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Studies on Cytogenetic Changes in Rats Fed Biologically Treated Diets

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Abstract: Cytogenetic and biological changes in rats fed diets containing, corn and soyabean were investigated as compared with biologically treated diet using *Trichoderma* ressei F-418 fungus. Also, the protective role of yeast was studied. Forty male rats were grouped into eight groups, the first group fed on corn and served as the control group. The second group fed on corn with soyabean (used as second control).The third, fourth and fifth groups fed on biologically treated corn with fungus and soyabean, in different ratio. the six, seventh and eighth groups fed on diets containg biologically treated corn, soyabean mixture and with 0.1% active dry *saccharomyces cerevisiae* 2.0×10^{10} CFU/g in different ratio for ten weeks. Rats in all groups were weekly weighed. After ten weeks feeding , rats were sacrificed and samples from bone marrow and testicles were collected for cytogenetic analysis. Results revealed obvious increase in the weight of all rats, especially those fed on biologically treated ration. Cytogenetic analysis revealed that the used biologically treated diets did not induce any mutagenitic activity in rat bon marrow and spenmatocytes cells as compared with the control groups.. It was concluded that, biologically treated diets with *Trichoderma ressei F-418 fungus* did not have any genotoxic activity on somatic and grem cells and they can be add in the ration of farm animals without any hazardous effect.

Key words: Rats · Corn · Soyabean · *Trichoderma reesei F-418* · *Saccharomyces Cerevisiae*

INTRODUCTION

Biological treatments using fungi is the most common procedures used for improving the nutritive value of poor quality roughages by increasing the crude protein content [1,2]. Diets contain corn as a source of carbohydrates, soyabean as a source of protein treated with *Trichoderma reesei F-418* fungus and *Saccharomyces cerevisiae* which play an important role as protective agent whereas it contains about 22 and 18% glucan and mannan oligosaccharides , respectively. It also, reduced the pathogenic bacteria in the digestive tract [3- 6] .

Sims *et al*. [7] reported that, there is no dangerous mutagenic effect due to using diet treated with fungus.

The protein source in diet led to prevention of ammonia toxicity [8,9] and increased animals weight [4,10] especially in diet supplemented with yeast .

The present study aimed to investigate cytogenetic changes in somatic and germ cells together with changes in body weight of rats fed on diets biologically treated with *Trichodrema reesei F-418.*

MATERALS AND METHODS

Animal: Forty male Albino rats, weighing about 100g were obtained from a closed random-bred colony at the National Research Centre.

Diets: Eight different diets were used. The ingredients used according to NRC[11] to formulate such diets for eight animals groups are shown in table 1. The diets in eight rations groups were discerned different treatments for corn, soyabean and active dry yeast 2.0×10^{10} CFU/g as follows; untreated corn for the first group (used as first control), untreated corn with soyabean for the second group (used as second control), corn treated with fungus and soyabean in different percentages for the 3rd, 4th and 5th groups, (used as biological treatments). Finally, the groups of biological treated corn and soyabean in different percentages and yeast in fixed weight 19/kg diets for 6th , 7th and 8th groups.

Tested Constituents of Diets: Corn grains and soyabean were purchased from local market at blended case.

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Table 1: Experimental diets containg treated or untreated and protective groups

Ingredients	Untreated diets (first control) G1	Second control 2 G2	Diet treated with fungus G3	Diet treated with fungus G4	Diet treated with fungus G5	Diet treated with fungus + yeast G6	Diet treated with fungus + yeast G7	Diet treated with fungus + yeast G8
Corn	1000	750						
Treated corn			750	850	950	750	800	850
Soya		250	250	150	50	150	100	50
Yeast						100	100	100
Total weigh (g)	1000	1000	1000	1000	1000	1000	1000	1000

Microorganisms: *Trichoderma reesei F- 418 Saccharomyces cerevisiae* F-707 were obtained from Microbial Chemistry Department, N.R.C, Egypt.

Preparation of Biological Diets: *Trichoderma reesei F-418* was cultured in PDA (potato dextrose agar) medium for 3days. After that it was crushed in 15ml of sterilized water with 0.01% tween 80 and shaked for 10 minutes, fungus spores were used as an inoculum at 10% (v/w) to inoculate cooled sterilized (autoclaved at 121°c for 30 minutes) conical flask contain 10g of corn or soyabean moistend with water to be about 65% humidity. The inoculated flasks were incubated at room temperature 28°C ± 2 for 5 days. The obtained growth was used to treat more corn or soyabean at 10% (w/w), then incubated for 5 days.

At the end of incubation period, the treated corn or soyabean were air dried then used in the formula diets.

Saccharomyces cerevisiae F-707 yeast in the active dry form 2×10^{10} CFU/g was supplemented at 0.01%. [11].

Experimental Design: Rats were classified to eight similar groups five rats each. They were kept in an ambient temperature of 25±3.2°C on light/dark cycle of 12/12 hours and supplied with fresh water *ad-libitum*. The animals of each group were fed on the corresponding previously menteioned diets (Table 1), for 10 weeks. Weighs were weekly recorded. At the end of trails rats were investigated for cytogenetic studies.

