



Original Research Article

Oral administration of probiotic *Lactobacillus casei* spp. *casei* ameliorates oxidative stress in rats

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ABSTRACT

The present study was conducted to determine the effect of *Lactobacillus casei* spp. *casei* in induced oxidative stress in rats. *L. casei* spp. *casei* was evaluated for probiotic attributes like acid and bile tolerance, surface hydrophobicity, antimicrobial activity etc. The probiotic culture was fed to rats in lyophilized and fermented milk form. Male Wistar rats were distributed into six groups, Group-FC (Fresh soybean oil control); Group-FF (Fresh soybean oil- fermented milk); Group-FL (Fresh soybean oil- probiotic culture); Group-OC (Oxidised soybean oil control); Group-OF (Oxidised soybean oil- fermented milk), Group-OL (Oxidised soybean oil- probiotic culture) and were fed for 90 days. Blood samples were collected at 0, 30, 60 and 90 days of feeding and checked for activity of oxidative markers like catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and lipid peroxidation. At the end of 90th day all rats were sacrificed, blood and liver were collected, and catalase, SOD, GPx activity, lipid peroxidation was estimated in liver. Feeding lyophilized culture or fermented milk to rats significantly ($P<0.05$) increased the activity of antioxidative enzymes. Similarly, lipid peroxidation in RBC and liver was also found to be significantly ($P<0.05$) high in rats which were fed with oxidized soybean oil and this increase was significantly ($P<0.05$) decreased after *L. casei* ssp. *casei* feeding to the rats. The results suggested that probiotic strain of *L.casei* ssp. *casei* can improve the antioxidant status and minimize the effects of oxidative stress in liver and RBC of rats.

Keywords

Probiotic
Lactobacillus casei spp.
casei,
oxidative
stress in rats

Introduction

The importance of oxidation in the body and in foods has been widely recognized. Oxidative metabolism is essential for the survival of cells. A side effect of this dependence is the production of free radicals and other reactive species that cause oxidative changes. When an excess of free radicals is formed, they can overwhelm

protective enzymes like superoxide dismutase, catalase and peroxidase, and cause destructive and lethal cellular effects by oxidizing membrane lipids, cellular proteins, DNA and enzymes, thus shutting cellular respiration. It has been recognized that oxidative stress plays a significant role in a number of age specific diseases e.g.

Alzheimer disease (Liu et al., 2003). Other pathological condition associated with evidence of significant free radical mediated injury includes atherosclerosis, diabetes, rheumatoid arthritis (Abuja et al., 2001; Halliwell and White., 2004; Hoelzl et al., 2005). Strong attention has recently been focused on the importance of the protective defense systems in living cells against damage caused by active oxygen and free radicals. Several endogenous antioxidants have been found to play an important role in non-enzymatic protection. In addition to vitamin E, vitamin C, carotene, ubiquinol natural defense systems, there is increasing antioxidants, which are candidates for disease prevention and for extending the life span of animals.

Probiotics have been defined as live microbial food supplements that benefit human health (McFarland, 2000; Salminen, 2001). The two strains of bacteria among lactic acid bacteria (LAB) recognized as most important to health are lactobacilli and bifidobacteria. Lactobacilli (which include *Lactobacillus acidophilus* and *Lactobacillus casei* among many others) are concentrated in small intestine and are most often implicated in assisting the establishment of a 'normal microflora' called as probiotics. Viable lactic acid bacteria of probiotic foods have several scientifically established and/or clinically proved health effects, such as reduction and prevention of diarrheas of different origin, improvement of the intestinal microbial balance by antimicrobial activity, alleviation of lactose intolerance symptoms, prevention of food allergy, enhancement of immune potency, and antitumorogenic activities (Salminen, 2001, Andersson et al., 2001, Kapila et al., 2007, 2013 and Kemgang et al., 2014). The reactive oxygen species scavenging ability of microorganisms has been investigated by many workers (Kaizu et al., 1993; Zommara

et al., 1994; 1996; Lin and Yen, 1999, Kapila et al., 2006). Moreover, some studies have shown that certain lactic acid bacteria possess antioxidative activity (Kaizu et al., 1993; Kullisaar et al., 2002; Kapila et al., 2006; Ou et al., 2009).

Lactobacilli, which are to be used as dietary adjuncts, must survive the extremely low pH of human stomach (Klaenhammer, 1982; Kim, 1988). Acid tolerant strains obtained through natural selection techniques by sequential exposure to hydrochloric acid (pH 3.5 to 7.0) exhibited growth advantages in stability, lactose utilization, protease activity, aminopeptidase activity and cell wall fatty acid profile over those of acid sensitive parents (Chou and Weimer, 1999). Bile tolerance is another important characteristic of probiotic lactic acid bacteria since this enables them to survive, to grow and to perform their beneficial action in the small intestine (Gilliland and Walker, 1990). Prakash et al. (1997) reported that organisms exhibiting higher cell surface hydrophobicity showed good adhesion index to epithelial cells and would have an added advantage to grow in gastrointestinal tract. Only bacteria that can survive passage through the stomach and can colonize the intestinal will exert any effect on the host. So, in the present study probiotic attributes of *Lactobacillus casei* ssp *casei* were investigated and was used to evaluate its antioxidative property in oxidized soyabean oil induced oxidative stress in rats. This could be one step further to understand the mechanisms and probiotic properties of *Lactobacillus casei* ssp *casei* in the development of strategic management to minimize the oxidative stress in cells.

