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# Antigenotoxicity of Dietary Coconut Oil

Clara Y. Lim-Sylianco,* A.P. Guevara,
and L. Sylianco-Wu
*Institute of Chemistry*
*College of Science*

## ABSTRACT

*Benzo(a)pyrene, dimethylnitrosamine, methylmethanesulfonate and tetracycline induced formation of micronucleated polychromatic erythrocytes indicating that these substances are genotoxic to bone marrow cells of the experimental mice.*

*Genotoxicity of these substances to germ cells was also observed when low fertility index and high percentage dead implants were induced in experimental mice.*

*When each genotoxin was administered to mice fed with diets containing 18% coconut oil for 23 days, the formation of micronucleated polychromatic erythrocytes was greatly reduced. Antigenotoxic activity of dietary coconut oil was very much greater than dietary soybean oil.*

*Germ cell genotoxicity of each genotoxin was also reduced when male mice fed the 18% coconut oil diet were used. When male mice treated with the genotoxin was mated with virgin females, fertility index was increased in the group fed with coconut oil diet. Percentage dead implants was reduced. The antigenotoxic activity of dietary coconut oil on germ cells far exceeds that of dietary soybean oil.*

*Dietary restriction of coconut oil diets enhanced the antigenotoxic activity of coconut oil in bone marrow cells and germs cells.*

*Among the triacylglycerols of coconut oil, trilaurin gave the best antigenotoxic activity in bone marrow cells. Trilaurin is the major triacylglycerol in coconut oil.*

* Author to whom correspondence should be addressed.

## INTRODUCTION

Genotoxins are substances that alter the structure of DNA, the genetic substance of the living cell. These substances can be genotoxic to both somatic cells and germ cells.

If the structural alterations in DNA of somatic ells are not repaired, cancer can be induced. Failure of repair of DNA alterations in germ cells can induce sterility and genetic disorders that can be transmitted from one generation to the next.

There are substances that can reduce the activity of genotoxins. These are known as antigenotoxins. Vitamins and mineral ions (Sylianco, 1985), amino acids (Sylianco and Guevara, 1989) and plant foods (Sylianco, 1991) have been shown to exhibit antigenotoxic activities.

This paper reports the studies that reveal antigenotoxic activity of dietary coconut oil.

## MATERIALS AND METHODS

Swiss Webster mice were obtained from the College of Veterinary Science, University of the Philippines, Diliman.

Fetal calf serum was purchased from Grand Island Biological Supply, Grand Island, New York.

Benzo(a)pyrene, dimethylnitrosamine, methylmethanesulfonate, tetracycline and triacylglycerols were supplied by Sigma Chemical Company, St. Louis, Missouri.

Fresh coconut oil was prepared by heating fresh coconut milk from matured coconuts.

Refined coconut oil and refined soybean oil were obtained from local grocery stores.

The composition of the diets are shown in Table 4.

The micronucleus test (Schmid, 1978) was used to study the formation of micronucleated polychromatic erythrocytes. Two administrations by oral gavage (30 hours and 6 hours before the animals were sacrificed) of each genotoxin were made to mice fed the different diets for 23 days. The animals were killed by cervical dislocation and the femur removed. The bone marrow cells were flushed out from the femur using fetal calf serum; the cell suspension centrifuged and the supernatant discarded. The cells were mounted on slides for staining with Giemsa and May-Grünwald stains. The

micronucleated polychromatic erythrocytes were counted under the microscope.

Genotoxicity to germ cells was studied using the method modified by Generoso (1980). For antigenotoxicity studied to germ cells, the same method was used. The genotoxin was administered to the male mouse at the start of the feeding period with the prepared diets. After six days the treated male was mated with 2 virgin females. Vaginal plugs were examined every morning. Plugged females were separated from the treated males. After 18 days, the pregnant females were dissected, the uterus carefully removed and the live and dead implants were recorded.

For one group, dietary coconut oil was fed for 23 days, *ad libitum*. Soybean oil diet as well as fat-free diets were also given *ad libitum*. For the group under dietary restriction, all diets were reduced by 30% (of the *ad libitum* consumption). The restricted feeding was conducted for 23 days.

The effect of triacylglycerols of coconut oil was studied using the micronucleus test of Schmid (1978).

## RESULTS AND DISCUSSION

The genotoxic activity indicated by the micronucleus test is the chromosome breaking effects of the genotoxin. When the chromatin material is fragmented by the genotoxin, some fragments will be left behind when the red blood cells expel the nucleus after telophase. The fragments left behind form micronuclei.

The chromosome breaking effects of benzo(a)pyrene, dimethylnitrosamine, methylmethanesulfonate and tetracycline are shown in Table 1. Each gave appreciable formation of micronucleated polychromatic erythrocytes indicating their genotoxic activity to bone marrow cells.

Germ cell genotoxicity of each mutagen was indicated by the low fertility index and high percentage dead implants as compared with the negative control (Table 2).

Bone marrow genotoxicity and germ cell genotoxicity of benzo(a)pyrene is expected because it is a well-known intercalating agent. It is also metabolized to a bay-region carbocation which can alkylate DNA bases. Dimethylnitrosamine is metabolized to give a carbonation which also alkylates DNA. Tetracycline can intercalate with DNA in a non-classical way. Methylmethanesulfonate is a direct alkylating agent of DNA.

The chromosome breaking potential of fresh coconut oil, refined coconut oil and soybean oil are compared (Table 3). Coconut oil, whether

freshly prepared or refined, does not possess chromosome breaking effects. Soybean oil, on the other hand, exhibits chromosome breaking potential. This could be attributed to the high content of linoleic acid, a polyunsaturated fatty acid which can readily form free radicals and peroxides which are very reactive with bases of DNA (Sevanaian et al., 1985). Unlike soybean oil, coconut oil is a saturated oil. The fat-free diet induced more chromosome breaking effects than those with either coconut oil or soybean oil. This suggests that the presence of lipids in some ways can reduce the chromosome breaking effects of genotoxins.

When the genotoxin was administered to the mice fed the coconut oil diet for 23 days, the formation of micronucleated polychromatic erythrocytes was greatly reduced. This suggests that antigenotoxic activity of coconut oil in bone marrow cells. This was not only shown with benzo(a)pyrene, dimethylnitrosamine, methylmethanesulfonate, but also with tetracycline. The antigenotoxic activity of coconut oil could be a consequence of its saturation. Saturated fatty acyl groups have been identified in an antigenotoxin isolated from fruits of *Mamordica charantia* Linn. (Guevara et al., 1990). The antigenotoxic activity of dietary coconut oil was greatly enhanced when restricted feeding was used. In restricted feeding, only 30% of the *ad libitum* consumption per day was given. Enhancement of the antigenotoxic activity of coconut oil was observed in dietary restriction (Tables 5, 6, 7 and 8). The mechanism of enhancement upon restriction of feeding has yet to be studied. There were reports of reduced formation of tumors upon caloric restriction. (Kuchevsky, 1990).

Not only bone marrow genotoxicity but also germ cell genotoxicity was reduced in groups fed the different diets with restriction as compared to those given diets *ad libitum* (Tables 10, 11, 12 and 13).

Among the triacylglycerols of coconut oil, trilaurin gave the best antigenotoxic activity in bone marrow cells (Table 14). Trilaurin is the most predominant triacylglycerol of coconut oil.

## CONCLUSION

Dietary coconut oil (fresh and refined) reduced the genotoxicity of benzo(a)pyrene, dimethylnitrosamine, methylmethanesulfonate and tetracycline. This was observed in bone marrow cells and germ cells. Dietary restriction enhanced its antigenotoxic activity. The antigenotoxic activity of dietary coconut oil surpassed that of dietary soybean oil.

Of the triacylglycerols in coconut oil, trilaurin exhibited the highest antigenotoxic activity in bone marrow cells.

## ACKNOWLEDGMENTS

Financial support from the Office of Research Coordination, University of the Philippines, is gratefully acknowledged.

