28-Day Continuous Dosing Study in Minipigs with a SALATRIM Structured Triacylglycerol Composed of Stearate, Acetate, and Propionate

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SALATRIM 23SO lot A026 belongs to a family of structured triacylglycerols with lower caloric availability (4.5–6.0 kcal/g) than corn oil (9 kcal/g). Male and female minipigs were divided into five groups of four pigs of each sex and fed diets containing 0%, 3%, 6%, and 10% SALATRIM or 10% corn oil, as a reference for the high fat content of the SALATRIM diets, for 28 days. Diets were supplemented with 2% corn oil to ensure adequate essential fatty acid availability. No effects on body weight, feed consumption, clinical observations, hematology, serum chemistry, bone mineral content, serum and liver levels of fat-soluble vitamins, and organ weights were detected. Focal hepatocellular vacuolation was observed in treated and control groups. The incidence was similar among groups. Becaue minimally increased severity of vacuolation was observed in only one male in each of the 10% SALATRIM and corn oil treatments, this appears to be a spurious finding or a nonspecific fat effect. SALATRIM 23SO lot A026 produced neither toxicologically nor nutritionally significant effects.

INTRODUCTION

SALATRIM 23SO lot A026 is a member of the SALATRIM family of structured triacylglycerols developed by Nabisco Foods Group, East Hanover, NJ. These fats provide lower caloric availability (4.5–6.0 kcal/g) than corn oil (9 kcal/g) (Finley et al., 1994a). SALATRIM 23SO lot A026 is produced by interesterification among triacetin, tripropionin, and hydrogenated soybean oil that contains a high concentration of stearic acid esterified to glycerol. The resulting fat contains a preponderance of acetic, propionic, and stearic acids esterified to glycerol.

SALATRIM fats should be hydrolyzed in the gastrointestinal tract similarly to typical dietary fats to yield monoacylglycerols, diacylglycerols, short-chain fatty acids, and stearic acid. Short-chain fatty acids and 2-monoacylglycerols are absorbed and enter normal metabolic pathways (Bugaut, 1987; Jensen et al., 1982; Rombeau et al., 1990), whereas free stearic acid and calcium and magnesium soaps of stearic acid are excreted in the feces (Benzonana and Desnuelle, 1968; Bliss et al., 1972; Gacs and Barltrop, 1977; Mattson et al., 1979; Sammons and Wiggs, 1960). The poor absorption of stearate and the lower number of calories provided by the metabolism of short-chain fatty acids compared to that of long-chain fatty acids result in lower caloric availability for SALATRIM fats compared with dietary fats such as corn oil. An *in vivo* metabolism study using rats as the experimental model supports the conclusion that SALATRIM fats are metabolized in a predictable manner, similar to other fats (Hayes et al., 1994a).

Because of their similarity to dietary fats and their predictable metabolism, members of the SALATRIM family should have no potential to produce toxicological effects. This hypothesis was confirmed in 13-week subchronic toxicity studies in rats with five SALATRIM fats made using hydrogenated canola oil or hydrogenated cottonseed oil as the stearic acid source (Hayes et al., 1994b–d). Three of these fats, SALATRIM 4CA lot A006, SALATRIM 23CA lot A014, and SALATRIM 32CA lot A015, were made using hydrogenated canola oil and represented SALATRIM fats with a high content of butyrate, acetate, or propionate, respectively. SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 represented SALATRIM fats made from hydrogenated canola oil and hydrogenated cottonseed oil, respectively, and contained approximately equal quantities of all three short-chain fatty acids.

In the 28-day repeated dosing minipig study reported here, SALATRIM 23SO lot A026 was chosen as a representative high-acetate SALATRIM fat containing acetic and propionic acid with hydrogenated soybean oil as the precursor fat. Because hydrogenated soybean oil contains fatty acids in addition to stearic acid, these fatty acids will also be minor components of this SALATRIM fat. The SALATRIM precursor fats contain the normal constituents of vegetable fats, such as phytosterols. As a result, these constituents may occur in small quantities in SALATRIM fats.

This 28-day repeated dosing study was designed to comply with the guidelines for toxicity testing developed by the FDA (U.S. Food and Drug Administration, 1982). Additional variables were added to the study design to address specific nutritional questions. For instance, serum and hepatic concentrations of the fat-soluble vitamins A and E were evaluated to determine if SALATRIM fats had a potential to alter absorption of these vitamins. Bone minerals were quantified to determine if SALATRIM fats altered mineral homeostasis. High dietary concentrations of SALATRIM fats were used to maximize the potential for producing toxicity. The dietary concentration was limited to 10% by weight because higher doses may

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Table 1. Total Fatty Acid Profile for SALATRIM 23SO Lot A026[a]

fatty acid name	fatty acid designation	wt %
stearic (octadecanoic)	C18:0	57.823 ± 1.000
acetic	C2:0	23.39 ± 1.12
palmitic (hexadecanoic)	C16:0	7.543 ± 0.027
propionic	C3:0	2.92 ± 0.11
arachidic (eicosanoic)	C20:0	0.425 ± 0.023
benhenic (docosanoic)	C22:0	0.252 ± 0.015
oleic (9-octadecenoic)	C18:1	0.289 ± 0.011
lignoceric (tetracosanoic)	C24:0	0.100 ± 0.006
myristic (tetradecanoic)	C14:0	0.084 ± 0.004
linoleic (9,12-octadecadienoic)	C18:2	0.018 ± 0.001
lauric (dodecanoic)	C12:0	0.016 ± 0.001

[a] Data represent the mean ± standard deviation for triplicate determinations.

produce marginal micronutrient deficiency by dilution of the diet. The U.S. Food and Drug Administration (1982) and others (Borzelleca, 1992) have recommended that dietary exposure be limited to 5% of the diet. SALATRIM was fed as high as 10% of the diet in this study to ensure that maximal doses were evaluated. As a precaution, all diets in this study (control basal diet, 3%, 6%, and 10% SALATRIM diets, and 10% corn oil diet) were supplemented with 2% corn oil to prevent possible essential fatty acid deficiency produced by dietary dilution resulting from the large quantities of SALATRIM added to the basal diet.

Recently, it has been reported that caprenin, a reduced-calorie triacylglycerol, produced increases in serum alanine aminotransferase (ALT) in male and female rats in a 28-day continuous dosing study (Webb et al., 1991). No effects on ALT have been noted in rat 90-day subchronic studies with five different SALATRIM fats (Hayes et al., 1994b–d). To determine if SALATRIM fats could produce transient changes in serum transaminases that would have gone undetected in the 90-day studies, ALT, aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT) were determined in a short-term rat study at 12 and 4 days before feeding SALATRIM 23SO lot A026 and at days 3, 6, 9, 13, and 17 after initiation of feeding (Hayes et al., 1994c). This study indicated that these three enzymes were not altered in rats by feeding SALATRIM. Finley et al. (1994b) have reported that high doses (45 and 60 g/day) of SALATRIM in humans caused slight increases in AST and ALT but still within normal ranges. A longer term study in which SALATRIM fats were fed at 60 g/day for 28 days indicated that the increases return to normal and are not of clinical significance (Finley et al., 1994c). To investigate any potential changes in these transaminases in this 28-day pig study, they were determined at 2 weeks and 3 days before dosing and at days 3, 7, 14, 21, and 29 after dosing.