Materials and Methods

Animals

Male albino rats of Wistar Strain (6-8

weeks) were obtained from Small Animal House of Haryana Agricultural University (HAU), Hisar, India. The experimental protocol was got approved from the Institute Ethics Committee of Animal Experiments (IEAE), National Dairy Research Institute (Deemed University), Karnal, India. The rats were fed with synthetic diet (43.3% Starch, 16.6% Casein, 10% Sucrose, 20 %fat, 3.5 % Mineral mixture, 1% Vitamin mixture, 0.25% Choline chloride, 0.3% Methionine). Vitamin and mineral mixture were prepared and blended in synthetic diet according to AOAC (1984).

Grouping of animals

Thirty six male rats were distributed (6 rats each) into six groups as described in table-1. Three groups were fed with 20% fresh soybean oil and alongwith either of three skim milk/fermented milk/lyophilized *L.casei*. Another three groups of rats were given diet containing 20% oxidized oil alongwith either of three i.e. skim milk/fermented milk/ lyophilized *L.casei*. The synthetic diet was supplemented either with skim milk or fermented milk or lyophilized *L.casei* at the rate of 5%. Oxidized soybean oil was prepared by heating fresh soybean oil at 60 °C for 15 days and peroxide value was estimated to 91meq.

Bacterial culture

Lactobacillus casei spp. *casei* was procured from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute (NDRI), Karnal, India. The culture was activated prior to use by sub culturing twice in MRS broth for 18 h at 37 °C (deMan et al., 1960). The culture was sub-cultured at least thrice prior to experimental use. Sub-cultured cells were harvested and washed with normal saline. The cell pellet so

obtained was resuspended in a media (10% skim milk, 1.5% gelatin and 8% sucrose) in a volume of 1/10th of original growth medium and lyophilized using a lyophiliser (Heto Lyo Lab 3000).

Fermented milk

Fermented milk was prepared by inoculating the culture *Lactobacillus casei* spp. *casei* in skimmed milk procured from the Experimental Dairy Unit, NDRI, Karnal. The milk was boiled at 90 °C for 30 minutes with constant stirring and then allowed to cool up to 30 °C. The culture was inoculated (1%) and kept for 18 hours on 37 °C. The pH value (4.98) and titratable acidity (0.7%) were within the range typically of normal dahi or yogurt. After fermentation, colony forming unit per gram of fermented milk was counted by plating.

Probiotic attributes of *Lactobacillus casei* spp. *casei* culture

Acid and bile tolerance

Tolerance of *Lactobacillus casei* spp. *casei* for low pH (Acid tolerance) was estimated in culture by using the protocol of Clark et al., (1997) with some modifications. In Brief, The MRS broth (deMan et al., 1960) was adjusted to pH 3.5, pH 2.5 and pH 2.0 with 1 M HCl. The MRS broth having its native pH of 6.5 was used as control. Survival of *Lactobacillus casei* spp. *casei* in acidic MRS broth was evaluated at 2h of incubation (log phase of growth) by plating cells on MRS agar.

Bile tolerance of *Lactobacillus casei* spp. *casei* culture was estimated as described by Gilliland et al., (1984). In brief, 0.5%, 1%, 1.5%, 2% of Ox-bile (Himedia Laboratories Pvt. Ltd, Mumbai, India) was supplemented (w/v) in MRS broth. The broth without Ox-

bile was taken as control. Actively growing *Lactobacillus casei* spp. *casei* culture was inoculated in MRS broth supplemented with and without Ox-bile and incubated for 3h at 37 °C. Survival of bacteria in MRS broth was evaluated by plating cultures on MRS agar.

Cell surface hydrophobicity

Cell surface hydrophobicity of *Lactobacillus casei* spp. *casei* was determined as described by Rosenberg et al., (1980). In brief, cells from actively grown culture in MRS broth was harvested, washed twice and resuspended in 5 ml of phosphate urea magnesium sulphate buffer (pH 6.5) and absorbance was read in spectrophotometer at 400nm λ_{max} . The initial optical density (OD_{initial}) of the cell suspension was adjusted to 1.0. To 3 ml of bacterial suspension slowly 0.6 ml of n-hexadecane or Xylene or Octane was added. The suspension was incubated at 37 °C for 10 min and vortexed for 2 min and kept in standing position so, hydrocarbon layer allowed to rise completely. After 1 h aqueous phase was removed and the final absorbance (OD_{final}) was read using Spectrophotometer. The decrease in the absorbance was taken as a measure of percent cell surface hydrophobicity and calculated by using formula Hydrophobicity (%) = [(OD_{initial}-OD_{final})/ OD_{initial}] × 100.

