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Effects of Dietary Fatty Acid and Protein Sources on Serum Protein Profiles and Liver Functional Enzyme Activities in Rats with DMBA-Induced Mammary Tumors*

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ABSTRACT

This study was conducted to examine the effects of dietary sources of fatty acids and protein on serum protein profiles, hepatic functional enzyme activities, mammary tumor incidence and tumor weight in 7,12-dimethylbenz(α)anthracene (DMBA)-treated rats. The sources of dietary fatty acids were 18n6 (rich in linoleic acid), 18n3 (rich in linolenic acid) and 22n3 (rich in DHA) ; sources of dietary protein were casein (C) and soy protein isolate (S). Mammary tumors (MTs) were chemically induced by DMBA (9 mg/100 g body weight) which was gastrically intubated at 7 weeks of age. Each experimental diet was given for the following 25 weeks. Casein-fed rats (group C) exhibited significantly higher levels of weight gain and FER (food efficiency ratio) than did group S. Group C showed higher levels of serum protein and globulin, and higher albumin/globulin (A/G) ratios than group S. Liver functional enzyme activities (GOT, GPT, ALP, LDH, γ-GT) and LDH/GOT ratios were not influenced by dietary protein. GPT activity was lower in the group given 18n3, and ALP activity was lower in the group given 18n6. The incidence and total number of MTs appeared to be lower in the group given 22n3 than in the group given 18n3 or 18n6, even though the average weight of MTs was highest in the group given 22n3. The average weight of MTs was higher in the C group than in the S group. MT incidence had a positive correlation with LDH activity and LDH/GOT ratio. The average weight of MTs had a negative correlation with serum albumin levels and A/G ratios, and a positive correlation with ALP activity. This research suggests that the measurement of serum protein profiles and liver functional enzyme activities may be utilized to monitor the development of mammary tumors.

KEY WORDS: dietary fatty acid, soy protein, mammary tumor, serum protein, liver functional enzyme activities.

INTRODUCTION

In Korea, breast cancer incidence is relatively low in comparison with Western society[1] and the mortality from breast cancer is lower than from other cancers. Breast cancer represents 18% of female cancers, ranking second after stomach cancer. The incidence of breast cancer tends to increase with age, particularly in women in their forties.[2] Due to a recent westernization of dietary habits, Koreans take more fats and animal foods than before. In Korea, natural parturition and breastfeeding have decreased and caesarean operations and infant formula feeding have increased from the past. As a result, the incidence of breast cancer has increased.[3]

The risk factors of breast cancer are related to female hormones, dietary factors, and environmental factors. Among the dietary factors, dietary fat is the most important risk factor: excessive intakes of fats, the degree of unsaturation of fatty acids, and the types of fatty acids in the n-series are of major concern for their contributions to breast cancers. An excessive intake of fats and polyunsaturated fatty acids promote carcinogenesis. The n6 fatty acids have shown promotion effects for cancer, while the n3 fatty acids have shown inhibition effects.[4,5]

Eicosapentaenoic acid (EPA, C20 : 5) and docosahexaenoic acid (DHA, C22 : 6) are well known as repressors of cancer production.[6] On the other hand, EPA and DHA are highly unsaturated and are susceptible to biomembrane damage by free radicals.[7] Linolenic acid (LNA, C18 : 3), which is also an n-3 fatty acid, has tumor inhibition effects similar to EPA and DHA,[8] and is less vulnerable to free radicals due to its lower degree of unsaturation. Perilla seed oil is rich in LNA, and is more readily available in the market than fish oil. Researchers have studied the inhibitory effects of the single sources of n3 fatty acids on cancer, but no study has been undertaken to date on the impact of degrees of unsaturation in the n3 series fatty acids on mammary tumor incidence. Some studies reported that intakes of soybean and soybean products repress cancer[9] and car-

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diovascular diseases,[10] while negative results were obtained by others.[11] In animal experiments, there are contradictory reports on soybeans and their products regarding cancer repressing effects.[12]

In Korea, in particular, there has not been sufficient research on the impacts of vegetable protein, especially soybean protein, on breast cancer promotion. In view of these facts, this study produced cancer in rats by injecting DMBA, and examined the effects of the mixed diets of n6 and n3 fatty acids on breast cancer. Serum protein profiles and liver functional enzyme activities were examined, as well as the correlations between these factors and tumor incidence.