MATERIALS AND METHODS

The in-life and necropsy phases of this 28-day repeated dosing study with Hanford minipigs were conducted at Hazleton Wisconsin, Inc. (HWI), Madison, WI, from November 18, 1992, through December 18, 1992. Histopathologic examination of the tissues from the minipigs was conducted at Experimental Pathology Laboratories, Inc., Research Triangle Park, NC.

Materials. SALATRIM 23SO lot A026 (also known as TAG A9300 lot A026) was supplied by Nabisco Foods Group (NFG), East Hanover, NJ. Data from gas chromatographic total fatty acid analyses of this SALATRIM fat conducted at EPL Bio-Analytical Services, Inc., Decatur, IL, are presented in Table 1. As expected, the predominant short- and long-chain fatty acids are acetic and stearic, respectively. A titratable acid value of 0.44 ± 0.01 wt % for this SALATRIM fat was determined by NFG using the American Oil Chemists' Society Official Method Ca 5a-40 (AOCS, 1990a). This indicates that the free fatty acid concentration in the acylglycerol mixture was low. The peroxide content of the fat was 0.95 ± 0.29 mequiv of peroxide/kg as determined by AOCS Official Method Cd 8-53 (AOCS, 1990b). Commercially available Mazola corn oil was used as the reference fat.

Dosing and Diets. Pigs were fed either SALATRIM 23SO lot A026 at 3%, 6%, or 10% (w/w) of the diet or corn oil at 10% (w/w) of the diet for 28 days. A control group was fed the basal diet alone. The 10% corn oil-fed group served as a reference for the high fat content of SALATRIM diets. All diets (including the diet used for the untreated control group) were supplemented with 2% (w/w) corn oil. This supplementation was believed necessary to avoid possible induction of essential fatty acid deficiency caused by dietary dilution with the test fat. SALATRIM and corn oil were mixed with corn oil-supplemented ground Lab Mini-Pig Chow Breeder Meal 5082 (Purina Mills, 1987). Each pig was given 500 g of the appropriate diet twice each day. Test diets were prepared biweekly and stored frozen (−20 ± 10 °C) until removed from the freezer and dispensed into food containers. After being removed from the freezer, diets were maintained at room temperature for 1–6 days (average 3.3 days) before being fed to the pigs. Drinking water was provided *ad libitum* during all phases of the study.

To confirm the presence of test and reference fats, diets were assayed for contents of corn oil and SALATRIM 23SO lot A026. Before initiation of treatment, homogeneity of the diet preparations was evaluated by gravimetric determination for the 3% and 10% SALATRIM diets and the 10% corn oil diet. Stability of the SALATRIM in the diet was evaluated using supercritical fluid chromatography for diets prepared for the 3% and 10% treatment groups. Four sets of samples were collected. One set was analyzed on the day of mixing. Three sets were stored in a freezer set to maintain a temperature of −20 ± 10 °C. Two of the stored sets were analyzed after 2 or 5 weeks of frozen storage. The third stored set was maintained frozen for 10 days followed by room temperature storage for 11 days and then analyzed. The results of these analyses indicated that the frozen diets were stable for up to 5 weeks but also demonstrated a lack of stability during 11 days of room temperature storage. Therefore, subsequent stability analyses were conducted using samples of the 3% and 10% diets prepared for the homogeneity assessment conducted prior to study initiation. These samples had been stored frozen (−20 ± 10 °C) for 63 days. They were removed from the freezer and analyzed by supercritical fluid chromatography on that day and then again after 2, 4, 7, 9, or 11 days of room temperature storage. In addition, analyses were conducted on the day of preparation for the 3%, 6%, and 10% SALATRIM diets (chromatographic analysis) and the 10% corn oil diet (gravimetric analysis) fed to the pigs.

Animals. Hanford minipigs (MINIPIG-HA) were from Charles River Laboratories, Inc. (Pittsfield, NH). The pigs were acclimated at HWI for 15 days prior to initiation of the study. Pigs were 3.5–7 months old and weighed 17.2–30.4 kg at initiation of treatment. Animal husbandry complied with the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (Consortium for Developing, 1988). Pigs were identified by ear tags and housed singly in stainless steel cages in an animal room set to maintain 18.5–22.2 °C and 50% ± 20% relative humidity, with a 12-h light/dark cycle.

Pigs were assigned to treatment groups using a blocked random assignment. For each sex, the body weights of 20 pigs were listed in ascending order. These weights were divided into four blocks. The first block contained the lowest five weights. The second block contained the next five lowest weights, etc., until each of the four blocks was filled. Within each block, one weight was randomly assigned to each of the five treatment groups in the study, yielding five groups of four pigs each.

Experimental Design. The five groups of four pigs per sex were as follows: (1) untreated controls that received basal diet only; (2) treated pigs fed SALATRIM 23SO lot A026 at either 3%, (3) 6%, or (4) 10% (w/w) of the diet; and (5) pigs fed corn oil at 10% (w/w) of the diet. As indicated previously, the basal diet and each of the SALATRIM and corn oil diets were supplemented with an additional 2% (w/w) corn oil.

Antemortem Data Collection. Pigs were observed twice daily for mortality and moribundity and once daily for signs of

Table 2. Clinical Pathology

serum chemistry	hematology
glucose	red blood cell count
urea nitrogen	hemoglobin
creatinine	hematocrit
total protein	mean corpuscular volume
albumin	mean corpuscular hemoglobin
globulin	mean corpuscular hemoglobin concentration
albumin/globulin ratio	platelet count
total bilirubin	prothrombin time
cholesterol	white blood cell count
high-density lipoprotein cholesterol	differential blood cell count
low-density lipoprotein cholesterol	blood cell morphology
aspartate aminotransferase	reticulocyte count smear[a]
alanine aminotransferase	
alkaline phosphatase	
γ-glutamyltransferase	
calcium	
inorganic phosphorus	
sodium	
potassium	
chloride	
triglycerides	

[a] Made but not examined.

Table 3. Tissues Collected at Necropsy

tissue	tissue
adrenals[a]	mammary gland (females)[a]
aorta[a]	muscle (thigh)
bone with bone marrow (left sixth rib)[a,b]	ovaries[a]
brain[a]	pancreas[a]
cecum[a]	parathyroid
colon[a]	pituitary[a]
duodenum[a]	prostate[a]
epididymides[a]	rectum[a]
esophagus	salivary gland (submandibular)
eyes	sciatic nerve
femur and bone marrow	spinal cord (cervical, thoracic, lumbar)
gall bladder[a]	spleen[a]
heart[a]	stomach[a]
ileum[a] (with Peyer's patches)	testes[a]
jejunum[a]	thymus
kidneys[a]	thyroid[a]
lesions[a]	trachea
liver[a]	urinary bladder
lungs	uterus[a]
lymph nodes (mesenteric[a] and mandibular)	

[a] Denotes tissue examined histopathologically. [b] In addition to the histopathologic examination, bone marrow smears were prepared for possible examination if deemed necessary.

poor health or abnormal behavior. Body weight was recorded before initiation of treatment, on the first day of treatment (day 1), weekly thereafter, and at necropsy. Individual feed consumption was measured daily during treatment.

