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A 4-Week Repeated Oral Dose Toxicity Study of Dairy Fat Naturally Enriched in Vaccenic, Rumenic and α-Linolenic Acids in Rats

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ABSTRACT: Few studies have focused on the toxicological risks of dairy fat intake. A standard dairy fat (SDF) with a 70% SFA content and a naturally enriched dairy fat (EDF) in vaccenic, rumenic and α-linolenic acids and low in SFA (54%) have been examined in a 4-week repeated dose oral toxicity study as a daily dose of 2000 mg/kg bw by gavage in rats. Comparisons were established with a third group of rats (control) which did not receive fat administration. Both fats were well tolerated, and no adverse events or mortality were observed during the treatment nor after a 2-week observation period. EDF and SDF did not cause significant differences with respect to a control group in body weight gain, food consumption, clinical observations, organ weight ratios, histopathological findings and most of the hematological and biochemical parameters including total cholesterol and cholesterol fractions in plasma. In rats treated with SDF, a significant increase of triglycerides was observed as compared to the control group. By contrast, in rats treated with EDF, a significant decrease in triglycerides was detected. EDF orally administered to rats was safe, and no treatment-related toxicity was detected. The results also suggest that EDF could protect against the increase of triglyceride concentrations in plasma.

KEYWORDS: dairy fat, vaccenic acid, rumenic acid, α-linolenic acid, safety evaluation, triglyceridemia

INTRODUCTION

Dairy fat is relatively more saturated than most natural fats and oils. This fact, together with the belief that milk is "fattening", appears to have led to the widespread conviction that dairy food is an obesity and heart disease factor, and that its consumption should be limited. However, dairy fat also naturally contains a number of components that could have healthy properties.[1,2]

Conjugated linoleic acid (CLA) is a term used for a mixture of positional and geometric isomers of linoleic acid that contain conjugated double bonds. *Cis*-9 *trans*-11 C18:2 (rumenic acid, RA) is the major CLA isomer in ruminant fat, representing about 70−90% of the total CLA. This isomer could confer a number of beneficial biological effects. RA healthy properties have been identified in a wide range of animal model and human cell lines and include anticarcinogenesis, immunomodulation and antiatherosclerosis.[2,3] Particularly noteworthy is the fact that RA is bioactive when supplied as a natural food component in the form of CLA-enriched milk fat.[3] Vaccenic acid (VA, *trans*-11 C18:1) is the precursor of RA in the mammary gland via δ-9 desaturase and the predominant *trans*-monoene that is naturally found in ruminant milk. Although there is considerable concern regarding the health risks associated with the consumption of *trans* fatty acids (FA), intake of ruminant *trans* FA would not be associated with a higher risk of cardiovascular diseases.[4,5] Even more, emerging results suggest that the consumption of VA may impart health benefits beyond those associated with CLA.[2,6] From these combined data the importance of improving VA and RA contents in dairy fats is encouraged.

Due to the increased consumer awareness of the link between diet and health, research has focused on altering the FA composition of milk to achieve a profile consistent with consumer perceptions and health recommendations.[1] Dairy fat FA composition can be significantly modified in a natural way through animal nutrition, offering the opportunity to respond to market forces and human health recommendations. The impact of ruminant nutrition on fat content and FA composition has been extensively reviewed[2,7] but few is known about the effects induced in humans by natural changes addressed toward a healthier composition in the dairy fat FA profile.[8,9] Furthermore, in animal models, relatively little information from dietary intervention studies with CLA-enriched dairy fat is available. Butters naturally enriched in VA and/or RA tended to reduce the atherogenic potential[10−12] of dairy fat in different animal models. Nevertheless, the putative effects of these bioactive compounds in dairy fat have to be considered in relation to the combined effects of the overall FA profile, amount and duration of dairy fat consumption.[2]

There has been a growing interest referred to the functional food aspect of milk fat. This involves designing dairy fat to improve its healthy properties[4,5] as well as to monitor its safety for human consumption. To date, limited toxicity studies have been published for high levels of dairy fat intake. Toxicology

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Table 1. Ingredients and Nutrient Composition of TMR and HEL Experimental Diets

item	ingredients, % of DM	
	TMR[a]	HEL[a]
alfalfa hay	35.5	34.7
beet pulp	5.1	5.0
corn	4.2	4.1
soybean meal	8.6	6.7
barley	11.4	4.3
corn distillers dried grains	10.8	10.6
wheat straw	17.3	16.9
green peas	3.4	
corn gluten feed	0.9	
malt sprouts	0.9	
sunflower meal	0.4	2.6
palm oil	0.1	
calcium soaps of palm fatty acids	0.9	
linseed		12.0
wheat middlings		2.6
sodium chloride	0.5	0.4
mineral−vitamin premix	0.1	0.1

[a] Dietary treatment of goats: TMR, total mixed ration, forage:concentrate ratio of 60:40; HEL, TMR supplemented with 12% (DM basis) of extruded linseed (high extruded linseed).

studies are necessary to assess the safety of a functional food before commercialization. The aim of this study was to evaluate the potential repeated dose (4 weeks) oral toxicity of a dairy fat low in saturated FA (less than 55%) and naturally enriched in VA, RA and other healthy FA as oleic and α-linolenic acids (EDF fat) as well as to compare the putative effects of this material in relation to non-dairy fat control treatment, and a standard dairy fat (SDF fat) low in *trans* and polyunsaturated FA in rats, in an experimental design in accordance with the European Union Guideline[13] and under Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.

MATERIALS AND METHODS

Test Substances. Two dairy fats obtained from two groups of goats (Murciana breed) fed with two different dietary treatments during 30 days were studied. The first group of goats was fed with a typical total mixed ration (TMR) with a forage concentrate ratio of 60:40 while the second group of goats received the same diet supplemented with 12% (DM basis) of extruded linseed (high extruded linseed [HEL]) (Table 1). Both diets were previously described by Gómez-Cortés et al.[14] After a period of 30 days of dietary treatment, bulk milks from both groups of goats were collected, and their dairy fat samples (SDF and EDF) were extracted, characterized and then used in the subsequent toxicological testing.

Characterization of Dairy Fat Samples. Dairy fat was extracted following the method of Luna et al.,[15] and fatty acid methyl esters (FAME) were prepared by base-catalyzed methanolysis of the glycerides (KOH in methanol) according to the ISO-IDF procedure.[16] After derivatization, FAME were fractionated according to the double bond geometry by silver ion solid phase extraction (Ag^+-SPE). Ag^+-SPE was developed using cartridges purchased from Supelco (Discovery Ag^+-Ion SPE, Bellefonte, PA, USA) as described by Kramer et al.[17] The total FA profile was recorded by analysis of FAME on a gas−liquid chromatograph (GC) (Agilent 6890 N Network System) with auto injector, fitted with a flame ionization detector onto a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm, Varian, Middelburg, The Netherlands). Helium was the carrier gas at an inlet pressure of 235.8 kPa and a split ratio of 1:100. Injector and detector temperature was 250 °C. Total time of the chromatographic program was 125 min, and the initial oven temperature was 160 °C. After 80 min, oven temperature was raised at 10 °C min^{-1} to 210 °C and then held for 40 min. Saturated, mono *cis* and mono *trans* FA fractions were analyzed as FAME on the same equipment and conditions but with a split ratio of 1:2. Calibration of the individual FA was carried out using the reference material CRM-164 obtained from the Community Bureau of Reference (Brussels, Belgium). The determination of the profile of CLA isomers was also supported by Ag^+-HPLC analysis in the conditions described by Gómez-Cortés et al.[14]

Repeated Dose (4-Week) Oral Toxicity Study. Wistar male and female rats (Charles River Inc., Marget, Kent, U.K.) were acclimated for 7 days prior to study initiation with an evaluation of health status. The rats were individually housed in polycarbonate cages with sawdust bedding and maintained in environmentally controlled rooms (22 ± 2 °C and 50% ± 10% relative humidity) with a 12 h light−dark cycle (light from 8 am to 8 pm). Food (A03 rodent diet, Scientific Animal Food and Engenieering, Villemoisson-sur-Orge, France) and water were available *ad libitum*. The rats were 56 days old at the initiation of treatment. Repeated dose (4 weeks) study was conducted in accordance with the European Union guidelines.[13] The study was undertaken in accordance with the ethics requirements and authorized by the Official Ethical Committee of the Complutense University.

