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EFFECTS OF SOYBEAN AND RICE BRAN OIL ON HEMATO-BIOCHEMICAL PARAMETERS IN MICE

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ABSTRACT

The study was conducted on "Swiss Albino" mice fed with additional supplementation of soybean oil and rice bran oil to observe the effects on hematological (total erythrocyte count and hemoglobin concentration) and biochemical parameters (total serum cholesterol, triglycerides, high density lipoproteins and uric acid). A total of 30, 6-8 weeks old mice were randomly divided into 3 equal groups (n=10) as A, B and C. Group A was considered as control (fed only commercial ration), group B was supplemented with rice bran oil and group C treated with soybean oil respectively in addition to commercial ration for 60 days. At the end of feeding trial the mice were sacrificed for analysis of hemato-biochemical parameters. The total erythrocyte count and hemoglobin concentration were increased significantly ($P<0.05$) in group B and C compared to control group A and the highest values was recorded in soybean oil group C. The total serum cholesterol, triglycerides, HDL and uric acid were increased significantly ($P<0.05$) in both rice bran oil and soybean oil group compared to control group. It is concluded that some hemato-biochemical parameters of blood in the mice are affected by rice bran and soybean oil enriched diet. Though, oils and fats are detrimental to health but to evaluate the effects of rice bran and soybean oil, further studies with more animals and some other parameters like liver function, kidney function may be conducted.

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INTRODUCTION

Edible oils are the esters of glycerol and various straight chain monocarboxylic acids, known as fatty acids rich in triglycerides, sterol, tocoferol, carotenes and pigments. Upon frying, edible oils gives rise to formation of free radicals and other harmful agents. As a consequence, the use of recycled oils which may generates high levels of cytotoxic products. It may promote the induction, development and progression of cardiovascular diseases. Cardiovascular disease is one of the major health problems in the world. It is dramatically increasing in the last 10 years (Yamada et al., 1997). Blood lipid profile determines the risk of cardiac disease. The major cause of this disease is due to high intake of cholesterol containing foods. Dietary saturated fatty acid increases blood cholesterol level, while polyunsaturated fatty acids (PUFAs) reduce blood cholesterol.

Food rich in fats and oils are responsible for various cardiovascular and liver diseases. The presence of cholesterol, saturated fatty acids (Wood et al. 1996) and trans fatty acids (Anon, 1997) in fats and oils increase the risk of coronary heart disease and atherosclerosis by increasing the blood cholesterol (Lichtenstein, 1998). Oils containing unsaturated fatty acids help to decrease the blood cholesterol as well as increase the level of LDL –cholesterol (Sugano et al. 1996, Sinha and Rahman, 1995 and Baron and Browner, 1998). Unsaturated fats tend to depress serum cholesterol while saturated fats appear to be hypercholesterolemic (Beveridge et al. 1995)**.** Ingestion of saturated fats increase serum cholesterol level as compared to unsaturated oils (Ramesha et al., 1980**)** but others (Triscari et al., 1978, Ide et al., 1978**)** reported the opposite. The hematological and biochemical constituents of blood are relatively constant. Variations occur due to age, sex, breed, climate, geographical location, nutritional status, seasons and present status of the individuals (Dukes, 1955). Any physical abnormalities or pathology is first reflected in the blood and body fluid. Hemato-biochemistry permits the study of specific pathological alteration of certain blood constituents.

The fatty acid profiles of the diet are reflected in the fatty acid pattern of plasma lipoproteins. Increasing the linolenate in the diet will increase the linolenate level of lipoprotein types and there by the polyunsaturated: saturated (p:s) ratio. Cholesterol is an important metabolic precursor for biosynthesis of steroid hormones. It acts as a special transport agent for unsaturated fatty acids (Orten and Neuhaus, 1970). Triglycerides are used for energy production, therefore two – third to three quarter of all the energy derived directly by the cells might be supplied with triglycerides (Guyton, 1971). The endogenous triglycerides which are synthesized by the liver and carries as bound to very low density lipoproteins (VLDL) is progressively removed from circulation by lipolysis which is enhanced by lipoprotein lipase enzyme attached to capillary endothelium of certain tissues. The HDL cholesterol in blood acts as reverse transport mediators accepting cholesterol from peripheral cells like arterial walls and taking to the liver, thus it is protective against ischemic heart diseases (Laurence and Bennett, 1992).

