Influence of Jojoba Meal Supplementation on Growth and Organ Function in Rats

Marnix M. Cokelaere,*,† Johan Buyse,‡ Paul Daenens,§ Eddy Decuypere,‡ Eduard Kühn,∥ and Maurits Van Boven§

Interdisciplinary Research Center, Katholieke Universiteit Leuven Campus Kortrijk, Universitaire Campus, E. Sabbelaan, B-8500 Kortrijk, Belgium, Laboratory of Physiology and Immunology of Domestic Animals, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium, Laboratory of Toxicology, Katholieke Universiteit Leuven, Van Evenstraat 4, B-3000 Leuven, Belgium, and Laboratory of Comparative Endocrinology, Katholieke Universiteit Leuven, Naamse Straat 61, B-3000 Leuven, Belgium

Supplementation of 3% of deoiled jojoba meal in the food of weanling rats for 11 weeks caused a food intake inhibition of about 20%. This caused retarded growth in jojoba-treated rats (JJ) and rats pair-fed to them (PF). Compared to JJ, the relative growth rate of PF was higher in the first part of the experiment but similar toward the end. Differences in growth rate between JJ and PF can be explained by increased T3 concentrations in the first period of the experiment, which cause a lower food efficiency. The T3 increase could be explained by a relative protein shortage. JJ rats showed hemolymph nodes in the mesentery, with erythrophagocytosis. Minor changes in hematological parameters and increased relative liver weight were probably due to protein deficiency. Biochemical parameters significant for liver, pancreas, and kidney function were normal.

INTRODUCTION

The jojoba shrub (*Simmondsia chinensis*) produces oil-containing nuts. After oil extraction, a protein-rich meal remains which is difficult to be used as animal feed because of the presence of glycosides, with simmondsin and simmondsin 2′-ferulate as the most important components (Elliger et al., 1973, 1974). Simmondsin and its analogues decrease food intake (Booth et al., 1974; Cokelaere et al., 1992a). In adult rats, the decreased food intake causes the same emaciation as in rats pair-fed to rats treated with pure simmondsin (Cokelaere et al., 1992a,b). The mechanism by which simmondsins cause decreased food intake is unknown. Some authors consider simmondsins to be toxic, probably after metabolism by gut microorganisms (Booth et al., 1974; Verbiscar et al., 1980). According to Williams (1980), HCN could be liberated during the metabolism of simmondsin in mice, although this could not be demonstrated in rats (Cokelaere et al., 1992b). Several experiments have demonstrated growth retardation in animals eating jojoba meal supplemented food (Booth et al., 1974; Manos et al., 1986; Verbiscar et al., 1980). The following experiments were designed to discriminate between growth retardation caused by the food intake inhibition itself and possible supplemental effects of jojoba meal.

EXPERIMENTAL PROCEDURES

Thirty weanling male Wistar rats of about 80 g were used during 11 weeks in normal laboratory circumstances (22 °C, light from 8 a.m. to 8 p.m., relative humidity 40–60%, water ad libitum). The rats were divided in three groups of 10 rats each: (a) Ten control rats received normal laboratory mash ad libitum (C). (b) Ten rats received ad libitum normal laboratory mash supplemented with 3% deoiled jojoba meal (JJ). (c) Ten rats received normal laboratory mash ad libitum the first week, but afterward, they were pair-fed to JJ, receiving exactly the same daily amount of food eaten by JJ in the preceding week. The deoiled jojoba meal was obtained by Soxhlet-extraction of press-cake with *n*-hexane for 8 h.