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**Table 1. Chromosome breaking effects induced by Benzo(a)pyrene (BAP), Dimethylnitrosamine (DMN), Methylmethanesulfonate (MMS) and Tetracycline (TET)**

| | No. of micronucleated polychromatic erythrocytes per thousand ± S.D. |
|---|---|
| Negative control (distilled water) | 1.02 ± 0.06 |
| Benzo(a)pyrene (BAP) (39 mg/kg body weight) | 10.23 ± 0.96 |
| Dimethylnitrosamine (DMN) (10 mg/kg body weight) | 9.43 ± 0.09 |
| Methylmethanesulfonate (MMS) (12.5 mg/kg body weight) | 8.21 ± 0.97 |
| Tetracycline (TET) (55 mg/kg body weight) | 11.64 ± 1.11 |

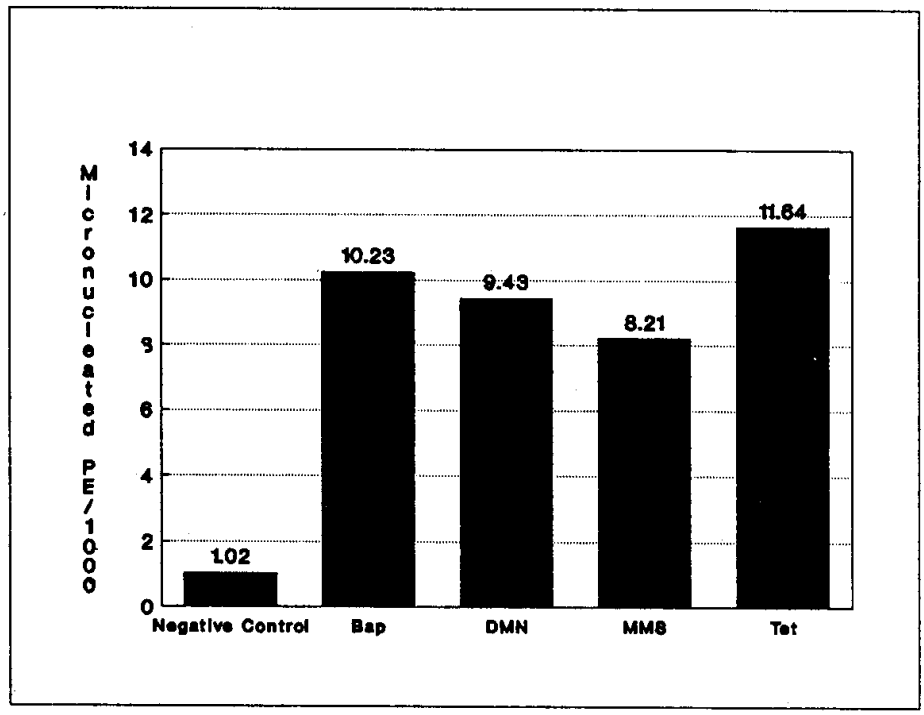


**Table 2. Germ cell genotoxicity induced by Benzo(a)pyrene (BAP), Dimethylnitrosamine (DMN), Methylmethanesulfonate (MMS) and Tetracycline (TET)**

| | *Fertility index %* | *Dead Implants %* |
|---|---|---|
| Benzo(a)pyrene | 10.9 | 79.6 |
| Dimethylnitrosamine | 20.9 | 65.4 |
| Methylmethanesulfonate | 22.1 | 86.4 |
| Tetracycline | 24.8 | 67.5 |
| Control, distilled water | 87.6 | 2.1 |

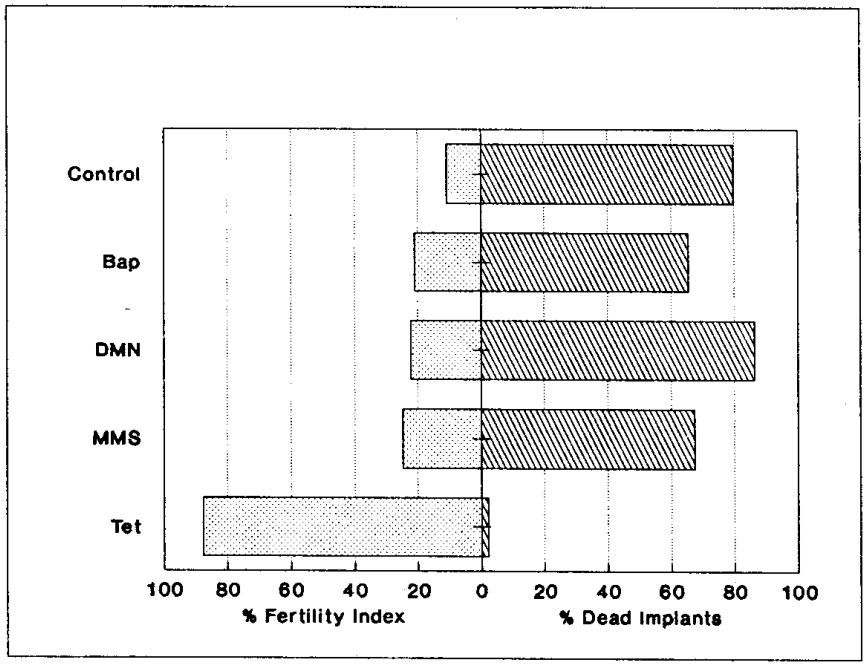


**Table 3. Chromosome breaking potential of the prepared diets and commercial diet[a]**

| | No. of micronucleated polychromatic erythrocytes per thousand ± S.D. |
|---|---|
| Commercial Diet[b] | 0.87 ± 0.56 |
| Fresh Coconut Oil Diet | 0.50 ± 0.17 |
| Refined Coconut Oil Diet 1[c] | 0.68 ± 0.14 |
| Refined Coconut Oil Diet 2[c] | 0.71 ± 0.11 |
| Soybean Oil Diet | 2.75 ± 0.67 |
| Fat-free Diet[d] | 3.31 ± 0.87 |

[a]after 23 days feeding of each diet
[b]dog pellets plus corn kernel
[c]two different brands of refined coconut oil were used
[d]alphacel was used instead of the oil component

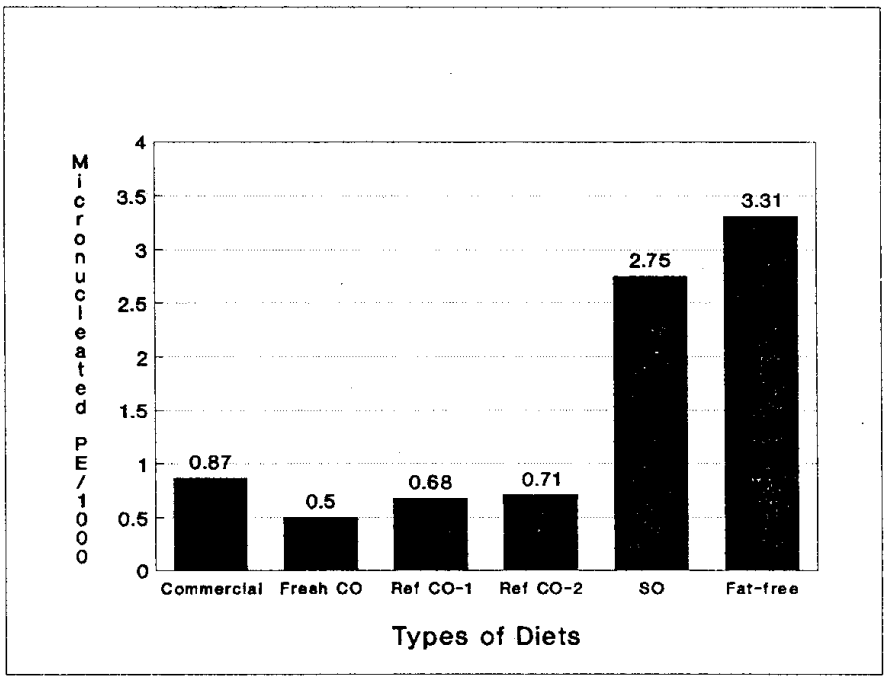


**Table 4. Composition of prepared diet**

| | |
|---|---|
| Cornstarch | 52% |
| Oil | 18% |
| Vitamin Free Casein | 25% |
| Salt Mixture | 4% |
| Vitamin Mixture | 1% |

Note: For the fat-free diet, alphacel was used instead of the oil component.