Information about hemato-biochemical parameters of commercial fed with different level of rice bran oil and soybean oil in mice under Bangladesh condition is scarce. The present study was designed to evaluate the effects of edible oils (Soybean oil and rice bran oil) on hemato-biochemical parameters in mice for observing the following objectives:

- The effect of rice bran and soybean oil on hematological parameters like total erythrocyte count (TEC) and hemoglobin (Hb) concentration of mice.
- The effect of rice bran and soybean oil on lipid profiles as total serum cholesterol, triglycerides and high density lipoprotein (HDL) in mice.
- To study the effect of rice bran and soybean oil on serum uric acid in mice.

MATERIALS AND METHODS

The experiment was conducted in the Department of Physiology, Bangladesh Agricultural University (BAU), Mymensingh. Twenty Swiss Albino mice *(Mus musculus)* aging 6-8 weeks and an average body weight of 20-25 gm were used. Before being used in the experiment, mice were adapted for 7 days in order for them to get used to the new environment. The mice were randomly divided into 3 equal groups (n=10) as A, B and C. Group A was considered control fed on standard broiler pellet (5 gm/mouse/day) and fresh drinking water. Group B and group C were fed on standard broiler pellet enriched with rice bran oil and soybean oil for a

period of 60 days. All groups were housed in a compartmentalized rectangular metallic cages (9x 11x7 cubic inches) wrapped with wire mesh. The cages were kept in well ventilated room at 28.2°C and a relative humidity of 70-80% with natural day light. The experimental laboratory was cleaned and washed at a regular interval.

Experimental diet

Commercial Broiler pellet (HI-PRO-VITE feed), rice bran oil and soybean oil were purchased from local market and were supplied as group wise. The diet was prepared on daily basis and supplied as 5 gm/mice/day and water was supplied *adlibitum* in all groups.

Group-A: Control and fed with broiler pellet

Group- B: Broiler pellet+ Rice bran oil (1000 gm : 25 ml)

Group-C: Broiler pellet + Soybean oil (1000 gm : 25 ml)

Collection of sample

On day 60, blood samples were collected by sacrificing the mice. The abdominal cavity and thoracic cavity were opened and the blood was collected by a syringe directly from the heart. About 2 ml of blood was collected in the sterile glass test tube. The blood containing syringe was placed in upright slanting position at room temperature for 6 hours. The tubes were then incubated overnight in the refrigerator (4°C). The serum samples were separated and centrifuged to get rid of unwanted blood cells where necessary. Serum samples were stored in capped tube at -20°C for until analysis.

Total Erythrocyte Count (TEC) and Hemoglobin

The counting and calculation of RBC were performed as per methods indicated by Ghai (1999) and the result was expressed in million/mm^3. The hemoglobin estimation was determined as per method described by Ghai (1999) and the result was expressed in gm/dl.

Biochemical studies

The biochemical parameters of serum like Total Cholesterol, Triglyceride, HDL and uric acid were estimated.

Determination of total serum cholesterol and triglycerides

The cholesterol was determined using the procedure described by Trinder (1969). The result was expressed in mg/dl. The triglyceride of blood serum was determined by Biochemistry Humalyzer-3000 (Human type, Germany) according to the technique described by Trinder (1969). The result was expressed in mg/dl.