The concentrations of simmondsin and simmondsin 2′-ferulate in the deoiled jojoba meal were 3.6 and 0.7%, respectively. These concentrations were measured by a HPLC method, following extraction of 1-g jojoba meal aliquots with methanol. The organic extract was diluted with eluens (methanol–distilled water 1:1) and injected into a 7-μm C_{18} column, 20 × 0.4 mm i.d. Pure standards of simmondsin and simmondsin 2′-ferulate were used to construct the calibration graph. Food uptake and weight evolution were measured twice a week until week 11. Relative growth rate is calculated by dividing the weight gain of each week by the weight of the previous week, and this multiplied by 100. Food conversion was calculated as gram of food needed for the gain of 1 g of body weight (BW). In week 4, blood was taken by tail clipping at 8 a.m., to determine the serum concentration of the thyroid hormones triiodothyronine (T3) and tetraiodothyronine (T4) with radio immuno assay (RIA) (antisera and standards obtained from Byk-Sangtec, Germany). (Detection limits, for T3, 0.063–8 ng/mL; for T4, 0.39–100 ng/mL. Intra-assay variability, for T3, 2%; for T4, 4%. Inter-assay variability, for T3, 8%; for T4 2.7%.) At the end of the experiment, the rats were killed by CO_2 asphyxiation. Liver, spleen, left kidney, left adrenal, left testis, prostate, and seminal vesicles were weighed and fixed in formalin. The stomach, duodenum, ileum, colon, thyroid gland, and lymph nodes from the mesentery were fixed in formalin and routinely processed for microscopic examination. Semiserial sections of each specimen were stained with hematoxylin and eosin. Blood was obtained by heart puncture to determine T3 and T4 concentrations as described above. Different biochemical and hematological parameters were measured by routine methods to investigate liver, pancreas, and kidney function. Statistical analysis of the results was done by analysis of variance (ANOVA), followed by *t*-test. Standard error of the mean (SEM) values are given in parentheses.

RESULTS AND DISCUSSION

Food intake, weight evolution, relative growth rate, and feed conversion data are summarized in Figures 1–4. Compared to C, 3% deoiled jojoba meal caused a decrease in food intake in JJ of about 20.9% (±0.9), with a maximum of 26.1% in week 2 and gradually decreasing to 16.6% in

† Interdisciplinary Research Center.
‡ Laboratory of Physiology and Immunology of Domestic Animals.
§ Laboratory of Toxicology.
∥ Laboratory of Comparative Endocrinology.

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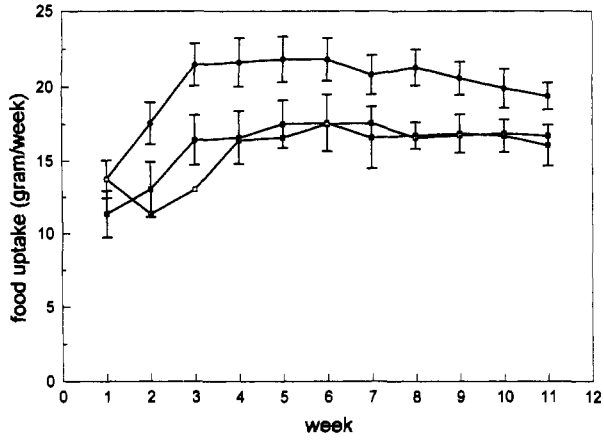

Figure 1. Food uptake (grams) during 11 weeks of experiment: (—) controls; (■) jojoba-treated animals; (□) animals pair-fed to jojoba-treated animals, mean ± SEM. No SEM are given for pair-fed animals, because they were given the same amount of food as the jojoba-treated animals 1 week before.

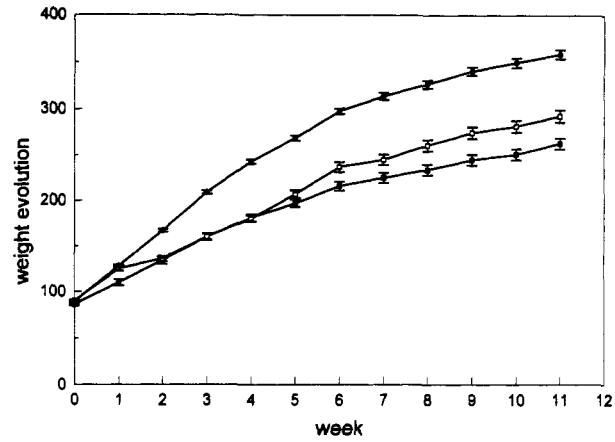

Figure 2. Body weight evolution (grams) during 11 weeks of experiment: (●) controls; (■) jojoba-treated animals; (□) animals pair-fed to jojoba-treated animals, mean ± SEM.