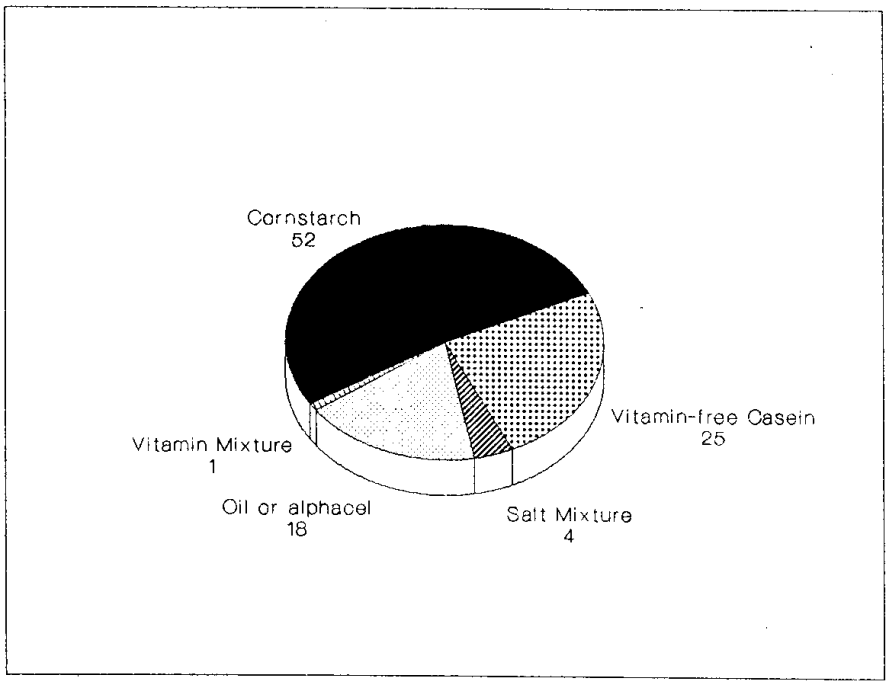


**Table 5. Effect of restricted feeding of coconut oil (CO) on bone marrow genotoxicity of Benzo(a)pyrene (Bap)**

| | No. of micronucleated polychromatic erythrocytes per thousand ± S.D. | |
|---|---|---|
| BEFORE DIETARY REGIMEN | 10.23 ± 0.96 | |
| AFTER DIETARY REGIME | *Ad libitum* | *Restricted* |
| Fresh CO diet | 1.07 ± 0.28 | 0.67 ± 0.16 |
| Refined CO diet 1[a] | 1.20 ± 0.06 | 1.12 ± 0.62 |
| Refined CO diet 2[a] | 1.33 ± 0.67 | 1.05 ± 0.56 |
| Soybean oil (SO) diet | 2.86 ± 0.56 | 2.64 ± 0.10 |
| Fat-free diet[b] | 3.67 ± 0.65 | 2.89 ± 0.66 |

[a]two different brands of refined coconut oil were used.
[b]alphacel was used instead of the oil component.

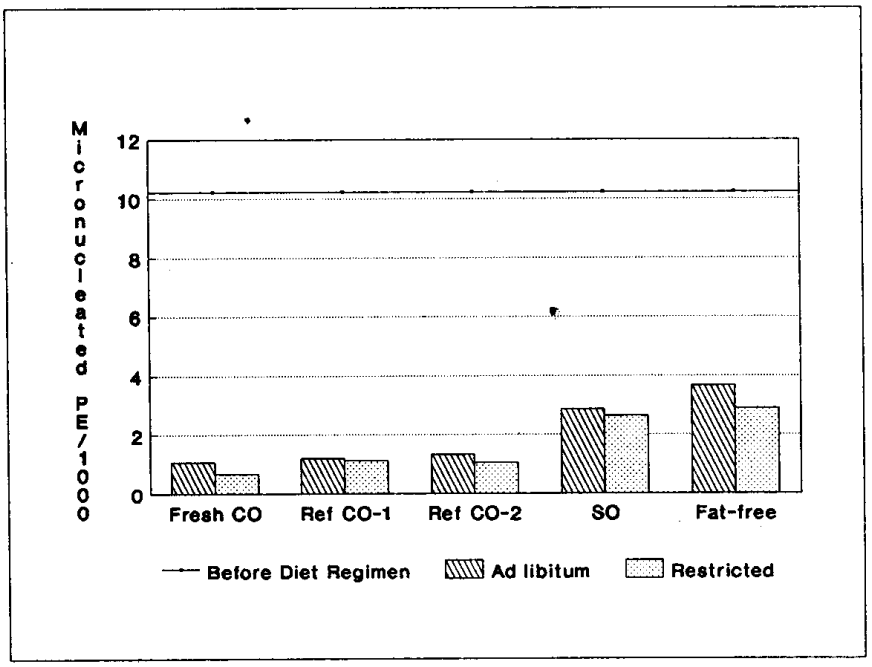


**Table 6. Effect of restricted feeding of coconut oil (CO) on bone marrow genotoxicity of dimethylnitrosamine (DMN)**

| | No. of micronucleated polychromatic erythrocytes per thousand ± S.D. | |
|---|---|---|
| BEFORE DIETARY REGIMEN | 9.43 ± 0.09 | |
| AFTER DIETARY REGIMEN | *Ad libitum* | *Restricted* |
| Fresh CO diet | 1.47 ± 0.28 | 0.87 ± 0.65 |
| Refined CO diet 1[a] | 1.50 ± 0.56 | 0.83 ± 0.54 |
| Refined CO diet 2[a] | 0.88 ± 0.09 | 0.78 ± 0.39 |
| Soybean oil (SO) diet | 4.67 ± 0.85 | 2.54 ± 0.67 |
| Fat-free diet[b] | 4.33 ± 0.78 | 3.22 ± 0.68 |

[a]two different brands of refined coconut oil were used.
[b]alphacel was used instead of the oil component.

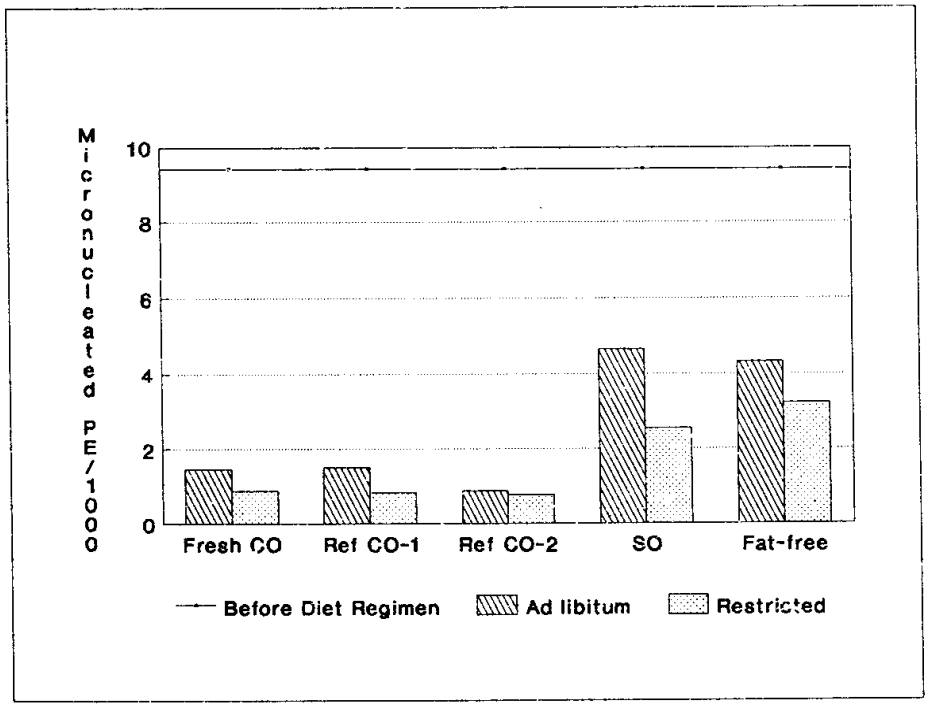


**Table 7. Effect of restricted feeding of coconut oil (CO) on bone marrow genotoxicity of Methylmethanesulfonate (MMS)**