The repeated dose (4 weeks) (limit test) study was conducted in 72 rats (36 males, 36 females) divided into six groups of 12 animals each (6 males and 6 females): group 1 (control), group 2 (SDF), group 3 (EDF), group 4 (satellite control), group 5 (satellite SDF) and group 6 (satellite EDF). Rats received a daily dose of either distilled water (groups 1 and 4) or 2,000 mg kg^{-1} of body weight of SDF (groups 2 and 5) or EDF (groups 3 and 6), orally once a day over 4 weeks. This dosage was selected on the basis of a preliminary study performed with single animals of both sexes in which no evidence of toxicity was observed at oral dosages of 500 and 1,000 mg kg^{-1} of body weight. Doses of the test (SDF or EDF) and control articles were administered by gavage at a volume of 2.5 mL kg^{-1} of body weight based on the individual animal body weights obtained on the day dosing. During dose administration, test article preparations were maintained in a water bath set to maintain approximately 40 °C to reach a liquid state of the test articles. The 800 mg mL^{-1} of SDF or EDF preparations were prepared immediately prior to administration (2.5 mL kg^{-1} of body weight or 0.5 mL per rat weighing 200 g). Animals were dosed at approximately the same time each day (approximately 4−6 h into light cycle). Food but not water was withheld from 4 h before until 2 h after control and test article administration. Animals were checked for clinical signs and mortality twice a day (a.m. and p.m.). All rats of groups 1, 2, and 3 were deprived of food for 18 h, weighed, euthanized by CO_2 inhalation, exsanguinated, and necropsied on day 29. All animals of the satellite groups (groups 4, 5 and 6) were kept a further 14 days without treatment to detect delayed occurrence, or persistence of, or recovery from toxic effects. All rats of groups 4, 5, and 6 were deprived of food for 18 h, weighed, euthanized by CO_2 inhalation, exsanguinated, and necropsied on day 42.

Observations. All animals were observed twice daily for general appearance, behavior, signs of morbidity and mortality (once before treatment and once daily thereafter). Rats were observed for their general condition and the condition of the skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, evaluated for respiration rate and palpated for masses. Behavioral parameters checked were abnormal movements (tremor, convulsion and muscular contractions), reactions to handling and behavior in open field (excitability, responsiveness to touch and to sharp noise), changes in ordinary behavior (changes in

Table 2. Hematological and Clinical Biochemistry Parameters

Hematological Parameters	
red blood cell count (RBC)	eosinophil count
hemoglobin	lymphocyte count
hematocrit	monocyte count
mean corpuscular vol (MCV)	basophil count
mean corpuscular hemoglobin (MCH)	platelet count
mean corpuscular hemoglobin concn (MCHC)	mean platelet vol (MPV)
red blood cell distribution width (RDW)	prothrombin time (28 days)
white blood cell count (WBC)	thromboplastin partial time (28 days)
band neutrophil count	fibrinogen (28 days)
neutrophil count	
Clinical Biochemistry Parameters	
glucose	alanine aminotranferase (ALAT)
urea	alkaline phosphatase
creatinine	triglyceride
total protein	cholesterol
total bilirubin	high density lipoproteins (HDL)
calcium	low density lipoproteins (LDL)
sodium	albumin (28 days)
potassium	lipoprotein A (28 days)
aspartate aminotransferase (ASAT)	

grooming, head shaking, and gyration), abnormal behavior (autophagy, backward motion) and aggression. Body weight, body weight gain and food and water consumption were measured daily, and at the end of the observation periods the rats were examined by necropsy, and the weights of the organs recorded.

Clinical Test Parameters. Blood samples for hematology and clinical chemistry evaluation were collected from the retro-orbital plexus from animals under light anesthesia induced by CO_2 inhalation after 4 weeks of treatment and 14 days of recovery. EDTA was used as an anticoagulant for hematology samples, and sodium citrate was used as an anticoagulant for clinical chemistry. Food was withheld for approximately 18 h before blood collection, and samples were collected early in the working day to reduce biological variation; water was provided *ad libitum*. Clinical pathology parameters (hematological and clinical biochemistry) were evaluated (Table 2). Most hematology variables were measured with a Coulter/CELL-DYN 3500 whole blood automated analyzer (Abbott, Chicago, IL). Blood cell smears were observed with an Olympus Microscopy BX41 (Olympus, Tokyo, Japan). Clinical chemistry parameters were evaluated with a spectrophotometer Konelab PRIME 30 (Thermofisher Scientific Inc., Waltham, MA, USA) and special biochemistry parameters with a clinical chemistry analyzer AU640 (Olympus, Tokyo, Japan). Coagulation parameters were analyzed with a coagulation analyzer Coatron M1 (Teco Medical Instruments, GMBH, Neufahrn, Germany).

Anatomical Pathology. All rats were euthanized by CO_2 inhalation and necropsied. The necropsy included a macroscopic examination of the external surface of the body, all orifices, the cranial cavity, the brain and spinal cord, the nasal cavity and paranasal sinuses, and the thoracic, abdominal, and pelvic cavities and viscera. Descriptions of all macroscopic abnormalities were recorded. Samples of the following tissues and organs were collected from all animals at necropsy and fixed in neutral phosphate-buffered 4% formaldehyde solution: adrenal glands, brain, heart, ileum, jejunum, cecum, colon, duodenum, rectum, stomach, esophagus, trachea, kidneys, liver, lungs, pancreas, spleen, skin, testicles with epididymes, ovaries with oviducts, bone marrow, thymus, thyroid and parathyroid glands, seminal vesicles, urinary bladder and uterus. The organ and body weight ratios were calculated. All organ and tissue samples for histopathological examination were processed, embedded in paraffin, cut at an approximate thickness of 2 to 4 μm, and stained with hematoxylin and eosin. Slides of all organs and tissues listed above were collected from all animals of the control and treated groups.

Statistical Analysis. All data are expressed as means ± standard error of the mean (SEM) of 6 determinations (i.e., 6 males and 6 females). Differences between control and treated groups were evaluated with a one-way analysis of variance (ANOVA) followed by Dunnett's test,[18] and differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Fatty Acid Composition of Dairy Fat. Table 3 shows the detailed FA profiles of the dairy fats tested. FA profile of the SDF resembles to a market standard butter with a saturated FA content around 70% and a *trans* FA content about 5% of total FAME, which was within the range commonly found in a conventional goat milk. In comparison, EDF was characterized by percentages of saturated FA lower than 55% and very high amounts of *trans* monounsaturated FA (higher than 13% of total FAME, mainly VA) (Table 3). This milk contained more than 3-fold of VA and RA than control sample and 5-fold of α-linolenic acid (*cis*-9 *cis*-12 *cis*-15 C18:3) percentage. Additionally levels of oleic acid (*cis*-9 C18:1) were also higher (an increase of more than 15%) in dairy fat coming from goats fed the linseed supplemented diet. From a nutritional point of view, the EDF showed an atherogenicity index (AI) that was half compared to SDF. Furthermore the omega-6/omega-3 index was clearly lower for the EDF (0.89 vs 6.55) (Table 3).

Repeat Dose (4 Weeks) Oral Toxicity in Rats. No mortality was observed. No treatment related changes in the general condition and external appearance were observed in male and female rats treated with SDF and EDF at 2,000 mg kg^{-1} of body weight daily dose. The development of the animals during the experimental period corresponded to their species and age.