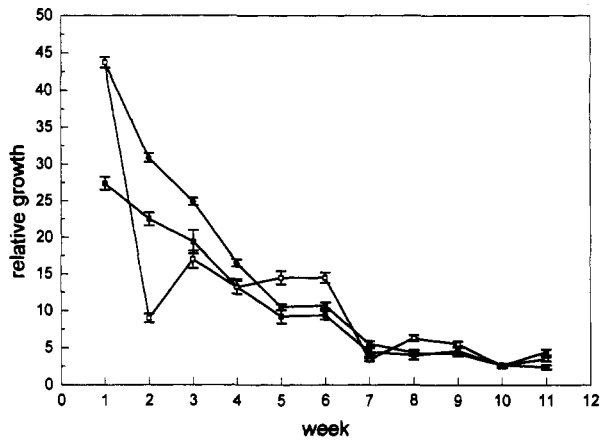

Figure 3. Relative growth rate during 11 weeks of experiment: (●) controls; (■) jojoba-treated animals; (□) animals pair-fed to jojoba-treated animals, mean ± SEM.

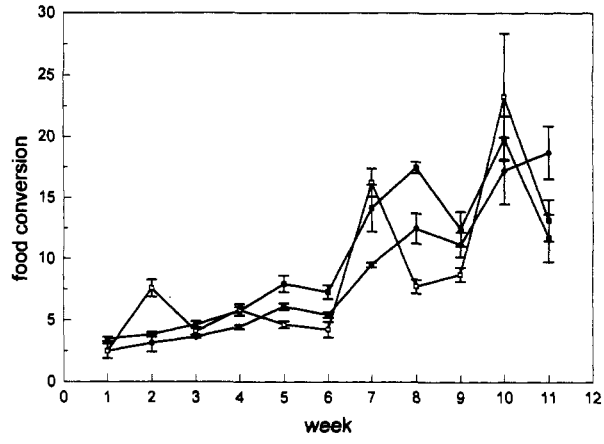

Figure 4. Food conversion during 11 weeks of experiment: (●) controls; (■) jojoba-treated animals; (□) animals pair-fed to jojoba-treated animals, mean ± SEM.

week 10, which means a decrease in food intake inhibition of 36.4% between weeks 2 and 10 (Figure 1). Although the calculated intake of simmondsin and simmondsin 2′-ferulate remained constant during the experiment (17 ± 0.5 and 3.3 ± 0.1 mg day^{-1} $animal^{-1}$ respectively), the simmondsin uptake decreased gradually from 111 to 66 mg day^{-1} (kg of BW)$^{-1}$. This was a decrease of 40.5%. The same pattern was seen for simmondsin 2′-ferulate [from 21.6 to 12.8 mg (kg of BW)$^{-1}$]. Several authors have demonstrated the food intake inhibition effect of simmondsin and to a smaller degree of simmondsin 2′-ferulate (Booth et al., 1974; Cokelaere et al., 1992a,b). Moreover, the decrease in food uptake inhibition during the experiment, expressed as percent of the original inhibition, fits very well with the decrease in simmondsin intake expressed per kilogram of BW (36.4 vs 40.5%). We conclude that the decrease in food uptake inhibition during the experiment is caused by a decrease in uptake of simmondsins, expressed per kilogram of BW. In former studies a food intake inhibition of about 27% in young rats of about 150 g of BW with 250 mg of TLC-pure simmondsin (kg of BW)$^{-1}$ was obtained (Cokelaere et al., 1992b). In the present experiments, the daily uptake of about 111 mg of simmondsin (kg of BW)$^{-1}$ in the first week, when rats weighed about 150 g, caused about 20% of food intake inhibition, the effect being relatively more pronounced than in the former experiment (Cokelaere et al., 1992b). However, in the present experiment, the food intake inhibitory effects of simmondsin 2′-ferulate and the bitter taste of the jojoba meal must also be taken into account. We therefore conclude that the difference in food intake inhibition between the present and former experiments can be explained by factors other than simmondsin, present in the deoiled jojoba meal.

The growth curves of C and JJ were diverging gradually, as did those of JJ and PF (Figure 2). At the end of the experiment, JJ rats weighed only 73.1% (±1.95%) (263 ± 7 g) of C rats (359 ± 4 g) and PF rats 81.4% (±1.95%) (293 ± 6 g). An age-related decrease in relative growth rate or growth speed was observed in all groups but with a different age-related pattern. Compared to C, relative growth of JJ was smaller during the first 4 weeks (Figure 3). Afterward, a similar level was reached with the exception of week 11, where a higher value was observed. PF rats also grew more slowly than C during weeks 2–4, but growth speed was again enhanced in the following weeks. Compared to JJ, relative growth of PF was lower the second week, similar in weeks 3, 4, 7, 10, and 11, and higher in weeks 5, 6, 8, and 9.