MATERIAL AND METHODS

1. Experimental diet

All experimental groups and experimental diets are listed in Table 1. The diets are based on AIN-93G (American Institute of Nutrition-93 Growth) animal diets.[13] The compositions of fatty acids in the diets of each experimental group are shown in Table 2. As sources of carbohydrate, corn starch (produced by Poongjeon Co.) and sucrose (produced by the Jeilchedang Co.) were used. As sources of protein, casein (ALACIDTM 730, New Zealand Milk Products, 98% protein) and soy protein isolates (SUPRO Protein Technologies International TM, 98% protein) were used. In order to identify the role of protein more clearly, we used AIN-93G containing 20% protein instead of AIN-93M (Maintenance) containing 15% protein. In order to exclude the effects of soybean, we used corn oil as a source of fat instead of soybean oil.

The quantity of fat in the experimental diet was fixed at 15% (30% of the total calories). Corn oil (Dongbang), palm oil (Lotte Samgang), perilla oil (Sigolnongsan), and fish oil (Dongwon) were used as sources of n6 and n3 fatty acids. We fixed the amount of linoleic acid to a minimum level of 4.4%, because Munos *et al.*[8] suggested that beyond 4.4% of linoleic acid, there is no effect on cancer incidence. The P/M/S ratios of each experimental diet were similar.

2. Experimental animals and breast cancer induction

Three-week old female Sprague-Dawley rats were used. The rats were housed in stainless-steel wire-bottomed cages, and were maintained at a temperature of 22 ± 2℃ with a 12-hour light and dark cycle (07 : 00 – 19 : 00). The rats were fed a commercial diet (Samyang Co.) until they became 50 days old (the age when cancers are produced most frequently).[14-17] The rats were then fasted for 24 hours, following which DMBA (Sigma Chemical Co.), dissolved in corn oil, was administered intragastrically in the amounts of 9 mg/100 g body weight. The animals were then randomly assigned to six groups and were fed different experimental diets for 25 weeks. Food and tap water were provided *ad libitum*. Body weight was measur-

Table 1. Composition of the experimental diet (g/kg diet)

	18n6-C	18n6-S	18n3-C	18n3-S	22n3-C	22n3-S[1)]
Corn starch	450	450	450	450	450	450
Sucrose	100	100	100	100	100	100
Protein						
Casein	200		200		200	
Soy protein		200		200		200
Fat source						
Corn oil	130	130	51	51	65	65
Palm oil	20	20	24	24	10	10
Perilla oil			75	75		
Fish oil					75	75
DL-methionine (Sigma)	3	3	3	3	3	3
Choline chloride (Sigma)	2.5	2.5	2.5	2.5	2.5	2.5
α-Cellulose (Sigma)	50	50	50	50	50	50
Vitamin mixture[2)]	10	10	10	10	10	10
Mineral mixture[3)]	35	35	35	35	35	35
tert-Butylhydroquinone[4)]	0.014	0.014	0.014	0.014	0.014	0.014

1) 18n6 (high C18 : 2 n6 fatty acid), 18n3 (high C18 : 3 n3 fatty acid), 22n3 (high C20 : 5 and C22 : 6 n3 fatty acid), C (Casein), S (soy protein)
2) Based on AIN-93 Vitamin Mixture (ICN)
3) Based on AIN-93 Mineral Mixture (ICN)
4) *tert*-Butylhydroquinone (Sigma)