Body Weight and Food Consumption. There were no significant differences in body weight (data not shown) or body weight gain (Figure 1 and Figure 2) among groups treated with EDF in comparison to the control and SDF groups at any time of the experimental period. All dairy fat (SDF and EDF) treated groups consumed similar amounts of food and water (data not shown) to those corresponding to control group.

Clinical Pathology. All hematological data were within normal limits, and differences between groups were not observed (Table 4). Clinical chemistry data showed no treatment-related alterations at the end of the 4-week treatment period (Table 5) except for triglycerides. In female and male rats, the treatment with SDF increased triglyceride concentrations as compared to control which received distilled water ($P < 0.001$ and $P < 0.05$, respectively) (Table 5). Compared to the control group and to the SDF group, triglyceride concentrations were significantly lower in female and male rats treated with EDF ($P < 0.01$ and $P < 0.001$, respectively). Triglyceride concentrations of control, SDF and EDF female rats were 131.17 ± 4.41, 190.00 ± 6.66 and 101.00 ± 6.66 mg dL^{-1}, respectively, and the values for control, SDF and EDF male rats were 142.67 ± 4.56, 177.50 ± 11.12 and

Table 3. Fatty Acid Profile (g/100 g of Total Fatty Acid Methyl Esters) Determined by GC and Ag+−HPLC of Standard Dairy Fat and Naturally Enriched Dairy Fat from Goats Fed with a Typical Total Mixed Ration (TMR) and a TMR Supplemented with 12 g of Extruded Linseed/100 g of DM Diets, Respectively[a]

	SDF	EDF		SDF	EDF
			SFA		
C4:0	2.85	2.82	C16:0 *iso*	0.21	0.15
C5:0	0.02	0.02	C16:0	23.59	16.68
C6:0	3.15	2.76	C17:0 *iso*	0.28	0.26
C7:0	0.04	0.03	C17:0 *anteiso*	0.33	0.24
C8:0	3.34	2.69	C17:0	0.45	0.39
C9:0	0.07	0.05	C18:0 *iso*	0.03	0.03
C10:0	9.73	7.26	C18:0	13.13	11.00
C12:0	3.69	2.47	C19:0	0.08	0.05
C13:0 *iso*	0.01	0.02	C20:0	0.21	0.12
C13:0 *anteiso*	0.02	0.02	C21:0	0.04	0.08
C14:0 *iso*	0.07	0.05	C22:0	0.07	0.04
C14:0	7.83	5.81	C23:0	0.02	0.02
C15:0 *iso*	0.17	0.12	C24:0	0.02	0.02
C15:0 *anteiso*	0.27	0.21	ΣSFA	70.35	53.96
C15:0	0.64	0.56	AI	2.00	0.93
			cis MUFA		
C10:1 + C12:0 *iso*	0.27	0.19	*cis*-8 C18:1	0.05	0.13
cis-9 C12:1 + C13:0	0.13	0.08	*cis* (9 + 10) C18:1	17.64	21.20
cis-9 C14:1	0.09	0.07	*cis*-11 C18:1	0.30	0.34
cis-9 C15:1	0.07	0.05	*cis*-12 C18:1	0.27	0.42
cis-7 C16:1	0.27	0.18	*cis*-13 C18:1	0.05	0.07
cis-9 C16:1	0.49	0.41	*cis*-14 C18:1	0.03	0.12
cis-10 C16:1	0.02	0.02	*cis*-15 C18:1	0.05	0.46
cis-11 C16:1	0.01	0.01	*cis*-16 C18:1	0.06	0.07
cis-12 C16:1	0.02	0.01	*cis*-11 C20:1	0.05	0.05
cis-13 C16:1	0.14	0.08	Σ*cis* MUFA	20.16	24.10
cis-9 C17:1	0.16	0.15			
			trans MUFA		
trans-5 C16:1	0.01	0.02	*trans*-5 C18:1	0.02	0.04
trans (6 + 7) C16:1	0.04	0.05	*trans* (6 + 7+8) C18:1	0.28	0.56
trans-8 C16:1	0.03	0.03	*trans*-9 C18:1	0.32	0.56
trans-9 C16:1	0.12	0.42	*trans*-10 C18:1	0.49	0.74
trans-10 C16:1	0.04	0.04	*trans*-11 C18:1	1.50	5.10
trans-11 C16:1	0.03	0.06	*trans*-12 C18:1	0.42	0.72
trans-12 C16:1	0.04	0.10	*trans*-13 C18:1	0.40	1.50
trans-13 C16:1	0.01	0.01	*trans*-14 C18:1	0.77	2.10
trans-14 C16:1	0.03	0.07	*trans*-15 C18:1	0.29	0.78
trans-15 C16:1	0.02	0.01	*trans*-16 C18:1	0.36	0.58
trans-4 C18:1	0.03	0.04	Σ*trans* MUFA	5.25	13.55
			Nonconjugated C18:2		
trans-11 *trans*-15	0.06	0.22	*trans*-11 *cis*-15	0.08	1.69
trans-9 *trans*-12	0.10	0.26	*cis*-9 *cis*-12	2.08	1.30
cis-9 *trans*-13 + *trans*-8 *cis*-12	0.14	0.40	*cis*-9 *cis*-15	0.09	0.09
trans-8 *cis*-13	0.10	0.26	other C18:2	0.07	0.03
cis-9 *trans*-12	0.06	0.11	Σnonconjugated C18:2	2.81	4.37
trans-9 *cis*-12	0.03	0.01			

Table 3. Continued

	SDF	EDF		SDF	EDF
			Conjugated C18:2 (CLA)		
trans-13 *trans*-15	0.002	0.003	12,14 (*cis-trans* + *trans-cis*)	0.001	0.024
trans-12 *trans*-14	0.004	0.040	*trans*-11 *cis*-13	0.006	0.108
trans-11 *trans*-13	0.002	0.045	*trans*-10 *cis*-12	0.003	0.006
trans-10 *trans*-12	0.005	0.005	*cis*-9 *trans*-11	0.424	1.538
trans-9 *trans*-11	0.006	0.017	*trans*-9 *cis*-11	0.011	0.013
trans-8 *trans*-10	0.002	0.003	7,9 + 8,10 (*cis-trans* + *trans-cis*)	0.055	0.109
trans-7 *trans*-9	0.000	0.003	*cis*-9 *cis*-11	0.002	0.001
trans-6 *trans*-8	0.002	0.001	ΣCLA	0.525	1.915
			Other PUFA		
cis-6 *cis*-9 *cis*-12 C18:3	0.02	0.02	C22:2 n-6	0.01	0.02
cis-9 *cis*-12 *cis*-15 C18:3	0.21	1.03	C20:5 n-3	0.02	0.07
cis-9 *trans*-11 *trans*-15 C18:3	0.00	0.07	C22:5 n-3	0.05	0.09
cis-9 *trans*-11 *cis*-15 C18:3	0.03	0.28	C22:6 n-3	0.05	0.05
C20:2 n-6	0.02	0.02	Σn-6	2.30	1.41
C20:3 n-6	0.02	0.01	Σn-3	0.35	1.59
C20:3 n-3	0.00	0.01	n-6/n-3 (omega-6/omega-3 index)	6.55	0.89
C20:4 n-6	0.14	0.05	Σother PUFA	0.57	1.70

[a] SDF, standard dairy fat; EDF, naturally enriched dairy fat; VA, *trans*-11 C18:1; RA, *cis*-9 *trans*-11 C18:2; α-linolenic acid, *cis*-9 *cis*-12 *cis*-15 C18:3; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid; MUFA, monounsaturated fatty acids; AI, atherogenicity index (C12:0 + 4 × C14:0 + C16:0)/(MUFA+ PUFA).