Food conversion was low and rather stable during the first 6 weeks but started to increase in all groups from week 7 on. The overall picture showed a higher, hence worse, food conversion ratio for the JJ group compared to the C group. Although the weekly food conversion data for the PF group was rather irregular, the overall food conversion data of this group were similar to those of the C group, whereas a higher overall food conversion was found for the JJ group compared to PF (Figure 4).

Table I summarizes the results of T3 and T4 measurements. In week 4, the T3 concentration of PF was lower than in C, while that of JJ remained at the same level as

Table I. Plasma Concentrations of T3 and T4 (Nanograms per Milliliter) of Growing Male Wistar Rats 4 and 11 Weeks after Weaning[a]

	week 4			week 11		
	T3	T4	T3/T4	T3	T4	T3/T4
C	$0.894^{a} \pm 0.004$	52.66 ± 2.71	$1.70^{a} \pm 0.053$	$0.294^{a} \pm 0.015$	$28.39^{ab} \pm 1.07$	$1.04^{a} \pm 0.046$
JJ	$0.875^{a} \pm 0.008$	54.68 ± 3.34	$1.61^{a} \pm 0.13$	$0.408^{b} \pm 0.004$	$25.03^{a} \pm 1.64$	$1.56^{b} \pm 0.17$
PF	$0.566^{b} \pm 0.004$	48.84 ± 3.49	$1.17^{b} \pm 1.09$	$0.460^{b} \pm 0.004$	$30.45^{b} \pm 1.46$	$1.50^{b} \pm 0.104$

[a] Controls (C), animals treated with 3% jojoba meal supplementation in food (JJ), and animals pair-fed to JJ (PF) (±SEM) (Anova followed by *t*-test). Values with different superscripts are significantly different.

Table II. Organ Weights in Percent of Body Weight of Male Wistar Rats at the End of the Experiment[a]

	liver	kidney	spleen	adrenal	testis	prostate	sem ves
C	$3.12^{a} \pm 0.13$	$0.21^{a} \pm 0.01$	$0.187^{a} \pm 0.006$	$0.009^{a} \pm 0.0005$	$0.489^{a} \pm 0.011$	$0.237^{a} \pm 0.010$	$0.351^{a} \pm 0.018$
JJ	$3.65^{b} \pm 0.23$	$0.29^{a} \pm 0.01$	$0.200^{a} \pm 0.008$	$0.008^{a} \pm 0.0008$	$0.553^{b} \pm 0.028$	$0.235^{a} \pm 0.017$	$0.335^{a} \pm 0.027$
PF	$3.27^{ab} \pm 0.07$	$0.30^{a} \pm 0.02$	$0.190^{a} \pm 0.007$	$0.0089^{a} \pm 0.0004$	$0.535^{b} \pm 0.030$	$0.282^{b} \pm 0.015$	$0.399^{a} \pm 0.024$

[a] Controls (C), animals treated with 3% jojoba meal supplementation in food (JJ), and animals pair-fed to JJ (PF) (±SEM) (Anova followed by *t*-test). Values with different superscripts are significantly different.