Blood was collected from the vena cava of each pig at 2 weeks and at 3 days before initiation of SALATRIM feeding and at days 3, 7, 14, 21, and 29 after initiation of feeding. The pigs were fasted overnight before blood collection. Hematology and clinical chemistry variables were determined on these samples as indicated in Table 2. Serum levels of *trans*-retinol (vitamin A) and α-tocopherol (vitamin E) also were evaluated for the samples collected 3 days prior to treatment and at day 29.

Blood samples for hematology were collected with 7.5% EDTA anticoagulant, plasma for the prothrombin assay was prepared from blood collected with 3.8% sodium citrate anticoagulant, and serum for the clinical chemistry and vitamin determinations was prepared from blood collected without anticoagulant. Hematology variables were determined using a Coulter Counter S-Plus IV whole blood automated analyzer. Prothrombin time was measured using a Coag-A-Mate X2 coagulation analyzer. Differential leukocyte count and blood cell morphology slides were prepared using a Geometric Data Hemastainer and read manually. Serum chemistry variables were determined using a Hitachi 704 random access chemistry analyzer except that low-density lipoprotein cholesterol (Friedewald et al., 1972) and globulin were calculated. Globulin was calculated by subtracting serum albumin from serum total protein. Serum *trans*-retinol (vitamin A) and α-tocopherol (vitamin E) concentrations were determined by high-performance liquid chromatographic methods (Driskell et al., 1982).

Postmortem Data Collection. After 28 days of treatment, all pigs were fasted overnight, anesthetized with ketamine and sodium pentobarbital, exsanguinated, and subjected to gross necropsy. Adrenals, brain, kidneys, liver, ovaries, spleen, testes, thymus, and thyroid were weighed. After the liver of each pig was weighed, the left lateral lobe was isolated and perfused with saline. After a section was collected for histopathology, the remainder of the lobe was sliced into sections thin enough to freeze easily, blotted dry with paper towels, wrapped in aluminum foil, and frozen in liquid nitrogen. Samples were stored in a freezer set at −70 ± 10 °C until analyzed by high-performance liquid chromatography for vitamins A and E (Kayden et al., 1983). The entire femur not used for histopathology was removed and stored frozen at −20 ± 10 °C. Dry weight and percent ash of femurs were determined. Each femur was assayed for calcium, phosphorus, strontium, and zinc concentrations by inductively coupled plasma spectrometry.

At necropsy, tissues listed in Table 3 were collected from all pigs and fixed in 10% phosphate-buffered formalin. Selected tissues from all pigs, as indicated in Table 3, were embedded, sectioned, stained with hematoxylin and eosin, and examined by light microscopy.

Statistical Analyses. Statistical analyses were conducted for the following: body weights, cumulative body weight gains; feed consumption; serum chemistry; hematology (except red blood cell morphology); serum and liver vitamin A and E concentrations; organ weights; organ-to-body weight percentages; organ-to-brain weight ratios and bone mineral analyses. Levene's test (Levene, 1960) was used to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations were used to stabilize the variance (Draper and Hunter, 1969). If necessary, the following transformations were conducted in sequence until homogeneity of variance was achieved: $\log_{10}$, square, square root, reciprocal, angular, and rank. Analysis of variance (ANOVA) (Winer, 1971) was performed on the homogeneous or transformed data. If ANOVA was significant, Dunnett's *t*-test (Dunnett, 1964) was used for pairwise comparisons between groups. Group comparisons were evaluated at the 5% two-tailed probability level. All statistically significant differences cited are based on comparisons with the untreated control group (group 1).

RESULTS

Diet Analysis. Pretest gravimetric analysis of diets for homogeneous dispersion of the fats demonstrated the diet mixing procedure yielded SALATRIM and corn oil diets with acceptable homogeneity.

Data from the chromatographic analyses of SALATRIM 23SO lot A026 in 3% and 10% SALATRIM diets stored frozen for 2 or 5 weeks showed acceptable stability (100% and 95.8% of theoretical, respectively, for the diets stored frozen for 5 weeks). Analyses of diets stored at room temperature showed a decrease in SALATRIM concentration over time as described below.

Analysis of diet samples stored frozen for 10 days and held at room temperature for 11 days showed a decline in SALATRIM to 73.7% and 78.5% of the theoretical concentration for the 3% and 10% diets, respectively. A time course study was done to determine the stability curve. In this study, 3% and 10% diets originally prepared for evaluation of diet homogeneity prior to initiation of the study and then stored in the freezer were used. Analysis of the diets after 63 days of frozen storage showed that the concentrations were 79.7% and 94.4% of theoretical for the 3% and 10% diets, respectively. Analysis of these diets at 2, 4, 7, 9, and 11 days after removal from frozen storage showed a linear decline in SALATRIM

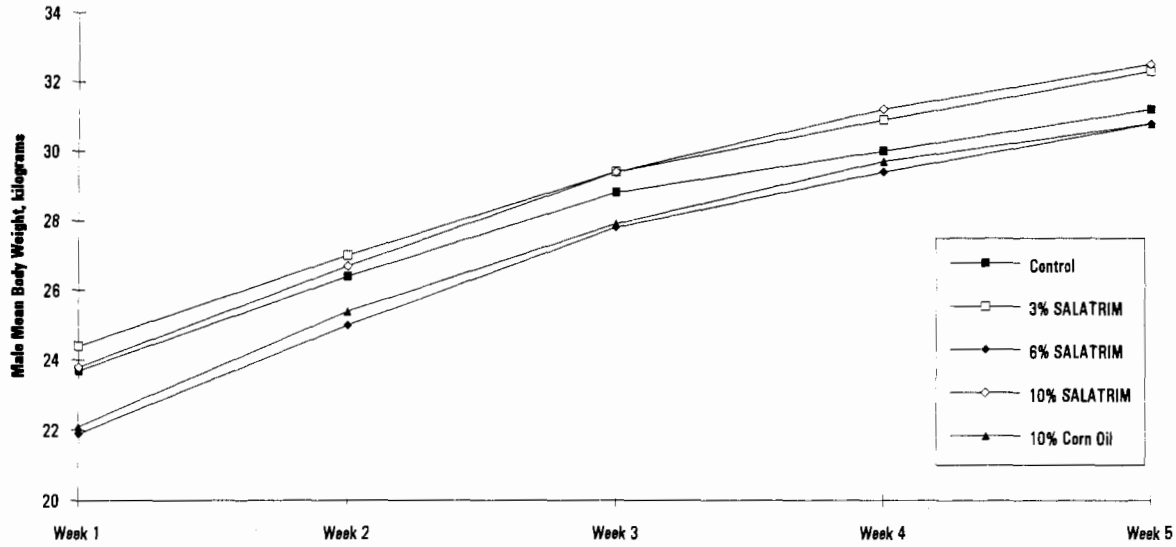

Figure 1. Male mean body weight (kg) vs study week. Data points represent the means of four pigs. Standard deviations (SD) are not shown for the sake of clarity of the figure. For each week, the SD was less than 18% of the mean. No statistically significant differences ($p \leq 0.05$) were noted when mean weights of pigs treated with SALATRIM and corn oil were compared with controls.