108.83 ± 7.03 mg dL^{-1}, respectively (Table 5). Total cholesterol concentrations were not affected after the treatment with SDF and EDF compared to control. After 14 days without treatment to detect delayed occurrence of potential toxic effects, there were no treatment related changes in hematological and clinical test parameters (data shown in Table 4 and Table 5). After the 14 day recovery period, the reduction and increase in triglyceride concentrations respectively observed in EDF and SDF groups were no longer apparent.

Anatomical Pathology. The necropsy performed on day 29 after the last dose of the SDF (group 2) and EDF (group 3) and on day 42 after 14 days without any treatment (groups 5 and 6) did not reveal any gross pathological changes or any differences in organ weights in comparison to the corresponding control groups. Mean organ weights and rate body weight and organ are presented in Table 6. After 4 weeks of treatment, there were no histopathological findings in the organs examined considered to be treatment related in male and female rats (data not shown). There were also no treatment related histopathological findings in the satellite groups (groups 4, 5 and 6) (data not shown).

To our knowledge, this study is the first published examination of a repeated dose (4 week) oral toxicity of two different dairy fats

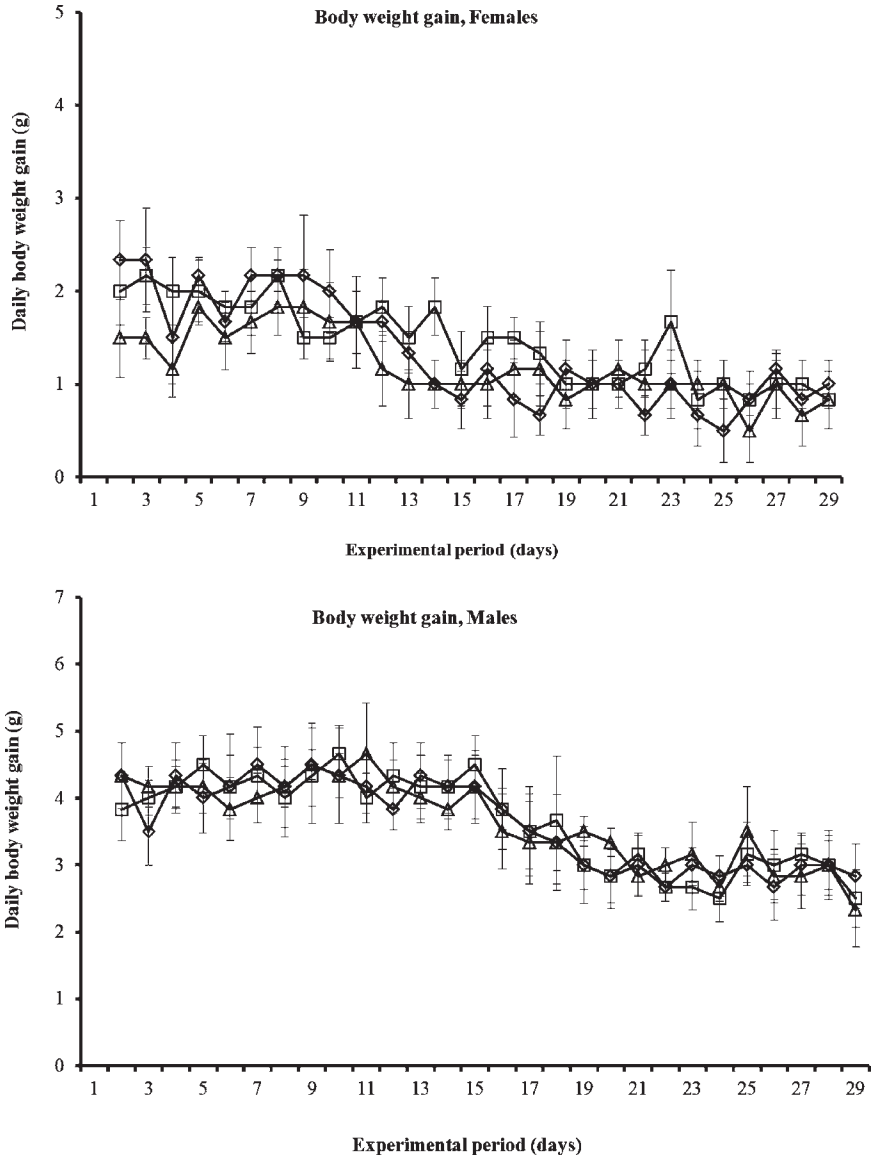

Figure 1. Daily body weight gain of rats exposed to repeated (4 weeks) oral doses of control (◇), standard dairy fat (SDF) (Δ) and naturally enriched dairy fat (EDF) (□) at 2,000 mg kg $^{-1}$ of body weight. Mean values, $n = 6$ animals/sex group, with bars equal to the SEM. Absence of bars means that the SEM was less than the size of the symbol.

with saturated FA content around 70% and a *trans* FA content about 5% (SDF) and with saturated FA content lower than 55% and very high amounts of *trans* monounsaturated FA (higher than 13% of total FAME, mainly VA) (EDF). Experimental data on the putative effects of both dairy fat treatments in relation to a no-fat control group were compared.

Attending to most of the different parameters evaluated (Tables 4, 5 and 6), both dairy fats (SDF and EDF) would not be associated with adverse effects. The increased triglyceride levels in the animals treated with SDF, in comparison with the control group, could be justified due to the high and repeated dose of fat administered. In contrast, the lack of statistically significant differences in biomarkers as HDL, LDL and total cholesterol between rats treated with SDF and control group would be one more piece of evidence supporting the idea that high consumption of conventional dairy fat with a 70% of SFA level would not promote hypercholesterolemia in rats, a risk factor used for the diagnosis of metabolic syndrome and cardiovascular diseases (CVD).

The most striking effect was a 38 and 53% decrease in triglyceride concentrations in EDF male and female rats respectively relative to SDF treated animals. Even more, in comparison with the control group, a 23% diminution of this biomarker in plasma was also observed, whatever the gender. Because of the complexity inherent to dairy fat, it is not an easy task to attribute these results to specific FA. From a nutritional point of view, EDF showed an AI that was half compared to SDF (0.93 vs 2). Also the omega-6/omega-3 index was clearly lower for the EDF compared to SDF (0.89 vs 6.55). The importance of the maintenance of this ratio below 4 in CVD and other chronic diseases has been recently remarked.[19]

Although nutritional recommendations have further highlighted that *trans* FA are linked to increase risk of CVD, the current research suggests the idea that the EDF containing a high