in C. T4 concentrations were similar for the three groups. The T3/T4 ratio was lower for PF than for C and JJ, which were similar. At the end of the experiment, much lower concentrations of T3 and T4 were seen in the three groups compared to those of week 4. Here, the T3 concentration of the C was lower than that of JJ and PF, the latter being similar. The T3/T4 ratio was similar for JJ and PF but higher than in C. The T4 concentration in PF was higher than in JJ but not different from C. The decrease of T3 in fasting animals is a known phenomenon (Kühn et al., 1985). This is seen in PF, eating about 20% food less than C. The T3/T4 ratio of PF also in lower than in C. This has been described in food-restricted rats (Harris et al., 1979) and could be explained by a lower 5′-monodeiodinase activity observed in fasted growing chickens (Decuypere et al., 1984). The T3 concentration and T3/T4 ratio in JJ were similar to C, in spite of the same food restriction as in PF. The higher T3 concentration and T3/T4 ratio in JJ compared to PF in week 4 could be explained by a lower protein availability caused by jojoba meal. Indeed, the presence of trypsin inhibitors in jojoba meal (Samac et al., 1981; Sanchez-Lucero et al., 1988; Storey et al., 1982) may decrease the protein digestion and cause a loss of essential amino acids (Khayambashi et al., 1966; Melmed et al., 1969). The presence of trypsin inhibitors in the jojoba-supplemented food together with the food intake inhibition may induce a relative protein shortage in actively growing rats. Protein malnutrition with concomitant food restriction causes a specific increase of T3 concentration in growing rats, compared to rats with simple food restriction (Rothwell et al., 1982; Tulp et al., 1979). This relative protein shortage may explain the worse food conversion ratio of the JJ group compared to C or PF. The higher T3 concentration should increase thermogenesis and provide an energy-balancing mechanism whereby unneeded nonprotein energy in the diet could be dissipated as heat (Tulp et al., 1979; Swick et al., 1983). The higher energy dissipation causes a decreased food efficiency in rats (Tulp et al., 1979). Similar results have been obtained in growing chicken, but some of the surplus energy is stored as body fat (Buyse et al., 1992; Keagy et al., 1987).

At the end of the experiment, lower T3 and T4 concentrations than in week 4 were seen in all rats. This can be explained by the normal evolution of T3 and T4 concentrations in young growing rats, where these concentrations increase for a few weeks before they decrease to normal adult levels (Dussault et al., 1975; Fisher et al., 1977; Tulp et al., 1979). On the other hand, the T3 concentrations of JJ and PF became equal but higher than in C. This phenomenon also has been described by Tulp et al. (1979). According to these authors, prolonged protein malnutrition in growing rats decelerates the decrease in T3 concentration compared to normally fed rats. In food-restricted rats the low T3 concentration of the early period does not decrease further. Therefore, the T3 concentration becomes lower in normally fed rats than in food-restricted rats whose T3 concentration arrived at the same level as in rats with both food restriction and protein malnutrition (Tulp et al., 1979).

It is argued that difference in growth rate between growing JJ and PF can be explained by a protein shortage caused by some components of the jojoba meal, which can cause less protein synthesis and differences in T3 concentration with concomitant differences in energy dissipation during the period of high growth rate. Trypsin inhibitors and tannins of the jojoba meal may cause the relative protein shortage. The role of simmondsin in this matter remains to be elucidated.

In previous experiments (Cokelaere et al., 1992b) it was demonstrated that TLC-pure simmondsin provokes a pronounced food intake inhibition in adult rats. The emaciation seen in these rats was not different from that seen in pair-fed rats after 4 weeks of treatment. This fits well with our present observations, where an equal T3 concentration and relative growth are seen in JJ and PF. Therefore, it is argued that the faster emaciation seen in jojoba-supplemented rats is restricted only to growing animals. However, this hypothesis needs to be proved by further experiments.

At autopsy, red mesenteric lymph nodes were seen in JJ. Other lymph nodes appeared to be normal, as did the other internal organs. The blood vessels in the mesentery of JJ were more dilated than in C or PF.

The weights of liver, left kidney, left adrenal, spleen, left testis, prostate, and seminal vesicles, expressed as percent of BW, are summarized in Table II. There were no differences for proportional spleen, kidney, adrenal, and seminal vesicles weight. Liver and testis were heavier in JJ than in C but not different from PF. Diets marginally deficient in protein induce an increase of proportional liver weight compared to pair-fed animals eating the same amount of food but with a normal protein content (Keagy et al., 1987). We assume that the increase in proportional liver weight was due to a mild protein deficiency in JJ. However, the role of the different components of the jojoba meal, including simmondsins, has to be elucidated. The influence of the jojoba meal on the testis weight was apparently due to its food restriction effect, because the increased testicular weight was seen both in JJ and in PF. The prostate was relatively heavier in PF than in C or JJ. However, the prostate weight can be influenced by the quantity of liquid it contained (Cokelaere et al., 1992b). Microscopic examination of hematoxylin and eosin stained sections showed a normal structure of liver, kidney,

adrenal, stomach, thyroid, caecum, and ileum. In the duodenal biopsies, epithelial lifting and loss of surface villous epithelial cells resulting in denudation of the superior third of some villi could be seen. A similar picture has been described following acute alcoholic intake (Millan et al., 1980) and in an early phase of reperfusion (Chiu et al., 1970). The alterations were, however, mild. Jojoba meal contains tannins (Sanchez-Lucero et al., 1988) which could possibly be responsible for the denudation of the duodenal mucosa.