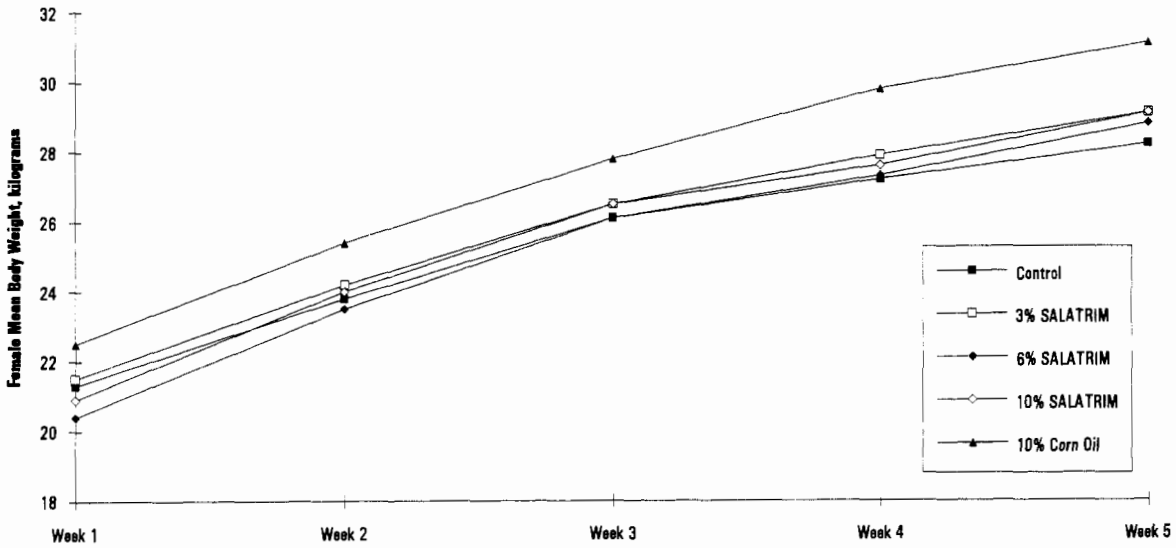

Figure 2. Female mean body weight (kg) vs study week. Data points represent the means of four pigs. Standard deviations (SD) are not shown for the sake of clarity of the figure. For each week, the SD was less than 18% of the mean. No statistically significant differences ($p \leq 0.05$) were noted when mean weights of pigs treated with SALATRIM and corn oil were compared with controls.

concentration (R^2 = 0.976 and 0.958 for 3% and 10% diets, respectively).

Analysis of the diets fed during the study was conducted to confirm proper diet preparation. These data indicated the diets contained the proper amounts of fats at the time of preparation.

Compound Consumption. The long period of frozen storage of the diets used for the time course stability study caused a decrease in diet concentrations of SALATRIM 23SO lot A026, especially in the 3% diet. Because diets with low concentrations of this SALATRIM fat were shown to be less stable than those with higher concentrations, the data from the time course study were not used to calculate compound consumption. The diets analyzed during the initial stability evaluation were those actually fed to the pigs. These diets were not stored frozen for an extended period of time. Therefore, it was considered appropriate to use the results from the initial stability evaluation to calculate compound consumption by assuming a linear decrease in concentration over time.

The average length of time that SALATRIM-containing diets were at room temperature prior to being consumed by the pigs was 3.3 days. Therefore, daily SALATRIM 23SO lot A026 consumption (corrected for the duration of room temperature storage) for the 3%, 6%, and 10% groups was calculated to be 1.0, 2.1, and 3.3 g/kg in males and 1.1, 2.3, and 3.7 g/kg in females during the study. Daily consumption of 10% corn oil averaged 3.7 g/kg in both sexes. These values for compound consumption were calculated using the diet stability data discussed above and are 92%, 93%, and 93% of the dose that would have been consumed by the pigs in the 3%, 6%, and 10% SALATRIM diets, respectively, if no decrease in diet concentration had occurred.

Antemortem Observations. No treatment-related effects were noted during daily physical examinations. All pigs survived to the scheduled terminal sacrifice.

In both sexes, mean body weights, body weight gains, and feed consumption for pigs in the groups receiving SALATRIM 23SO lot A026 and corn oil were comparable to untreated control pigs. Weekly body weight data are presented in Figures 1 and 2.

Clinical Pathology. Hematological and clinical chemistry evaluations revealed no treatment-related effects when pigs receiving SALATRIM 23SO lot A026 and corn oil were compared with controls. Occasional statistically

Table 4. Selected Serum Enzyme Levels in Pigs[a]