Antimicrobial activity

The agar spot assay as described by Fleming et al. (1975) was followed to evaluate the antimicrobial activity of *Lactobacillus casei* spp. *casei*. The indicator organisms used were *B. cereus* (NCDC-66), *S. aureus* (NCDC-109), *E. faecalis*, *L. mesenteriodes* LY, *E. coli* V517. The pathogenic cultures used in this study were obtained from National Collection for Dairy Cultures,

National Dairy Research Institute, Karnal, India. *Bacillus cereus* (NCDC-66), *Staphylococcus aureus* (NCDC-109), *Enterococcus faecalis*, *Leuconostoc mesenteriodes* and *Escherichia coli* V517 were maintained in Brain Heart infusion broth. Tryptone glucose yeast extract broth was used for growing *Leuconostoc mesenteriodes* LY.

Antioxidative activity

The antioxidative activity of *Lactobacillus casei* spp. *casei* was determined by using thiobarbituric acid (TBA) method (Kaizu et al., 1993) which is based on inhibition of linoleic acid peroxidation by intracellular cell free extract (Kapila et al., 2006). Linoleic acid was used as the source of unsaturated fatty acid. Intracellular cell free extract of *Lactobacillus casei* was prepared by sonicating 10 mg of wet harvested cells in normal saline for 2 min at 0 °C. The contents were centrifuged at 3000× g for 10 min and supernatant was taken as intracellular cell free extract.

Feeding trials in rats and assessment of oxidative markers

Rats distributed into six groups (Group-1 to Group-6) were fed for a period of up to 3 months (90 days) as described earlier in section-2.2. Orbital venous blood from rats of each group was collected at 30th, 60th and 90th days of feeding in a heparinised tube (2 IU/ml). The plasma from blood was separated and cells were washed three times in normal saline (0.9% NaCl) and RBC was lysed by adding cold distilled water. The erythrocyte lysate was stored at -70°C and used to estimate catalase, superoxide dismutase (SOD), Glutathione peroxidase and lipid peroxidation (Malondialdehyde, MDA). Haemoglobin content in blood was estimated immediately after collection.

The *in vivo* experiment was terminated at 90th day by sacrificing all rats and liver was excised, washed with normal saline and stored at -70 °C. Homogenate of liver in isotonic solution was prepared by following the protocol of Cohen et al. (1970). Protein in homogenate was estimated according to the method of Lowry et al., (1951). Homogenate was used for estimation of catalase, superoxide dismutase (SOD), Glutathione peroxidase and lipid peroxidation (Malondialdehyde).

Catalase assay in RBC and Liver lysate

Catalase activity in RBC and liver lysate was estimated using the protocol of Aebi (1984). Briefly, to diluted lysate in phosphate buffer, H₂O₂ was added to a final concentration of 30 mM. The decrease in absorbance per min was recorded at λ_{\max} of 240nm (Specord 200, Analytikjena, Germany) against blank prepared in absence of H₂O₂. The difference in absorbance (ΔA_{240}) per unit time was the measured by using an extinction coefficient of 0.00394 litres mM⁻¹ mm⁻¹.

For catalase assay, liver homogenate was further processed as described by Cohen *et al.* (1970). In brief, to an aliquot of the liver homogenate, ethanol was added to a final concentration of 0.17 M (0.01 ml ethanol/ml homogenate) and incubated for 30 min in ice. After 30 min incubation, Triton X-100 (10%) was added to produce 100 fold dilution of the original homogenate.

Superoxide dismutase (SOD) assay in RBC and Liver lysate

SOD activity in RBC and Liver lysate was estimated by using protocol of Marklund and Marklund (1974). In brief, 200µl of lysate was diluted in phosphate buffer to make final volume of 2.0ml. Total volume was made to 3 ml by adding 0.8 ml of Tris-

HCl buffer (50 mM Tris, 1mM diethylene triamine pentaacetic acid, pH 8.2) and 0.2 ml of 2 mM Pyrogallol. A blank was prepared without addition of pyrogallol in the reaction system. The increase in absorbance per minute was read at 240nm (Δ_{\max}) against blank. The inhibition of pyrogallol auto-oxidation was brought about by superoxide dismutase, which was used for the determination of enzyme activity. A unit of enzyme was defined as the amount of enzyme that inhibits the reaction by 50 percent.

Glutathione peroxidase (GPx) assay in RBC and Liver lysate

GPx activity was estimated by using protocol as described by Lawrence and Burk (1976). To 100µl of lysate, reaction mixture (50 mM potassium phosphate buffer, 10 mM EDTA, 10 mM NaN₃, 2 mM NADPH, 1 EU/ml glutathione reductase, 10 mM GSH, pH 7.0) was added to make a final volume of 900 µl and incubated at room temperature for 1 min. Reaction was initiated by adding 100 µl of cumene hydroperoxide (1.5 mM) and change in absorbance per 5 min was recorded at λ_{\max} of 340nm. The enzyme activity was calculated using extinction coefficient of 6.22 x 10³ litres mol⁻¹ cm⁻¹.