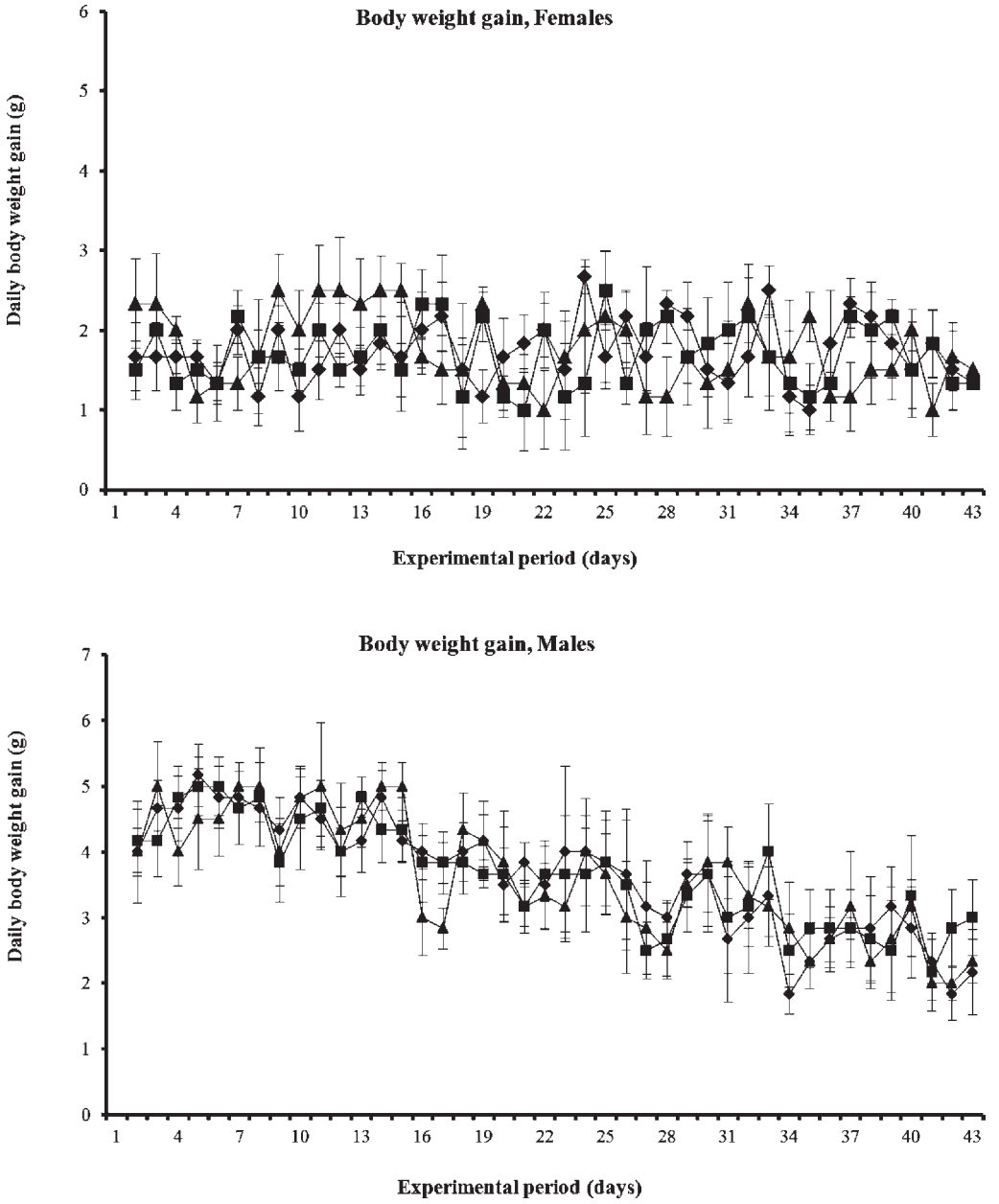

Figure 2. Daily body weight gain of rats exposed to repeated (4 weeks) oral doses of control (◆), standard dairy fat (SDF) (▲) and naturally enriched dairy fat (EDF) (■) at 2,000 mg kg $^{-1}$ of body weight and moreover observed 14 days after treatment (satellite groups). Mean values, $n = 6$ animals/sex group, with bars equal to the SEM. Absence of bars means that the SEM was less than the size of the symbol.

amount of monounsaturated FA (Table 3) protects against triglyceridemia. The present study is consistent with recent results from an acute oral safety study of another dairy fat obtained from ewes fed with diets supplemented with oil or seed rich in linoleic or α-linolenic acids,[20] in that rats treated with a dairy fat rich in RA plus VA significantly decreased triglycerides in plasma whereas this feeding did not result in any detrimental metabolic effects or negative influence in any toxicological parameters. Furthermore, clinical evidence indicates that, relative to industrially derived *trans* FA, a natural source of *trans* FA (such as VA-enriched butter) in the human diet can elicit either neutral or beneficial health effects on blood lipid variables among healthy individuals.[21,22] Furthermore, a very recent study[5] concluded that a limited increase of VA together with a decrease of saturated FA in dairy products was associated with an improvement in some cardiovascular risk factors.

RA exhibits several health-promoting attributes, including antiatherogenic activities in animal models.[3] Dairy fat naturally enriched in VA and RA has also been shown to be antiatherogenic in experimental animals,[2,9,23] with the effect of VA due to the ability of mammalian cells to use VA in the endogenous synthesis of RA by δ-9 desaturase. The rate of the conversion of VA to RA has been estimated to range from 20 to 30% in humans.[24]

Regardless of the presence of RA, we know very little about the potential bioactivity of VA on blood triglyceridemia. Nevertheless Wang et al.[25] have shown that 3-week feeding with a diet enriched in purified VA reduces plasma triglycerides by 40% without any concomitant change in total cholesterol, LDL or

Table 4. Hematological Parameters in Rats after Repeated (4 Week) Oral Dose of Standard Dairy Fat and Naturally Enriched Dairy Fat at 2,000 mg kg^{-1} of Body Weight per Day[a]

	group 1: control		group 2: SDF		group 3: EDF		group 4: satellite control		group 5: satellite SDF		group 6: satellite EDF	
param[b]	female	male	female	male	female	male	female	male	female	male	female	male
RBC ($\times 10^6/\mu$L)	8.57 ± 0.14	8.87 ± 0.12	8.75 ± 0.11	8.96 ± 0.14	8.48 ± 0.07	9.22 ± 0.15	8.85 ± 0.27	9.36 ± 0.25	8.50 ± 0.10	9.62 ± 0.16	8.55 ± 0.16	9.32 ± 0.24
hemoglobin (g/dL)	15.73 ± 0.20	16.28 ± 0.23	15.83 ± 0.31	15.85 ± 0.31	15.52 ± 0.27	16.77 ± 0.37	16.60 ± 0.51	16.62 ± 0.28	15.67 ± 0.16	17.03 ± 0.21	15.48 ± 0.30	16.75 ± 0.40
hematocrit (%)	47.68 ± 0.85	48.28 ± 0.96	46.07 ± 1.09	47.65 ± 0.41	46.95 ± 0.88	50.80 ± 1.52	48.20 ± 1.17	49.61 ± 0.80	46.10 ± 0.43	50.43 ± 0.54	44.92 ± 0.99	48.30 ± 1.08
MCV (fL)	55.93 ± 0.85	54.47 ± 0.40	52.87 ± 1.43	53.53 ± 0.85	55.43 ± 1.06	54.92 ± 0.91	55.10 ± 1.03	53.23 ± 0.64	54.15 ± 0.76	52.10 ± 0.40	52.67 ± 1.01	52.63 ± 0.48
MCH (pg)	18.42 ± 0.19	18.27 ± 0.03	18.10 ± 0.38	18.50 ± 0.23	18.32 ± 0.27	18.37 ± 0.19	18.62 ± 0.19	17.92 ± 0.18	18.43 ± 0.21	17.60 ± 0.15	18.03 ± 0.27	18.02 ± 0.17
MCHC (g/dL)	32.97 ± 0.20	33.52 ± 0.24	34.20 ± 0.50	34.60 ± 0.56	32.95 ± 0.38	33.18 ± 0.35	33.83 ± 0.35	33.57 ± 0.27	33.95 ± 0.23	33.74 ± 0.17	34.43 ± 0.26	34.38 ± 0.32
RDW (%)	18.45 ± 0.22	18.92 ± 0.14	18.52 ± 0.27	17.83 ± 0.34	19.12 ± 0.54	18.65 ± 0.16	18.42 ± 0.32	18.88 ± 0.59	17.13 ± 0.53	19.58 ± 0.33	17.30 ± 0.44	19.25 ± 0.28
WBC ($\times 10^3/\mu$L)	5.45 ± 0.12	8.62 ± 0.50	6.67 ± 1.06	8.40 ± 0.24	5.15 ± 0.39	8.80 ± 0.67	6.75 ± 0.72	9.88 ± 0.77	5.54 ± 0.18	8.34 ± 0.64	5.49 ± 0.28	10.02 ± 0.57
banded neutrophils ($\times 10^3/\mu$L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
neutrophils ($\times 10^3/\mu$L)	1.75 ± 0.24	2.62 ± 0.19	2.47 ± 0.41	2.59 ± 0.20	1.81 ± 0.22	3.12 ± 0.27	2.04 ± 0.19	2.90 ± 0.20	1.62 ± 0.05	2.35 ± 0.13	1.87 ± 0.16	2.81 ± 0.28
eosinophils ($\times 10^3/\mu$L)	0.07 ± 0.01	0.11 ± 0.06	0.11 ± 0.01	0.15 ± 0.06	0.14 ± 0.09	0.10 ± 0.01	0.08 ± 0.01	0.07 ± 0.03	0.10 ± 0.00	0.07 ± 0.02	0.07 ± 0.02	0.10 ± 0.03
lymphocytes ($\times 10^3/\mu$L)	3.53 ± 0.31	5.89 ± 0.34	4.27 ± 0.71	5.54 ± 0.30	3.39 ± 0.22	5.37 ± 0.48	4.72 ± 0.55	6.78 ± 0.68	3.77 ± 0.18	5.88 ± 0.37	3.54 ± 0.13	6.91 ± 0.44
monocytes ($\times 10^3/\mu$L)	0.07 ± 0.01	0.06 ± 0.02	0.08 ± 0.02	0.07 ± 0.01	0.03 ± 0.01	0.17 ± 0.07	0.07 ± 0.02	0.10 ± 0.02	0.03 ± 0.01	0.12 ± 0.02	0.04 ± 0.01	0.15 ± 0.04
basophils ($\times 10^3/\mu$L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
platelets ($\times 10^3/\mu$L)	770.17 ± 30.27	739.50 ± 16.13	895.00 ± 46.77	761.33 ± 17.52	783.17 ± 24.27	762.67 ± 27.94	951.83 ± 51.76	887.00 ± 35.88	868.33 ± 50.89	900.83 ± 38.43	930.83 ± 35.37	843.00 ± 36.13
MPV (fL)	7.43 ± 0.08	7.93 ± 0.07	7.40 ± 0.08	7.58 ± 0.10	7.68 ± 0.11	7.83 ± 0.13	8.28 ± 0.13	8.20 ± 0.07	8.23 ± 0.13	8.72 ± 0.17	8.37 ± 0.08	8.47 ± 0.14