	week −2	week −1	day 3	day 7	day 14	day 21	day 29
Males							
aspartate aminotransferase (AST), IU/L							
control	26 ± 5.1	57 ± 37.8	40 ± 10.8	36 ± 9.7	47 ± 44.6	81 ± 58.3[d]	28 ± 12.4
3% SALATRIM	28 ± 8.7	66 ± 55.1	32 ± 17.3	27 ± 10.4	45 ± 34.4	28 ± 4.9	21 ± 3.9
6% SALATRIM	32 ± 8.2	212 ± 336.2[c]	30 ± 13.2	30 ± 13.0	49 ± 13.1	28 ± 3.0	20 ± 9.0
10% SALATRIM	58 ± 24.7[b]	45 ± 21.6	26 ± 7.1	48 ± 43.9	26 ± 1.0	44 ± 30.2	21 ± 1.8
10% corn oil	33 ± 8.3	32 ± 8.5	32 ± 10.7	59 ± 53.7	44 ± 21.1	37 ± 16.4	45 ± 33.2
alanine aminotransferase (ALT), IU/L							
control	49 ± 6.1	49 ± 10.4	49 ± 8.7	50 ± 10.0	50 ± 12.0	54 ± 11.9	42 ± 8.3
3% SALATRIM	42 ± 5.5	38 ± 9.2	36 ± 7.0	36 ± 6.6	38 ± 8.6	36 ± 5.6	35 ± 6.9
6% SALATRIM	37 ± 4.7[b]	40 ± 13.9	35 ± 8.1	34 ± 10.3[b]	42 ± 9.1	37 ± 9.1	28 ± 19.5
10% SALATRIM	43 ± 4.2	36 ± 4.7	37 ± 1.3	38 ± 5.3	39 ± 4.7	37 ± 5.9	34 ± 3.4
10% corn oil	51 ± 2.6	41 ± 3.6	45 ± 7.7	50 ± 6.6	45 ± 7.9	48 ± 17.2	41 ± 5.9
γ-glutamyltransferase (GGT), IU/L							
control	28 ± 7.0	32 ± 10.4	30 ± 7.3	31 ± 9.3	30 ± 6.7	30 ± 7.9	28 ± 9.5
3% SALATRIM	31 ± 15.5	35 ± 12.1	30 ± 11.4	30 ± 10.4	34 ± 18.3	30 ± 8.2	29 ± 10.5
6% SALATRIM	26 ± 9.2	31 ± 12.8	28 ± 8.5	27 ± 7.2	26 ± 11.6	28 ± 7.2	25 ± 5.7
10% SALATRIM	26 ± 12.0	29 ± 14.7	25 ± 12.0	32 ± 22.9	29 ± 12.0	28 ± 11.0	24 ± 12.8
10% corn oil	34 ± 10.5	40 ± 8.7	38 ± 14.8	38 ± 19.8	39 ± 14.2	36 ± 12.9	34 ± 14.6
Females							
aspartate aminotransferase (AST), IU/L							
control	26 ± 3.0	64 ± 67.1	41 ± 26.2	27 ± 1.5	38 ± 11.0	129 ± 198.9[e]	22 ± 5.1
3% SALATRIM	24 ± 0.5	44 ± 25.4	32 ± 18.9	28 ± 4.9	44 ± 41.8	26 ± 6.8	62 ± 72.6
6% SALATRIM	27 ± 12.0	45 ± 29.0	42 ± 16.3	22 ± 2.8	31 ± 11.5	44 ± 13.7	30 ± 17.5
10% SALATRIM	26 ± 3.8	36 ± 13.3	41 ± 21.9	28 ± 15.7	23 ± 3.6	39 ± 21.7	22 ± 7.2
10% corn oil	26 ± 2.9	38 ± 16.1	46 ± 15.8	33 ± 9.5	41 ± 15.4	28 ± 8.4	34 ± 27.0
alanine aminotransferase (ALT), IU/L							
control	47 ± 13.0	40 ± 6.2	42 ± 3.4	40 ± 4.7	48 ± 4.2	50 ± 11.4	42 ± 4.1
3% SALATRIM	44 ± 1.7	41 ± 2.1	41 ± 4.9	44 ± 3.9	42 ± 3.0	42 ± 2.9	46 ± 9.7
6% SALATRIM	41 ± 6.2	38 ± 7.4	38 ± 7.0	35 ± 3.7	42 ± 3.9	40 ± 3.5	39 ± 5.1
10% SALATRIM	46 ± 7.4	43 ± 8.5	46 ± 5.8	41 ± 9.9	41 ± 6.6	38 ± 9.1	37 ± 7.3
10% corn oil	40 ± 8.3	40 ± 6.4	46 ± 6.5	42 ± 8.8	48 ± 16.1	39 ± 8.8	41 ± 10.2
γ-glutamyltransferase (GGT), IU/L							
control	35 ± 6.6	40 ± 6.2	38 ± 9.7	43 ± 16.9	44 ± 10.7	40 ± 11.1	36 ± 8.7
3% SALATRIM	32 ± 9.8	36 ± 9.3	36 ± 12.1	40 ± 17.6	37 ± 14.2	36 ± 14.4	34 ± 13.5
6% SALATRIM	28 ± 13.7	34 ± 14.3	29 ± 11.8	30 ± 12.5	36 ± 12.1	31 ± 12.5	30 ± 13.2
10% SALATRIM	28 ± 7.3	32 ± 9.5	29 ± 6.7	22 ± 8.2	31 ± 5.9	28 ± 5.4	26 ± 4.0
10% corn oil	28 ± 7.8	31 ± 10.5	28 ± 10.3	30 ± 10.7	24 ± 18.1	29 ± 8.1	27 ± 11.1

[a] Data represent the mean ± standard deviation of values for four pigs per sex per group. [b] Statistically significant difference compared to control; $p \leq 0.05$. [c] Individual values were 43, 716, 52 and 36. [d] Individual values were 154, 46, 101, and 24. [e] Individual values were 427, 35, 17, and 37.

significant differences between the treated and control groups were considered to be unrelated to treatment because they occurred during the pretest evaluations, did not occur consistently during the several evaluation intervals of the study, or did not occur in a dose-related fashion.

As indicated earlier, serum levels of alanine aminotransferase (ALT), aspartate aminotransferase, (AST), and γ-glutamyltransferase (GGT) were of special interest in this study. The mean serum levels of these enzymes in male and female pigs are presented in Table 4. The variability of AST levels in individual animals was large during the study. The reason for this variability is unknown. At 2 weeks prior to initiation of treatment, serum levels of AST were significantly higher for males in the group destined to be fed 10% SALATRIM diet and serum levels of ALT were lower for males destined to be fed 6% SALATRIM diet. Serum ALT was also significantly lower for males fed the 6% SALATRIM diet at the day 7 interval. No other statistically significant differences between the control group and the SALATRIM and corn oil groups were noted for any of the three enzymes at any interval during the study.

Because the concentration of fat in the SALATRIM and corn oil diets was higher than that of the untreated control diet, serum lipid values were also of particular interest in this study. Mean serum lipid data for males and females are shown in Table 5. The cholesterol level of the group of females given 6% SALATRIM at day 3 was lower than that of controls. Also at day 3, the low-density lipoprotein levels of females given 6% or 10% SALATRIM or 10% corn oil were lower than those of controls. At day 29, cholesterol and high-density lipoprotein cholesterol were higher in female pigs fed 10% corn oil diets when compared with controls. These differences were not observed at other intervals during the study.

Fat-Soluble Vitamins and Bone Mineral Determinations. Serum and liver vitamin A and E data are presented in Figures 3 and 4. Mean serum vitamin A was significantly higher than control in males fed 6% SALATRIM 23SO lot A026. Mean liver vitamin E was significantly lower than controls in females fed 6% SALATRIM. These differences were not considered to be biologically significant or related to treatment because of the lack of dose response and the lack of effect in both sexes.

Prothrombin time, an indicator of vitamin K status, was unaffected by exposure to either SALATRIM 23SO lot A026 or corn oil.

No differences in percent ash or bone concentrations of calcium, phosphorus, or strontium were noted between treated and control pigs of either sex in this study. These data are presented in Table 6.

Organ Weights and Macroscopic Pathology. There were no differences between the organ weights of pigs fed SALATRIM 23SO lot A026 and untreated control pigs. Macroscopically, no treatment-related effects were observed in any of the pigs treated with either SALATRIM or corn oil. Microscopically, a slight increase in severity of focal vacuolation in hepatocytes was noted for one male fed 10% corn oil and one male fed 10% SALATRIM (see

Table 5. Serum Lipid Data in Pigs[a]