Determination of HDL

The concentration of serum HDL cholesterol was estimated with the incubation of supernatant of serum sample and reagent mixture in Reflectron® Humalyzer 3000 (Human type, Germany) and then placing the mixture in the Reflection® against the blank reagent. The result was expressed in mg/dl.

Uric acid

Both reagent and sample brought at room temperature and mixed 1.0 ml reagent with 25µl sample in test tube. Let waited for 10 minutes and poured the mixture in cuvette. The spectrophotometer was fixed at 500 nm and recorded the reading. Used reagent contains different types of enzymes. Uric acid is oxidized by uricase to allantoin with the formation of hydrogen peroxide. This hydrogen peroxide oxidized 4-aminopyrine to quinoneimine which is proportional to the concentration of uric acid in the sample. The reading was divided by standard value and result was multiplied by 6mg/dl. So the result was expressed as mg/dl.

Statistical analysis

The hematological and biochemical parameters of mice corresponding to the different levels of rice bran oil and soybean oil supplementation are compared and performing by Student t' Test (SPSS, 16 version).

RESULTS AND DISCUSSION

The experiment was conducted to study the effects of soybean and rice bran oil on the hematological (TEC, Hb) and biochemical parameters (total Serum Cholesterol, triglyceride, HDL, uric acid) in mice.

Total erythrocyte count (million/cubic millimeter)

Total erythrocyte count is presented in Table 1 and Figure 1. The highest TEC was recorded in soybean oil group C (8.55 ± 0.23 million/cu.mm.) followed by rice bran group B (7.95 ± 0.24 million/cu.mm.) and control group A (7.75 ± 0.12 million/cu.mm.). The soybean oil treated group statistically significant ($P<0.05$) from the other two groups. The present finding is not consistent with Ahmed et al. (1994) who reported that TEC is not affected by dietary treatment in Japanese quails. The result of total erythrocyte count is also partially in agreement with Aletor et al. (1991) who reported that of TEC differ significantly among the different treated groups in broiler chickens.

Hemoglobin concentration (gm %)

Hemoglobin concentration in different groups of mice is presented in Table 1 and Figure 2. The highest hemoglobin concentration was recorded in soybean oil treated group C (8. 66 ± 0.29 gm%) followed by rice bran oil group B (7.93 ± 0.17 gm%) and control groups A (7.16 ± 0.12 gm%). The soybean oil treated group statistically significant ($P<0.05$) from the other group. The present finding is not consistent with Ahmed et al. (1994) who reported that hemoglobin concentration of Japanese quails affected by dietary treatment. A sex and age relating difference in Hb concentration (Holmes et al. 1933) has been reported.

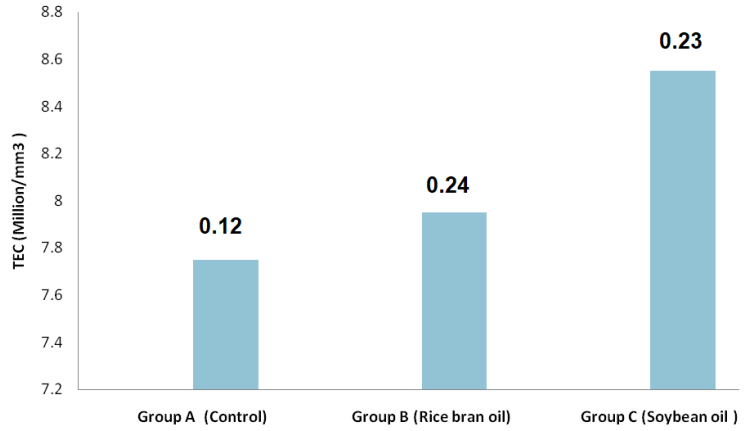

Figure 1. Effects of additional supplementation of soybean and rice bran oil on total Erythrocyte Count (million/cu.mm) in mice (n=10). The superscript value above mark indicates the standard error