| | No. of micronucleated polychromatic earythrocytes per thousand ± S.D. | |
|---|---|---|
| BEFORE DIETARY REGIMEN | 8.21 ± 0.97 | |
| AFTER DIETARY REGIMEN | *Ad libitum* | *Restricted* |
| Fresh CO diet | 1.07 ± 0.39 | 0.63 ± 0.28 |
| Refined CO Diet 1[a] | 1.11 ± 0.87 | 0.72 ± 0.32 |
| Refined CO Diet 2[a] | 1.45 ± 0.54 | 0.92 ± 0.21 |
| Soybean oil (SO) diet | 5.43 ± 0.96 | 5.23 ± 0.87 |
| Fat-free diet[b] | 6.78 ± 0.78 | 5.33 ± 0.98 |

[a]two different brands of refined coconut oil were used.
[b]alphacel was used instead of the oil component.

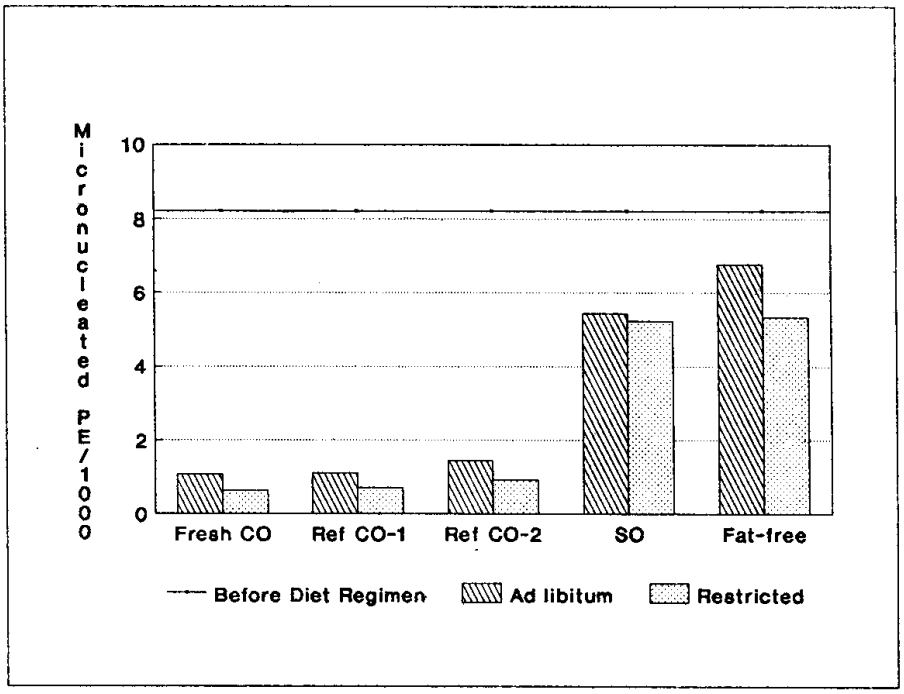


**Table 8. Effect of restricted feeding of coconut oil (CO) on bone marrow genotoxicity of Tetracycline (Tet)**

| | No. of micronucleated polychromatic erythrocytes per thousand ± S.D. | |
|---|---|---|
| BEFORE DIETARY REGIMEN | 11.64 ± 1.11 | |
| AFTER DIETARY REGIMEN | *Ad libitum* | *Restricted* |
| Fresh CO diet | 2.07 ± 0.28 | 0.33 ± 0.14 |
| Refined CO diet 1[a] | 1.87 ± 0.87 | 0.58 ± 0.13 |
| Refined CO diet 2[a] | 0.67 ± 0.03 | 0.64 ± 0.21 |
| Soybean oil (SO) diet | 4.00 ± 0.56 | 3.45 ± 0.44 |
| Fat-free diet[b] | 5.21 ± 0.97 | 4.28 ± 0.33 |

[a]two different brands of refined coconut oil were used.
[b]alphacel was used instead of the oil component.

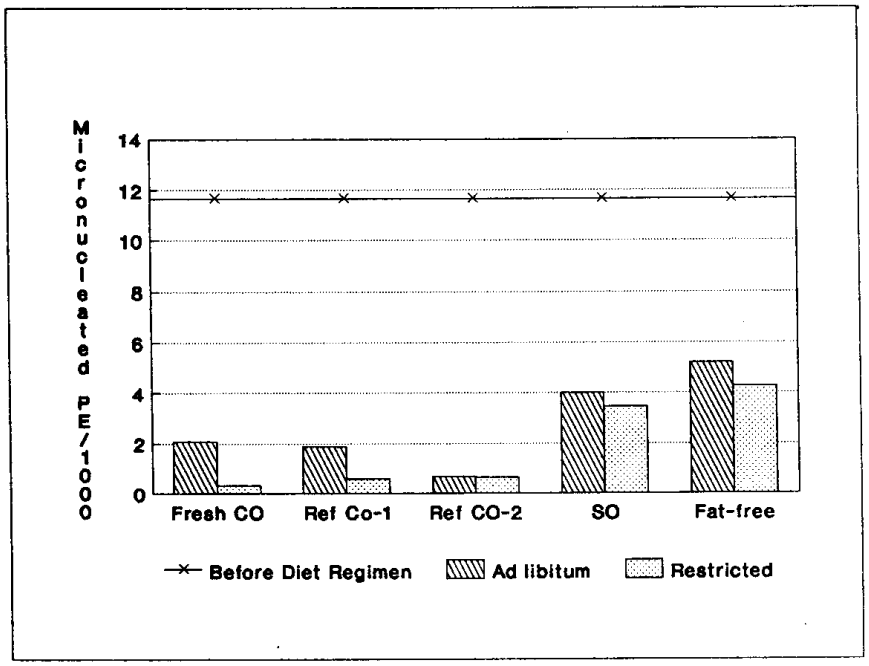


**Table 9. Fertility index and dead implants with different diets given *ad libitum* for 23 days**

| | *Fertility index %* | *Dead implants %* |
|---|---|---|
| Commercial diet[a] | 72.8 | 5.4 |
| Fresh Coconut oil diet | 84.3 | 2.7 |
| Refined coconut oil diet 1[b] | 82.6 | 2.7 |
| Refined coconut oil diet 2[b] | 79.9 | 3.1 |
| Soybean oil diet | 34.6 | 10.9 |
| Fat-free diet[c] | 29.8 | 33.2 |

[a]dog pellets plus corn kernel
[b]two different brands of refined coconut oil were used
[c]alphacel was used instead of the oil component.