Lipid peroxidation (malondialdehyde) assay in RBC and Liver lysate

The extent of lipid peroxidation, an index of oxidative stress was measured as thiobarbituric acid reactive substances formed (TBARS). Lipid hydroperoxides were measured by TBA test as described by Asakawa and Matsushita (1979). One hundred microlitre of lysate was diluted in 100µl ferric chloride (10mM), 1.5 ml glycine HCl buffer (0.2 M) and 1.5 ml of TBA reagent (34.68 mM TBA, 10.40 mM SDS). The mixture was heated in boiling

water bath for 15 min and after cooling 2 ml of chloroform and 1 ml of acetic acid was added, mixed and centrifuged. Absorbance of supernatant was read at Δ_{\max} of 532 nm against blank processed without lysate. The molar extinction coefficient ($1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$) was used to calculate the amount of malonaldehyde produced.

Statistical Analysis

The results were expressed as mean \pm standard error of mean. Significance was tested by employing analysis of variance (ANOVA) and comparison between means was made by critical difference (C.D.) value. For computation of data, software application programmes like Microsoft Excel and Systat 7.0 were used.

Results and Discussion

Probiotic attributes of *Lactobacillus casei* spp. *casei*

Acid and bile tolerance

Lactobacillus casei spp. *casei* (log cfu per ml culture) showed lower viability in MRS broth at pH 2.0 than at pH 2.5 and 3.5 after 2 h of incubation. Similarly, addition of Ox-bile up to 1.5% in MRS broth did not affect the viability of the cells in comparison to culture in their absence (Table-2). Addition of 2% Ox-bile to MRS broth results in significant ($P < 0.05$) decrease in cell viability (log cfu per ml culture) compared to control but this decrease was non-significant to cells cultured in presence of Ox-bile up to 1.5% (Table-2). The results indicated acid and bile tolerance of *Lactobacillus casei* spp. *casei*.

Cell surface hydrophobicity

Cell surface hydrophobicity is an indicator

of colonization potential of the organism to intestinal lumen. In the present study, cell surface hydrophobicity of *Lactobacillus casei* spp. *casei* was measured in n-hexadecane, Xylene and Octane (Table-2). *Lactobacillus casei* shows maximum hydrophobicity in octane (24.4 ± 1.30) which was significantly ($P < 0.05$) higher than n-hexadecane (7.0 ± 0.80) than Xylene (8.7 ± 0.9).

Antimicrobial activity

Antimicrobial activity of *Lactobacillus casei* spp. *casei* was seen in agar plates of *B. cereus* (NCDC-66), *S. aureus* (NCDC-109), *E. faecalis*, *L. mesenteriodes* LY and *E. coli* V517. The zone of inhibition was taken as measure of antimicrobial potential and it was found that the area of zone of inhibition were maximum (21-30mm) for *B. cereus* (NCDC-66), *E. faecalis*, and *E. coli* V517 (Table-2). Similarly, *Lactobacillus casei* spp. *casei* was also found to be antimicrobial against *Staphylococcus aureus* (NCDC-109) and *Leuconostoc mesenteriodes* (11-20 mm zone of inhibition; Table-2).

Antioxidative activity

Intracellular cell free extract of *Lactobacillus casei* spp. *casei* showed 72.0 ± 3.20 percent inhibition of lipid peroxidation in linoleic acid. This shows possible antioxidant property of probiotic strain of *Lactobacillus casei* spp. *casei*.

Antioxidative property of *Lactobacillus casei* spp. *casei* in rat RBC

Catalase activity in RBC

On day zero, catalase activity was similar among all the groups (data not shown). After 30 days of feeding catalase activity significantly ($P < 0.05$) increased in FF (fed

fresh soybean along with fermented milk) in comparison to groups-FC, FL & OC. On 60th day of feeding the catalase activity increased almost two fold in all the groups significantly as compared to group-FC, but the increase was more pronounced in groups fed either probiotic culture or fermented milk. The similar pattern continued on 90th day in the groups fed probiotic culture either in fermented form or lyophilized form. The results indicated that feeding of *Lactobacillus casei* spp. *casei* in lyophilized form or in the form of fermented milk enhanced the catalase activity both in normal and oxidative stress condition.

Superoxide Dismutase activity in RBC

Up to 30 days of feeding the activity of SOD in RBC remained equal in all the groups. After 60 days of feeding SOD activity in RBC was significantly ($P<0.05$) increased in rats fed with *Lactobacillus casei* spp. *casei* fermented milk or lyophilised culture along with oxidised soybean oil (Fig-2). Similar trend continued on 90th day, but there was decrease in SOD units. SOD results showed that feeding of fresh soybean did not cause much change in the enzyme activity whereas feeding of oxidized soybean resulted in increased SOD activity and the increase was more in groups fed probiotic culture and fermented milk.