	week −2	week −1	day 3	day 7	day 14	day 21	day 29
			Males				
triglycerides, mg/dL							
control	45 ± 21.7	40 ± 15.8	51 ± 11.5	41 ± 11.9	36 ± 10.8	26 ± 7.9	22 ± 11.7
3% SALATRIM	49 ± 13.1	52 ± 9.7	47 ± 8.6	48 ± 18.5	52 ± 10.8	32 ± 11.6	34 ± 11.6
6% SALATRIM	53 ± 22.5	28 ± 3.7	70 ± 39.1	62 ± 28.7	57 ± 8.0	57 ± 24.4	44 ± 19.7
10% SALATRIM	64 ± 28.1	45 ± 21.0	68 ± 45.7	61 ± 21.4	61 ± 15.0	40 ± 19.3	41 ± 23.0
10% corn oil	29 ± 11.8	32 ± 19.1	65 ± 35.8	59 ± 26.2	54 ± 25.4	24 ± 14.6	26 ± 11.5
cholesterol, mg/dL							
control	98 ± 8.8	99 ± 12.5	92 ± 8.1	95 ± 6.2	96 ± 10.1	99 ± 5.7	90 ± 14.1
3% SALATRIM	102 ± 14.0	104 ± 21.3	104 ± 9.6	107 ± 11.2	106 ± 13.7	111 ± 11.8	106 ± 11.4
6% SALATRIM	109 ± 16.5	94 ± 18.0	94 ± 4.7	110 ± 9.3	109 ± 5.2	108 ± 11.4	112 ± 8.2
10% SALATRIM	112 ± 15.6	110 ± 22.7	103 ± 4.0	114 ± 9.2	109 ± 10.6	117 ± 12.1	116 ± 11.0
10% corn oil	96 ± 14.5	98 ± 21.9	94 ± 9.0	110 ± 16.6	107 ± 14.7	111 ± 9.9	83 ± 21.8
high-density lipoprotein cholesterol, mg/dL							
control	46 ± 2.1	48 ± 5.4	47 ± 4.5	55 ± 5.7	44 ± 5.9	50 ± 3.1	42 ± 6.6
3% SALATRIM	43 ± 5.9	48 ± 8.1	50 ± 4.3	57 ± 7.9	48 ± 8.1	52 ± 7.6	49 ± 4.3
6% SALATRIM	43 ± 9.2	45 ± 12.6	47 ± 2.5	59 ± 8.9	52 ± 4.5	56 ± 8.0	54 ± 6.4
10% SALATRIM	48 ± 4.4	48 ± 4.8	52 ± 2.2	66 ± 1.4	54 ± 2.2	60 ± 2.2	58 ± 4.7
10% corn oil	41 ± 3.8	43 ± 5.9	50 ± 5.9	66 ± 10.8	55 ± 8.9	60 ± 12.2	38 ± 18.8
low-density lipoprotein cholesterol, mg/dL							
control	44 ± 10.8	42 ± 5.2	35 ± 6.3	32 ± 3.5	44 ± 6.2	44 ± 3.9	44 ± 6.4
3% SALATRIM	49 ± 7.1	46 ± 13.7	44 ± 5.5	40 ± 5.7	48 ± 5.7	52 ± 6.2	50 ± 7.5
6% SALATRIM	56 ± 15.7	44 ± 10.8	34 ± 9.9	38 ± 8.4	46 ± 5.4	40 ± 13.4	48 ± 8.4
10% SALATRIM	51 ± 12.4	53 ± 18.3	37 ± 6.5	36 ± 7.1	42 ± 9.7	49 ± 6.9	50 ± 4.2
10% corn oil	50 ± 15.8	48 ± 21.5	31 ± 5.2	33 ± 7.8	41 ± 5.0	47 ± 9.0	40 ± 7.6
			Females				
triglycerides, mg/dL							
control	36 ± 11.2	33 ± 13.1	42 ± 10.0	58 ± 10.4	58 ± 11.1	45 ± 11.7	31 ± 3.6
3% SALATRIM	46 ± 3.4	28 ± 7.8	63 ± 30.8	65 ± 14.4	38 ± 8.0	38 ± 7.3	50 ± 7.7
6% SALATRIM	44 ± 14.7	47 ± 17.3	68 ± 30.3	63 ± 6.9	57 ± 7.2	50 ± 7.1	48 ± 12.6
10% SALATRIM	38 ± 10.4	42 ± 11.1	67 ± 17.7	49 ± 8.7	63 ± 19.1	106 ± 116.4[c]	47 ± 9.9
10% corn oil	44 ± 9.4	38 ± 9.5	79 ± 6.7	71 ± 24.4	71 ± 19.2	36 ± 9.7	41 ± 16.8
cholesterol, mg/dL							
control	112 ± 11.5	113 ± 9.0	116 ± 7.3	115 ± 5.7	113 ± 6.3	115 ± 4.0	106 ± 5.9
3% SALATRIM	127 ± 4.8	122 ± 14.9	114 ± 10.7	120 ± 7.7	118 ± 8.3	119 ± 7.9	120 ± 10.1
6% SALATRIM	100 ± 17.2	107 ± 9.8	98 ± 3.8[b]	108 ± 10.3	106 ± 10.3	109 ± 12.4	107 ± 10.5
10% SALATRIM	104 ± 15.5	114 ± 15.5	105 ± 7.2	106 ± 13.0	111 ± 8.3	111 ± 10.3	113 ± 12.7
10% corn oil	124 ± 10.2	110 ± 10.7	107 ± 8.9	117 ± 2.6	122 ± 9.7	125 ± 13.3	130 ± 6.7[b]
high-density lipoprotein cholesterol, mg/dL							
control	50 ± 6.1	43 ± 4.2	52 ± 5.9	59 ± 5.9	50 ± 5.8	52 ± 5.4	46 ± 6.5
3% SALATRIM	45 ± 8.0	46 ± 5.8	52 ± 4.0	61 ± 6.9	47 ± 7.6	52 ± 8.8	53 ± 2.9
6% SALATRIM	45 ± 7.8	45 ± 11.1	53 ± 14.2	61 ± 8.9	50 ± 8.3	54 ± 8.5	52 ± 7.9
10% SALATRIM	43 ± 2.2	46 ± 2.6	52 ± 3.5	58 ± 3.1	51 ± 1.5	54 ± 9.6	54 ± 5.7
10% corn oil	48 ± 4.5	48 ± 6.6	56 ± 6.4	68 ± 4.3	58 ± 10.5	64 ± 9.8	64 ± 7.1[b]
low-density lipoprotein cholesterol, mg/dL							
control	64 ± 9.2	63 ± 4.1	56 ± 3.3	45 ± 6.9	51 ± 3.2	54 ± 2.9	54 ± 8.3
3% SALATRIM	73 ± 13.4	70 ± 17.4	50 ± 10.9	47 ± 15.0	64 ± 15.0	60 ± 13.5	56 ± 11.4
6% SALATRIM	46 ± 6.8	53 ± 10.2	32 ± 9.3[b]	34 ± 1.7	45 ± 5.0	44 ± 4.4	45 ± 3.3
10% SALATRIM	54 ± 13.5	60 ± 14.5	40 ± 5.0[b]	38 ± 10.7	48 ± 6.6	36 ± 23.0	50 ± 9.6
10% corn oil	67 ± 9.6	56 ± 7.9	36 ± 4.7[b]	35 ± 2.9	50 ± 5.6	54 ± 4.9	57 ± 8.8

[a] Data represent the mean ± standard deviation of values for four pigs per sex per group. [b] Statistically significant difference compared to control; $p \leq 0.05$. [c] Individual values were 30, 41, 278, and 73.

Table 7). Focal vacuolation in the hepatocytes was noted in all groups of pigs in this study, but these were the only two pigs with a severity grade of mild (all others were minimal). Since the slight increase in severity of focal hepatocellular vacuolation was only observed in two male pigs and was not observed in any females, it appears to be a spurious finding or perhaps a nonspecific fat effect because it occurred only in pigs given 10% fat diets (corn oil or SALATRIM). Histopathologic findings in all other organs were similar for SALATRIM, corn oil, and control pigs.