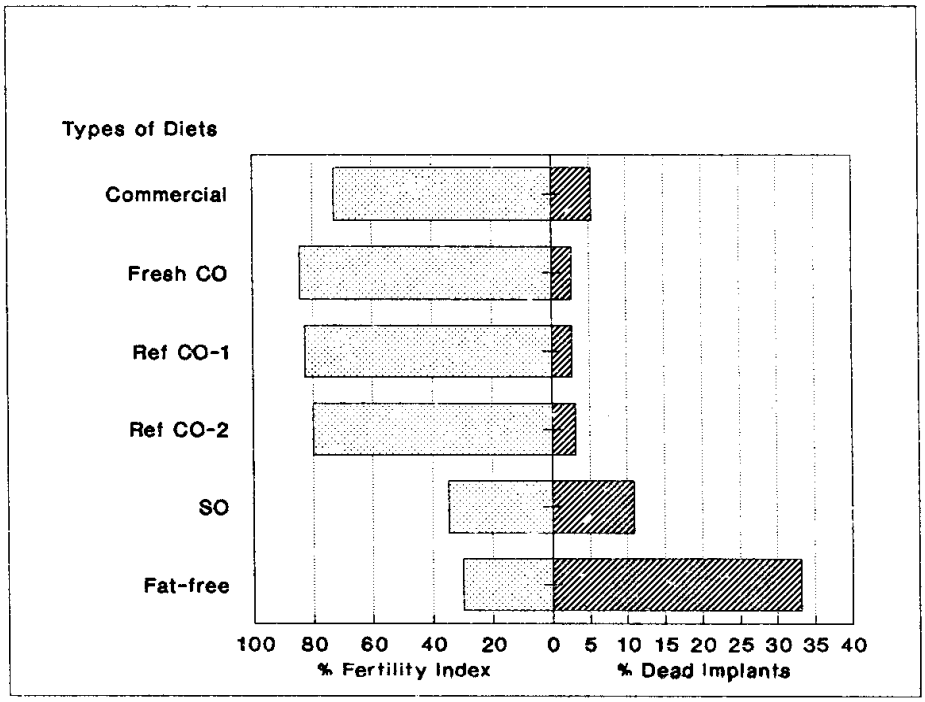
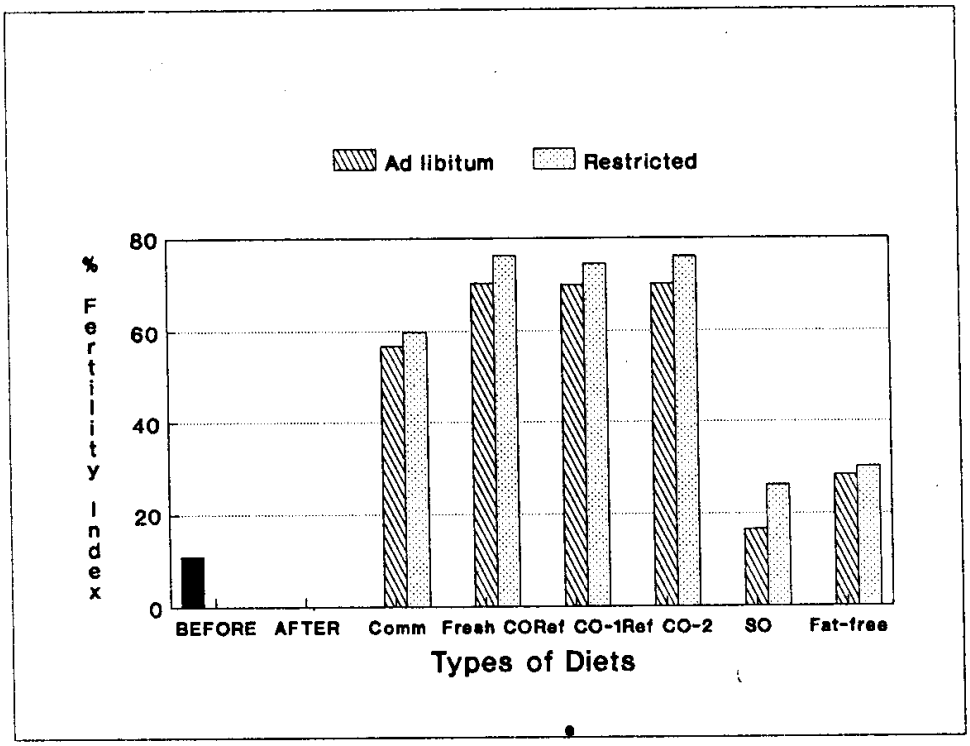


**Table 10. Effect of restricted feeding on germ cell genotoxicity of Benzo(a)pyrene (Bap)**

| | *Fertility Index %* | | *Dead Implants %* | |
|---|---|---|---|---|
| BEFORE DIETARY REGIMEN | 10.9 | | 79.6 | |
| AFTER DIETARY REGIMEN | *Ad libitum* | *Restricted* | *Ad libitum* | *Restricted* |
| Commercial Diet[a] | 56.7 | 59.8 | 10.6 | 8.1 |
| Fresh CO Diet | 70.3 | 76.1 | 2.3 | 1.1 |
| Refined CO Diet 1[b] | 69.8 | 74.3 | 2.6 | 1.1 |
| Refined CO diet 2[b] | 70.1 | 75.9 | 3.1 | 1.6 |
| SO Diet | 16.5 | 26.2 | 46.9 | 23.5 |
| Fat-free Diet[c] | 28.4 | 30.1 | 79.4 | 61.1 |

CO=coconut oil
SO=soybean oil
[a]dog pellets plus corn kernel
[b]two different brands of refined coconut oil were used
[c]alphacel was used instead of the oil component.


Ad libitum
Restricted
% Fertility Index
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Fresh CO
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SO
Fat-free
Types of Diets

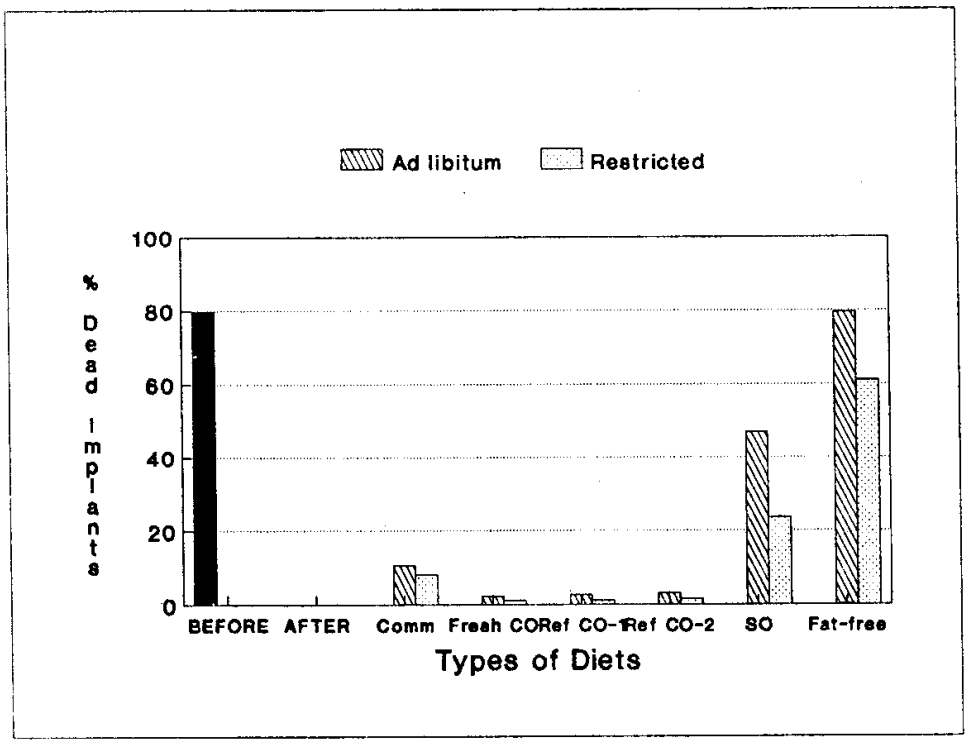
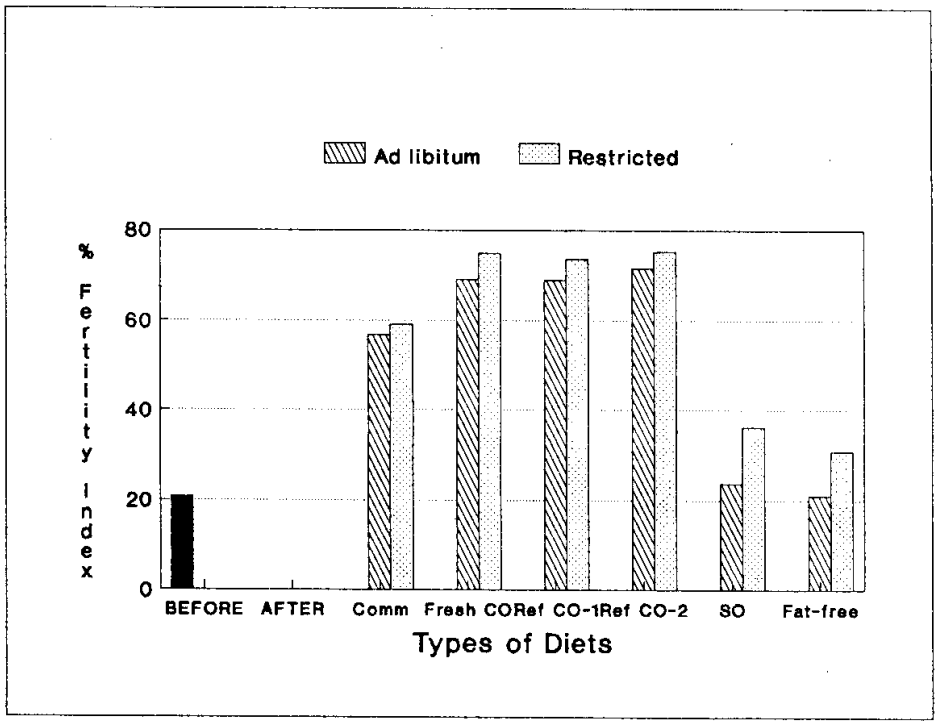