Glutathione Peroxidase (GPx) activity in RBC

Feeding rats with soybean oil with and without probiotic culture resulted in non-significant change in GPx activity in all the groups up to 30 days (Fig-3). After 60 days of feeding statistically significant ($P<0.05$) increase was reported in rats fed probiotic culture and fermented milk in comparison to control group, except group FF, in which the increase was non-significant. On the 90th

day of feeding, GPx activity increased 1.5 to 3 fold from day 60th but the groups OC, OF & OL which were maintained on oxidized oil did not show variation in GPx activity whereas on the other hand, groups maintained on fresh soybean with probiotic and fermented milk i.e. FF and FL showed significant ($P<0.05$) variation in comparison to its control group i.e. FC.

Lipid peroxidation in RBC and Liver

Lipid peroxidation in RBC of all groups of rats was measured on 90th day of feeding (Table-3). There was slight increase in amount of MDA produced as a measure of lipid peroxidation in the rats fed with oxidised soybean oil alone with standard chow (OC) as compared to rats fed with fresh soybean oil (FC). Rats of group- FL and OF, showed significantly ($P<0.05$) low level of lipid peroxidation compared to rats of their respective controls. Decline in the levels of TBARS was more in rats fed fresh soybean oil supplemented with either *Lactobacillus casei* spp. *casei* or fermented milk.

Feeding fresh and oxidised soybean oil to rats (OC) resulted in higher level of TBARS in rat liver. Feeding *Lactobacillus casei* spp. *casei* to rats in the form of fermented milk (Group-FF,OF) or lyophilised culture (Group-FL,OL) for 90 days resulted in significantly ($P<0.05$) lowering level of lipid peroxidation products in liver cells

Antioxidative property of *Lactobacillus casei* spp. *casei* in rat Liver

Antioxidative enzyme activities in liver

Catalase activity was measured in homogenised liver tissue of sacrificed rats after 90 days of feeding. Catalase activity of group-OC increased significantly on feeding

oxidized oil compared to rats of group-FC which were given fresh soybean oil (Table-4). It indicated that oxidative stress increased the catalase activity. There was significant increase in catalase activity in groups which were fed oxidized oil along with probiotic culture and fermented milk and similar trend in catalase activity was observed in groups maintained on fresh soybean oil supplemented with probiotic culture and fermented milk but the extent of increase was not as high as reported in oxidized soybean oil fed groups with probiotics.

Whereas SOD activity decline in oxidized oil fed group but it improved on feeding *Lactobacillus casei* spp. *casei* or fermented milk but the increase was non-significant. In case of fresh soybean oil fed groups significant ($P < 0.05$) effect was seen only in group-FF which was given fermented milk. However no effect was observed in liver GPx activity in all the groups on feeding different diets.

Live microbial supplementation in diet, intended to positively impact the host by balancing the natural microflora of the gastrointestinal tract (Hammes and Vogel, 1995). During last decade, much interest has been generated worldwide to assess the beneficial effect of LAB, particularly lactobacilli in reducing the risk of heart diseases and to be used as probiotics (Taranto *et al.*, 2000).

Gilliland and Walker (1990) reported that a lactic culture of human origin which assimilates cholesterol, grows well in presence of bile, low pH and produces bacteriocin, can be selected for use as a dietary adjunct. In the present study survival of *Lactobacillus casei* spp. *casei* at pH 2.5 is significant as ingestion with food or dairy products raises the pH in stomach to 3.0 or

higher (Martini *et al.*, 1987). In similar studies, Goldin *et al.*, (1992) reported survival of *Lactobacillus* GG at pH 3.0 which facilitate bacteria to reach small intestine. Once bacteria reach the small intestinal tract, their ability to survive depends on their resistance to bile (Gilliland *et al.*, 1984) as bile entering the duodenal section of the small intestine has been reported to reduce the survival of bacteria. In the present study at 0.5, 1, 1.5 and 2 percent Ox-bile salt concentrations the *L. casei* showed no log reduction after 3h of treatment. Thus, *L. casei* is potentially poised to show better survival through gastro-intestinal tract, especially if consumed along with milk or within a food matrix as milk has been shown to be an excellent vehicle for probiotic bacteria probably due to its high buffering capacity (Haung and Adams, 2004).

Once bacteria reached large intestine by passing bile salt cell surface hydrophobicity of organism is indicator of colonization potential to intestinal lumen. The strain under study was also evaluated for its cell surface hydrophobicity towards different hydrocarbons like n-hexadecane, n-octane and xylene. The strain showed variable degree of hydrophobicity. The strain of *Lactobacillus casei* spp. *casei* has relatively more affinity for n-octane (24.4%) followed by n-hexadecane and xylene. The variation in hydrophobicity to solvents and among strains has been explained by the fact the adhesion depends upon the origin of strains as well as surface properties (Morata *et al.*, 1998). Ouwehand *et al.*, (2000) reported *Lactobacillus* GG showed highest adhesion (32%), while *L. casei* strain shirota exhibited low level of adhesion to intestinal mucus.

The antimicrobial activity of *L. casei* against pathogens revealed that the zone of inhibition was more in case of *Bacillus*

ceruus NCDC66, *Enterococcus faecalis* and *E coli* V517. Kivanc (1990) in a study on antibacterial properties of cell free filtrate from *L. casei* strains isolated from Dahi, Cheddar cheese and Shrikhand also showed antibacterial activity against both gram negative and gram positive bacteria.