In PF, the red pulp of the spleen showed a mild congestion and the presence of numerous pigmented macrophages. This was also seen in JJ. The investigation of the red lymph nodes of JJ revealed a normal architecture with normal capsule and peripheral sinus. In the cortex, normal structured B-follicles were seen with a slightly developed germinal center. In the sinuses of the medulla, numerous iron-loaded macrophages were distinguished. Some of these macrophages were also found in the center of the B-follicles and in the peripheral sinuses. They showed signs of erythrophagocytosis. Furthermore, some of the lymph nodes showed numerous red blood cells in their sinuses. The red lymph nodes appeared like hemolymph nodes, as described at the renal hilus in rats (Luk et al., 1973; Kazeem et al., 1982). It is known that a dilatation of mesenterial vessels can cause an invasion of erythrocytes in the mesenteric lymph nodes with subsequent erythrophagocytosis (Sasaki et al., 1990). Therefore, it is concluded that the red mesenteric lymph nodes of JJ were caused by the pronounced dilatation of the mesenterial vessels, with resulting invasion of erythrocytes and erythrophagocytosis. The reason for this vasodilatation remains unknown. Cholecystokinin (CCK), however, is a known dilator for intestinal vessels (Chou et al., 1977; Richardson et al., 1977). It is also a peptide with strong anorexigenic capacities (Cooper et al., 1990). CCK is liberated as a result of trypsin inhibition (Melmed et al., 1969; McLaughlin et al., 1983). Therefore, it is not excluded that the uptake of 3% jojoba meal produced an increased liberation of CCK by its trypsin inhibitors and tannins and probably by the simmondsins or their metabolites as well. This CCK could be responsible for (part of) the food intake inhibition and vasodilatation with subsequent hemolymph node formation in JJ. CCK is also known to induce a dilatation of hepatic arteries with increased blood content (Richardson et al., 1977), and it cannot be excluded that the observed increased liver weight could be induced by this phenomenon. This possibility is currently under investigation. Most authors (Booth et al., 1974; Verbiscar et al., 1980), however, dedicate the food intake inhibition to a toxic effect of some jojoba components, including simmondsins, probably after generation of a more lipophilic metabolite such as 2-hydroxy-3-methoxyphenylacetonitrile (HMPA). The generation of HMPA has never been demonstrated in the animal body and remains to be proved. On the other hand, it has been demonstrated that a maximal food intake inhibiting effect of pure simmondsin is seen during the first hour after oral intake (Cokelaere et al., 1992a). Therefore, it seems to be improbable that simmondsin has to be metabolized before it induces food intake inhibition. The exact mechanism inducing a decreased food intake remains to be elucidated.

Tables III and IV show the values of the biochemical and hematological parameters, obtained at the end of the experiment. Compared to C, JJ showed an increased mean corpuscular volume (MCV) and a decreased red cell distribution width (RDW) (coefficient of variation of RBC volume distribution: standard deviation/mean × 100). Compared to PF, there was a lower hemoglobin concentration, lower mean corpuscular hemoglobin concentration (MCHC), and a lower number of white blood cells (WBC), although these parameters are unchanged compared to C. Furthermore, there was a decrease of the uric acid concentration in JJ compared to C but not to PF and of the glutamic-oxaloacetic transaminase (GOT) and lactate dehydrogenase (LDH) concentrations in JJ compared to C and PF. The urea concentration was increased in JJ compared to PF but unchanged compared to C. No reticulocytes have been observed. The values obtained in our experiments were about the normal ranges published for normal rats (Baker, 1980; Benirschke, 1978; Harkness et al., 1989).