Ad libitum
Restricted
% Dead implants
100
80
60
40
20
0
BEFORE
AFTER
Comm
Fresh CO
Ref CO-1
Ref CO-2
SO
Fat-free
Types of Diets

**Table 11. Effect of restricted feeding on germ cell genotoxicity of Dimethylnitrosamine (DMN)**

| | *Fertility Index %* | | *Dead Implants %* | |
|---|---|---|---|---|
| BEFORE DIETARY REGIMEN | 20.8 | | 65.4 | |
| AFTER DIETARY REGIMEN | *Ad libitum* | *Restricted* | *Ad libitum* | *Restricted* |
| Commercial Diet[a] | 56.7 | 59.1 | 5.2 | 4.6 |
| Fresh CO Diet | 69.1 | 74.8 | 4.9 | 2.2 |
| Refined CO Diet 1[b] | 68.9 | 73.6 | 4.6 | 2.1 |
| Refined CO Diet 2[b] | 71.6 | 75.2 | 5.4 | 3.4 |
| SO Diet | 23.7 | 36.2 | 53.1 | 48.7 |
| Fat-free Diet[c] | 21.1 | 30.0 | 73.2 | 59.0 |

CO=coconut oil
SO=soybean oil
[a]dog pellets plus corn kernel
[b]two different brands of refined coconut oil were used
[c]alphacel was used instead of the oil component.


Ad libitum
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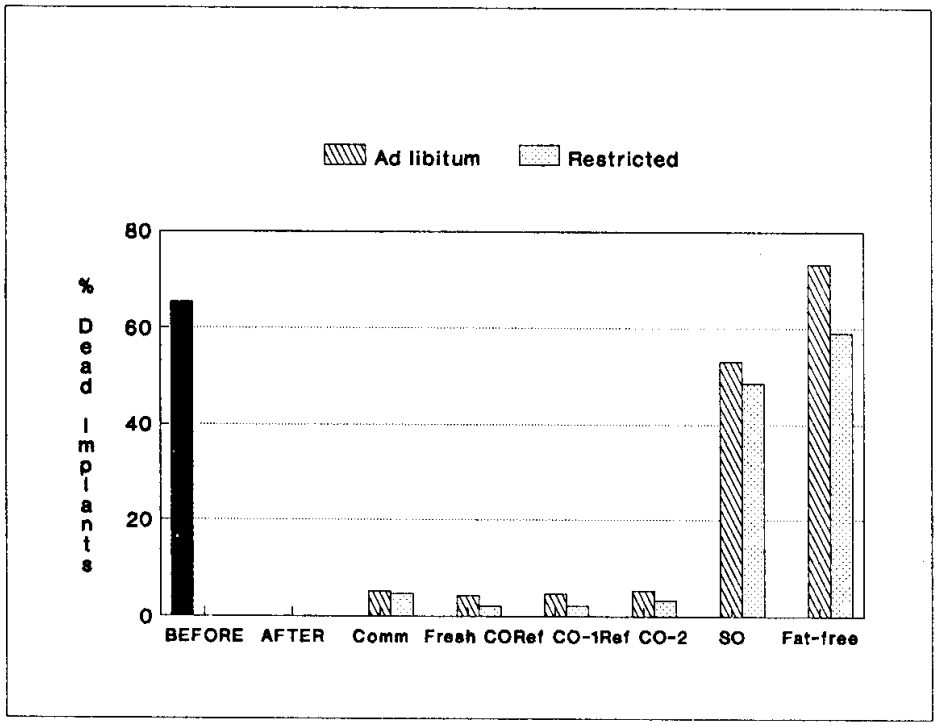
Fat-free
Types of Diets


Ad libitum
Restricted
% Dead implants
80
60
40
20
0
BEFORE
AFTER
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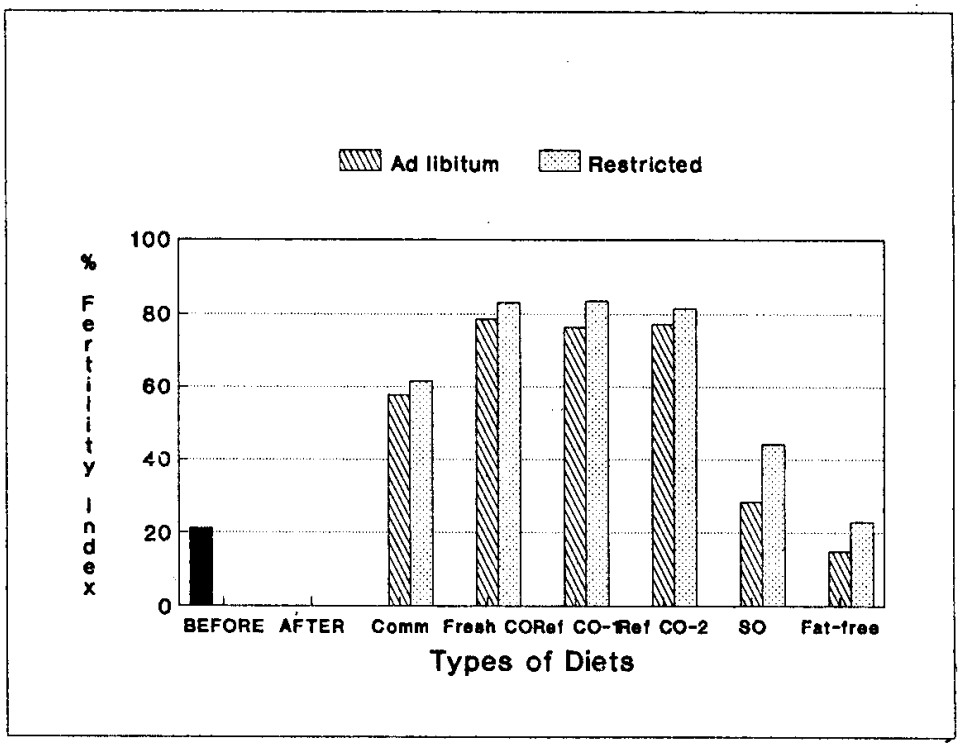
Fresh CO
Ref CO-1
Ref CO-2
SO
Fat-free

**Table 12. Effect of restricted feeding on germ cell genotoxicity of methylmethanesulfonate (MMS)**

| | *Fertility Index* | | *Dead Implants* | |
|---|---|---|---|---|
| BEFORE DIETARY REGIMEN | 21.1 | | 86.4 | |
| AFTER DIETARY REGIMEN | *Ad libitum* | *Restricted* | *Ad libitum* | *Restricted* |
| Commercial Diet[a] | 57.6 | 61.4 | 8.6 | 5.7 |
| Fresh CO Diet | 78.6 | 83.1 | 3.2 | 2.1 |
| Refined CO Diet 1[b] | 76.3 | 83.5 | 2.8 | 1.5 |
| Refined CO Diet 2[b] | 77.3 | 81.5 | 2.4 | 1.6 |
| SO Diet | 28.3 | 44.2 | 56.4 | 46.8 |
| Fat-free Diet[c] | 14.8 | 22.8 | 67.9 | 52.2 |

CO=coconut oil
SO=soybean oil
[a]dog pellets plus corn kernel
[b]two different brands of refined coconut oil were used.
[c]alphacel was used instead of the oil component.