[a] Data are expressed as mean ± SEM ($n = 6$) in each sex group. Differences between EDF group and control or SDF groups were not significant. [b] RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; WBC, white blood cell count; MPV, mean platelet volume.

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Table 5. Clinical Chemistry in Rats after Repeated (4 Week) Oral Dose of Standard Dairy Fat and Naturally Enriched Dairy Fat at 2000 mg kg^{-1} of Body Weight per Day[a]

	group 1: control		group 2: SDF		group 3: EDF		group 4: satellite control		group 5: satellite SDF		group 6: satellite EDF	
param[b]	female	male	female	male	female	male	female	male	female	male	female	male
glucose (mg/dL)	117.50 ± 8.04	106.83 ± 3.63	103.17 ± 3.53	123.17 ± 4.08	107.17 ± 8.09	113.67 ± 3.01	96.00 ± 3.56	98.00 ± 2.80	94.83 ± 3.13	88.83 ± 1.62	88.67 ± 2.26	94.00 ± 2.85
urea nitrogen (mg/dL)	45.67 ± 0.61	41.83 ± 1.25	44.33 ± 1.43	41.17 ± 2.46	44.50 ± 1.48	39.50 ± 1.67	38.00 ± 2.52	37.83 ± 1.54	43.17 ± 1.45	39.33 ± 1.02	39.17 ± 1.45	41.17 ± 1.56
creatinine (mg/dL)	0.68 ± 0.02	0.65 ± 0.02	0.65 ± 0.02	0.58 ± 0.03	0.67 ± 0.03	0.65 ± 0.02	0.65 ± 0.04	0.57 ± 0.02	0.63 ± 0.02	0.63 ± 0.02	0.58 ± 0.02	0.58 ± 0.03
albumin (g/dL)	4.03 ± 0.09	3.63 ± 0.02	3.95 ± 0.05	3.55 ± 0.08	4.03 ± 0.06	3.73 ± 0.06	4.25 ± 0.35	3.60 ± 0.06	4.18 ± 0.03	3.78 ± 0.07	4.22 ± 0.05	3.72 ± 0.06
total protein (g/dL)	7.93 ± 0.12	6.67 ± 0.08	7.42 ± 0.09	6.70 ± 0.22	7.52 ± 0.16	6.92 ± 0.13	7.65 ± 0.24	6.83 ± 0.27	8.17 ± 0.22	7.28 ± 0.18	8.17 ± 0.16	7.32 ± 0.17
total bilirubin (mg/dL)	0.30 ± 0.06	0.22 ± 0.02	0.17 ± 0.02	0.23 ± 0.03	0.25 ± 0.03	0.17 ± 0.02	0.22 ± 0.03	0.25 ± 0.02	0.27 ± 0.03	0.23 ± 0.02	0.25 ± 0.03	0.25 ± 0.02
calcium (mg/dL)	10.53 ± 0.14	10.58 ± 0.08	10.75 ± 0.08	10.39 ± 0.05	10.81 ± 0.10	10.82 ± 0.31	10.45 ± 0.51	10.58 ± 0.12	10.96 ± 0.10	10.46 ± 0.18	10.85 ± 0.18	11.06 ± 0.22
sodium (mequiv/L)	143.67 ± 1.28	143.67 ± 0.42	144.33 ± 1.28	142.17 ± 0.70	141.50 ± 2.25	144.83 ± 1.01	144.00 ± 1.77	144.67 ± 1.05	146.50 ± 0.96	143.00 ± 1.48	144.33 ± 0.95	146.50 ± 0.67
potassium (mequiv/L)	6.53 ± 0.84	6.48 ± 0.23	6.40 ± 0.18	5.98 ± 0.25	6.45 ± 0.33	6.32 ± 0.21	6.60 ± 0.16	6.65 ± 0.09	6.22 ± 0.08	6.67 ± 0.10	6.35 ± 0.27	6.60 ± 0.09
ASAT (unit/L)	140.67 ± 8.30	164.50 ± 9.31	151.00 ± 11.64	148.67 ± 6.89	127.83 ± 8.36	133.17 ± 10.62	140.00 ± 6.73	163.00 ± 10.55	165.33 ± 9.65	165.33 ± 13.55	161.83 ± 14.68	139.17 ± 5.24
ALAT (unit/L)	43.17 ± 2.27	46.50 ± 2.49	51.33 ± 4.48	47.50 ± 2.57	37.83 ± 4.99	39.17 ± 2.80	34.67 ± 3.81	44.50 ± 3.31	43.00 ± 3.93	44.33 ± 1.05	39.50 ± 2.60	43.50 ± 1.84
alkaline phosphatase (unit/L)	222.00 ± 14.83	454.17 ± 25.45	262.33 ± 28.60	397.83 ± 20.66	256.83 ± 20.35	382.33 ± 20.22	155.83 ± 16.23	334.33 ± 21.56	219.67 ± 24.49	357.00 ± 23.24	170.50 ± 18.02	448.50 ± 29.99
triglyceride (mg/dL)	131.17 ± 4.41	142.67 ± 4.56	190.00 ± 6.66 (i)*** [c]	177.50 ± 11.12 (i)*	101.00 ± 6.66 (i)** (ii)***	108.83 ± 7.03 (i)** (ii)***	130.83 ± 10.67	157.67 ± 13.38	166.83 ± 12.35	182.67 ± 11.17	164.33 ± 11.63	167.33 ± 10.41
total cholesterol (mg/dL)	77.83 ± 8.30	61.67 ± 2.23	73.33 ± 4.03	66.67 ± 2.39	75.83 ± 1.51	68.33 ± 2.23	78.17 ± 6.56	68.00 ± 6.22	73.00 ± 3.99	74.00 ± 4.07	74.33 ± 3.41	80.00 ± 4.52
HDL (mg/dL)	52.10 ± 4.66	39.07 ± 1.12	49.70 ± 1.55	43.08 ± 1.64	53.63 ± 2.23	41.96 ± 1.05	53.75 ± 3.07	43.82 ± 4.76	45.78 ± 2.86	42.88 ± 1.58	46.68 ± 2.55	48.55 ± 2.77
LDL (mg/dL)	14.32 ± 0.85	15.50 ± 0.95	15.05 ± 1.31	15.37 ± 0.69	14.23 ± 1.23	15.08 ± 1.19	14.37 ± 1.08	15.67 ± 1.02	15.40 ± 0.58	15.92 ± 0.77	14.72 ± 0.78	15.55 ± 0.94
lipoprotein A	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2	<2
prothrombin time (seg)	25.45 ± 0.48	24.63 ± 0.55	26.47 ± 0.54	25.38 ± 0.29	25.48 ± 0.21	25.68 ± 0.30	21.23 ± 0.54	24.12 ± 0.65	20.80 ± 0.72	23.88 ± 0.29	21.17 ± 0.33	23.27 ± 0.25
thromboplastin partial time (seg)	27.02 ± 1.56	23.33 ± 0.83	28.10 ± 0.70	24.77 ± 0.94	25.67 ± 1.12	24.97 ± 0.47	23.43 ± 2.28	19.40 ± 1.65	25.70 ± 0.50	22.50 ± 2.19	24.50 ± 1.89	22.05 ± 1.05
fibrinogen (mg/dL)	302.50 ± 18.42	355.50 ± 27.39	279.83 ± 11.48	424.50 ± 33.61	317.50 ± 19.96	350.67 ± 24.93	275.67 ± 32.85	266.00 ± 26.95	249.33 ± 11.76	276.67 ± 19.62	218.00 ± 27.18	324.17 ± 24.60