Cytogenetic Studies: Chromosomal analysis: Treated and control rats were sacrified by cervical dislocation, two hours before sacrifice, rats were injected with 6mg of colchicine kg.b.w. femurs were removed and the bone marrow cells were aspirated using the saline solution. Metaphase spreads were prepared using method of Brewen and Preston [13]. Fifty metaphase spreads per Rat were analyzed, for scoring the different types of chromosomal abarretions.

The spematocytes cells were prepared according to Green wood and Nikulin [14], for meiotic chromosomal analysis.

Statistical Analysis: Data are expressed as mean ± S.E according to using chi-square test [15].

RESULTS

Genotoxic Effect: Tables 2 and 3 show the effect of feeding of rats on the present diets for ten weeks. Results indicated no abnormal cytogenetical changes due to feeding on all diets..

Somatic Chromosomal Aberrations: Chromosomal aberrations such as gab, break, deletion, fragment, centromeric attenuation and endomitosis were not significantly different between the experimental and the two control groups(Table 2).

Germ Cell Chromosomal Aberrations: Table 3 shows the incidence of detected in spermatocytes of rats fed on different experimental diets for 10 weeks. It was noted that all types of spermatocytes aberrations (X-Y) univalent, autosanal univalent and chain aberrations are not significantly differ among all groups of rats .

Changes in Body Weight: Table 4 shows the mean weight of treated groups, Statistical analysis revealed a significant ($P<0.05$) increase of weight in 3rd, 4th and 7th groups when compared to the mean weight of the first control group. Also, there is a significant($P<0.05$) increase of mean weight in 4th and 7th groups as comparing with the second control group, while weights of rats in groups 5th, 6th and 8th increased significantly ($P<0.05$) as comparing to group four.

Finally, there is significant ($P<0.05$) difference between sixth and seventh group.

Table 2: Frequency of occurrence of chromosomal aberration of somatic cells in control and fed biologically treated diets in male rats.

Treatment	Groups	Gap	Break	Deletion	Fragment	Centromeric attenuation	Endomi-tosis	Total aberration
Corn	First control G1	0.4 ± 0.24	0.4 ± 0.2	1.00 ± 0.31	0.2 ± 0.2	1.2 ± 0.2	0.8 ± 0.2	4 ± 0.39
Corn(750g) + soyabean(250g)	Second control G2	0.2 ± 0.2	0.2 ± 0.2	0.8 ± 0.2	-	0.6 ± 0.24	0.6 ± 0.24	0.24 ± 0.26
Treated corn (750g) + soya bean (250g)	G3	0.4 ± 0.24	0.2 ± 0.24	0.8 ± 0.2	0.2 ± 0.2	1.6 ± 0.24	1.2 ± 0.2	4.4 ± 0.25
Treated corn (850g) + soya bean (150g)	G4	0.4 ± 0.2	0.4 ± 0.24	1.2 ± 0.2	-	0.6 ± 0.4	1.00 ± 0.3	4.6 ± 0.26
Treated corn (950g) + soya bean (50g)	G5	0.2 ± 0.2	0.4 ± 0.24	1.00 ± 0.3	1.4 ± 0.2	0.6 ± 0.4	0.6 ± 0.24	4.2 ± 0.19
Treated corn (750g) + soya bean (150g) + yeast (100g)	G6	0.4 ± 0.2	0.2 ± 0.2	1.00 ± 0.00	-	0.8 ± 0.2	0.6 ± 0.24	3.00 ± 0.16
Treated corn (800g) + soya bean (100g) + yeats (100g)	G7	0.4 ± 0.24	0.6 ± 0.24	0.8 ± 0.2	-	1.00 ± 0.000	0.6 ± 0.24	3.4 ± 0.15
Treatment corn (850g) + soya bean (50g) + yeast (100g)	G8	0.2 ± 0.2	0.4 ± 0.24	0.6 ± 0.24	-	1.2 ± 0.2	0.6 ± 0.24	3.4 ± 0.20
L.D.S at < 0.05	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

All values were expressed as mean ± S.E

Table 3: Frequency of occurrence of chromosomal aberration of germ cells in control and fed biologically treated diets in male rats.