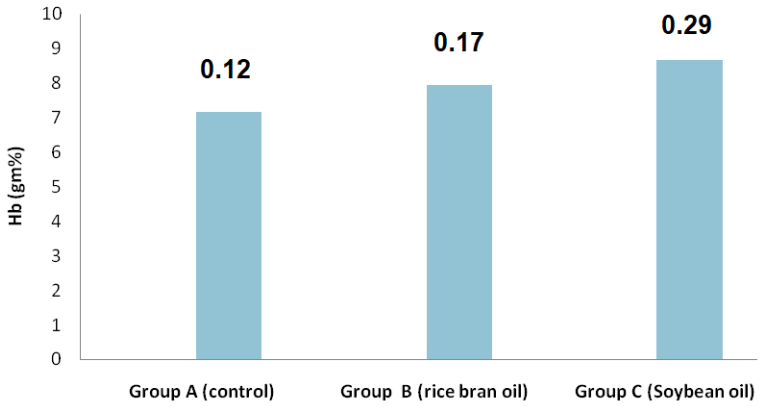

Figure 2. Effects of additional supplementation of soybean and rice bran oil on hemoglobin concentration (gm %) in mice (n=10). The superscript valueabove mark indicates the standard error.

Table 1. Effects of additional supplementation of soybean and rice bran oil on hematological parameters (mean ± SE) in mice (n=10). Values with different superscript letter in same raw differ significantly (P<0.05)

Haematological parameters	Mean ± SE			P- value
	Group A (control)	Group B (rice bran oil)	Group C (soybean oil)	
RBC (million/mm^3)	7.75 ± 0.12^a	7.95 ± 0.24^b	8.55 ± 0.23^c	0.05
Hb (gm %)	7.16 ± 0.12^a	7.93 ± 0.17^b	8.66 ± 0.29^c	

Effect on Biochemical parameters

Total serum cholesterol (mg/dl)

The effects of rice bran and soybean oil supplementation with ration on total cholesterol are shown in Table 2 and Figure 3. The total cholesterol values increased with addition of soybean oil supplemented group C and rice bran oil supplemented group B compared to control group A. The highest total cholesterol was recorded in soybean oil treated group C (146.78 ± 3.72 mg/dl) followed by rice bran oil group B (134.07 ± 4.33 mg/dl) and control group A (130.07 ± 1.57).

Table 2. Effects of of additional supplementation soybean and rice bran oil on biochemical parameters (mean ± SE) in mice (n=10)

Biochemical parameters	Mean ± SE			P-value
	Group A (Control)	Group B (Rice bran oil)	Group C (Soyabean oil)	
Cholesterol(mg/dl)	130.55 ± 1.57^a	134.07 ± 4.33^b	146.78 ± 3.72^c	
Triglycerides(mg/dl)	42.77 ± 2.64^a	55.09 ± 3.23^b	69.75 ± 4.01^c	0.05
HDL (mg/dl)	52.42 ± 3.83^a	69.18 ± 1.24^b	69.50 ± 1.74^b	
Uric acid (mg/dl)	3.16 ± 0.18^a	4.72 ± 0.05^b	5.32 ± 0.13^c	

Values with different superscript letter in same raw differ significantly (P<0.05)

The soybean oil treated group statistically significantly differ (P<0.05) from the other groups. The present finding coincided with the findings of Fernandez et al. (1996) who reported that concentration of cholesterol concentration increased significantly in waster rats fed on diet enriched with soybean oil but inconsistent with the findings of Kamei et al. (1995) who observed decreased plasma cholesterol in rats fed with hydrogenated soybean oil. Kahlon et al. (1990) reported that significant total cholesterol reductions in hamsters fed stabilized rice bran diets compared with those fed cellulose control diet.