Table 2. Fatty acid composition of experimental diets

Fatty acid	18n6	18n3	22n3
C12 : 0	0.11[1)]	0.13	0.05
C14 : 0	0.26	0.24	2.08
C16 : 0	15.66	13.94	17.28
C16 : 1	0.10	0.06	8.80
C18 : 0	2.46	2.37	3.93
C18 : 1 (n9)	26.79	23.77	21.50
C18 : 2 (n6)	50.33	26.91	25.92
C18 : 3 (n3)	0.48	30.71	0.59
C20 : 0	0.41	0.24	0.20
C20 : 1	–	–	0.51
C20 : 4 (n6)	–	–	0.92
C20 : 5 (n3)	–	–	2.70
C22 : 6 (n3)	–	–	13.65
ΣSFA	18.89	16.92	23.54
ΣMUFA	26.90	23.83	30.80
ΣPUFA	50.81	57.62	43.76
P/S ratio	2.69	3.41	1.86
n6/n3	104.85	0.88	1.58
PI[2)]	51.97	88.93	156.87

1) Expressed as % distribution of fatty acid methyl esters
2) Peroxidizability index (PI) = monoenoic acid × 0.025 + dienoic acid × 1 + trienoic acid × 2 + tetraenoic acid × 4 + pentaenoic acid × 6 + hexaenoic acid × 8

ed once a week, and food intake was recorded every other day.

3. Blood and tissue sampling

After fasting for 16 hours, the rats were anesthetized with ethyl ether, and blood was collected from the heart. The serum was separated by centrifugation at 1000 × g for 15 minutes and was stored at −75℃ until analysis. The liver, kidney, spleen were removed promptly, washed with cold saline solution and weighed. All the tissues suspected to contain breast tumors were removed, and their numbers and weights were recorded; the tissues were kept in 10% neutral buffered formaldehyde solution for histological confirmation of the tumors.

4. Biochemical analysis

Levels of serum albumin and total protein were determined by using commercial kits (Shinyang Pharmaceutical Co., Korea). Globulin level was estimated from total protein minus albumin level. Glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and γ-glutamyl transpeptidase (γ-GT) activities were determined by using commercial kits (Asan Pharmaceutical Co., Korea).

5. Statistical analysis

Data were expressed as mean ± S.E. and statistical analysis was performed by ANOVA and the students' t-test using the SPSS (Statistical Package for Social Science for windows release 9.0). Pearson's correlation coefficients were used to determine the relationship between the parameters.

RESULTS AND DISCUSSION

1. Effect of dietary fat and protein sources on body weights, food intakes, and organ weights

Weight gain and food efficiency ratio (FER) are shown in Table 3. Weight gain and FER were not different among the groups taking different dietary fatty acids. This result is similar to that of another study,[17] which indicated that the different sources of fat did not influence the weights of experimental animals. On the other hand, there was a research that the group fed fish oil as a major sources of fat showed a reduction in body weights, compared to the group fed beef tallow or soybean oil.[18]

The soy protein-fed group in the present study showed a lower weight gain ($p < 0.01$) and a lower FER ($p < 0.001$) than the casein-fed group. This result is similar to other research[19] which found that animals fed soy protein and whey protein weighed less than those fed casein. When the same amount of calories were given, the animals taking vegetable protein weighed less than those taking animal protein.[20-22]

Organ indices of each experimental group are shown in Table 3. In this experiment, organ indices were not different according to the dietary fatty acid composition.

Table 3. Effect of experimental diets on weight gain, food intake, food efficiency ratio (FER) and organ indices