		Univalent			
Treatment	Groups	X - Y	Autosomal	Chain	Total aberration
Corn	First control				
G1	0.8 ± 0.2	0.8 ± 0.2	-	1.6 ± 0.2	
Corn(750g) + soyabean(250g)	Second control G2	1.00 ± 0.00	0.4 ± 0.24	-	5.00 ± 0.9
Treated corn (750g) + soya bean (250g)	G3	1.2 ± 0.2	0.8 ± 0.2	-	2.00 ± 0.27
Treated corn (850g) + soya bean (150g)	G4	1.4 ± 0.24	0.6 ± 0.2	0.2 ± 0.2	2.2 ± 0.27
Treated corn (950g) + soya bean (50g)	G5	1.00 ± 0.000	0.6 ± 0.2	0.2 ± 0.2	1.8 ± 0.17
Treated corn (750g) + soya bean (150g) + yeast (100g)	G6	0.2 ± 0.2	0.2 ± 0.2	-	1.4 ± 0.28
Treated corn (800g) + soya bean (100g) + yeats (100g)	G7	1.00 ± 0.000	0.6 ± 0.24	-	1.6 ± 0.2
Treatment corn (850g) + soya bean (50g) + yeast (100g)	G8	0.8 ± 0.2	0.8 ± 0.2	1.6 ± 0.20	1.6 ± 0.2
L.D.S at < 0.05	N.S	N.S	N.S	N.S	N.S

All values were expressed as mean ± S.E

Table 4: Mean values for weights in grams of control and biologically treated diets in male rats

Treatment	Groups	Duration of weight: First week	Second week	Third week	Fourth week	Fifth week	Sixth week	Seventh week	Eight week	Ninth week	Tenth week
Corn	First control G1	96.2±2.88	100.8±5.86	105.6±5.76	117.6±6.39	139±8.461	168.8±12.76	173.6±16.00	195.6±9.85	206.2±9.70	214^{a}±4.48
Corn + 750 g soyabean 250g	Second control G2	89.2±4.28	99.6±5.45	105.4±5.97	132.2±7.47	134.8±7.95	156±7.91	174.4±4.80	181.6±4.20	190.6a±2.48	204.8±1.8
Treated corn (750g) + soya bean (250g)	G3	101.4±4.8	118.2±7.03	113.2±5.17	125.4±5.74	116.6±5.3	156±6.6	149.2±6.86	154.6±6.66	159.8±7.35	174.8^{b}±7.61
Treated corn (850g) + soya bean (150g)	G4	107.4±2.39	100±3.70	100.8±4.5	107.6±4.37	103±4.86	139.4±5.44	129.6c±5.64	127.8±6.12	153.6^{c}±7.20	146.8^{c}±2.95
Treated corn (950g) + soya bean (50g)	G5	94±6.05	101.6±5.42	112±5.02	124±5.13	127±6.72	176.6±10.48	173.6^{a}±8.48	191.6±10.16	207.2±10.64	193.2±9.07
Treated corn (750g) + soya bean (150g) + yeast (100g)	G6	96.6±8.84	110.6±8.62	130.6±9.6	140.8±9.99	152±10.37	141.6^{a}±7.54	153.6^{a,b}±8.00	169.4±6.5	177.8±5.19	204.6±4.27
Treated corn (800g) + soya bean (100g) + yeats (100g)	G7	84.8±2.78	115.2±10.75	128.6±12.24	156.8±13.64	149.4±12.34	141d±12.28	158.2±11.49	172.2^{ad}±14.77	175.2^{ad}±13.68	165.4±15.8
Treatment corn (850g) + soya bean (50g) + yeast (100g)	G8	98.4±5.35	122±7.1	138.2±8.84	156.2±8.18	160.8±8.08	166.8±8.89	193.2^{ab}±15.39	202.6±12.09	203.8±12.91	199.2±12.70

The different letteres (a,b,c,d) in the same column are significantly different at level (P<0.05). All values were expressed as mean ± S.E.

DISCUSSION

In the present studies diet biological treated with *Trichoderam ressei F-418.* Fungs had no hazardous effect on rat chromosomes as shown by the non significant differences among groups fed biological treated and the two control groups. This result may be explained in light of that treated diets contain no hazard substances due to its treatment with fungus as well as upgrading the nutritive value of the diet with consequent increasing of its protein contents [15]. This finding agree with that obtained by Kholif [2] who showed that feeding of goats on roughages diets treated with different strains of fungi led to increase of milk production and its constituents. Also, some other studies demonstrated, that carbohydrate (corn) are well known to have a protective role against urea toxication [8,9] with a pronounced improvement of the genetic material in animals fed diet treated with th*e T. reesei F-418*. [3].

Data obtained in table 2 referred that biologically treated corn or soyabean resulted in an increased body weight of rats as compared with the rates fed on untreated diets. This improvement in body weight may be attributed to the enhancement of nutritive values through biological treatment and also due the digestive enzymes produces by the fungus in the treated diet i.e. cellulase, xylanase amylase and proteinase [16]. More obvious increases were seen when diets were supplemented with yeast and this may be due to the effect of yeast on the upgrading immunity and also due to contents of many viatamins i.e. β-complex and amino acids [4, 10, 17, 18].

In conclusion, diets contained corn as source of carbohydret, corn biologically treated with fungus, soyabean as a source of protein), yeast (as source of vitamins). Have no hazardous effect on chromosomal aberration of somatic and germ cells. Moreover, this diet improved body weight of animals, so this results encourage feeding with biologically treated diet with *T. resei F-418* in farm animals.

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