</sup> <sub>1,2</sub>	19.8±2.9 <sup>b</sup> <sub>2</sub>	19.9±1.7 <sup>b</sup> <sub>2</sub>
	20:5n-3	0.3±0.2 <sup>b</sup> <sub>1</sub>	0.4±0.2 <sup>a,b</sup> <sub>1</sub>	0.4±0.2 <sup>a,b</sup> <sub>1</sub>	0.6±0.1 <sup>a,b</sup> <sub>1</sub>	0.6±0.3 <sup>a,b</sup> <sub>1</sub>	0.7±0.2 <sup>a</sup> <sub>1</sub>	0.7±0.3 <sup>a</sup> <sub>1</sub>
	22:5n-3	1.9±0.4 <sup>d</sup> <sub>1</sub>	2.4±0.5 <sup>c,d</sup> <sub>1</sub>	2.7±0.7 <sup>b,c,d</sup> <sub>1</sub>	4.3±1.0 <sup>a</sup> <sub>1</sub>	3.2±0.7 <sup>a,b,c</sup> <sub>1</sub>	3.5±0.9 <sup>a,b,c</sup> <sub>1</sub>	3.6±0.9 <sup>a,b</sup> <sub>1</sub>
	22:6n-3	1.0±0.5 <sup>a,b</sup> <sub>1</sub>	1.0±0.3 <sup>a,b</sup> <sub>2</sub>	0.9±0.3 <sup>b</sup> <sub>1</sub>	1.5±0.7 <sup>a</sup> <sub>1,2</sub>	1.1±0.3 <sup>a,b</sup> <sub>2</sub>	1.2±0.4 <sup>a,b</sup> <sub>2</sub>	1.0±0.1 <sup>a,b</sup> <sub>2</sub>

Values are presented as mean ± S.D.

Superscript letters in a row not in common for each diet are significantly different at p<0.05

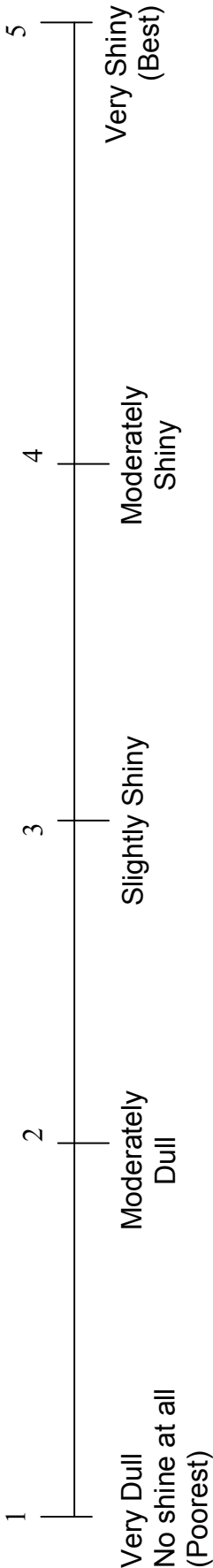
Subscript numbers in a column not in common for each day among the diets are significantly different at p< 0.05

## **APPENDIX B**

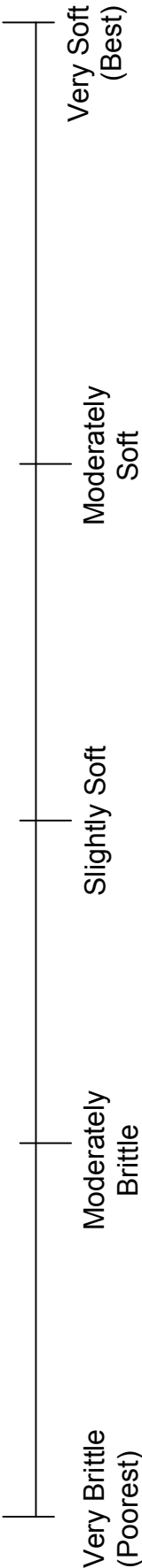
**Scoring Sheets Used for SIA and STMA Graded Evaluations**

Integer Evaluations: Please circle a whole number  
Tick Mark Evaluations: Please indicate a tick mark between integer values

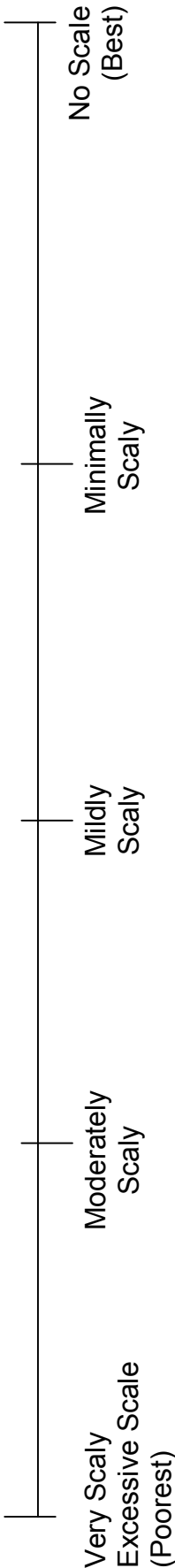
**GLOSSINESS**



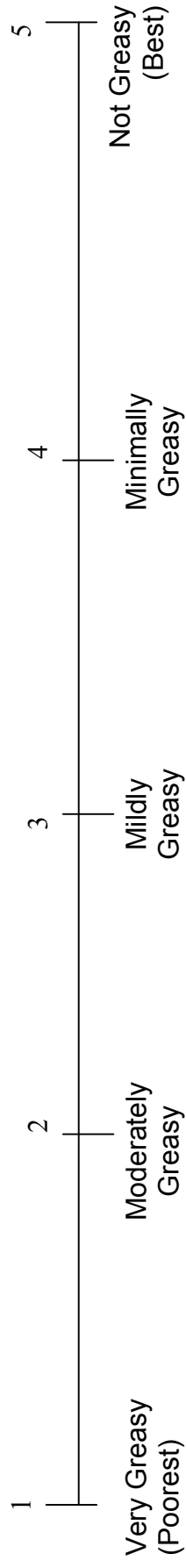
**SOFTNESS**



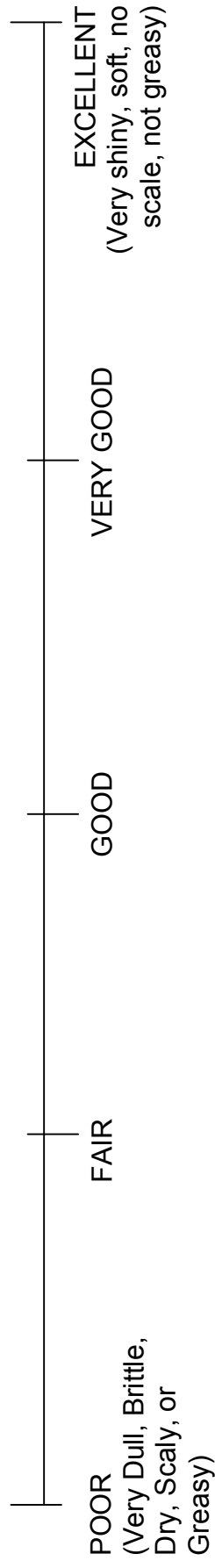
**SCALE**



## GREASINESS



## OVERALL QUALITY



## VITA

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**Publications:** Hester, S., Bauer, J., Rees, C., Kennis, R., Zoran, D., Bigley, K., Wright, A., Kirby, N. Evaluation of Corneometry (Skin Hydration) and Transepidermal Water Loss Measurements in Two Canine Breeds. J. Nutrition, (In Press, August 2004).

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