Ad libitum
Restricted
% Fertility Index
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Fresh CO
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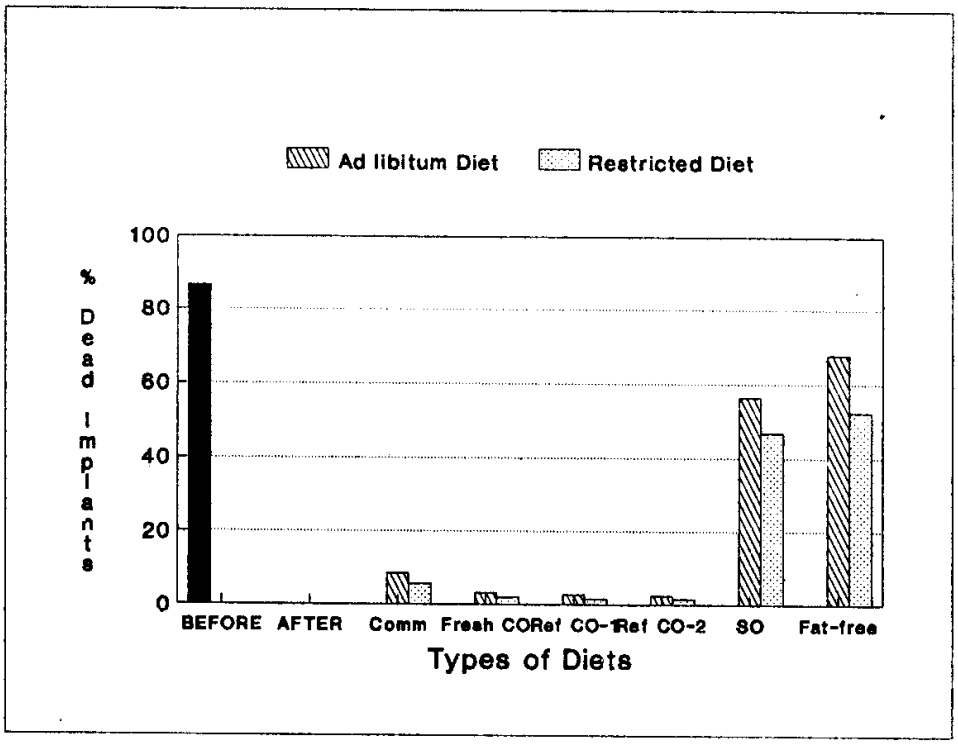
SO
Fat-free
Types of Diets


Ad libitum Diet
Restricted Diet
% Dead Implants
100
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BEFORE
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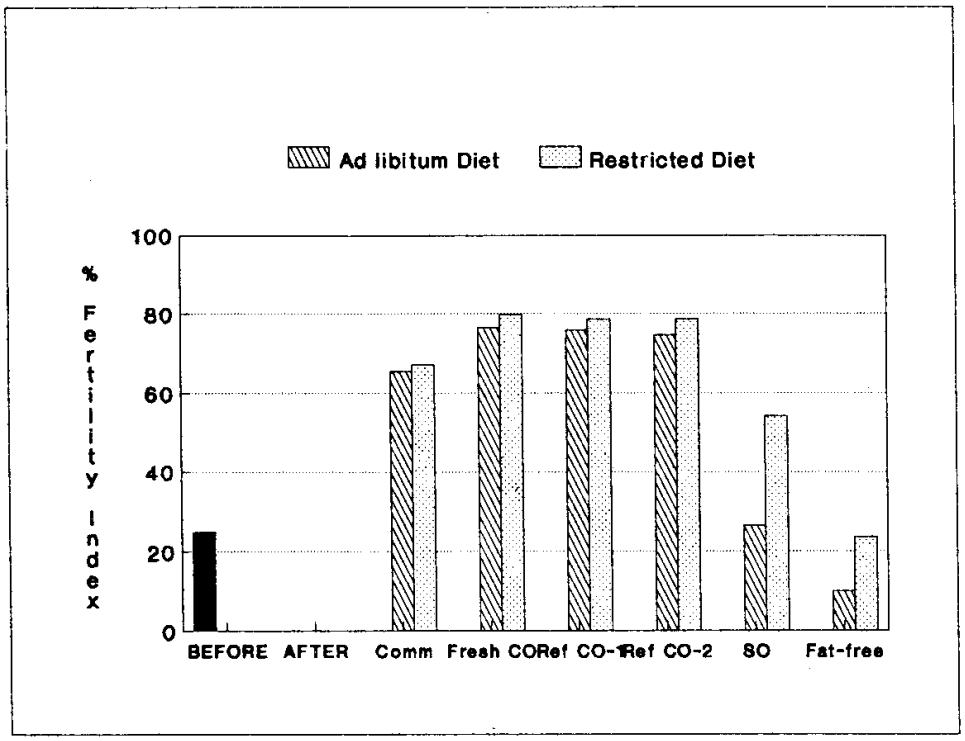
Comm
Fresh CO
Ref CO-1
Ref CO-2
SO
Fat-free
Types of Diets

**Table 13. Effect of restricted feeding on germ cell genotoxicity of tetracycline (Tet)**

| | *Fertility Index %* | | *Dead Implants %* | |
|---|---|---|---|---|
| AFTER DIETARY REGIMEN | 24.8 | | 67.5 | |
| AFTER DIETARY REGIMEN | *Ad libitum* | *Restricted* | *Ad libitum* | *Restricted* |
| Commercial Diet[a] | 65.4 | 67.1 | 8.8 | 5.7 |
| Fresh CO Diet | 76.4 | 79.8 | 5.6 | 3.4 |
| Refined CO Diet 1[b] | 75.9 | 78.6 | 6.9 | 4.2 |
| Refined CO Diet 2[b] | 74.7 | 78.6 | 10.8 | 4.2 |
| SO Diet | 26.5 | 54.2 | 45.7 | 21.6 |
| Fat-free Diet[c] | 10.1 | 23.7 | 54.1 | 45.2 |

CO=coconut oil
SO=soybean oil
[a]dog pellets plus corn kernel
[b]two different brands of refined coconut oil were used
[c]alphacel was used instead of the oil component.