Antioxidative activity of the intracellular extract of the *L. casei* determined by inhibition of linoleic peroxidation showed 72.04% inhibition of linoleic acid peroxidation. Similarly, Lin and Yen (1999) examined radical scavenging activity of yoghurt organisms based on linoleic peroxidation assay, *Streptococcus thermophilus* and *L.delbrueckii* ssp *bulgaricus* demonstrated an antioxidative effect on the inhibition of linoleic acid peroxidation. From the results, it is clearly evident that on feeding high oil /fat diets supplemented with fermented milk or probiotic culture, increased the catalase activity. The increase of catalase activity in probiotic organisms fed group has been reported earlier (Zommara et al., 1996). These workers observed that whey from bovine skim milk fermented with *B. longum* increased the activity of catalase in RBC lysate of rats. Likewise, Rajpal and Kansal (2009) reported that the catalase activity in RBC increased on probiotic Dahi feeding in rats. The effect of feeding fermented milk and culture had little impact on SOD activity. Although groups which were fed with fermented milk / culture showed higher values in comparison to control but activities were statistically non-significant. But these values were significant in case of groups fed with oxidized oil supplemented with fermented milk and probiotic *L. casei* ssp.*casei*. The increase in GSHPx activity in probiotic organisms fed group was also observed earlier (Zommara et al., 1996).

The results clearly show that feeding of

lactobacilli either in the form of fermented milk or in lyophilized form exhibited an increase in activity of hepatic catalase. Whereas in SOD, an increase in activity was observed only in fermented milk fed group, while no effect on the activity of hepatic GSHPx was observed. The present observations are in agreement with the findings of Zommara et al., (1996). These workers reported that the activity of catalase in liver was elevated on cultured product diets but, activities of the hepatic SOD and GSHPx were not affected by the type of diet. However, Rajpal and Kansal (2009) observed that the SOD activity in liver was stimulated by feeding probiotic dahi to rats. Husain and Somani (1997) suggested that increased plasma CAT/SOD and GPx/SOD depicts tissue's ability to cope with oxidative stress.

In the present investigation when ratio of CAT/SOD was calculated in RBC after 90 days of feeding experimental diet (Data not shown), it was observed that the ratio increased on feeding probiotics either in fermented form or lyophilized form. Effect of feeding experimental diets on lipid hydroperoxides in RBC and liver shows decreased lipid peroxidation significantly ($P<0.05$) compared to rats fed on only oxidized soybean oil. The present findings are in agreement with the findings of Zommara et al., (1998) reported reduction in TBARS in RBC on feeding fermented bovine whey preparation. Likewise, the probiotic dahi (*L. acidophilus* and *L.casei*) significantly suppressed streptozotocin-induced oxidative damage in pancreatic tissues by inhibiting the lipid peroxidation (Yadav et al.,2008). Certainly, systemic investigations are now needed to establish molecular aspects and mechanisms (including anti-atherogenic) underlying the positive effect of this potential probiotic strain *Lactobacillus casei* spp. *casei*.

Table.1 Grouping of animals

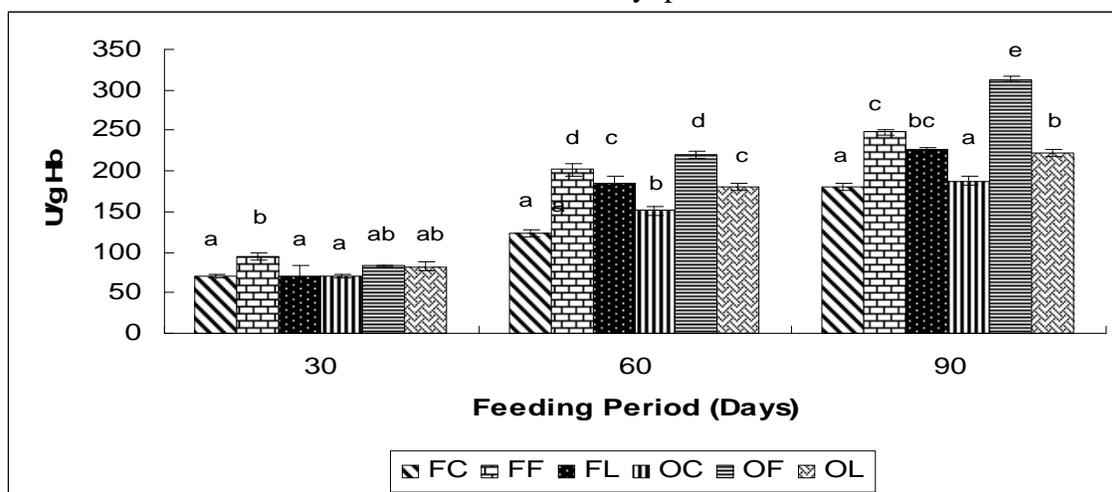
Groups	Diet
FC	Synthetic diet with 20% fresh soybean oil + skim milk
FF	Synthetic diet with 20% fresh soybean oil+ fermented milk (using <i>L casei</i> ssp <i>casei</i>)
FL	Synthetic diet with 20% fresh soybean oil + lyophilized culture (<i>L. casei</i> ssp. <i>casei</i>)
OC	Synthetic diet with 20% oxidized soybean oil +skim milk
OF	Synthetic diet with 20% oxidized soybean oil+ fermented milk (using <i>L casei</i> ssp <i>casei</i>)
OL	Synthetic diet with 20% oxidized soybean oil +lyophilized culture(<i>L. casei</i> ssp. <i>casei</i>)