Table III. Hematological Parameters at the End of the Experiment[a]

	C	JJ	PF
RBC, 10^{12}/L	6.77[a] ± 0.44	6.41[a] ± 0.39	7.69[b] ± 0.33
hemoglobin, g/dL	13.02[a] ± 0.87	12.58[a] ± 0.89	15.67[b] ± 0.64
hematocrit, %	36.91 ± 2.45	36.90 ± 2.35	42.67 ± 1.89
MCV, fL	54.18[a] ± 0.74	57.5[b] ± 0.42	55.64[a] ± 0.47
MCH, pg	19.18 ± 0.50	19.75 ± 0.72	20.36 ± 0.31
MCHC, g/dL	35.36[a] ± 0.8	34.25[a] ± 1.24	36.34[b] ± 0.43
RDW, %	19.59[a] ± 1.17	13.35[b] ± 0.21	19.71[a] ± 1.37
WBC, 10^9/L	5.39[ab] ± 0.72	3.51[a] ± 0.62	7.31[b] ± 1.27
lymphocytes, % of WBC	86.26 ± 0.92	87.57 ± 1.93	86.05 ± 1.52
monocytes, % of WBC	7.71 ± 0.93	7.39 ± 0.77	7.35 ± 0.65
granulocytes, % of WBC	6 ± 1	5.04 ± 1.26	6.60 ± 1.13

[a] Controls (C), animals treated with 3% jojoba meal supplementation in food (JJ), and animals pair-fed to JJ (PF) (±SEM) (Anova followed by t-test). Values with different superscripts are significantly different.

Table IV. Biochemical Parameters at the End of the Experiment[a]

	C	JJ	PF
glucose, mg/dL	142.6 ± 5.5	144.2 ± 2.9	130.9 ± 7.6
iron, μmol/L	42.79 ± 2.5	44.9 ± 2.3	47.5 ± 1.4
uric acid, mg/dL	2.06[a] ± 0.19	1.87[b] ± 0.11	1.91[a,b] ± 0.08
urea, mg/dL	43.7[ab] ± 1.08	44.2[b] ± 1.7	40.1[a] ± 0.9
creatinine, mg/dL	0.63 ± 0.03	0.61 ± 0.22	0.65 ● 0.04
bilirubin (total), mg/dL	0.38 ± 0.01	0.38 ± 0.01	0.41 ± 0.02
protein (total), mg/dL	6.88 ± 0.06	6.80 ± 0.07	6.81 ± 0.06
GOT, units/L	136.17[a] ± 5.97	103[b] ± 5.11	142.67[a] ± 12.73
GPT, units/L	48.92 ± 5.25	38 ± 1.82	50.08 ± 9.87
LDH, units/L	3370[a] ± 210	2037[b] ± 136	3580[a] ± 503
alc phosphatase, units/L	180 ± 11	221 ± 33	160 ± 10
γ-GT, units/L	1.17 ± 0.32	1.11 ± 0.43	0.83 ± 0.24
lipase, units/L	<10–61	<10–61	<10–89
amylase, units/L	5679 ± 205	5883 ± 679	6230 ± 265
Na^+, mmol/L	143.6 ± 0.6	143.4 ± 0.3	143.1 ± 0.5
Cl^-, mmol/L	99.1 ± 0.47	99.3 ± 0.75	98.4 ± 0.62
phospate, mmol/L	2.08 ± 0.15	1.91 ± 0.14	2.25 ± 0.07
Ca, mmol/L	2.67 ± 0.2	2.66 ± 0.04	2.69 ± 0.02
HCO_3^-, mmol/L	21.97 ± 0.31	18.45 ± 0.47	19.69 ± 0.42

[a] Controls (C), animals treated with 3% jojoba meal supplementation in food (JJ), and animals pair-fed to JJ (PF) (±SEM) (Anova followed by t-test). Values with different superscripts are significantly different.

There were no indications for a malabsorption with insufficiency of folic acid or vitamin B_{12} (unchanged bilirubin and normal iron and LDH concentrations). Pernicious anemia was unlikely (low RDW, low MCHC), as was hemolytic anemia (low RDW, low LDH, normal iron and bilirubin concentrations). There were no signs of liver, pancreas, or kidney malfunction or hypothyroidism. It is known that protein deficiency can induce anemia (Delmonte et al., 1964; Reissmann, 1964). Taking into account the discussion on relative protein shortage in JJ, it is argued that the slight changes in the hematological parameters could be provoked by this phenomenon, although the increased erythrophagocytosis in the me-

senteric lymph nodes could be involved as well. Further investigations are needed to elucidate this problem.

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