[a] Data are expressed as mean ± SEM ($n = 6$) in each sex group. [b] ASAT, aspartate amino transferase; ALAT, alanine amino transferase; HDL, high density lipoprotein; LDL, low density lipoprotein. [c] (i) Significantly different from control, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. (ii) Significantly different from SDF, $^{***}P < 0.001$.

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Table 6. Mean Organ Weights and Rate Body Weight/Organ in Rats after Repeated (4 Week) Oral Dose of Standard Dairy Fat and Naturally Enriched Dairy Fat at 2,000 mg kg^{-1} of Body Weight per Day[a]

	group 1: control		group 2: SDF		group 3: EDF		group 4: satellite control		group 5: satellite SDF		group 6: satellite EDF	
param	female	male	female	male	female	male	female	male	female	male	female	male
body wt (g)	226.33 ± 1.54	307.17 ± 2.82	222.50 ± 3.42	306.83 ± 5.85	229.00 ± 1.69	307.67 ± 2.03	269.67 ± 2.36	368.17 ± 3.96	270.17 ± 4.56	367.83 ± 1.99	268.17 ± 3.09	369.00 ± 5.64
increase body wt (g)	37.50 ± 1.36	101.67 ± 3.39	33.67 ± 2.76	101.33 ± 6.09	40.67 ± 1.80	101.83 ± 2.27	72.83 ± 2.23	153.33 ± 4.18	73.66 ± 4.58	151.67 ± 2.11	71.50 ± 2.77	153.33 ± 6.02
brain wt (g)	1.80 ± 0.01	1.85 ± 0.01	1.76 ± 0.02	1.83 ± 0.02	1.77 ± 0.01	1.82 ± 0.01	1.74 ± 0.06	1.90 ± 0.05	1.82 ± 0.02	1.89 ± 0.05	1.76 ± 0.06	1.85 ± 0.05
rate body wt/brain (%)	0.796 ± 0.006	0.604 ± 0.006	0.79 ± 0.02	0.599 ± 0.011	0.772 ± 0.010	0.591 ± 0.009	0.645 ± 0.028	0.516 ± 0.015	0.674 ± 0.014	0.513 ± 0.011	0.659 ± 0.021	0.503 ± 0.014
thymus wt (g)	0.427 ± 0.032	0.473 ± 0.043	0.384 ± 0.031	0.433 ± 0.008	0.37 ± 0.04	0.406 ± 0.011	0.373 ± 0.026	0.425 ± 0.028	0.416 ± 0.020	0.433 ± 0.034	0.419 ± 0.044	0.449 ± 0.019
rate body wt/thymus (%)	0.188 ± 0.013	0.154 ± 0.014	0.172 ± 0.013	0.141 ± 0.004	0.161 ± 0.018	0.132 ± 0.006	0.142 ± 0.010	0.115 ± 0.008	0.154 ± 0.008	0.118 ± 0.009	0.156 ± 0.016	0.121 ± 0.006
heart wt (g)	0.671 ± 0.016	0.940 ± 0.022	0.653 ± 0.009	0.867 ± 0.032	0.664 ± 0.014	0.915 ± 0.017	0.646 ± 0.022	0.913 ± 0.034	0.705 ± 0.030	0.888 ± 0.022	0.645 ± 0.012	0.841 ± 0.014
rate body wt/heart (%)	0.296 ± 0.006	0.306 ± 0.008	0.294 ± 0.008	0.282 ± 0.009	0.290 ± 0.004	0.297 ± 0.006	0.239 ± 0.008	0.248 ± 0.010	0.261 ± 0.011	0.241 ± 0.006	0.241 ± 0.005	0.228 ± 0.005
right lung wt (g)	0.621 ± 0.035	0.686 ± 0.043	0.565 ± 0.023	0.666 ± 0.029	0.609 ± 0.029	0.641 ± 0.032	0.585 ± 0.006	0.839 ± 0.059	0.589 ± 0.017	0.759 ± 0.027	0.590 ± 0.021	0.713 ± 0.049
rate body wt/right lung (%)	0.274 ± 0.015	0.223 ± 0.013	0.254 ± 0.009	0.217 ± 0.009	0.266 ± 0.013	0.208 ± 0.015	0.217 ± 0.003	0.228 ± 0.017	0.218 ± 0.008	0.206 ± 0.007	0.220 ± 0.008	0.193 ± 0.011
left lung wt (g)	0.325 ± 0.009	0.439 ± 0.029	0.308 ± 0.013	0.447 ± 0.033	0.364 ± 0.022	0.425 ± 0.009	0.364 ± 0.013	0.438 ± 0.018	0.358 ± 0.022	0.39 ± 0.02	0.379 ± 0.015	0.399 ± 0.019
rate body wt/left lung (%)	0.144 ± 0.004	0.143 ± 0.011	0.138 ± 0.005	0.147 ± 0.013	0.16 ± 0.01	0.138 ± 0.004	0.135 ± 0.005	0.119 ± 0.004	0.133 ± 0.010	0.105 ± 0.005	0.142 ± 0.006	0.108 ± 0.004
liver wt (g)	7.68 ± 0.10	11.47 ± 0.26	5.72 ± 1.04	10.38 ± 0.27	7.42 ± 0.24	11.00 ± 0.24	7.43 ± 0.33	12.07 ± 0.24	7.46 ± 0.15	11.90 ± 0.18	7.58 ± 0.44	11.73 ± 0.25
rate body wt/liver (%)	3.39 ± 0.03	3.73 ± 0.08	2.56 ± 0.46	3.38 ± 0.04	3.24 ± 0.12	3.58 ± 0.12	2.76 ± 0.13	3.28 ± 0.08	2.76 ± 0.07	3.23 ± 0.04	2.83 ± 0.16	3.18 ± 0.05
spleen wt (g)	0.490 ± 0.009	0.619 ± 0.005	0.520 ± 0.025	0.618 ± 0.018	0.560 ± 0.038	0.593 ± 0.012	0.505 ± 0.015	0.668 ± 0.031	0.524 ± 0.024	0.612 ± 0.044	0.517 ± 0.025	0.653 ± 0.035
rate body wt/spleen (%)	0.216 ± 0.004	0.202 ± 0.003	0.234 ± 0.012	0.201 ± 0.003	0.245 ± 0.017	0.193 ± 0.006	0.187 ± 0.006	0.182 ± 0.009	0.195 ± 0.011	0.166 ± 0.012	0.193 ± 0.009	0.177 ± 0.007
pancreas wt (g)	0.493 ± 0.097	0.479 ± 0.090	0.596 ± 0.115	0.434 ± 0.127	0.589 ± 0.096	0.486 ± 0.086	0.517 ± 0.100	0.615 ± 0.125	0.556 ± 0.134	0.755 ± 0.161	0.472 ± 0.141	0.692 ± 0.172
rate body wt/pancreas (%)	0.217 ± 0.041	0.156 ± 0.029	0.268 ± 0.052	0.139 ± 0.039	0.258 ± 0.044	0.158 ± 0.040	0.192 ± 0.038	0.168 ± 0.035	0.204 ± 0.046	0.205 ± 0.044	0.175 ± 0.052	0.189 ± 0.049
right kidney wt (g)	0.698 ± 0.032	0.958 ± 0.027	0.629 ± 0.023	0.886 ± 0.017	0.638 ± 0.018	0.923 ± 0.007	0.716 ± 0.016	0.979 ± 0.065	0.710 ± 0.026	0.971 ± 0.028	0.640 ± 0.034	0.911 ± 0.041
rate body wt/right kidney(%)	0.309 ± 0.014	0.312 ± 0.010	0.282 ± 0.008	0.289 ± 0.005	0.278 ± 0.008	0.300 ± 0.004	0.266 ± 0.007	0.266 ± 0.017	0.264 ± 0.012	0.264 ± 0.008	0.239 ± 0.013	0.246 ± 0.007
left kidney wt (g)	0.644 ± 0.011	0.928 ± 0.026	0.601 ± 0.017	0.868 ± 0.019	0.611 ± 0.015	0.900 ± 0.005	0.681 ± 0.012	0.956 ± 0.018	0.668 ± 0.021	0.944 ± 0.017	0.646 ± 0.032	0.900 ± 0.028
rate body wt/left kidney (%)	0.285 ± 0.004	0.302 ± 0.010	0.270 ± 0.005	0.283 ± 0.005	0.267 ± 0.007	0.293 ± 0.004	0.253 ± 0.006	0.260 ± 0.005	0.247 ± 0.009	0.257 ± 0.005	0.241 ± 0.012	0.244 ± 0.006
right adrenal gland wt (g)	0.039 ± 0.002	0.033 ± 0.003	0.035 ± 0.002	0.033 ± 0.003	0.035 ± 0.003	0.027 ± 0.002	0.039 ± 0.002	0.033 ± 0.003	0.039 ± 0.003	0.028 ± 0.002	0.037 ± 0.001	0.033 ± 0.003
rate body wt/right adrenal gland (%)	0.017 ± 0.001	0.011 ± 0.001	0.016 ± 0.001	0.011 ± 0.001	0.015 ± 0.001	0.009 ± 0.001	0.015 ± 0.001	0.009 ± 0.001	0.015 ± 0.001	0.008 ± 0.001	0.0137 ± 0.0003	0.009 ± 0.001
left adrenal gland wt (g)	0.036 ± 0.004	0.033 ± 0.003	0.041 ± 0.002	0.031 ± 0.001	0.038 ± 0.002	0.026 ± 0.002	0.042 ± 0.002	0.038 ± 0.001	0.041 ± 0.003	0.034 ± 0.003	0.037 ± 0.003	0.036 ± 0.003
rate body wt/left adrenal gland (%)	0.016 ± 0.002	0.011 ± 0.001	0.018 ± 0.001	0.010 ± 0.001	0.017 ± 0.001	0.009 ± 0.001	0.016 ± 0.001	0.0103 ± 0.0003	0.015 ± 0.001	0.009 ± 0.001	0.014 ± 0.001	0.010 ± 0.001
right testicle wt (g)		1.67 ± 0.03		1.60 ± 0.04		1.56 ± 0.03		1.75 ± 0.02		1.70 ± 0.01		1.71 ± 0.03
rate body wt/right testicle (%)		0.546 ± 0.013		0.522 ± 0.015		0.508 ± 0.017		0.475 ± 0.008		0.462 ± 0.004		0.465 ± 0.010
right epididyme (g)		0.650 ± 0.057		0.549 ± 0.031		0.582 ± 0.039		0.708 ± 0.032		0.64 ± 0.06		0.613 ± 0.045
rate body wt/right epididyme (%)		0.21 ± 0.02		0.180 ± 0.012		0.189 ± 0.019		0.192 ± 0.009		0.173 ± 0.016		0.165 ± 0.010