	Weight gain (g/day)	Food intake (g/day)	FER	Liver index (g/100 g bw)	Kidney index (g/100 g bw)	Spleen index (g/100 g bw)
Group[1)]						
18n6-C	1.04 ± 0.06^{bc}	11.64 ± 0.29^{ab}	0.089 ± 0.004^{c}	2.29 ± 0.10[2)]	0.56 ± 0.0^{ab}[3)]	0.165 ± 0.007
18n6-S	1.12 ± 0.03^{cd}	12.79 ± 0.39^{b}	0.088 ± 0.002^{bc}	2.13 ± 0.09	0.51 ± 0.01^{a}	0.149 ± 0.008
18n3-C	1.19 ± 0.05^{d}	12.42 ± 0.53^{ab}	0.096 ± 0.001^{c}	2.09 ± 0.08	0.51 ± 0.01^{a}	0.152 ± 0.008
18n3-S	0.76 ± 0.03^{a}	12.32 ± 0.39^{ab}	0.062 ± 0.003^{a}	2.40 ± 0.02	0.68 ± 0.04^{c}	0.199 ± 0.031
22n3-C	1.01 ± 0.04^{bc}	11.21 ± 0.37^{a}	0.091 ± 0.004^{c}	2.49 ± 0.02	0.58 ± 0.02^{abc}	0.167 ± 0.012
22n3-S	0.94 ± 0.06^{b}	11.95 ± 0.41^{ab}	0.078 ± 0.005^{b}	2.45 ± 0.16	0.63 ± 0.04^{bc}	0.191 ± 0.024
Significance	$p < 0.001$	$p < 0.05$	$p < 0.001$	NS[4)]	$p < 0.01$	NS
Fatty acid						
18n6	1.08 ± 0.03	12.21 ± 0.28	0.089 ± 0.002	2.21 ± 0.07	0.56 ± 0.02	0.156 ± 0.006
18n3	1.00 ± 0.08	12.38 ± 0.33	0.081 ± 0.005	2.22 ± 0.11	0.58 ± 0.03	0.172 ± 0.015
22n3	0.97 ± 0.04	11.63 ± 0.29	0.084 ± 0.003	2.47 ± 0.06	0.61 ± 0.03	0.182 ± 0.016
Significance	NS	NS	NS	NS	NS	NS
Protein						
Casein	1.09 ± 0.03^{B}[5)]	11.84 ± 0.25	0.092 ± 0.002^{B}	2.27 ± 0.07	0.55 ± 0.02^{A}	0.160 ± 0.005
Soy protein	0.95 ± 0.04^{A}	12.36 ± 0.23	0.077 ± 0.003^{A}	2.32 ± 0.09	0.61 ± 0.02^{B}	0.177 ± 0.013
Significance	$p < 0.01$	NS	$p < 0.001$	NS	$p < 0.05$	NS

1) Statistical significance was calculated by one-way ANOVA
2) Mean ± S.E.
3) Values with different superscript are significantly different at $\alpha = 0.05$ level by Duncan's multiple range test
4) Not significant
5) Values with different superscript are significantly different at $\alpha = 0.05$ level by t-test.

This result was not consistant with other studies[23] that fish oil increases the organ weight. According to Kim's[24] study, use of fish oil resulted in increased liver size compared to sesame oil and perilla oil. Jones[25] reported that use of fish oil, which contains highly unsaturated fatty acids, resulted in less accumulated body fat than saturated fatty acids and mono unsaturated fatty acid did.

The casein-fed group showed low kidney indices. Liver and spleen indices were not significantly influenced by dietary protein sources in this experiment. This result does not agree with a previous study which showed that the animals given vegetable protein had relatively lower weights of the pancreas, kidneys, and testicles, compared to the group fed animal protein sources.[26] We suggest that in our research the different types of protein did not influence the organ indices because we provided sufficient methionine which is a limiting amino acid in soy protein.

2. Effect of dietary fat and protein sources on incidences and weights of mammary tumors

The effects of dietary fatty acids and protein on the rate of mammary tumor incidence, and on the number and weight of tumors, are shown in Table 4. The 18n6 group showed the highest MT incidence, followed by the 18n3 group and then the 22n3 group. The total weight of MTs was highest in the 18n6 group, followed by the 22n3 group and then the 18n3 group. The total number of MTs was lowest in the 22n3 group, compared to the 18n3 group and the 18n6 group. The average weight of MTs was highest in the 22n3 group, followed by the 18n6 group and then the 18n3 group. These results show that the n3 group has a higher tendency to inhibit the production of tumors, but to increase the weights of individual tumors, than the n6 group; it appears that the n3 group fatty acids repress the initiation phase, thereby reducing the production of tumors, but in the promotion phase the n3 fatty acids stimulate the already produced tumors.

The casein-fed group showed a slightly higher MT incidence and higher total weight of MTs than the soy protein-fed group. However, there was no difference in the total number of MTs between the groups. Average weight of MTs was lower in the soy-fed group than the casein-fed one. Therefore, although we may conclude that soy protein does not result in reduced breast tumor incidence, the consumption of soy protein resulted in a lowered average weight of MTs. The use of soy protein seemed to result in repressed tumor growth, in comparison with casein.