Table-2 Probiotic characteristics of *Lactobacillus casei* ssp *casei* (mean ± standard deviation, n = 3).

Acid tolerance*				Bile tolerance (%)*					
pH 6.5	pH 3.5	pH 2.5	pH 2.0	C	0.5%	1%	1.5 %	2%	
9.14±0.5	7.93±0.4	7.87±0.6	4.80±0.7	8.24±0.7	7.67±0.5	7.30±0.7	7.28±0.3	7.15±0.5	
%Hydrophobicity			Antioxidative activity						
n-Hexadecane	Xylene	Octane	% Linoleic inhibition						
7±0.8	8.75±0.9	24.4±1.3	72.04±3.2						
Antimicrobial activity									
<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Leuconostoc mesenteriodes</i>	<i>E. coli</i>					
NCDC66	NCDC109			V517					
++	+		++	+					

*Viable cell count (log cfu mL⁻¹) after exposure to pH (6.5,3.5,2.5, 2.0) after 2 h and bile% (0.5, 1, 1.5, 2) after 3 h at 37⁰C; +; 11.20mm inhibitory zone; ++: 21-30mm inhibitory zone.

Fig.1 Catalase activity in RBC lysate of rats fed on fresh or oxidized oil supplemented with skim milk/fermented milk/ lyophilized culture

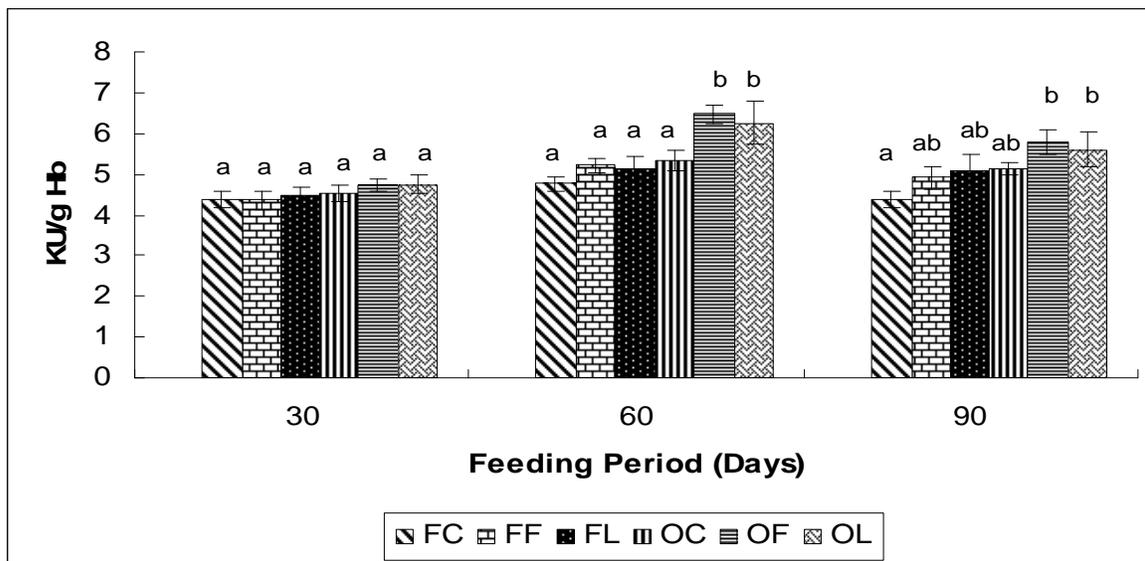


Catalase activity is expressed in U/mg Hb.

1 Unit (U) is equivalent to 1 μmol of H₂O₂ consumed per min per mg of haemoglobin at 25°C.

The values expressed as means ± SEM for 6 rats per group. The values with the same symbols are statistically in par within a row whereas, those with different symbols are significantly different at P < 0.001.