Triglycerides (mg/dl)

Triglycerides (TG) concentration is presented in Table 2 and Figure 4. The highest triglyceride was recorded in soybean oil treated group C (69.75 ± 4.01) mg/dl) followed by rice bran oil treated group B (55.09 ± 3.23 mg/dl) and control group A (42.77 ± 1.57 mg/dl). The soybean oil treated group showed significantly higher (P<0.05) triglyceride than other groups. The result are similar to that of Fernandez et al. (1996) who found that triglycerides were significantly increased in wistar male rat fed with (soybean oil). Rice bran diet consumption has been reported to result insignificant plasma triglycerides reduction in animals (Suzuki 1982, Newman et al., 1992, Kahlon et al., 1992b) and in humans (Kestin et al., 1990, Hegsted. 1992).

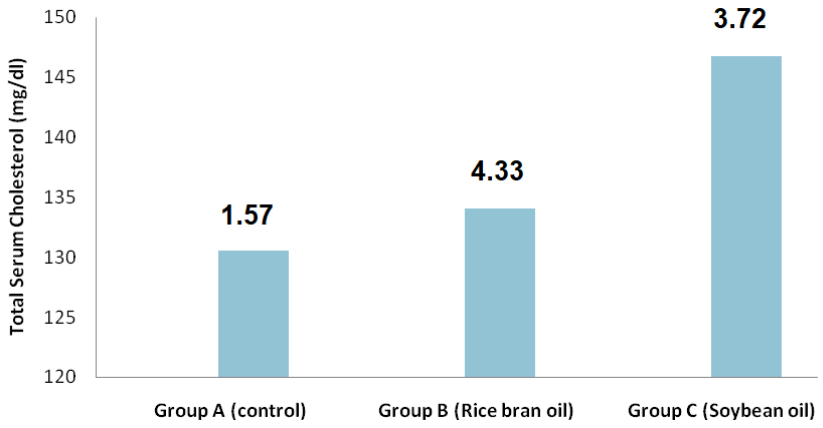

Figure 3. Effects of additional supplementation soybean and rice bran oil on total serum cholesterol (mg/dl) in mice (n=10). The superscript value above mark indicates the standard error.

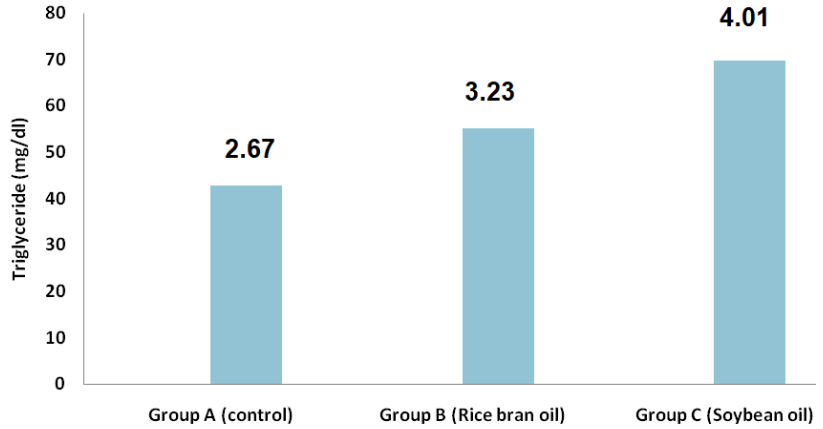

Figure 4. Effects of additional supplementation soybean and rice bran oil on triglycerides (mg/dl) in mice (n=10). The superscript value above mark indicates the standard error.