DISCUSSION

On the basis of its similarity to fats normally found in the food supply, its predictable metabolism (Hayes et al., 1994a), and the results of toxicity testing in rats with five other SALATRIM fats (Hayes et al., 1994b–d), SALATRIM 23SO lot A026 was not expected to cause toxicological effects. This 28-day continuous dosing study using Hanford minipigs as the animal model was conducted to test this hypothesis in a second animal species.

There were no significant differences between the body weights of control pigs and pigs treated with SALATRIM 23SO lot A026 or corn oil. Feed consumption values for pigs fed SALATRIM or corn oil diets were comparable to those of controls throughout the study.

Hematology and clinical chemistry data revealed no treatment-related effects for pigs fed SALATRIM 23SO lot A026 or corn oil compared to controls. During this study, the high dietary concentrations of SALATRIM and corn oil fed did not alter serum triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), or total cholesterol. This study is consistent with the previous SALATRIM rat studies that indicate neither short-term nor long-term changes in serum transaminases (Hayes et al., 1994b–d). This is in contrast to the results of a short-term rat study with caprenin, a low-calorie fat, in which elevated serum alanine aminotransferase (ALT) was noted (Webb et al., 1991). It is also in contrast to the very slight, but reversible, increases in serum ALT and aspartate aminotransferase (AST) seen when humans consumed SALATRIM at levels of 60 and 45 g/day for up to 28 days (Finley et al., 1994b,c).

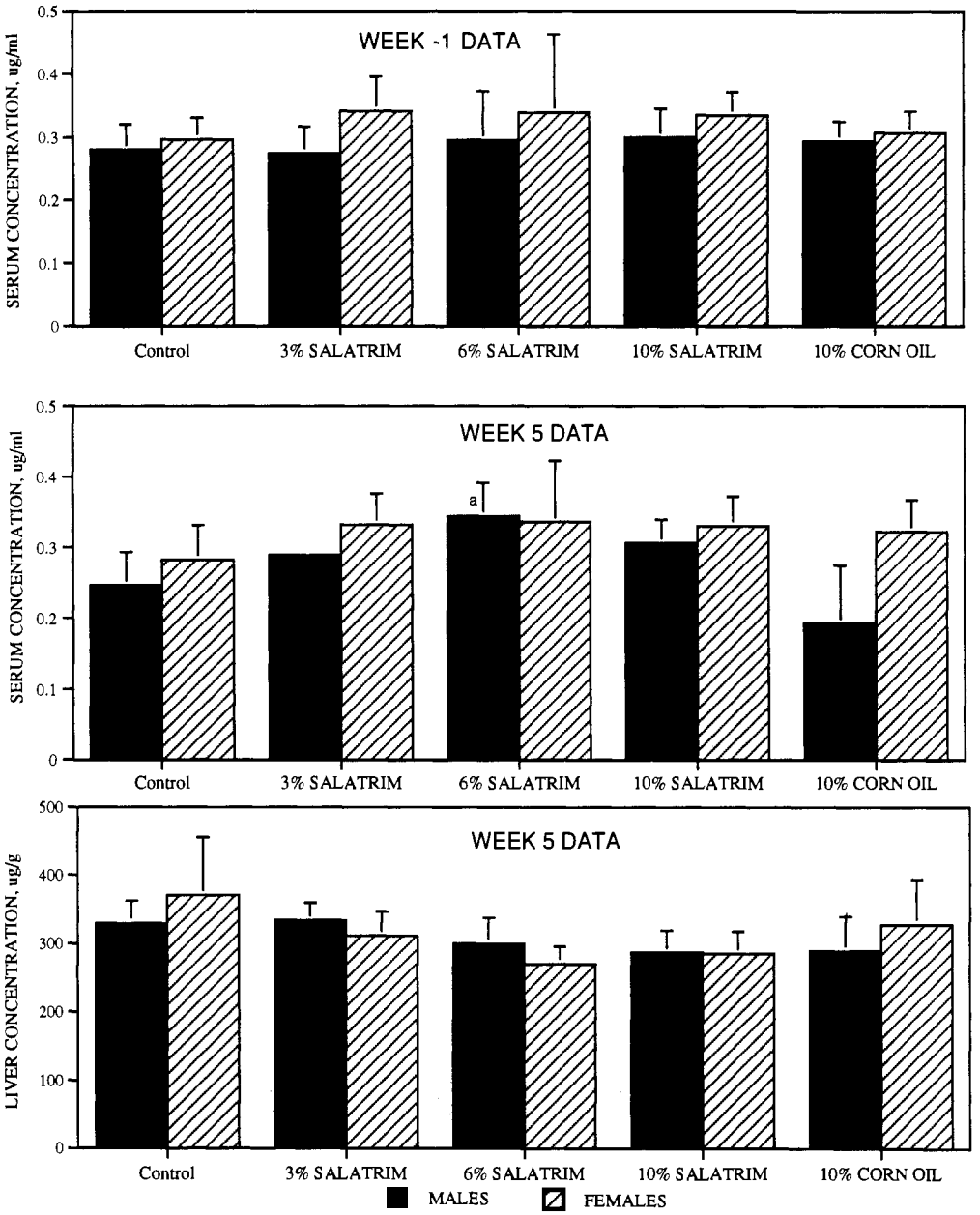

Figure 3. Serum (weeks −1 and 5) and liver (week 5) concentrations of vitamin A (*trans*-retinol) vs treatment. Data points represent mean ± SD of the mean for four pigs. Data significantly different from control group ($p \leq 0.05$) are noted with an "a".

Transient increases in ALT and AST have been reported in rats fed diets containing 30% or 36.5% total fat for 24 days (Krajcovicova-Kudlackova and Dibak, 1985). The reversible increases in ALT and AST noted in the SALATRIM clinical studies were possibly also related to high fat consumption. The absence of long- or short-term increases in serum transaminases in this 28-day minipig study with SALATRIM 23SO lot A026 and in the 90-day subchronic rat studies with five other SALATRIM fats (Hayes et al., 1994b–d) is probably related to the lower total dietary fat content (approximately 15%) compared with the studies of Krajcovicova-Kudlackova and Dibak (1985). In the studies conducted by those researchers, the diets fed to control rats for comparison to the high-fat-treated rats contained 10% or 15% fat.

Serum and liver vitamins A and E were not significantly altered in pigs treated with SALATRIM and corn oil compared to controls. Evaluation of prothrombin time as an indicator of vitamin K status indicated no effect on vitamin K status in pigs. No significant effects on serum or liver levels of fat-soluble vitamins have been noted in previous studies in rats with five other SALATRIM fats (Hayes et al., 1994b–d). Overall, it appears that SALATRIM fats do not substantially alter fat-soluble vitamin absorption.