Table 4. Mammary tumor incidence, weight and number of each experimental group

	MT incidence (MT-bearing rat/total rats)	Total weight of MT	Total Number of MT	Average weight of MT (Weight/ Number of MT)
Group				
18n6-C	5/ 8 (50%)	21.77	14	1.56
18n6-S	6/ 8 (75%)	61.22	24	2.55
18n3-C	5/ 8 (62.5%)	29.94	15	2.00
18n3-S	3/ 6 (50%)	20.31	11	1.85
22n3-C	3/ 6 (50%)	50.33	5	10.07
22n3-S	2/ 8 (25%)	6.79	2	3.40
Fatty acid				
18n6	11/16 (68.8%)	82.99	38	2.18
18n3	8/14 (57.1%)	50.25	26	1.93
22n3	5/14 (35.7%)	57.12	7	8.16
Protein				
Casein	13/22 (59.1%)	102.04	34	3.00
Soy protein	11/22 (50%)	88.32	37	2.39

3. Effect of dietary fat and protein sources on serum protein profiles

The total serum protein, albumin, globulin, and albumin/globulin ratio (A/G ratio) are shown in Table 5. Serum protein profiles were not influenced by dietary fatty acids. The casein-fed group showed significantly higher total serum protein and globulin levels than the soy-fed group. The A/G ratio was high in the soy protein-fed group. Serum protein profiles by mammary tumor incidence are shown in Table 7. Serum protein, albumin and globulin levels were slightly higher in tumor-bearing rats, compared to rats without tumors. Nagase's study[27] showed that the breast tumor incidence was low in animals which could not synthesize albumin genetically. As results, we suggest that excessive intakes of protein and animal protein stimulate the synthesis of serum protein level, and may increase tumor production.

4. Effect of dietary fat and protein sources on liver functional enzyme activities in the serum

GOT, GPT, ALP, LDH, γ-GT activities and the LDH/GOT ratios are shown in Table 6. The 18n3 group showed the lowest activity of GPT, and the 18n6 group showed the lowest activity of ALP. Generally speaking, the 18n3 group showed low activity levels for liver functional enzymes. The source of protein did not have any apparent influence on the activities of all GOT, GPT, ALP, LDH, γ-GT activities and LDH/GOT ratios.

The activities of liver functional enzymes related to mammary tumor incidence are shown in Table 7. LDH activity and the LDH/GOT ratio were significantly higher in MT-bearing rats. The reason that the liver functional enzyme activities are measured is to identify the metastases of tumors to the liver. There are reports that there was no change[28] in GOT and GPT activities when tumors were found, and that the activities of GOT and GPT increased in the last phase of the tumors.[29] In general, the activity of GOT increases higher than GPT activity does, and LDH activity is shown to be higher in cancer patients,[30] and the LDH/GOT ratio increases sharply in patients with malignant tumors; it is also known that γ-GT and ALP activities increase and at the same time ALP isoenzyme appears in cancer patients.[28,31,32]

In our experiment, the indices of liver functional enzyme activities in MT-bearing rats compared to the rats without tumors showed a significantly higher LDH/GOT ratio, and higher activities of LDH. The results of our experiment were consistent with the other studies mentioned above. Accordingly, we may say that the mammary tumors in our experimental animals would have spread to other regions.

Table 5. Effect of experimental diets on serum protein, albumin, globulin and A/G ratio

	Protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	A/G ratio
Group[1)]				
18n6-C	6.86 ± 0.47[2)]	2.43 ± 0.12	4.43 ± 0.39[ab3)]	0.56 ± 0.03
18n6-S	6.26 ± 0.23	2.46 ± 0.09	3.80 ± 0.21[ab]	0.66 ± 0.06
18n3-C	7.28 ± 0.50	2.52 ± 0.19	4.76 ± 0.38[b]	0.54 ± 0.04
18n3-S	6.25 ± 0.33	2.46 ± 0.09	3.67 ± 0.24[a]	0.66 ± 0.06
22n3-C	6.84 ± 0.51	2.40 ± 0.27	4.44 ± 0.33[ab]	0.55 ± 0.06
22n3-S	6.11 ± 0.14	2.31 ± 0.13	3.80 ± 0.19[ab]	0.62 ± 0.06
Significance	NS[4)]	NS	$p < 0.05$	NS
Fatty acid				
18n6	6.56 ± 0.27	2.44 ± 0.07	4.12 ± 0.23	0.61 ± 0.03
18n3	6.84 ± 0.34	2.54 ± 0.15	4.30 ± 0.28	0.62 ± 0.05
22n3	6.39 ± 0.23	2.34 ± 0.13	4.05 ± 0.19	0.59 ± 0.04
Significance	NS	NS	NS	NS
Protein				
Casein	7.02 ± 0.28[B5)]	2.45 ± 0.10	4.56 ± 0.21[B]	0.55 ± 0.02[A]
Soy protein	6.20 ± 0.13[A]	2.43 ± 0.09	3.77 ± 0.12[A]	0.66 ± 0.03[B]
Significance	$p < 0.05$	NS	$p < 0.001$	$p < 0.01$

1) Statistical significance was calculated by one-way ANOVA 2) Mean ± S.E.
3) Values with different superscript are significantly different at $\alpha = 0.05$ level by Duncan's multiple range test 4) Not significant
5) Values with different superscript are significantly different at $\alpha = 0.05$ level by t-test.

Table 6. Effect of experimental diets on liver functional enzyme activities and LDH/GOT ratio in Serum

	GOT (IU/l)	GPT (IU/l)	ALP (K-A U)	LDH (Wroblewski-U)	γ-GT (mU/ml)	LDH/GOT
Group[1)]						
18n6-C	44.46 ± 6.42[2)]	3.60 ± 0.73[b3)]	6.11 ± 0.91[a]	897.72 ± 270.16	12.03 ± 5.37	18.23 ± 3.11
18n6-S	40.99 ± 3.81	1.75 ± 0.36[a]	6.61 ± 0.49[a]	1213.99 ± 290.32	5.28 ± 1.38	27.56 ± 4.28
18n3-C	34.59 ± 3.03	1.12 ± 0.46[a]	8.42 ± 1.94[ab]	603.63 ± 148.02	5.33 ± 1.03	18.31 ± 5.14
18n3-S	37.09 ± 2.76	1.22 ± 0.51[a]	7.81 ± 1.51[ab]	528.65 ± 105.95	5.75 ± 2.63	14.09 ± 2.33
22n3-C	38.66 ± 3.49	1.81 ± 1.05[a]	13.19 ± 4.40[b]	754.44 ± 241.31	10.80 ± 3.44	18.75 ± 4.89
22n3-S	44.90 ± 5.40	1.79 ± 0.44[a]	8.17 ± 0.99[ab]	882.89 ± 319.94	11.07 ± 2.34	17.07 ± 4.67
Significance	NS[4)]	$p < 0.05$	$p < 0.05$	NS	NS	NS
Fatty acid						
18n6	42.73 ± 3.64	2.61 ± 0.45[B]	6.31 ± 0.50[A]	1055.86 ± 195.88	8.39 ± 2.65	22.90 ± 2.82
18n3	35.67 ± 2.05	1.16 ± 0.33[A]	8.16 ± 1.25[AB]	571.50 ± 93.26	5.54 ± 1.34	16.50 ± 3.06
22n3	42.50 ± 1.88	1.80 ± 0.46[AB]	10.10 ± 1.82[B]	833.48 ± 211.04	10.97 ± 1.87	17.71 ± 3.31
Significance	NS	$p < 0.05$	$p < 0.05$	NS	NS	NS
Protein						
Casein	39.32 ± 2.87	2.21 ± 0.47	8.68 ± 1.38	751.52 ± 127.47	9.55 ± 2.29	18.38 ± 2.44
Soy protein	41.35 ± 2.50	1.64 ± 0.24	7.47 ± 0.57	906.68 ± 163.87	7.71 ± 1.33	20.07 ± 2.61
Significance	NS	NS	NS	NS	NS	NS