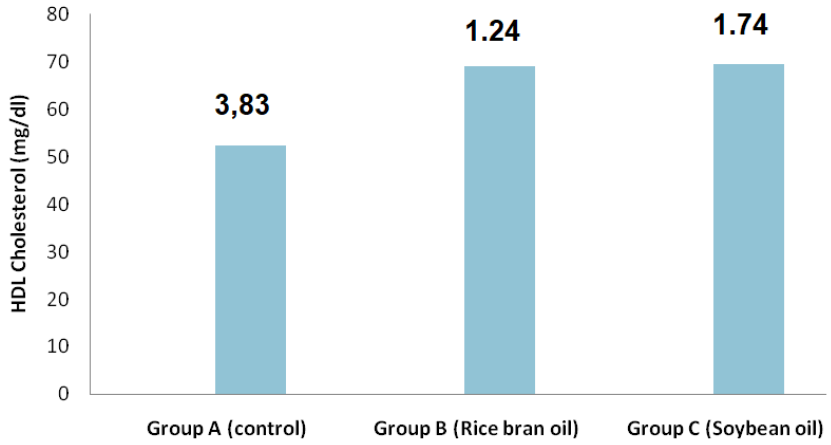

Figure 5. Effects of additional supplementation soybean and rice bran oil on high density lipoproteins (mg/dl) in mice (n=10). The superscript value above mark indicates the standard error.

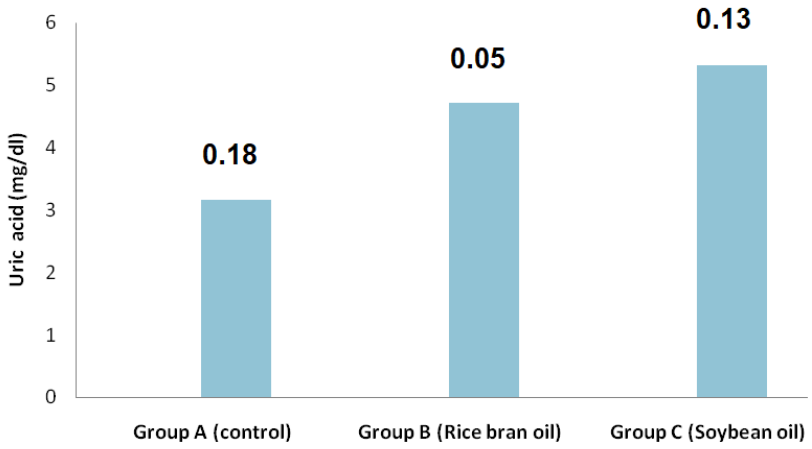

Figure 6. Effects of additional supplementation soybean and rice bran oil on uric acid (mg/dl) in mice (n=10). The superscript value above mark indicates the standard error.

High density lipoproteins (mg/dl)

The high density lipoproteins (HDL) value of different groups of mice is presented in Table 2 and Figure 5. The highest high density lipoprotein was recorded in treated soybean oil group C (69.50 ± 1.74 mg/dl) followed by rice bran oil B (69.18 ± 1.24 mg/dl) and control group A (52.42 ± 3.83 mg/dl). The soybean and rice bran oil treated group showed significantly higher HDL ($P < 0.05$) compared to control group. Though higher value was recorded in soybean oil supplemented group one but the differences with rice bran oil is ($P>0.05$) insignificant. This result is in agreement with earlier report Leplaix et al. (1996) who showed that dietary soybean oil induced an increase concentration of HDL. The result is also supported by Koh (1987), Verma et al. (1995).

Uric acid (mg/dl)

Uric acid concentration is presented in Table 2 and Figure 6. The highest uric value was recorded in treated in soybean oil group C (5.32 ± 0.13 mg/dl) followed by rice bran oil treated oil group B (4.72 ± 0.05 mg/dl) and control group A (3.16 ± 0.18 mg/dl). The soybean and rice bran oil treated group showed significantly higher ($P<0.05$) compared to control groups. The result is similar to that of Fernandez et al. (1996) who reported that uric acid concentration increased significantly in waster rats fed on diet enriched with soybean oil but inconsistent with the findings of Kamei et al. (1995) who observed decreased uric acid concentration in rats fed with hydrogenated soybean oil.

CONCLUSION

Hemato-biochemical parameters of blood in the mice are significantly affected by rice bran and soybean oil enriched diet.

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