Ad libitum Diet
Restricted Diet
% Fertility Index
100
80
60
40
20
0
BEFORE
AFTER
Comm
Fresh CO
Ref CO-1
Ref CO-2
SO
Fat-free

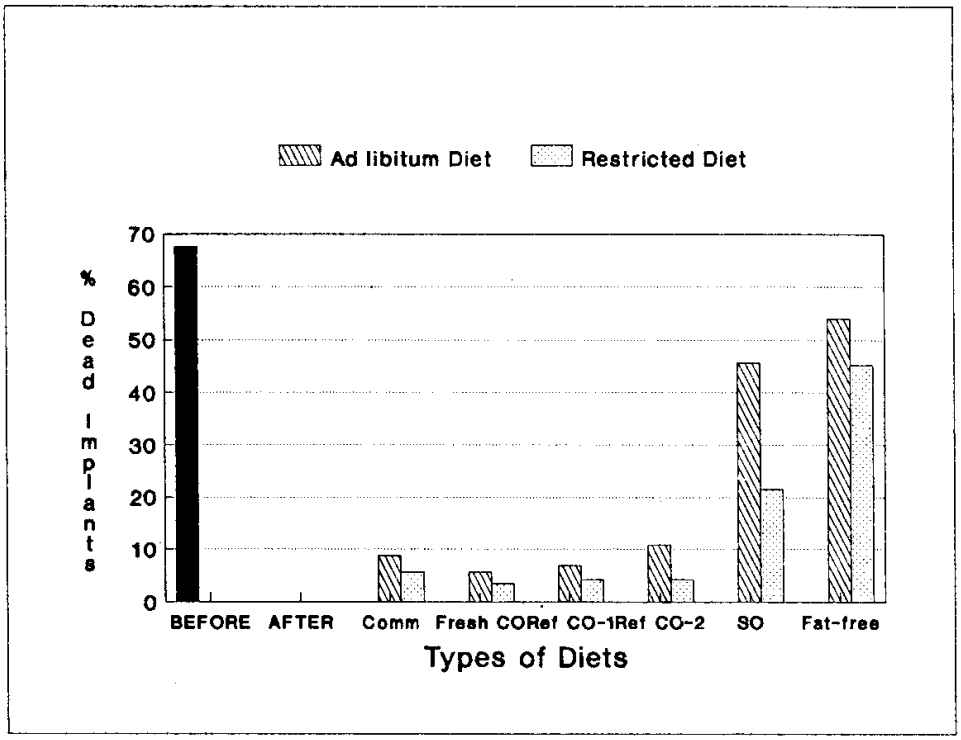
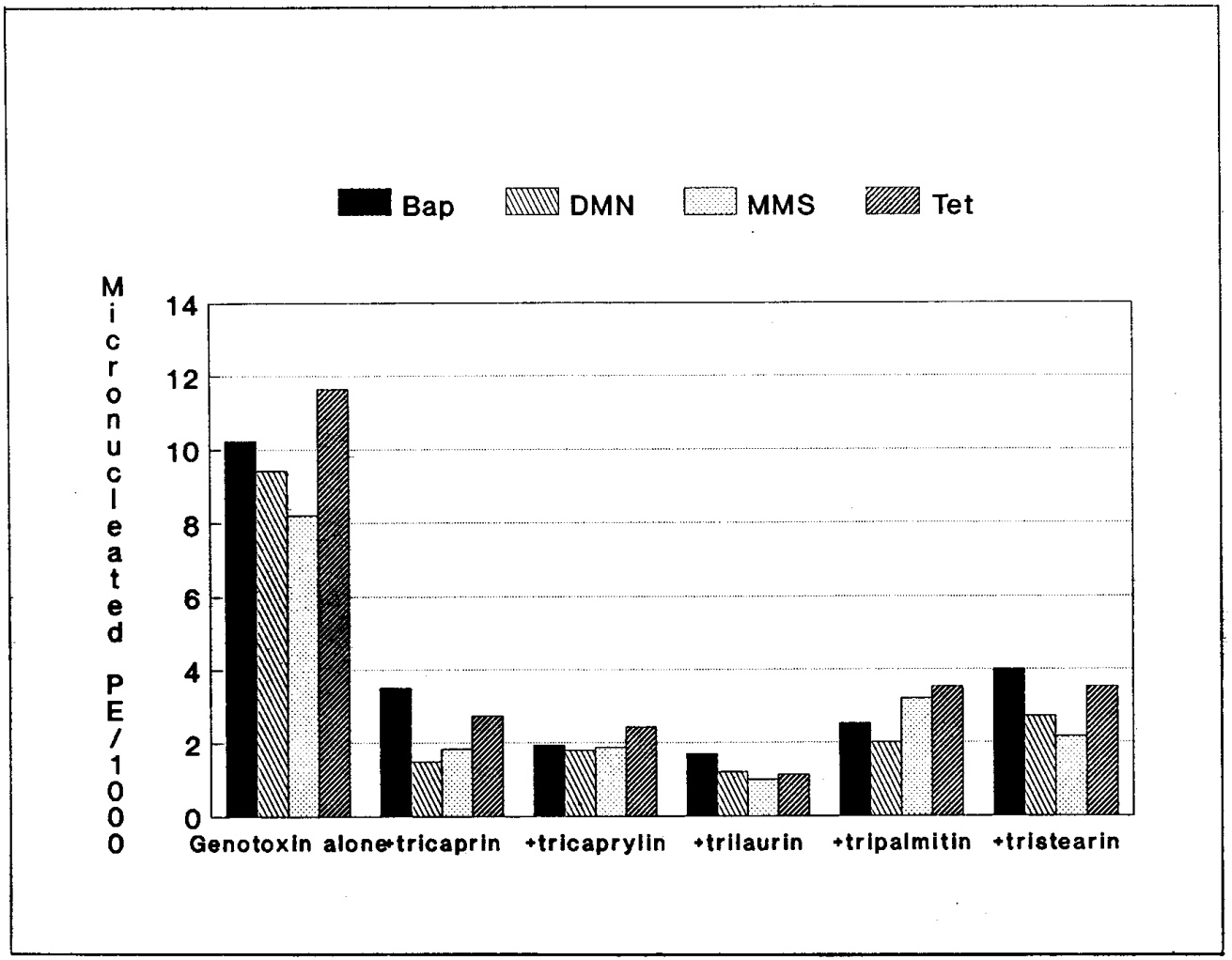

Ad libitum Diet
Restricted Diet
% Dead Implants
70
60
50
40
30
20
10
0
BEFORE
AFTER
Comm
Fresh CO
Ref CO-1
Ref CO-2
SO
Fat-free
Types of Diets

**Table 14. Effect of triacylglycerols of coconut oil on bone marrow genotoxicity of Benzo(a)pyrene (Bap), Dimethylnitrosamine (DMN), Methylmethanesulfonate (MMS) and Tetracycline (Tet)**

| | No. of micronucleated polychromatic erythrocytes per thousand ± S.D. | |
|---|---|---|
| Benzo(a)pyrene alone | 10.23 ± 0.96 | |
| plus tricaprin | | 3.50 ± 0.73 |
| plus tricaprylin | | 1.93 ± 1.07 |
| plus trilaurin | | 1.70 ± 0.02 |
| plus tripalmitin | | 2.53 ± 1.04 |
| plus tristearin | | 4.00 ± 0.41 |
| Dimethylnitrosamine alone | 9.43 ± 0.69 | |
| plus tricaprin | | 1.50 ± 1.14 |
| plus tricaprylin | | 1.79 ± 0.45 |
| plus trilaurin | | 1.20 ± 0.09 |
| plus tripalmitin | | 2.00 ± 0.27 |
| plus tristearin | | 2.71 ± 0.90 |
| Methylmethanesulfonate alone | 8.21 ± 0.97 | |
| plus tricaprin | | 1.83 ± 1.21 |
| plus tricaprylin | | 1.88 ± 0.66 |
| plus trilaurin | | 1.00 ± 0.58 |
| plus tripalmitin | | 3.20 ± 1.20 |
| plus tristearin | | 2.17 ± 0.59 |
| Tetracycline alone | 11.64 ± 1.11 | |
| plus tricaprin | | 2.73 ± 0.38 |
| plus tricaprylin | | 2.43 ± 0.87 |
| plus trilaurin | | 1.13 ± 0.24 |
| plus tripalmitin | | 3.53 ± 1.02 |
| plus tristearin | | 3.53 ± 0.69 |


Bap
DMN
MMS
Tet
Micronucleated PE/1000
14
12
10
8
6
4
2
0
Genotoxin alone
+tricaprin
+tricaprylin
+trilaurin
+tripalmitin
+tristearin