1) Statistical significance was calculated by one-way ANOVA 2) Mean ± S.E.
3) Values with different superscript are significantly different at $\alpha = 0.05$ level by Duncan's multiple range test 4) Not significant

Table 7. Serum protein profiles and liver functional enzyme activities by mammary tumor incidence

	Mammary tumor		Significance[1]
	Non-bearing rats	Tumor-bearing rats	
Protein (mg/dl serum)	6.29 ± 0.13[2]	6.87 ± 0.27	NS[3]
Albumin (mg/dl serum)	2.37 ± 0.04	2.52 ± 0.11	NS
Globulin (mg/dl serum)	3.93 ± 0.12	4.35 ± 0.22	NS
A/G ratio	0.61 ± 0.03	0.60 ± 0.04	NS
GOT (IU/lserum)	39.22 ± 2.34	41.35 ± 2.90	NS
GPT (IU/lserum)	1.86 ± 0.41	1.96 ± 0.33	NS
ALP (K-A unit)	7.41 ± 0.66	8.62 ± 1.24	NS
LDH (Wroblewski-U)	611.47 ± 77.30[a]	1021.76 ± 174.56[b]	$p < 0.05$
γ-GT (mU/ml)	7.16 ± 1.64	10.06 ± 1.89	NS
LDH/GOT	15.41 ± 1.84[a]	22.59 ± 2.75[b]	$p < 0.05$

1) Statistical significance was calculated by t-test
2) Mean ± S.E.
3) Not significant

Table 8. Correlation coefficient between serum protein profiles, liver functional enzyme activities and mammary tumor

	Mammary tumor (MT) incidence	Number of MT	Average weight of MT
Serum			
Protein	0.276	0.215	-0.117
Albumin	0.172	0.171	-0.453**
Globulin	0.248	0.174	0.086
A/G ratio	-0.029	0.042	-0.384*
GOT	0.087	-0.068	-0.111
GPT	0.029	-0.012	-0.277
ALP	0.127	-0.048	0.847***
LDH	0.304*	0.160	-0.170
γ-GT	0.196	-0.085	0.231
LDH/GOT	0.312*	0.349*	-0.190

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

5. Correlation of serum protein profiles and liver functional enzyme activities, with mammary tumors

Correlations between serum protein profiles and liver functional enzyme activities, with mammary tumors, are shown in Table 8. Serum albumin level and A/G ratios show a negative correlation with the average weight of MTs. The lower the serum albumin level, the heavier the weights of mammary tumors became. The MT incidence had a positive correlation with LDH activity and the LDH/GOT ratio. Particularly, the LDH/GOT ratio showed a positive correlation with the number of MTs. And average weight of MTs showed a very strong positive correlation with ALP activity and a negative correlation with serum albumin level.

SUMMARY AND CONCLUSION

According to the results of this research, dietary fatty acids did not influence serum protein profiles. The casein-fed group showed significantly higher levels of serum protein and globulin than the soy protein-fed group. Liver functional enzyme activities and LDH/GOT ratio were not influenced by dietary protein. The GPT and ALP activities were significantly low in the 18n3 group.

The n3 fatty acids had more inhibitory effects than the n6 fatty acids on tumor incidence. 22n3 fatty acids suppressed tumor incidence but stimulated the growth of tumors once they were produced. This implies that the highly unsaturated fatty acids damage the antioxidation system, thereby stimulating MT growth. Therefore, in the n3 group of fatty acids, 18n3 fatty acids are recommended over 22n3 fatty acids for the suppression of the incidence and promotion of mammary tumors. Soy protein is also recommended instead of animal protein for repression of the growth of mammary tumors.

A lower serum albumin level is associated with a higher average weight of MTs. LDH activity and the LDH/GOT ratio were significantly higher in MT-bearing rats. The heavier the average weight of MTs became, the higher the ALP activity increased. In conclusion, in the case of breast tumors, the measurement of serum protein profiles and liver functional enzyme activities may be utilized to monitor the development of mammary tumors.

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