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Table 6. Continued

param	group 1: control		group 2: SDF		group 3: EDF		group 4: satellite control		group 5: satellite SDF		group 6: satellite EDF	
	female	male	female	male	female	male	female	male	female	male	female	male
left testicle wt (g)		1.61 ± 0.03		1.591 ± 0.035		1.55 ± 0.03		1.69 ± 0.02		1.67 ± 0.02		1.60 ± 0.04
rate body wt/left testicle (%)		0.525 ± 0.011		0.519 ± 0.013		0.505 ± 0.018		0.458 ± 0.005		0.454 ± 0.006		0.434 ± 0.012
left epididyme wt (g)		0.583 ± 0.048		0.593 ± 0.041		0.637 ± 0.049		0.668 ± 0.036		0.600 ± 0.062		0.590 ± 0.044
rate body wt/left epididyme (%)		0.190 ± 0.017		0.195 ± 0.016		0.207 ± 0.023		0.181 ± 0.009		0.163 ± 0.017		0.160 ± 0.011
bone marrow wt (g)	0.066 ± 0.002	0.068 ± 0.002	0.077 ± 0.006	0.069 ± 0.007	0.064 ± 0.005	0.071 ± 0.004	0.061 ± 0.007	0.070 ± 0.008	0.063 ± 0.008	0.073 ± 0.006	0.059 ± 0.009	0.061 ± 0.009
rate body wt/bone marrow (%)	0.029 ± 0.001	0.022 ± 0.001	0.035 ± 0.003	0.023 ± 0.002	0.028 ± 0.002	0.023 ± 0.002	0.023 ± 0.003	0.019 ± 0.002	0.023 ± 0.003	0.020 ± 0.002	0.022 ± 0.003	0.017 ± 0.002

[a] Data are expressed as mean ± SEM ($n = 6$) in each sex group. Differences between EDF group and control or SDF groups were not significant.

glucose/insulin metabolism in rats under normolipidemic or hyperlipidemic conditions, therefore supporting a beneficial effect on CVD risk. Its contention was that the hypolipidemic benefits of VA treatment were not caused by indirect bioconversion to RA but rather by the direct dietary supplementation of VA. This effect of VA was attributed to a reduction of hepatic *de novo* lipogenesis pathways.[26]

The present study has evaluated the potential repeated dose (4 weeks) (2,000 mg kg^{-1} of body weight, per day) oral toxicity of a dairy fat naturally enriched in VA, RA and other healthy FA as oleic and α-linolenic acids (EDF). Based on the current results, the oral dose of EDF assayed represents a level of nonobservable toxic effects (NOAEL). Overall, no EDF-related toxicity has been observed after 4-week oral repeated limit dose (2,000 mg kg^{-1} of body weight), suggesting a very low potential oral toxicity with a promising use, due to its ability to reduce triglycerides in plasma, as a dietary supplement with healthy effects in humans.

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