There were no apparent effects on the concentrations of ash, calcium, phosphorous, strontium, or zinc in femurs of pigs treated with SALATRIM 23SO lot A026. In previous studies with SALATRIM and corn oil in rats, slight changes were observed in the bone levels of zinc and strontium (Hayes et al., 1994b–d). The changes in rats fed diets containing 10% SALATRIM and 10% corn oil are not considered toxicological effects because they appear to be directly related to the high level of unsaturated fatty acids in the corn oil diets and the lower level of unsaturated fatty acids in the SALATRIM diets. Lukaski and Johnson (1992) concluded that diets containing high levels of polyunsaturated fatty acids depress zinc status, as well as other minerals, in the rat. The authors indicated that high-linoleate diets have been reported to significantly depress zinc concentrations in the tibia. The absence of bone mineral changes in pigs fed 10% SALATRIM 23SO lot A026 may be related to dose level. In the rat studies, the 10% SALATRIM and corn oil diets resulted in daily

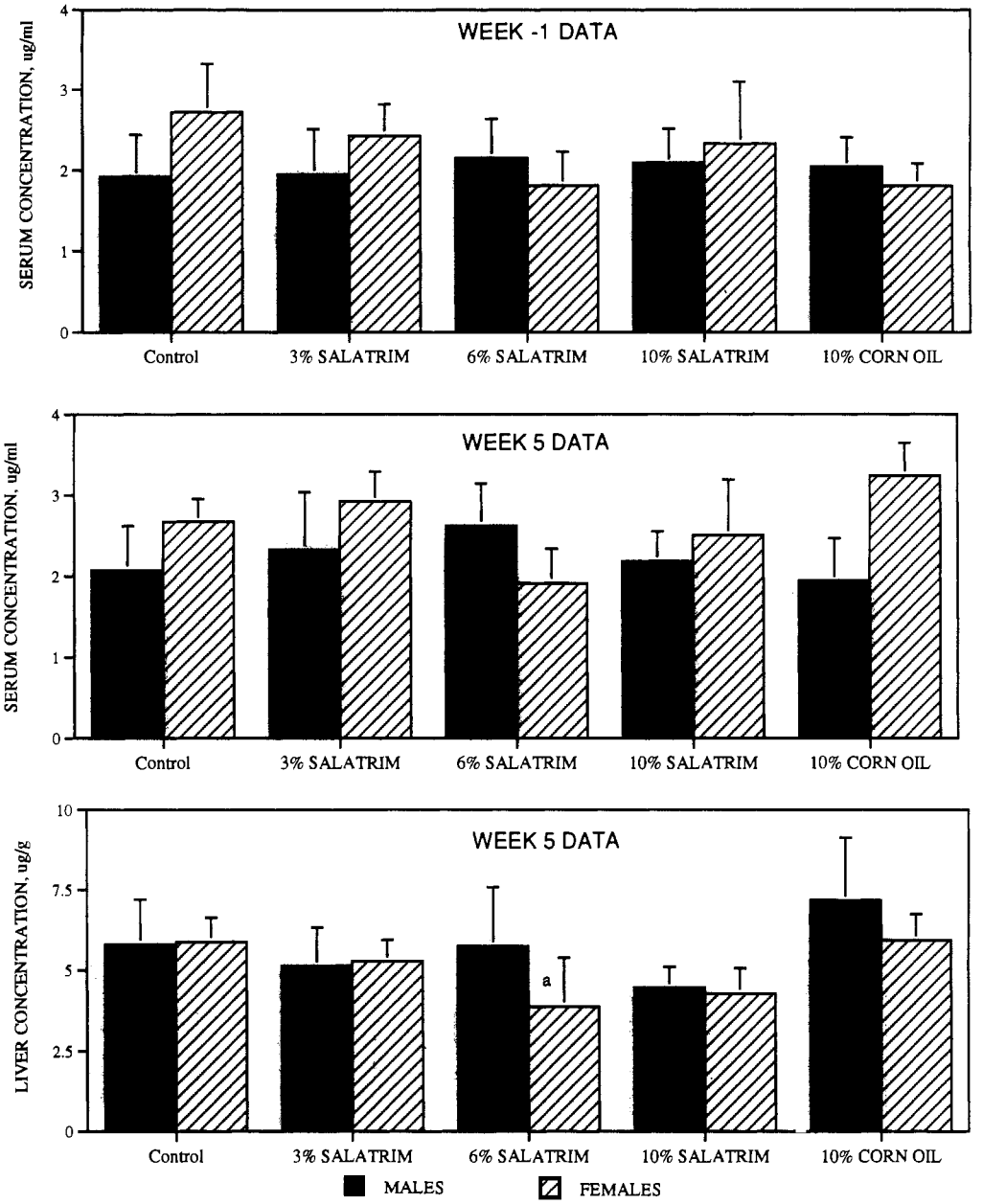

Figure 4. Serum (weeks −1 and 5) and liver (week 5) concentrations of vitamin E (α-tocopherol) vs treatment. Data points represent mean ± SD of the mean for four pigs. Data significantly different from control group ($p \leq 0.05$) are noted with an "a".

Table 6. Bone Mineral Data[a]

	calcium (mg/g)	phosphorous (mg/g)	strontium (μg/g)	zinc (μg/g)	ash (%)
		Males			
control	231 ± 7.4	112 ± 2.5	56.9 ± 2.35	118 ± 9.6	64.5 ± 1.26
10% SALATRIM	237 ± 4.3	115 ± 1.9	62.1 ± 5.63	127 ± 2.6	64.9 ± 1.04
10% corn oil	236 ± 5.7	113 ± 3.5	59.3 ± 3.45	124 ± 4.9	64.9 ● 0.87
		Females			
control	239 ± 4.3	115 ± 2.1	60.1 ± 4.67	127 ± 7.9	66.1 ● 1.69
10% SALATRIM	239 ± 6.1	115 ± 3.2	62.0 ± 4.55	130 ± 7.4	65.2 ± 1.69
10% corn oil	238 ± 4.5	115 ± 2.9	59.7 ± 5.14	124 ± 4.4	65.9 ± 1.52

[a] Data represent the mean ± standard deviation of values for four pigs per sex per group. [b] Statistically significant difference compared to control; $p \leq 0.05$.

dose levels of approximately 6–8 g/kg, while 10% diets fed to the pigs resulted in daily dose levels of approximately 3–4 g/kg.

Organ weights, organ-to-body weight percentages, and organ-to-brain weight ratios were comparable in treated and control pigs. There were no macroscopic pathology observations considered to be related to treatment. Mild focal vacuolation of the hepatocytes was observed in all groups of pigs, but the severity grade was higher only for one male fed 10% corn oil and for one male fed 10% SALATRIM 23SO lot A026. Because the incidence was similar among groups and the increased severity was observed in only one pig in each of the 10% fat diets, the finding appears to be spurious or perhaps a nonspecific fat effect.

The data confirm the hypothesis based on the scientific literature, structure/activity relationships, and results of testing with similar SALATRIM fats that this SALATRIM does not produce toxicologically significant effects in pigs

Table 7. Incidence and Severity of Focal Hepatocellular Vacuolization[a]

	diet concentrations				
		SALATRIM			
grade	control	3%	6%	10%	10% corn oil
		Males			
minimal	2	3	2	2	0
slight/mild	0	0	0	1	1
moderate	0	0	0	0	0
moderately severe	0	0	0	0	0
severe/high	0	0	0	0	0
total incidence	2	3	2	3	1
		Females			
minimal	3	3	3	3	1
slight/mild	0	0	0	0	0
moderate	0	0	0	0	0
moderately severe	0	0	0	0	0
severe/high	0	0	0	0	0
total incidence	3	3	3	3	1

[a] Livers were evaluated from four pigs per sex per group.

when fed for 28 days at average daily doses in males and females of up to 3.3 and 3.7 g/kg, respectively.

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