Fig.2 Superoxide dismutase activity in RBC lysate of rats fed on fresh or oxidized oil supplemented with skim milk / fermented milk/lyophilized culture

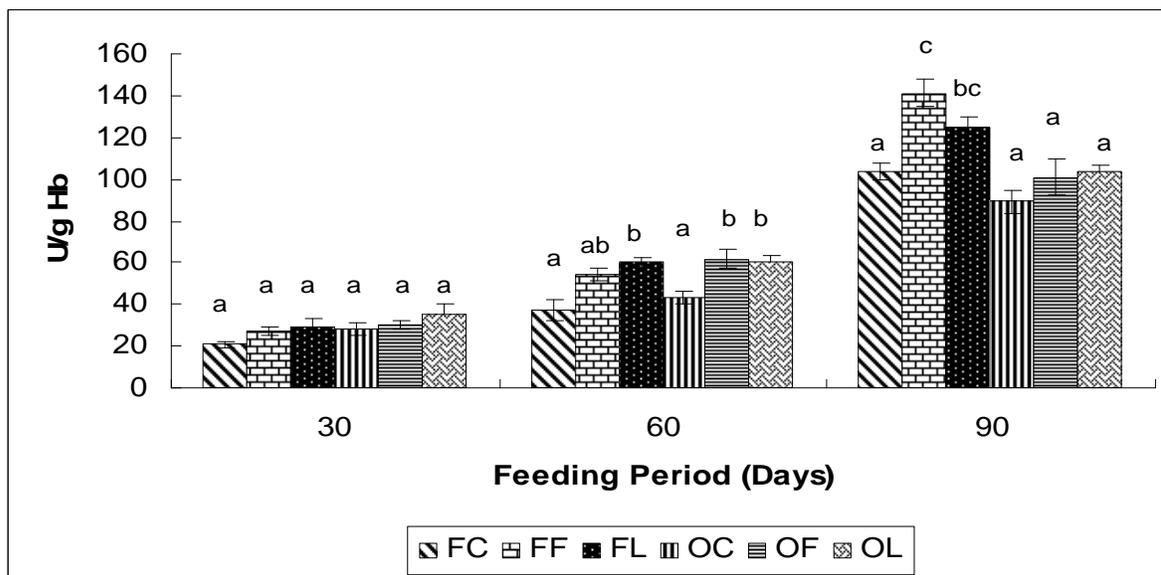


SOD activity is expressed in U/g Hb.

1 Unit (U) is equivalent to the amount of enzyme that inhibits the reaction by 50 per cent.

The values expressed as means \pm SEM for 6 rats per group. The values with the same symbols are statistically at par within a row whereas; those with different symbols are significantly different at $P < 0.01$.

Fig.3 Glutathione peroxidase activity in RBC lysate of rats fed on fresh or oxidized oil supplemented with skim milk / fermented milk / lyophilized culture



Glutathione peroxidase (GPx) activity is expressed in U/g Hb.

1 Unit (U) is equivalent to 1 μ mol NADPH oxidized per min.

The values expressed as means \pm SEM for 6 rats per group. The values with the same symbols are statistically at par within a row whereas, those with different symbols are significantly different at $P < 0.001$.

Table.3 Levels of TBARS in RBC and liver of rats fed on fresh or oxidized oil supplemented with skim milk/fermented milk/ lyophilized probiotic culture

Groups	TBARS	
	RBC (nmoles/ml)	Liver (nmoles MDA/g tissue)
FC	9.083 ± 0.527 ^b	85.67 ± 4.71 ^b
FF	6.292 ± 0.637 ^{ab}	35.67 ± 6.43 ^a
FL	3.500 ± 0.376 ^a	40.33 ± 5.76 ^a
OC	9.708 ± 0.350 ^c	90.25 ± 6.83 ^b
OF	6.125 ± 0.601 ^a	47.67 ± 4.60 ^a
OL	7.417 ± 0.455 ^{bc}	49.83 ± 5.56 ^a

The values expressed as means ± SEM for 6 rats per group. The values with the same symbols are statistically at par within a row, whereas those with different symbols are significantly different at P < 0.05.

Table.4 Antioxidative enzyme activities in liver of rats fed on fresh or oxidized oil supplemented with skim milk/ lyophilized probiotic culture/fermented milk

Liver enzyme	GROUPS					
	FC	FF	FL	OC	OF	OL
Catalase # (U/mg protein)	414.79 ^a ±4.7	557.29 ^b ±16.7	767.70 ^c ±7.7	689.73 ^{bc} ±28.7	1007.33 ^d ±9.6	951.39 ^d ±28.0
SOD @ (U/mg protein)	133.73 ^a ±17.0	202.9 ^b ±29.1	140.1 ^{ab} ±17.6	110.0 ^a ±9.4	144.1 ^{ab} ±12.2	157.0 ^{ab} ±6.5
GSHPx * (KU/mg protein)	8.0 ^a ±0.7	7.0 ^a ±0.6	7.6 ^a ±0.4	6.54 ^a ±0.3	7.87 ^a ±0.7	7.58 ^a ±0.4

1 Unit (U) is equivalent to 1 µmole of H₂O₂ consumed per minute per milligram of protein at 25°C

@ 1 Unit (U) is equivalent to the amount of enzyme that inhibit the reaction by 50 per cent.

* 1 Kilo Unit (KU) is equivalent to 1 µmole NADPH oxidized per minute.

The values expressed as means ± SEM for 6 rats per group. The values with the same symbols are statistically at par within a row whereas, those with different symbols are significantly different at P < 0.01.

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